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Viral Co-infections are Common and are Associated with Higher Bacterial Burden in Children with *C. difficile* Infection

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Abstract

Clostridium difficile infections in children are increasing. In this cohort study, we enrolled 62 children with diarrhea and *C. difficile*. We performed polymerase chain reaction (PCR) assays to detect viral agents of gastroenteritis and quantify *C. difficile* burden. Fifteen (24%) children diagnosed with *C. difficile* infection had a concomitant viral co-infection. These patients tended to be younger and had a higher *C. difficile* bacterial burden than children with no viral co-infections (median difference=565 957cfu/mL p=0.011), but were clinically indistinguishable. The contribution of viral co-infection to *C. difficile* disease in children warrants future investigation.

Keywords

C. difficile; children; viral gastroenteritis; norovirus

INTRODUCTION

Clostridium difficile is the most common cause of healthcare-associated diarrhea in adults in the United States (1), and is a major cause of morbidity and mortality in adults (2, 3), and children (4, 5). Rates of *C. difficile* infection (CDI) in hospitalized children are increasing (6-9). However, *C. difficile* and its toxins can be detected in up to 70% of asymptomatic young children (10-12). Rates of positive *C. difficile* assays are similar in stools of asymptomatic young children and children with diarrhea (13, 14). Therefore, guidelines warn physicians that “detection of *C. difficile* toxin cannot be assumed to be the causative agent for diarrhea in children before adolescence, particularly young children” (15, 16).

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Bacterial fecal cultures are often performed in a child with diarrhea and concern for *C. difficile*; however, with the absence of easily available viral studies, rates of viral co-infections are still unknown and the relationship between *C. difficile* colonization, *C. difficile* infection, and viral infections is unknown. Previous case reports suggest that viral gastrointestinal infections may be common in children with CDI (17, 18), as do prospective studies assessing etiologies of diarrhea in children (13, 19). Similarly, in adults, *C. difficile* has been detected with increased frequency during confirmed viral gastroenteritis outbreaks (20, 21). The contribution of viral co-infections to *C. difficile* disease severity and outcome has not been addressed.

We conducted this cohort study to identify the rates of viral co-infections in children diagnosed with CDI, and recognize any potential differentiating clinical features or differences in outcomes between children with and without viral co-infections. We hypothesized that viral co-infections are common in children with CDI and are associated with a more severe presentation.

MATERIALS AND METHODS

STUDY POPULATION

We conducted this prospective cohort study in St Louis Children's Hospital (SLCH), St. Louis, MO, and obtained approval from the Institutional Review Board of the Washington University School of Medicine. We approached all children with diarrhea and a positive *C. difficile* polymerase chain reaction (PCR) from the microbiology laboratory from 1 July 2011 to 5 July 2012, and obtained written informed consent from their caregivers.

CLINICAL DATA

We collected demographic and clinical data regarding the diarrhea at time of diagnosis (onset, number of bowel movements per day, stool consistency according to the Bristol stool chart (22), pain (on a scale of 1-10), nausea/vomiting, and laboratory findings). We also recorded outcomes including time to diarrhea resolution, diarrhea persistence at 5 days of therapy, intensive care unit admissions, severe disease (defined as white blood cell count (WBC) 15000 cells/ μ L or serum creatinine 1.5 times baseline (23)), severe complicated (defined as hypotension, shock, ileus or megacolon (23)), and documented recurrence on all our patients.

STOOL COLLECTION

Laboratory personnel immediately stored stool samples from children with positive *C. difficile* PCR at -80°C .

LABORATORY ASSAYS performed on all samples

Nucleic Acid Extraction—We used the NucliSENS® EasyMAG™ automated system software 1.0.2 specific A protocol (bioMérieux, Marcy L'Etoile, France), to extract total nucleic acid according to the manufacturer's instructions. 200 μ L of stool eluate was added to 2 mL of lysis buffer, followed by 100 μ L of magnetic silica for a final volume of 110 μ L of nucleic acid extract.

Polymerase Chain Reactions—All PCRs were performed using 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA).

Viral Gastroenteritis PCR: We performed multiplex TaqMan real-time reverse-transcription polymerase chain reactions (RT-PCR) for: norovirus genogroups 1 and 2, sapovirus, astrovirus, adenovirus group F, and rotavirus. Primers, probes and PCR conditions were based on those published previously by Grant *et al.* (24) and Freeman *et al.* (25). Positive and negative controls were included in each run.

Quantitative *tcdB* DNA PCR: We performed SYBR Green-based real-time *tcdB* PCR as described by Wroblewski *et al* (26), with slight modification (27). We validated the linearity of the *tcdB* PCR using *tcdB* cloned into plasmid pHIS1525 (MoBiTec Inc., Boca Raton, FL) as a DNA standard. Linear regression allowed calculations of *C. difficile* concentration ($\text{cfu/mL} = 7000 \times 10^{(\text{CT}-32.4)/-5.2}$) (data not shown).

STATISTICAL ANALYSIS

We used non-parametric Wilcoxon rank-sum test to compare the two groups, and Chi-square test and, when appropriate, Fisher's exact test, for categorical data comparison. We used log rank test to compare time to diarrhea resolution in cases with viral co-infections and those without. A two-tailed *p* value of <0.05 was considered significant.

RESULTS

Of 74 patients identified with a positive *C. difficile* PCR, seven had no available stools and families of two were unreachable. Of the 65 patients we approached, 64 were consented and enrolled. Two children were excluded because our *tcdB* PCR was negative while the microbiology laboratory's *C. difficile* PCR was positive. Our final cohort included 62 children.

Subjects had a median age of 10.3y (IQR= 3.3-14.9). Age distribution included one (2%) infant <1y, 11 (18%) patients 1-3y, and 50 (81%) patients >3y of age 28. 28 (45%) patients were males. 37 (60%) were on immunosuppressives at the time of diagnosis (27 (43%) were on steroids, and 35 (56%) on chemotherapeutic agents). 57 (92%) had received antibiotics within the 90days prior to admission. 24 (39%) patients had a malignancy, solid organ transplantation, or a bone marrow transplantation, 8 (13%) had a diagnosis of inflammatory bowel diseases. Enrollment per season was as follows: 14 children in the summer, 18 children in the fall, 17 children in the winter, and 13 children in the spring. 39 (63%) children had a concomitant fecal bacterial culture performed.

Of the 62 children with positive *C. difficile* testing, 55 (89%) were initially treated for CDI and 7 (11%) were not. Age did not differ between children who received CDI therapy and those who did not (median 10.2y compared to 10.7y (*p*=0.81)). Median time from diarrhea onset to diagnosis was 3d.

We found a virus capable of causing gastroenteritis in 15 (24%) children. Norovirus genogroup 2 was the most common virus encountered concomitantly with *C. difficile* (10

(16%) children), followed by sapovirus (3 (5%) children). Norovirus genogroup 1 and astrovirus were identified in one case each. We did not find any rotavirus or enteric adenovirus in our cohort. Two (29%) of the seven untreated children had a concomitant viral co-infection.

We attempted to identify clinical features differentiating children with *C. difficile* and viral co-infections from those without viral co-infections. The two groups of patients were clinically indistinguishable, and their median time to diarrhea resolution on CDI therapy was 3 days, regardless of the viral co-infection status (**Table**). We found no differences in severity or outcomes between the two groups (**Table**). *C. difficile* bacterial burden was significantly higher in patients with viral co-infections (*C. difficile* concentration median difference=565 957 cfu/mL, $p=0.011$) (**Figure**).

Although patients with *C. difficile* and viral co-infections tended to be younger (median 3.9y compared to 10.7y, $p=0.13$), the high rate of viral co-infections was not specific to the younger age-group. When we excluded children <3y of age, traditionally described to have high levels of *C. difficile* colonization, 11 (22%) of 50 older children had a viral co-infection. Treating physicians were unlikely to differentiate between the two groups: 13 (87%) cases with viral co-infections were treated for CDI, compared to 42 (89%) cases with no viral co-infections. When patients who did not receive therapy were excluded, 13 (24%) of the 55 patients diagnosed and treated for CDI had a viral co-infection.

DISCUSSION

Though concomitant *C. difficile* and viral infections has been described previously (18, 19), no study has systematically investigated the rate of viral co-infections in CDI or compared their clinical presentation to children without viral co-infections. In our cohort, we found that 24% of children diagnosed with CDI have a concomitant viral gastroenteritis. This finding raises questions regarding the pathogenicity of *C. difficile* in children and the role viruses play in this setting. Viruses may predispose children to develop CDI or vice-versa, or there may be features of the host, such as state of the mucosal immune response or the intestinal microbiome, that predispose to both infections.

The current study confirms our previous findings that *C. difficile* bacterial burden does not correlate with clinical presentations or outcome measures in CDI (27). Children with viral co-infections had higher fecal *C. difficile* concentrations than those without co-infections; however, their clinical illnesses were similar. It has been postulated that viral gastroenteritis exacerbates the effects of *C. difficile* because of alteration of intestinal epithelial homeostasis (18). The observation of higher *C. difficile* fecal bacterial burden in children with viral co-infection suggests that viruses may create favorable conditions for *C. difficile* to multiply and potentially cause disease, possibly through perturbation of the intestinal microbiota or innate host defenses. Indeed, viral gastroenteritis is associated with decreased intestinal microbial diversity and a shift in relative abundance of bacterial phyla, similar to that seen with antibiotic exposure, the major risk factor for *C. difficile* infection (28). Alternatively, virus-induced changes in stool composition may occur affecting the *tcdB* PCR

results, thus increasing the rate of *C. difficile* detection, and leading to an increased diagnosis of CDI in children with viral gastroenteritis.

Nosocomial outbreaks of norovirus originally attributed to *C. difficile* raised the possibility that *C. difficile* may be a colonizer or an innocent bystander (20, 21). It is possible that children with viral gastroenteritis are misdiagnosed with CDI and do not in fact require CDI therapy. An association between viral agents of gastroenteritis and CDI rather than a misdiagnosis of CDI in the setting of viral gastroenteritis may also be possible. An alternate possibility is that asymptomatic *C. difficile* carriers are more likely to develop viral gastroenteritis. A longitudinal case control study following children colonized with *C. difficile* for acquisition of viral gastroenteritis may address this question.

A limitation of our study is the lack of a longitudinal study design. Such a study following children prior to *C. difficile* acquisition would allow delineation of the timing of *C. difficile* and viral acquisition in relation to symptom onset. We cannot therefore generate a clear cause-and-effect hypothesis. It is possible that viral gastroenteritis leads to a misdiagnosis and overtreatment of CDI. It is also possible that viral gastroenteritis may predispose children colonized with *C. difficile* toward the development of symptomatic *C. difficile* infection.

In summary, we found that rates of viral co-infections in stools of children diagnosed and treated for *C. difficile* infections are high, and are associated with an elevated *C. difficile* fecal bacterial burden, suggesting an interaction between viral agents of gastroenteritis and *C. difficile*. With the development of new multiplexed nucleic acid-based technologies for diagnosis of gastroenteritis, dual identification of *C. difficile* and a viral pathogen is likely to be a common finding. Management of these children will be challenging. Identification of host-derived biomarkers that are specific for *C. difficile* may help clarify which of these children are likely to benefit from *C. difficile* targeted therapy. Longitudinal studies are needed to clarify the role of viral infection on the progression from asymptomatic colonization to *C. difficile*-associated disease.

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FIGURE. Bacterial burden in 15 children with *C. difficile* and viral co-infections compared to 47 children with *C. difficile* and no viral co-infections

A. Children with *C. difficile* and no viral co-infection (upward triangle) had a higher *tcdB* cycle threshold (CT) value than those with viral co-infections (downward triangle).

B. Children with *C. difficile* and no viral co-infection (upward triangle) had a lower *C. difficile* concentration than those with viral co-infections (downward triangle).

Each triangle represents an individual patient's value. Bars represent medians and interquartile ranges. *p value of the two-tailed Wilcoxon rank-sum test.

TABLE

Characteristics of 16 patients with a positive *C. difficile* assay and viral co-infections compared to 49 patients with no viral co-infections.

	<i>C. difficile</i> +, virus + n=15	<i>C. difficile</i> +, no virus n=47	P value
Age in years, median (IQR)	3.9 (2.3-12.4)	10.7 (5.5-15.7)	0.13 *
Male, n (%)	4 (37)	24 (51)	0.14 †
Number of stools/day, ¶ median (IQR)	6 (4-8)	5 (3-10)	0.75 *
Bristol score, ¶ median (IQR)	7 (7-7)	7 (6-7)	0.11 *
Pain on a scale of 1-10, ¶ median (IQR)	5 (3-7)	5 (2-8)	0.94 *
Maximum temperature in °Celsius, median (IQR)	37.2 (37-37.6)	37.4 (37-38.2)	0.28 *
Nausea/ vomiting, n (%)	11 (73)	27 (57)	0.36 †
WBC in 10 ³ cells/µL, median (IQR)	4.3 (1.2-10.6)	5.7 (0.7-13.9)	0.93 *
Serum creatinine 1.5 times baseline, n(%)	1 (7)	8 (17)	0.43 †
Severe (WBC 15000 cells/µL or creatinine 1.5 times premorbid level) n(%)	2 (13)	4 (8)	0.65 †
Severe complicated (hypotension, shock, ileus or megacolon) n(%)	0 (0)	2 (4)	1.0 †
Treated for CDI, n (%)	13 (81)	42 (86)	0.7 †
Treated with vancomycin, n (%)	4/13 (31)	19/42 (45)	0.52 †
Time to diarrhea resolution in days, median (IQR)	3 (3-6)	3 (2-9)	0.56 ‡
Diarrhea persistence at 5days of therapy, n (%)	6/12 (50)	16/42 (38)	0.52 †
Intensive care unit admission, n (%)	1 (7)	8 (17)	0.43 †
Documented recurrence, n (%)	1 (7)	8 (17)	0.43 †
<i>C. difficile</i> concentration (cfu/mL), median (IQR)	661291 (243223-1352208)	95334 (9775,448486)	0.011 *

IQR= interquartile range; CDI= *Clostridium difficile* infection; cfu= colony forming unit; WBC= white blood cell count

* p value of a two-tailed Wilcoxon rank-sum test

† p value of Fisher's exact test

‡ p value of the log rank test

¶ On the day of diagnosis