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Distribution and risk factor analysis for *Clostridium difficile*-associated diarrhea among hospitalized children over one year of age

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ABSTRACT

Importance: *Clostridium difficile*-associated diarrhea (CDAD) is a severe type of antibiotic-associated diarrhea (AAD). However, the risk factors for CDAD in children with AAD have not yet been clarified.

Objective: To investigate the distribution and risk factors for CDAD among hospitalized children in Beijing Children's Hospital.

Methods: Stool samples from 197 children with AAD were tested for the *C. difficile* pathogenic genes (*tcdA, tcdB, tcdC, tcdD, tcdE, cdtA,* and *cdtB*) using polymerase chain reaction between January 2011 and January 2014. Children who tested positive for *tcdA* or *tcdB* were included in the CDAD group, and those remaining comprised the non-CDAD group.

Results: The rate of CDAD among the 197 children with AAD was 42.6% (84/197). The age distribution was 1–15.6 years, among which the majority of children (54.8%, 46/84) were aged 1–4 years. Differences in the CDAD-positive rates among AAD children belonging to different age groups were not statistically significant. Univariate analysis revealed that the duration of antibiotic therapy, the length of hospitalization prior to diarrhea, and gastrointestinal tract operations were significant risk factors ($P < 0.05$). Children with CDAD underwent more antibiotic therapy and had longer periods of hospitalization prior to diarrhea onset than children in the non-CDAD group. Using multivariate regression analysis, hospitalization for ≥ 10 days prior to diarrhea was found to be an independent risk factor for CDAD.

Interpretation: This study revealed that the length of hospitalization (≥ 10 days) prior to diarrhea was an independent risk factor for CDAD in children with AAD.

KEYWORDS
Antibiotics, Children, *Clostridium difficile*, Diarrhea

INTRODUCTION

Clostridium difficile is an anaerobic, Gram-positive, spore-forming bacillus. It is an opportunistic pathogen, and it is the most common cause of antibiotic-associated diarrhea (AAD).[1] Approximately 15%–25% of AAD cases and over 95% of pseudomembranous colitis cases are caused by *C. difficile*.[2] When the intestinal environment is destroyed (such as following the use of antibiotics), colonized or orally ingested *C. difficile* can multiply significantly and release toxins including toxin A, toxin B, and binary toxin, thereby causing acute inflammation of the gastrointestinal (GI) tract and leading to *Clostridium difficile*-associated diarrhea (CDAD). The pathogenic genes of *C. difficile*

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include toxin A gene (*tcdA*), toxin B gene (*tcdB*), accessory genes (*tcdC*, *tcdD*, and *tcdE*), and binary toxin genes (*cdtA* and *cdtB*).[2]

The severity of CDAD varies from mild to severe, possibly leading to pseudomembranous colitis, toxic megacolon, bowel perforation, multiple organ failure, and even death.[3,4] With widespread antibiotic use, CDAD incidence in adults and children is increasing and gaining more attention from healthcare providers.

Studies on CDAD have been carried out in many countries, but documentation of CDAD in children is rare. In this study, we performed *C. difficile* toxin gene tests among children with AAD at Beijing Children's Hospital between January 2011 and January 2014 and aimed to identify the risk factors associated with CDAD to provide a basis for *C. difficile* detection and prevention in China.

METHODS

Ethical approval

This study was approved by the Ethics Committee of Beijing Children's Hospital. Informed consent to participate in the study and for publication was obtained from the children's guardians. [Correction added on 17 March 2020, after first online publication. The Ethical approval section was added.]

Sample size

According to the literature, the incidence of CDAD among hospitalized patients was 3.25/10 000–12.80/10 000.[5,6] Considering the severity of the disease and the high antibiotic use in hospitalized children in our hospital, we assumed the incidence of CDAD among hospitalized children at Beijing Children's Hospital would be 12/10 000. OpenEpi (http://www.openepi.com) was used to calculate the sample size. Based on a 95% confidence interval (*CI*) for the estimates: Sample size $n = [DEFF \times Np(1-p)] / [(d^2 / Z^2_{1-a/2} \times (N-1) + p \times (1-p)]$. A sample size of 163 was required, but to allow for contingency we increased this by over 20%, to a total of 197.

Study subjects

This was a retrospective study. AAD refers to diarrhea that cannot be explained by other causes than the use of antibiotics.[7] Based on previously established AAD diagnostic guidelines,[8] the inclusion criteria were: children with diarrhea (> 3 times/day, and lasting for more than 2 days), who were treated with antibiotics during or within a month before diarrhea onset.

Currently, there is no clear distinction for *C. difficile* positive stool specimens of children under 1 year of age between colonization and infection, so cases in children less than 1 year of age were ruled out. Fecal specimens from the same child for repeated examination or positive detection of other bacteria (such as *Shigella, Salmonella, Escherichia coli, Proteus, Staphylococcus aureus*) or virus (such as rotavirus) were also ruled out. In addition, abnormal anatomy, inappropriate diet, or diarrhea caused by other drugs were ruled out.

According to the diagnosis, treatment, and prevention guidelines for *C. difficile* infections released in 2013 in the United States,[9] CDAD is defined as the acute onset of diarrhea with documented toxigenic *C. difficile* or its toxin when there is no other documented cause for diarrhea.

Molecular methods

Bacterial genomic DNA was extracted from the stool samples of the 197 children using a QIAamp DNA Stool Mini Kit (Qiagen, Duesseldorf, Germany). Each sample was tested for toxins (*tcdA*, *tcdB*, *cdtA*, and *cdtB*) using polymerase chain reaction (PCR). Samples found to be positive for any of the aforementioned toxin genes were further tested for the presence of *tcdC*, *tcdD*, and *tcdE* genes. PCR primers and reaction conditions were used in accordance with previous reports by Herrera-Cáceres et al,[10] Spigaglia et al,[11] and Terhes et al.[12] Cases that tested positive for the *tcdA* or *tcdB* genes by PCR were considered to be CDAD and that tested negative were considered non-CDAD.

Statistical analyses

Statistical analyses were performed with the SPSS19.0 package (SPSS Inc., Chicago, IL, USA). The normal quantitative data were expressed as the mean ± standard deviation (SD), and between-group comparisons were performed using the Student's *t*-test. The non-normal quantitative data were expressed as the median (interquartile range), and between-group comparisons were performed using the rank sum test. Numerical data were expressed as the number of cases (%), and the between-group comparisons were performed using the Chi-square test. Multivariate logistic regression results were expressed as the odds ratio (*OR*) and the 95% *CI*. $P < 0.05$ was considered statistically significant.

RESULTS

Toxin gene test results

In total, 197 children with AAD treated at Beijing Children's Hospital were enrolled in this study between January 2011 and January 2014. Among these children, 42.6% (84/197) were positive for CDAD, including 34 cases that were both *tcdA* and *tcdB* positive (A+B+), 39 cases that were *tcdA* negative and *tcdB* positive (A−B+), and 11 cases that were *tcdA* positive and *tcdB* negative (A+B−). No samples were positive for *cdtA* or *cdtB*. Among the 84 positive samples, 42, 42, and 38 were positive for *tcdC*, *tcdD*, and *tcdE*, respectively.

Clinical characteristics of patients with CDAD

Among the 197 children with AAD, the positive detection rate of CDAD was 47.9% (34/71) in early childhood (1–3 years), 40.0% (18/45) in preschool children (3–6 years), and 42.0% (34/81) in school age children (> 6 years). There was no significant difference in the positive detection rate of CDAD among different age groups (P = 0.740) (Table 1).

TABLE 1 Comparison of the demographic and clinical characteristics of the 197 children with AAD

Variables	CDAD group (n = 84)	Non-CDAD group (n = 113)	Statistics	P
Male/female, n	50/34	65/48	0.079*	0.778
Age (years)	3.6 (1.8–8.4)	4.7 (2.3–9.2)	−1.203†	0.229
1–≤2	22 (26.2)	24 (21.2)	1.253*	0.740
2–≤3	12 (14.3)	13 (11.5)		
3–≤6	18 (21.4)	27 (23.9)		
>6	32 (38.1)	49 (43.4)		
Duration of diarrhea (days)	7.5 (4.0–17.8)	9.0 (5.0–16.0)	−0.538†	0.591
<14	58 (69.0)	81 (71.7)	4.601*	0.100
14–60	25 (29.8)	25 (22.1)		
>60	1 (1.2)	7 (6.2)		
Length of hospital stay (days) prior to diarrhea onset	17.0 (8.5–26.8)	7.0 (3.0–12.0)	−6.901†	<0.001
<10	22 (26.2)	79 (69.9)	36.865*	<0.001
≥10	62 (73.8)	34 (30.1)		
Severe pre-existing diseases	47 (56.0)	70 (61.9)	0.718*	0.397
White blood cell counts ($\times 10^9$/L)	5.0 (1.1–9.8)	4.4 (1.2–9.6)	−0.514†	0.607
<4	35 (41.7)	54 (47.8)	0.756*	0.685
4–10	29 (34.5)	34 (30.1)		
>10	20 (23.8)	25 (22.1)		
Hemoglobin (g/L)	101.4 ± 19.9	102.2 ± 23.3	−0.260‡	0.795
30–≤60	1 (1.2)	1 (0.9)	0.189*	0.664
60–≤90	28 (33.4)	39 (34.5)		
90–≤120	38 (45.2)	44 (38.9)		
>120	17 (20.2)	29 (25.7)		
Albumin (g/L)	34.5 (30.6–39.9)	35.4 (29.2–39.8)	−0.100†	0.920
<35	44 (52.4)	53 (46.9)	0.579*	0.447
≥35	40 (47.6)	60 (53.1)		

Data are presented as median (interquartile range) or n (%) or mean ± SD. *χ^2; †Z; ‡ t value. AAD, antibiotic-associated diarrhea; CDAD, *Clostridium difficile*-associated diarrhea.

Among the 84 children with CDAD, 50 were boys and 34 were girls, aged between 1.1–15.6 years. The positive detection rate of CDAD was 43.5% (50/115) in boys and 41.5% (34/82) in girls, which showed no significant difference (P = 0.778) (Table 1). The distribution of *C. difficile* toxins showed no statistically significant correlation with sex or age group (P = 0.912, P = 0.125, respectively).

The severity of CDAD among the 84 cases differed, with bowel movement frequency ranging from between 3 and 22 times/day. Stool characteristics were as follows: watery stools, 30 cases (35.7%); pasty stools, 32 cases (38.1%); mucus-like stools with yellow/green color, 14 cases (16.7%); and blood/pus in stools or mucus-like blood/pus in stools, 8 cases (9.5%). There were 58 cases (69.0%) with acute diarrhea, 25 cases (29.8%) with persistent diarrhea, and 1 case (1.2%) with chronic diarrhea. In total, 58 (69.0%) children had a fever with a body temperature ranging between 37.5°C and 40.2°C, 11 (13.1%) presented with abdominal pain, 8 (9.5%) with nausea/vomiting, and 4 (4.8%) with abdominal distention. The diarrhea lasted from 2 to 90 days, with a median of 7.5 (4.0–17.8) days. The duration of antibiotic therapy prior to diarrhea onset was between 2 and 35 days, with a median of 9 (6–14.8) days. Among the cases, 39 (46.4%) were receiving single antibiotic treatment and 45 (53.6%) were receiving multiple antibiotic treatments, including 38 cases receiving a combination with two types of antibiotics, seven receiving a combination of three types of antibiotics, 42 receiving cephalosporins combined with other types, one receiving macrolides combined with sulfonamides, and two receiving carbapenems combined with glycopeptides.

Univariate analysis

Among all of the variables, 15 variables were analyzed using the Chi-square test: sex, severe pre-existing conditions (including tumors, immunodeficiency, organ transplantation, inflammatory bowel diseases), the use of chemotherapy drugs, glucocorticoids, antacid medications, combined antibiotic therapy before the onset of diarrhea, numbers of antibiotics used, types of antibiotics (cephalosporins, penicillins, macrocyclic lactones, carbapenems, sulfonamides, glycopeptides, and nitro imidazoles), and GI tract operations (including invasive GI intubation and GI surgery). Seven other variables, including duration (in days) of antibiotic therapy, length of hospital stay before diarrhea, duration of diarrhea, age, white blood cell count, hemoglobin levels, and serum albumin levels, were analyzed using the rank sum test and the t test. Some variables were subdivided into groups and analyzed using the Chi-square test (Tables 1, 2).

As a result, three risk factors were identified: GI tract operation (P = 0.035), duration of antibiotic therapy ≥ 10 days prior to diarrhea onset (P = 0.029), and the length of hospital stay ≥ 10 days prior to diarrhea (P < 0.001).

Children with CDAD had longer durations of antibiotic therapy and longer hospital stays before diarrhea than non-CDAD children. The median duration of antibiotic therapy before diarrhea was 9 (6–14.8) days in children with CDAD and 6 (3–12) days in non-CDAD children. The median duration of the hospital stay was 17 (8.5–26.8) days in children with CDAD and 7 (3–12) days in non-CDAD children. There were no significant differences ($P > 0.05$ for all variables) between groups in terms of sex, age, severe pre-existing conditions, the use of chemotherapy, glucocorticoids and antacid medicines, the types and dosages of the antibiotics, the duration of diarrhea, white blood cell count, hemoglobin levels, and serum albumin levels (Tables 1, 2).

TABLE 2 Characteristics of the treatment of the 197 children with AAD

Variables	CDAD group ($n = 84$)	Non-CDAD group ($n = 113$)	Statistics	P
Duration of antibiotic therapy (days) prior to diarrhea onset	9.0 (6.0–14.8)	6.0 (3.0–12.0)	−3.307*	0.001
<10	46 (54.8)	79 (69.9)	4.769†	0.029
≥10	38 (45.2)	34 (30.1)		
Combined antibiotic therapy	45 (53.6)	54 (47.8)	0.645†	0.422
Types of antibiotics				
Cephalosporins	70 (83.3)	93 (82.3)	0.036†	0.850
Penicillins	2 (2.4)	3 (2.7)	0.015†	0.904
Macrocyclic lactones	21 (25.0)	24 (21.2)	0.387†	0.534
Carbapenems	14 (16.7)	17 (15.0)	0.096†	0.757
Sulfonamides	31 (36.9)	32 (28.3)	1.633†	0.201
Glycopeptides	11 (13.1)	16 (14.2)	0.046†	0.830
Nitroimidazoles	1 (1.2)	3 (2.7)	0.044†	0.834
Numbers of antibiotics used				
1	32 (38.1)	40 (35.4)	1.440†	0.487
2–3	40 (47.6)	62 (54.9)		
≥4	12 (14.3)	11 (9.7)		
Chemotherapy drugs	51 (60.7)	54 (47.8)	3.235†	0.072
Glutocorticoids	49 (58.3)	54 (47.8)	2.148†	0.143
Antacid medicine	28 (33.3)	35 (31.0)	0.123†	0.725
GI tract operation	21 (25.0)	15 (13.3)	4.436†	0.035

Note: Data are presented as median (interquartile range) or n (%). *Z value; †χ^2 value. AAD, antibiotic-associated diarrhea; CDAD, *Clostridium difficile*-associated diarrhea.

Multivariate analysis

The three significant variables identified in the univariate analysis (duration of antibiotic therapy ≥ 10 days prior to diarrhea, length of hospital stay ≥ 10 days prior to diarrhea, and GI tract operations) were included in multivariate logistic regression analysis. As shown in Table 3, a length of hospital stay ≥ 10 days prior to diarrhea was an independent risk factor for CDAD ($P < 0.001$).

DISCUSSION

CDAD is a severe type of AAD. The proportion of CDAD cases among AAD patients is reported to be 15%–25%.[2] With the widespread prescription of antibiotics, the frequency of CDAD is increasing. According to the results reported by Zilberberg et al,[5] in 3739 hospitals in the United States, the CDAD frequency among hospitalized children increased from 7.24/10 000 in 1997 to 12.80/10 000 in 2006, with an annual rate of increase of 9%. The number of CDAD cases in Korean children increased from 30 in 2008 to 96 in 2011, equivalent to an increase from 0.5/100 000 to 1.7/100 000.[13] Recent epidemiological studies of CDAD in Shanghai and Guangzhou in China reported only a few scattered cases. Wang et al[14] studied 283 and 216 inpatients from different wards in the same pediatric hospital and found that the positive rates of CDAD were 37.8% and 27.8%, respectively. A study by Cheng et al[15] showed that the positive detection rate of CDAD was 22.2% (128/577) among 577 children with AAD. Our study showed that the CDAD positive detection rate among children with AAD was 42.6% (84/197), which was higher than that reported by previous studies. This may be related to the different prevalence of CDAD in different regions or the different detection methods used by laboratories.

The symptoms associated with *C. difficile* infection are mainly caused by the release of the A, B, and binary CDT toxins. Toxin A is an enterotoxin that can cause a large amount of intestinal fluid secretion. Toxin B is a cytotoxin that can directly damage the cells of the intestinal wall and cause degeneration and necrosis of intestinal mucosa cells. Toxins A and B are encoded by genes *tcdA* and *tcdB*, which together with the genes *tcdC*, *tcdD*, and *tcdE*, form the pathogenicity locus (PaLoc) on the *C. difficile* chromosome, which is 19.b kb in length.[2,16,17] The binary toxins CDTa and CDTb are encoded by the *cdtA* and *cdtB* genes, which are located outside of the PaLoc. Among the 84 CDAD children in the present study, there were 34 cases positive for both toxins A and B (A+B+), 39 A−B+ cases, and 11 A+B− cases, none of the cases were positive for cdtA or cdtB. Among the 84 positive cases, 42 (50.0%), 42 (50.0%), and 38 (45.2%) cases were positive for *tcdC*, *tcdD*, and *tcdE*, respectively. In this study, it was found that A+B+ strains of *C. difficile* were predominant in 2011 and 2013, whereas A−B+ strains were predominant in 2012, indicating the different prevalence of epidemic strains of *C. difficile* over time in the same region. The reported prevalence of A−B+ strains differs between

TABLE 3 Multivariate logistic regression analysis for CDAD risk factors

Variables	*B*	SE	Wald	*P*	*OR*	95%*CI*
Length of hospital stay ≥10 days prior to diarrhea onset	2.218	0.408	29.621	<0.001	9.192	4.135–20.434
Duration of antibiotic therapy ≥10 days prior to the diarrhea onset	−0.600	0.412	2.119	0.145	0.549	0.245–1.231
GI tract operation	0.633	0.418	2.292	0.130	1.884	0.830–4.277

CDAD, *Clostridium difficile*-associated diarrhea; GI, Gastrointestinal; SE, Standard error; *OR*, Odds ratio; *CI*, Confidence interval.

studies. According to Kuijper et al,[18] 27 cases of CDAD occurred in a hospital with 800 beds in the Netherlands, of which 24 (88.9%) were A−B+ strains. According to Sato et al,[19] 10 cases of CDAD that occurred in a Japanese cancer hospital were A−B+ strains, accounting for 66.7% (10/15). In this study, among the 46 children with CDAD in 2012, 37 (80.4%) were infected with A−B+ strains, similar to previous reports in the literature. In 2011, 11 of the 25 children with CDAD were infected with A+B− strains. To ensure the accuracy of the test, each specimen was tested repeatedly and sequenced. Among the 11 children infected with strain A+B−, the stool characteristics mainly showed watery stools, with seven such cases, along with four cases of pasty stools, and no cases of mucopurulent bloody stools, and the clinical symptoms were consistent with the characteristics of toxin A.

It is currently accepted that there are three CDAD risk factors: patient factors (immunological status and pre-existing conditions), treatment factors (long-term hospital stay and long-term medical interventions), and GI tract microbiota disturbance (caused by antibiotic therapy or other medications, as well as the GI tract operations).[20] The use of broad spectrum antibiotics is considered a high risk factor for CDAD. Almost all antibiotics can potentially lead to CDAD, in particular, clindamycin, penicillin, cephalosporins, and fluoroquinolones are high risk factors.[20] The long-term use and combination of antibiotics are positively correlated with the risk of CDAD.[21] Other risk factors for CDAD include severe pre-existing conditions (tumors, immunodeficiency, organ transplantation, and inflammatory bowel diseases), GI tract operations (gastric intubation, jejunal intubation, and GI tract surgery), long-term hospital stays, antacid drug use (proton pump inhibitors and H_2 receptor inhibitors), and exposure to chemotherapy drugs and glucocorticoids.[22-25] There are few studies on the risk factors of CDAD in China. Our study showed that long-term hospitalization before the onset of diarrhea was an independent risk factor for CDAD. Long-term hospitalization can also increase the chances of various hospital-associated infections, including those contracted by contact with patients that have CDAD, therefore, the risk of *C. difficile* infection increases along with the duration of hospitalization.

Antibiotic therapy, which is considered as the highest risk factor for CDAD, did not appear to be a significant factor among the findings of this study. There was no significant difference in the type or quantity of antibiotics between children with CDAD and those with AAD caused by other factors. Univariate analysis showed that the duration of antibiotic therapy before diarrhea was different between the two groups, but multivariate analysis showed that it was not an independent risk factor. It is worth considering that, on the one hand, the subjects of this study were all children with AAD, and antibiotics were the essential cause of AAD, therefore, antibiotic factors were not reflected in the comparison between CDAD and AAD. More risk factors may be found if children not undergoing antibiotic treatment were added to the control group. However, the majority of children in this study received less than three types of antibiotics, and it has been reported that the use of more than three antibiotics in hospitalized children is a risk factor for CDAD.[26] Therefore, further enlargement of the sample size is needed to explore the role of antibiotics, especially multiple antibiotics, in CDAD. Another limitation of this study was the lack of PCR-ribosomal typing of *C. difficile* strains.

In conclusion, this study showed that children with CDAD underwent more antibiotic therapy, had longer periods of hospitalization prior to the onset of diarrhea, and experienced a higher rate of GI tract operations than children in the non-CDAD group. Using multivariate regression analysis, a length of hospitalization ≥ 10 days prior to diarrhea was found to be an independent risk factor for CDAD.

CONFLICT OF INTEREST

None.

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