Risk Factors for Recurrent *Clostridium difficile* Infection in Children: A Nested Case-Control Study

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Objective To identify risk factors for recurrent *Clostridium difficile* infection (RCDI) in children.

Study design A nested case-control study was performed to identify RCDI risk factors using a pediatric cohort of inpatients and outpatients diagnosed with *Clostridium difficile* infection by *tcdB* polymerase chain reaction (PCR) at an academic children's hospital between December 9, 2012, and June 30, 2014. Strict inclusion criteria were adopted to limit selection bias related to inappropriate inclusion of patients with probable *C difficile* colonization.

Results Thirty children with RCDI were compared with 94 children with non-RCDI. Statistically significant associations were identified between RCDI and malignancy (OR 2.8, 95% CI 1.0-7.4, P = .044), tracheostomy tube dependence (OR 5.2, 95% CI 1.1-24.7, P = .037), and *tcdB* PCR cycle threshold (OR 0.87, 95% CI 0.78-0.97, P = .01) using multivariable logistic regression modeling. The receiver operator characteristic curve for PCR cycle threshold as a predictor of RCDI demonstrated area under the curve = 0.67. The highest predictive rate (75%) for RCDI was demonstrated at cycle threshold cutpoint ≤ 20. The difference between sensitivity (64%) and specificity (68%) was minimized at cycle threshold cutpoint ≤ 23. Compared with controls with non-RCDI, children excluded because of probable *C difficile* colonization had a similar cycle threshold value (27.5 vs 27.2, P = .77).

Conclusions Malignancy and tracheostomy tube dependence were identified as RCDI risk factors. Although RCDI was associated with positivity at a lower *tcdB* PCR cycle threshold, the clinical utility of cycle threshold as a tool to predict recurrence was limited. Better methods to predict RCDI are needed to prioritize pediatric populations to target for RCDI prevention efforts. *(J Pediatr 2015;167:384-9)*.

Clostridium difficile infection (CDI) is diagnosed increasingly among pediatric patients.[1-5] Recurrence complicates 12%-25% of pediatric CDI cases.[1,6,7] Risk factors for recurrent CDI (RCDI) in children have been recently described.[6,7] Previous studies have exclusively[7] or predominantly[6] focused on inpatients. However, recent population-based pediatric CDI studies suggest that community-associated CDI accounts for 65%-85% of CDIs in children, but only a minority of children with community-onset of CDI symptoms are hospitalized for management of CDI.[1,8]

Findings from epidemiologic studies describing pediatric CDI can be challenging to interpret because of high asymptomatic colonization rates among many groups of children, including infants and young children,[4] hospitalized children,[9] and children with cancer[10] or inflammatory bowel disease.[11] Use of highly sensitive polymerase chain reaction (PCR) testing[12] for CDI at the majority of US children's hospitals further complicates interpretation of positive *C difficile* testing in pediatric populations. Many US children's hospitals do not have policies in place to optimize testing strategies and CDI surveillance among populations of children who are more likely to be colonized than infected with *C difficile*.[13] Our preliminary data suggest that many children at our institution who test positive for *C difficile* by PCR may have other reasons for diarrhea, such as viral illness, concomitant use of laxatives and stool softeners, and underlying gastrointestinal conditions.[14] These features complicate reliable identification of children with symptomatic CDI and bias epidemiologic investigation of pediatric CDI. The objective of this study was to understand risk factors for RCDI in a combined inpatient/outpatient pediatric cohort with strict exclusion of patients testing positive for *C difficile* by PCR who have a strong probability of asymptomatic colonization and an alternate explanation for their CDI symptoms.

CDI	*Clostridium difficile* infection
PCR	Polymerase chain reaction
RCDI	Recurrent CDI

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Methods

We conducted a retrospective cohort study of all patients receiving care at Ann & Robert H. Lurie Children's Hospital of Chicago, a 288-bed university-affiliated hospital that provides tertiary care to children in the greater Chicago area. The Institutional Review Board at Lurie Children's waived informed consent for this study. The clinical microbiology laboratory restricts testing for toxigenic *C difficile* by the *tcdB* (toxin B gene) PCR Xpert *C difficile* assay (Cepheid, Sunnyvale, California)[15] to unformed stools obtained from children ≥12 months old and in whom a *tcdB* PCR test was not processed in the previous 7 days. Study patients included children who tested positive by *tcdB* PCR between December 9, 2012, and June 30, 2014. Laboratory-identified CDI episodes were identified through MedMined (Care Fusion, San Diego, California), an electronic infection surveillance tool. A clinical CDI, defined as a laboratory-identified CDI in a patient with documented diarrhea or ileus, was confirmed by manual review of patient symptoms from the electronic medical record. Although the clinical microbiology laboratory rejects formed stools, documentation of at least one diarrheal stool (or ileus) in the medical record by the healthcare provider was required to confirm a clinical CDI.

Once clinical CDIs were identified and the cohort was assembled, a nested case-control study was performed to identify risk factors for RCDI. Cases included all cohort patients with a clinical CDI who experienced a CDI recurrence. A CDI recurrence was defined as a subsequent clinical CDI occurring within 8 weeks of the day the previous CDI was diagnosed.[16] In patients with multiple recurrences, only the first CDI episode occurring during the cohort period was included. Three unmatched controls with their first episode of CDI per each case of RCDI were selected. Although RCDI is defined as that recurring within 8 weeks of a previous CDI, our prior work suggests that CDI relapse (ie, a subsequent CDI caused by the same strain as the previous) can occur up to several months after a previous CDI.[17] Thus, the currently accepted 8-week cutoff for defining RCDI may misidentify many RCDIs as new CDIs. Therefore, patients who experienced a second CDI within 8-20 weeks of their initial CDI were ineligible to serve as either a case or control and were excluded from the study entirely. Controls also were excluded if they had probable asymptomatic colonization with *C difficile*. Because of the high rate of *C difficile* colonization and near lack of clinical CDI in infants,[4] all children <12 months old were excluded. Controls also were excluded if they did not receive antibiotic therapy for CDI, if they were concomitantly receiving stool softeners or laxatives at the time of CDI diagnosis, or if they had a previous negative *tcdB* PCR test during the same episode of diarrhea. Testing for additional gastrointestinal pathogens was at the discretion of the healthcare provider, and controls were excluded if additional testing identified a concomitant bacterial, viral, or parasitic gastrointestinal pathogen. Finally, rates of *C difficile* resistance to metronidazole and vancomycin are quite low,[18] and clinical response rates to metronidazole and vancomycin for mild or moderate CDI in adults are quite high.[19] Therefore, controls also were excluded if they failed to respond to CDI antibiotic therapy.

All clinical and demographic data were extracted manually from the electronic medical record. The medical records of all patients in the cohort were reviewed for at least 20 weeks following their CDI diagnosis. Potential risk factors included age, sex, comorbidities, proton pump inhibitor exposure within 7 days preceding CDI diagnosis, any inpatient or outpatient systemic antibiotic exposure within 30 days preceding CDI diagnosis, CDI antibiotic therapy, any additional non-CDI inpatient or outpatient systemic antibiotic exposure within 8 weeks following CDI diagnosis, and cycle threshold of the *tcdB* PCR assay. CDI cases were classified as hospital-onset healthcare facility-associated, community-onset healthcare facility-associated, indeterminate, community-associated, and recurrent using SDs.[16]

Continuous variables were summarized and reported as means and SDs. Categorical variables were summarized and reported as frequencies and percentages. PCR cycle threshold values of various groups were compared using the nonparametric Wilcoxon rank-sum test. ORs and 95% CIs were calculated using logistic regression analyses. All potential RCDI risk factors were assessed using bivariate logistic regression modeling. Using a model entry P value criterion of $P \leq .10$, potential risk factors were then entered into a multivariable logistic regression model. A manual stepwise selection procedure was used to determine a final model. To avoid potential multicollinearity between malignancy and immunocompromised status, immunocompromised status was assessed in a separate model using the same stepwise selection procedures. However, because all patients with malignancy were immunocompromised, we only report results from the malignancy model here. Because of significant variability in antibiotic exposure among patients (ie, specific antibiotics received, number of unique classes of antibiotics received, and timing/duration of antibiotic exposure), any antibiotic exposures within 30 days prior to CDI diagnosis and any antibiotic exposures within 8 weeks after CDI diagnosis were each collapsed into individual variables. These variables incorporating all antibiotic exposures during those time periods were eligible for inclusion in the multivariable regression model. Two-sided P values of <.05 were considered statistically significant. Analyses were performed using SAS v 9.4 (SAS Institute Inc, Cary, North Carolina).

Results

During the 18-month cohort period, the microbiology laboratory reported 292 positive *tcdB* PCR tests among 214 patients. Among these 214 patients, 30 (14%) experienced a CDI recurrence within 8 weeks. Ninety patients were excluded as controls, including 41 patients with probable *C difficile* colonization. After patient exclusion, the 94 patients