RESEARCH ARTICLE

Detection of *Clostridioides difficile* by Real-time PCR in Young Children Does Not Predict Disease

Barbara A. Pahud, MD, MPH,[a] Ferdaus Hassan, PhD,[a] Christopher J. Harrison, MD,[a] Natasha B. Halasa, MD,[b] James D. Chappell, MD,[b] Janet A. Englund, MD,[c] Eileen J. Klein, MD, MPH,[c] Peter G. Szilagyi, MD,[d] Geoffrey A. Weinberg, MD,[d] Ashley K. Sherman, MA,[a] Christopher Polage, MD, PhD,[e,f] Mary E. Wikswo, MPH,[g] L. Clifford McDonald, MD,[g] Daniel C. Payne, PhD, MSPH,[g] Rangaraj Selvarangan, BVSc, PhD[a]

ABSTRACT

OBJECTIVES: Diagnosing *Clostridioides difficile* infections in young children with high asymptomatic colonization is challenging. We compared the frequency of *C difficile* detection by polymerase chain reaction (PCR) in healthy control (HC) children with those with acute gastroenteritis (AGE) and evaluated fecal-lactoferrin and organism load as possible indicators of true *C difficile* infection disease.

METHODS: Stool was collected from children <2 years old with AGE and from HCs. *C difficile* was detected by real-time PCR, and lactoferrin was measured by enzyme-linked immunosorbent assay. Clinical data were obtained via interviews and chart review. Mann–Whitney *U* test and χ^2 tests were used for group comparisons.

RESULTS: Of 524 stools collected from 524 children (250 with AGE, 274 HCs), *C difficile* was detected less in children with AGE (14%, 36 of 250) than in HCs (28%, 76 of 274) stools ($P < .0001$). Among infants <1 year old ($n = 297$), *C difficile* was detected in 18% of children with AGE versus 32% of HCs ($P < .005$), and among children 1 to 2 years old ($n = 227$), *C difficile* was detected in 10% of children with AGE versus 21% of HCs ($P < .02$). There was no significant difference in *C difficile* PCR cycle threshold values between children with AGE and HCs or lactoferrin levels in *C difficile* PCR-positive versus -negative stools.

CONCLUSIONS: HC children <2 years of age had higher rates of *C difficile* detection by PCR than children with AGE; *C difficile* detection by real-time PCR alone is not a reliable means to diagnose *C difficile* disease in children <2 years old.

[a]*Children's Mercy Hospital Kansas City and University of Missouri, Kansas City, Kansas City, Missouri;* [b]*Department of Pediatrics, School of Medicine, Vanderbilt University, Nashville, Tennessee;* [c]*Department of Pediatrics, Seattle Children's Hospitals, Seattle, Washington;* [d]*School of Medicine and Dentistry, University of Rochester, Rochester, New York;* [e]*Department of Pathology and Laboratory Medicine, University of California–Davis Medical Center, Sacramento, California;* [f]*Clinical Microbiology Laboratory, Duke University Health System and Duke University, Durham, North Carolina; and* [g]*Centers for Disease Control and Prevention, Atlanta, Georgia*

www.hospitalpediatrics.org
DOI:https://doi.org/10.1542/hpeds.2020-0012

Address correspondence to Barbara A. Pahud, MD, MPH, Pediatric Infectious Diseases, Children's Mercy Hospital Kansas City, 2401 Gillham Rd, Kansas City, MO 64108. E-mail: bapahud@cmh.edu

HOSPITAL PEDIATRICS (ISSN Numbers: Print, 2154-1663; Online, 2154-1671).

FINANCIAL DISCLOSURE: Dr Pahud has been an investigator on clinical trials funded by GlaxoSmithKline and Alios Biopharma and Janssen. She has received honoraria from Pfizer, Sequirus, and Sanofi Pasteur for service on advisory boards and for nonbranded presentations. Dr Englund has received research support to her university from GlaxoSmithKline. Dr Halasa has received support in the form of vaccine donations and hemagglutination inhibition testing from Sanofi. Dr Harrison is an investigator on an Astellas-sponsored fidaxomicin research study and vaccine studies sponsored by GlaxoSmithKline and Pfizer for which his institution receives research grant funding. Dr Polage has received research grants from BioFire Diagnostics; consulting fees from BioFire Diagnostics, Singulex, Seres, Meridian Bioscience, and SlipChip; speaking fees from Medavera; and test materials from Cepheid, TechLab, and Alere; the other authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Supported by the Centers for Disease Control and Prevention (grant 5U01IP000460-02).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

Dr Pahud conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted; Dr Hassan participated in designing the study and drafting the initial manuscript and conducted and supervised all laboratory testing; Dr Harrison assisted with study design, data analysis, and manuscript revision; Ms Sherman analyzed the data and assisted with manuscript revision; Drs Englund and Klein participated in interpretation of the results and manuscript revision; Dr Payne assisted in the design of the study and participated in interpretation of the results, manuscript revision, and oversight of the network; Dr Selvarangan conceptualized and designed the study, supervised laboratory testing, and participated in interpretation of results, manuscript revision, and approval of manuscript; and all authors approved the final manuscript as submitted.

Clostridioides difficile, formerly "*Clostridium difficile*," manifests differently in children and adults. Although several risk factors for disease are similar (eg, antibiotic exposure, underlying malignancy),[1] asymptomatic detection, called colonization in this article (presence of pathogen in absence of disease), is more frequent in young children.[2,3] Epidemiological studies indicate high rates of *C difficile* detection in children <3 years old, ranging from 80% in neonates to 60% in infants <1 month old, decreasing to 10% in the second year of life, and reaching adult-like prevalence (0%–3%) by 3 years of age.[4–7] Young children are not widely thought to be as susceptible to true disease associated with *C difficile* (referred to as infection) in part because of lack of receptor for *C difficile* toxin,[8] making the clinical significance of detection an ongoing debate. Despite the general belief that most detections in young children primarily indicate colonization, some testing in young children continues in clinical settings. The literature has not clearly differentiated *C difficile* colonization from *C difficile*–caused diarrheal disease or infection.[2,9–11] The terminology "*C difficile* infection" has been used in published studies to represent colonization, disease associated with *C difficile*, or both.[11,12] More importantly, continued *C difficile* testing in young children, often as part of multiplex gastrointestinal pathogen panel polymerase chain reaction (PCR) assays, can lead to positive results for *C difficile*, leading to antibiotic treatment based on a test whose results have unclear clinical implications.

The American Academy of Pediatrics (AAP) recommends against routine *C difficile* stool testing in infants <12 months old because of high colonization rates but recommends testing in children >3 years on the basis of similar criteria for adults.[8] The difficulty for clinicians is that, for children 1 to 3 years old with diarrhea, the AAP guideline states testing can be considered after excluding other causes of diarrhea. The new Infectious Diseases Society of America guideline also does not recommend testing children aged 1 to 2 years unless other causes are ruled out.[13]

There is no laboratory confirmatory test that differentiates infection associated with *C difficile* from colonization. PCR-based methods are preferred because of rapid turnaround and high analytical sensitivity.[12,14–16] However, PCR has limitations, with false-positives resulting from detection of the *C difficile* toxin gene even when the gene is not actively producing toxin or remaining detectable after treatment. Thus, a positive PCR result does not differentiate infection from colonization, presenting clinical challenges in diagnosing children <3 years old.[17] Differing interpretations can be found in literature regarding the meaning of a positive PCR result in a child with[18,19] or without diarrhea.[12] The emergence of multiplex molecular-based assays to detect gastrointestinal pathogens has increased detection rates of *C difficile*. Positive results are frequently interpreted as infection regardless of age or symptoms, adding unnecessary burden to the health care system, including isolation precautions for hospitalized children and antibiotic overuse.

A reliable biomarker could assist in differentiating infection from colonization. In adults, fluoroquinolone-resistant North American pulsed-field gel electrophoresis type 1 (NAP1) strains have been associated with more severe disease and hypervirulence.[20] Thus far, NAP1 strains appear uncommon in children. Another potential disease indicator in adults is high *C difficile* burden, suggested by a low *C difficile* toxin B PCR cycle threshold (Ct) value.[21] The Ct is the PCR cycle number when the fluorescence of the PCR product can be detected above the background signal. A Ct value is inversely proportional to pathogen load; for example, a Ct value of 37 indicates lower pathogen load, whereas a Ct value of 20 indicates a higher pathogen load. In adults, the potential role of *C difficile* toxin B PCR Ct value to predict infection and poor outcome has been reported.[21] Finally, fecal levels of lactoferrin, a neutrophil-derived protein that closely correlates with bowel mucosal injury, has also been proposed as a potential biomarker of *C difficile* infection in children and adults.[22–24] Our goal was to compare *C difficile* detection rates in children <2 years of age with acute gastroenteritis (AGE) to rates in healthy controls (HCs) as well as evaluate NAP1 strains, PCR Ct values, and lactoferrin concentrations as potential biomarkers.

METHODS

The New Vaccine Surveillance Network (NVSN) includes 7 medical institutions across the United States conducting active, prospective surveillance for pediatric AGE hospitalizations and emergency department (ED) visits. NVSN methods have previously been published.[25,26] The test results and data reported in this article are derived from a randomly selected subset of patients previously described.[27] We tested stools collected at enrollment and analyzed parental interview information and selected data from medical chart reviews from 4 NVSN sites (Seattle, Kansas City, Memphis, and Rochester). The NVSN protocol was approved by the institutional review board at each site and the institutional review board at the Centers for Disease Control and Prevention. Written informed consent was obtained before collection of data and stool.

Study Population

This study included only the subset of enrolled NVSN subjects who were <2 years old and had been prospectively enrolled per the original NVSN protocol between January 1 and December 31, 2012, into 1 of 2 groups.

AGE Group

Per the original NVSN protocol, patients were eligible if there were no reported pre-existing chronic conditions and they presented with AGE (either diarrhea ≥3 episodes or vomiting ≥1 episode within a 24-hour period or both for ≤10 days' duration) for hospital admission or in the ED. Children presenting with vomiting alone without diarrhea were excluded from this study (Fig 1). Stool samples were collected within 10 days of AGE symptom onset.

HC Group

Per the original NVSN protocol, HCs had no known pre-existing chronic conditions and no diarrhea or vomiting within 14 days of enrollment. HCs were enrolled from

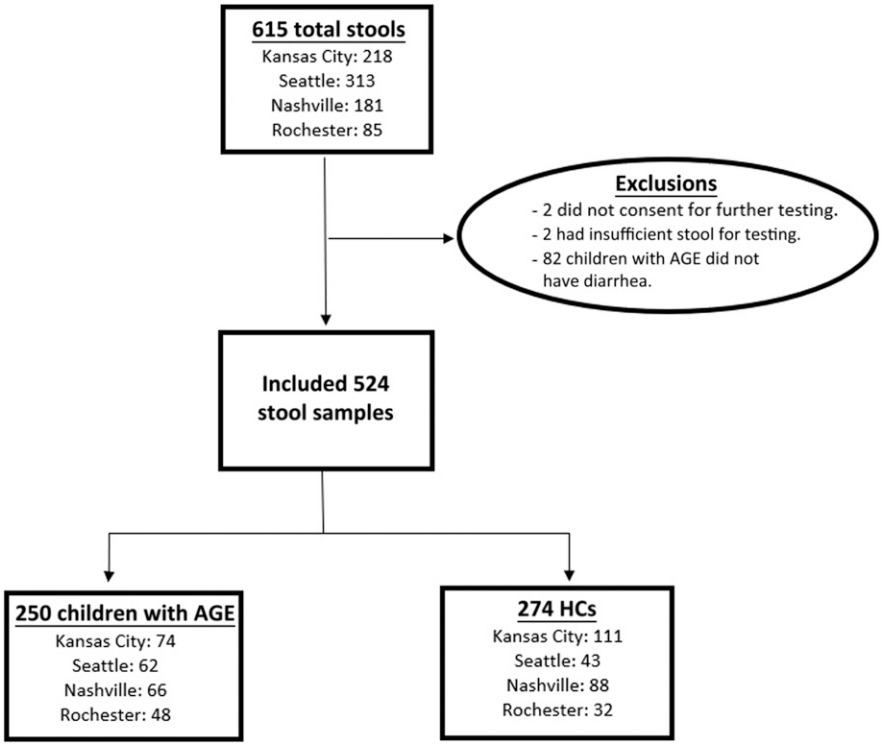

FIGURE 1 Eligibility diagram. Overall, 615 samples were available for testing from 4 NVSN sites. After we excluded 86 stool samples, 524 samples were included in the final analysis (Kansas City = 185, Nashville = 154, Seattle = 105, Rochester = 80).

outpatient clinics during routine well-child visits. Stool specimens were collected within 5 days of enrollment.

Sample Preparation and C difficile Testing

Whole stool samples were tested by US Food and Drug Administration–cleared Xpert *C difficile*/Epi PCR assay (Cepheid Inc, Sunnyvale, CA) per manufacturer's instructions. *C difficile*/Epi PCR is a multiplex real-time PCR for qualitative detection of toxigenic *C difficile* and its toxins. These toxins include toxin B gene (tcdB), binary toxin gene (cdt), and tcdC gene deletion at nucleotide 117 (which identifies a strain as being 027/NAP1/B1). PCR testing is valid in frozen stools. Frozen stools were used for testing as previously described.[27] PCR testing is valid in frozen and thawed stool samples. Results were reported as toxigenic *C difficile* positive and 027 presumptive negative, *C difficile* positive and 027 presumptive positive, and *C difficile* negative and 027 presumptive negative. Samples with invalid or error results were tested 1 additional time.

Lactoferrin Measurement

Thawed stool was used to measure lactoferrin by *IBD-SCAN* enzyme-linked immunosorbent assay (Tech Laboratory, Blacksburg, VA) on the same day, per manufacturer's instructions. Enzyme-linked immunosorbent assay testing is valid in frozen and thawed stool samples. Lactoferrin values >7.5 μg/mL (g) feces were considered elevated.

Analyses

Descriptive statistics, including means, medians, SDs, interquartile ranges, and proportions, were used to summarize the data. The χ^2 tests were used to compare groups on categorical variables. The *t* tests and Wilcoxon Rank Sum tests were used to look for group differences in continuous variables. Because lactoferrin values were not normally distributed, their natural log values were used for all analyses. A whisker box plot was created to demonstrate potential differences in Ct values and lactoferrin levels between groups. Logistic regression models stratified by *C difficile* status were used to examine whether lactoferrin and breastfeeding were predictors of AGE. SAS version 9.4 (SAS Institute, Inc, Cary, NC) and SPSS version 23 (IBM SPSS Statistics, IBM Corporation) were used for analysis.

RESULTS

Study Population

A total of 615 <2-year-old children enrolled initially in the larger NVSN overall study met eligibility requirements for this substudy (Fig 1). However, 82 of 615 children with AGE were excluded because of having only vomiting without diarrhea, 5 were excluded for having inconclusive *C difficile* results, 2 were excluded for not consenting to further testing, and 2 were excluded for having insufficient sample. The final cohort (n = 524) included 250 children with AGE and 274 HCs.

Demographics and Host Factors

Demographic information of HCs and AGE groups can be found in Table 1. HC children were significantly younger than children with AGE. The increased proportion of female children in the HC group was not significant (P = .054). The HC group contained more African American children (P = .034), and the AGE group contained more Hispanic children (P = .004). Previous antibiotic use (oral or systemic) within 30 days of enrollment was reported in 12% (31 of 250) of the AGE group, but this question was not asked of HCs. Being breastfed or receiving breast milk was reported equally in the AGE and HC group (73% both groups). Ongoing breastfeeding at the time of enrollment was more common in the HC group (AGE: 20% [49 of 250] versus HCs: 26% [71 of 274], P = .086), and day care attendance was more common in children with AGE (AGE: 22% [56 of 250] versus HCs: 16% [44 of 274], P = .065), but these differences were not statistically significant.

C difficile Detection

Overall, *C difficile* was more frequently detected in HC children (28% vs 14%; P < .001) (Table 2). Among infants <1 year old, significantly more HCs tested *C difficile* positive (32% vs 18%; P < .005), and this was also true for children 1 to 2 years of age (21% vs 10%; P < .02). Of the

TABLE 1 Demographics of Study Population

	AGE (n = 250)	HC (n = 274)	P[a]	Total (n = 524)
Age, mean (SD), mo	11 (6.8)	9 (6.0)	.006	10 (6.4)
Sex, female, n (%)	104 (42)	137 (50)	.054	241 (46)
Race, n (%)			.034	
White	113 (46)	112 (41)	—	225 (43)
African American	78 (32)	116 (42)	—	194 (37)
Other	59 (22)	46 (17)	—	105 (20)
Ethnicity, n (%)			.004	
Hispanic	73 (29)	51 (19)	—	124 (24)
Insurance, n (%)			.504	
Public	178 (72)	209 (76)	—	387 (74)
Private	60 (24)	58 (21)	—	118 (23)
Other	12 (4)	7 (3)	—	19 (3)

—, not applicable.
[a] A t test was used for age comparison; all other comparisons used χ^2 tests.

250 children with AGE, 66% reported ≤3 days of diarrhea, and 34% had >3 days of diarrhea at time of enrollment; however, they had similar rates of *C difficile* positivity (15% and 13%, respectively). Lactoferrin levels did not differ statistically in *C difficile*–positive versus –negative status (P = .79). Sixteen percent (5 of 31) of children with AGE whose parents reported antibiotic exposure (within 30 days of enrollment) were *C difficile* positive compared with 14% (30 of 215) of the children with AGE without recent antibiotic exposure (P = .746).

C difficile NAP1 Strains

Three NAP1 strain types were identified in positive *C difficile* samples. Two were from HC subjects (ages 4 and 7 months), and 1 was from a child with AGE (16 months) who also reported antibiotic use within 30 days of enrollment.

C difficile Ct Values

Among the *C difficile*–positive samples, there was no difference in *C difficile* PCR Ct values between AGE and HC groups (Table 2). PCR Ct values among hospitalized children who tested positive versus those seeking care at an ED also were not statistically significantly different (P = .482).

Lactoferrin Results

Lactoferrin testing was only performed on 308 samples that had sufficient residual sample for testing (AGE = 137, HC = 171). Children with AGE had significantly higher levels of lactoferrin (P = .002) (Fig 2A). Children breastfeeding at the time of the study (n = 40) had statistically higher lactoferrin levels (n = 268) (P < .001) (Fig 2B). No differences in lactoferrin levels were observed when analyzing groups on the basis of whether the stool PCR result was *C difficile* positive (n = 82) or negative (n = 226) (P = .788) (Fig 2C). In the *C difficile*–positive group, lactoferrin was not a significant predictor of AGE status with and without adjusting for breastfeeding status. In the *C difficile*–negative group, lactoferrin was a significant predictor of AGE status even after adjusting for breastfeeding status (P = .001).

DISCUSSION

Recognizing the difficulty of differentiating infection from colonization in infants who tested positive for *C difficile*, the AAP recommends against routine *C difficile* stool testing in children <1 year of age unless the infant has a severe motility disorder or is in an outbreak situation. Although our findings support this recommendation for children <12 months, our data reveal that infection associated with *C difficile* cannot be differentiated from colonization in children 1 to 2 years old with a positive C *difficile* real-time PCR test result. HC children in both age groups had C *difficile* detected approximately twice as often as children with AGE. Our results question the predictive value of real-time PCR testing among these age groups and raise uncertainty as to the etiologic role of *C difficile* in symptomatic, diarrheal children up to 2 years old. In our results, it is suggested that neither Ct values nor lactoferrin levels help distinguish colonization from infection in our populations. High lactoferrin levels were associated with breastfeeding and with AGE in children who were *C difficile* PCR negative, yet neither a low Ct value (higher abundance of C *difficile* in the sample) nor a high lactoferrin level correlated with clinical disease or C *difficile* PCR positivity status. Because lactoferrin is associated with bowel mucosal injury, the lack of an association between lactoferrin and C *difficile* positivity further supports the broader conclusion that C *difficile* positivity

TABLE 2 *C difficile* Detection and Ct Values in Children With AGE Versus HCs by Age

	AGE (n = 250)	HC (n = 274)	P	Total
C difficile positive, n (%)				
All	36 (14)	76 (28)	<0.001	112 (21)
<1 y (n = 297)	24 (18)	52 (32)	0.005	76 (26)
1–2 y (n = 227)	12 (10)	24 (21)	0.023	36 (16)
Ct values for *C difficile*–positive samples, n = 112				
Mean (SD)	30 (4.6)	30 (4.3)	—	—
Median (IQR)	28.3 (26.2–34.6)	30 (26.0–33.1)	.663	—

IQR, interquartile range; —, not applicable.

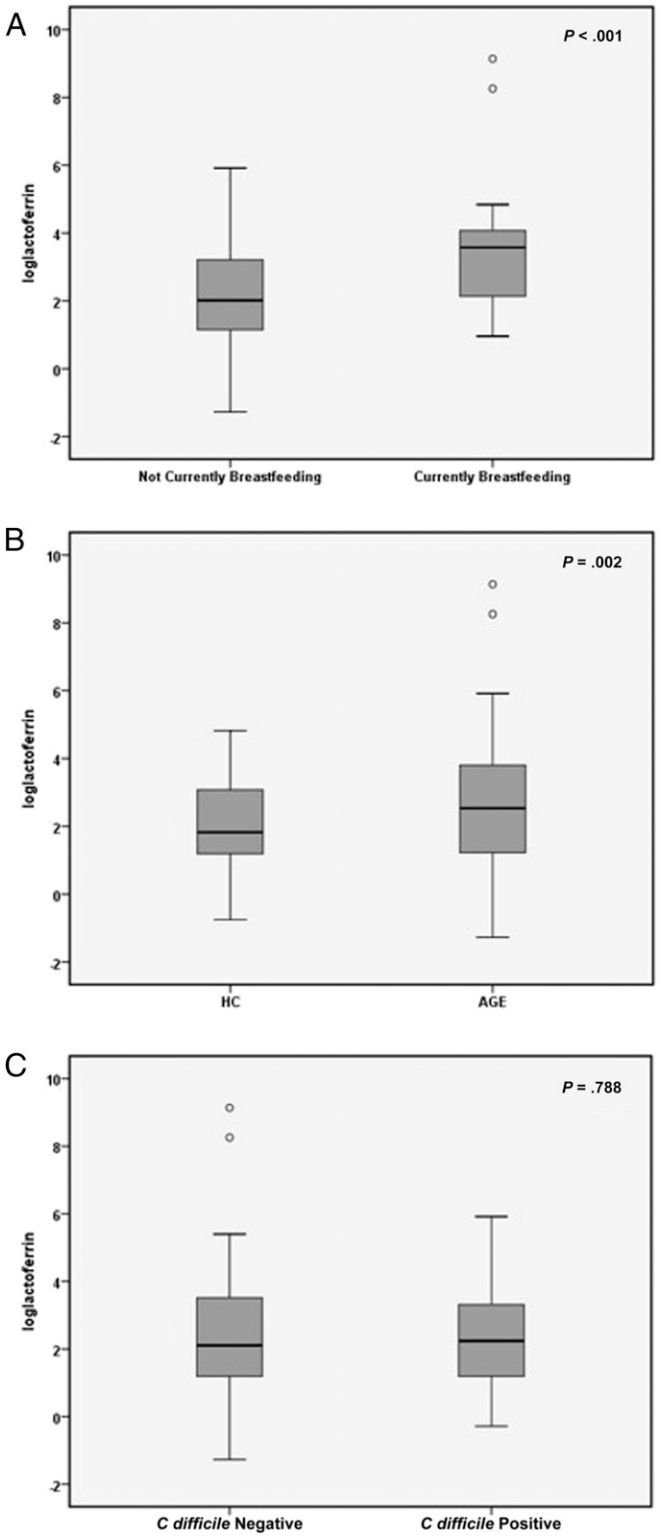

FIGURE 2 Lactoferrin comparison by boxplots by (A) breastfeeding status, (B) HC versus AGE, and (C) *C difficile* status.

is not clinically meaningful in this population.

Our overall finding was that 14% of children with AGE and 28% of HC children had C *difficile* detected by PCR, which is consistent with other studies. Nicholson et al[28] found that 16% of Tennessee pediatric inpatients or ED patients <6 years old who received care for diarrhea and/or vomiting tested positive for C *difficile* by PCR in 2008 to 2011. Eight percent of HCs aged 0 to 51 months tested positive. González-Del Vecchio et al[29] reported no statistically significant difference among children with diarrhea <2 years old when comparing those that tested positive for *C difficile* to those testing negative.

Leibowitz et al[17] studied children aged 1 to 18 years who tested positive for *C difficile* with or without diarrhea who were recruited from the pediatric oncology inpatient and outpatient setting. A higher positivity rate for *C difficile* toxin B (tcdB) by PCR was noted in the asymptomatic group (24%) versus the symptomatic group (19%), although it was not statistically significant ($P = .31$). Although these results suggest colonization is also common in older children and adolescents, this chronically ill population differs from our immunocompetent cohort because of more frequent medical care visits and likely higher antibiotic exposures.

In an active surveillance study from 10 states participating in the Emerging Infections Program in 2010 to 2011,[14] all positive C *difficile* test results (by toxin or molecular assay) were assessed from laboratories serving the pediatric population within defined areas. The highest incidence of positivity was observed in children 1 to 2 years old (66.3 per 100 000).[12] Unlike our findings, Wendt et al[12] concluded that the detection of C *difficile* in their population (of which only 72% reported diarrhea within 1 day of stool collection) represented infection (ie, *C difficile* disease) because the clinical presentation, severity, and outcomes in many of these 1- to 3-year-olds were similar to the 3- to 18-year-old

groups. The recurrence rate was 11%, and antibiotic use in the 2 weeks before testing was documented in 33% of those that tested positive for C *difficile*. Although no HCs were evaluated, Wendt et al[12] suggested they would have expected milder clinical symptomology in the younger children if these simply represented colonization. Information on C *difficile* copathogens was available for 57% of their subjects. Only 3% were found to be coinfected with other diarrheal pathogens, mostly among children ages 2 to 9. It appears that some cases of *C difficile*–positive test results did not have diarrhea in this study and that a copathogen might explain the diarrhea in some of the younger children.

With our findings, we suggest that other etiologies be considered even when *C difficile* is detected in symptomatic children <2 years old given that young children have multiple AGE episodes in the first few years of life. In the era before multiplex stool testing, ≤50% of these diarrheal illnesses had no detectable pathogen by conventional testing.[30] Indeed, viral agents have been detected in half to two-thirds of US pediatric AGE hospital admissions and ED visits, making viral testing more likely to yield a true etiology than *C difficile* testing alone.[8,25] With our findings, we suggest that treatments specifically targeting *C difficile* in young children who tested positive rarely be used.

The wide spectrum of symptomatic and asymptomatic *C difficile* infections is not fully understood. Researchers of most adult studies find low colonization rates and have speculated that host factors may explain this wide spectrum.[31–33] For example, relatively high colonization of *C difficile* has been reported in adult long-term care facilities and statistically associated with previous *C difficile*–associated disease ($P < .001$) and previous antibiotic use ($P = .017$).[34] Among our asymptomatic HC children <2 years old, we observed a higher positivity rate than adults; more than one-quarter of these infants and children yielded a positive *C difficile* test result without an apparent effect of recent antibiotic use among children with diarrhea. This indicates that *C difficile* colonization in healthy young children may be part of their nonpathogenic gut microbiome. Whether a putative or protective health outcome later in life is related to this carriage is unknown. Questions regarding potential relationships between maternally derived immunologic protection, age-dependent commensal microbiotic relationships, and/or the immunologic and structural characteristics of the young gut that may support *C difficile* colonization without producing symptoms deserve further study.

Given the lack of a true gold standard to confirm *C difficile* disease in children, we evaluated a laboratory assay that could help differentiate colonization from disease in children <2 years old. Researchers of a pediatric study suggested lactoferrin as a potential tool to distinguish between disease and colonization.[23] Our data suggest that lactoferrin can be an indicator of gut inflammation and injury because it was elevated in children presenting with AGE compared with HC children without diarrhea. However, a caveat regarding lactoferrin surrounds its known association with breastfeeding. The highest lactoferrin levels in our subjects were associated with breastfeeding regardless of whether they were in the AGE or HC group. For nonbreastfed children, modestly elevated lactoferrin levels were associated with any diarrheal disease, not just for *C difficile*–positive AGE. Our data do not support lactoferrin as a useful biomarker for differentiating disease versus colonization in children <2 years of age. Still, some suggest lactoferrin may be protective against disease during *C difficile* infection by reducing toxin production.[35]

Our findings were strengthened by the prospective, multicenter nature of our study design as well as the comparison of otherwise healthy children with AGE and HC children from the same community setting. The study was limited by the absence of information on antibiotic use in HC children and specific details about antibiotic use in those with AGE. Additionally, we did not test for other bacterial diarrheal pathogens, include stool samples from children >2 years old, or use the previously considered gold standard test (ie, cell culture cytotoxic assays) in this study because they are no longer used in the clinical setting and they are labor intensive. The HC group was younger than the AGE group, which may have skewed the *C difficile* detection rate to more carriage in the younger HC group than in the slightly older AGE group. However, clinically, a 2-month mean difference (Table 1) should not account for a doubled detection rate in HC. Finally, our sample size was not sufficient to power detailed stratifications of children who tested positive versus those who tested negative by PCR.

CONCLUSIONS

In conclusion, positive *C difficile* PCR results in children <2 years old are indicative of colonization, not infection, and are not sufficient as the sole diagnostic proof of *C difficile* as the cause of a current diarrheal episode. Neither lactoferrin nor PCR Ct values help differentiate infection from colonization. Our study findings make us question the sole use of the rapid, increasingly available, and highly sensitive (but not disease-specific) PCR tests to accurately confirm *C difficile* as an etiologic pathogen in children <2 years old.

Acknowledgments

We acknowledge the staff at participating NVSN sites for their work on data collection and study implementation, the patients, and their families. We especially are grateful for the staff at the Kansas City site, Karisa Deculus, Ashley Willingham, Mindy Spano, and Karen Snyder, for their work to help complete this project.

REFERENCES

1. Schwab EM, Wilkes J, Korgenski K, Hersh AL, Pavia AT, Stevens VW. Risk factors for recurrent Clostridium difficile infection in pediatric inpatients. *Hosp Pediatr*. 2016;6(6):339–344
2. Quesada-Gómez C, Vargas P, López-Ureña D, Gamboa-Coronado MM, Rodríguez-Cavallini E. Community-acquired Clostridium difficile NAP1/027-associated diarrhea in an eighteen month old child. *Anaerobe*. 2012;18(6):581–583
3. See I, Mu Y, Cohen J, et al. NAP1 strain type predicts outcomes from

Clostridium difficile infection. *Clin Infect Dis*. 2014;58(10):1394–1400

4. Jangi S, Lamont JT. Asymptomatic colonization by Clostridium difficile in infants: implications for disease in later life. *J Pediatr Gastroenterol Nutr*. 2010; 51(1):2–7
5. Cerquetti M, Luzzi I, Caprioli A, Sebastianelli A, Mastrantonio P. Role of Clostridium difficile in childhood diarrhea. *Pediatr Infect Dis J*. 1995;14(7): 598–603
6. Al-Jumaili IJ, Shibley M, Lishman AH, Record CO. Incidence and origin of Clostridium difficile in neonates. *J Clin Microbiol*. 1984;19(1):77–78
7. Bryant K, McDonald LC. Clostridium difficile infections in children. *Pediatr Infect Dis J*. 2009;28(2):145–146
8. Schutze GE, Willoughby RE; Committee on Infectious Diseases; American Academy of Pediatrics. Clostridium difficile infection in infants and children. *Pediatrics*. 2013;131(1):196–200
9. Cares K, El-Baba M. A 9-month-old presenting with rectal prolapse. *Gastroenterology*. 2016;150(1):42–43
10. Kim J, Smathers SA, Prasad P, Leckerman KH, Coffin S, Zaoutis T. Epidemiological features of Clostridium difficile-associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics*. 2008;122(6):1266–1270
11. Borali E, Ortisi G, Moretti C, et al. Community-acquired Clostridium difficile infection in children: a retrospective study. *Dig Liver Dis*. 2015;47(10):842–846
12. Wendt JM, Cohen JA, Mu Y, et al. Clostridium difficile infection among children across diverse US geographic locations. *Pediatrics*. 2014;133(4): 651–658
13. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis*. 2018;66(7):e1–e48
14. Berry N, Sewell B, Jafri S, et al. Real-time polymerase chain reaction correlates well with clinical diagnosis of Clostridium difficile infection. *J Hosp Infect*. 2014;87(2):109–114
15. Tartof SY, Yu KC, Wei R, Tseng HF, Jacobsen SJ, Rieg GK. Incidence of polymerase chain reaction-diagnosed Clostridium difficile in a large high-risk cohort, 2011-2012. *Mayo Clin Proc*. 2014; 89(9):1229–1238
16. Kociolek LK, Sandora TJ. National variability in surveillance, testing, and infection prevention for Clostridium difficile infection in pediatric populations. *Am J Infect Control*. 2013; 41(10):933–935
17. Leibowitz J, Soma VL, Rosen L, Ginocchio CC, Rubin LG. Similar proportions of stool specimens from hospitalized children with and without diarrhea test positive for Clostridium difficile. *Pediatr Infect Dis J*. 2015;34(3):261–266
18. Crews JD, Anderson LR, Waller DK, Swartz MD, DuPont HL, Starke JR. Risk factors for community-associated Clostridium difficile-associated diarrhea in children. *Pediatr Infect Dis J*. 2015;34(9):919–923
19. Denno DM, Shaikh N, Stapp JR, et al. Diarrhea etiology in a pediatric emergency department: a case control study. *Clin Infect Dis*. 2012;55(7):897–904
20. Kociolek LK, Gerding DN. Clinical utility of laboratory detection of Clostridium difficile strain BI/NAP1/027. *J Clin Microbiol*. 2016;54(1):19–24
21. Reigadas E, Alcalá L, Valerio M, Marín M, Martin A, Bouza E. Toxin B PCR cycle threshold as a predictor of poor outcome of Clostridium difficile infection: a derivation and validation cohort study. *J Antimicrob Chemother*. 2016;71(5):1380–1385
22. Nicholson MR, Crews JD, Starke JR, Jiang ZD, DuPont H, Edwards K. Recurrent Clostridium difficile infection in children: patient risk factors and markers of intestinal inflammation. *Pediatr Infect Dis J*. 2017;36(4):379–383
23. D¹browskal S, Demkow U, Podsiad³y E. [Presence of lactoferrin in faeces as the indicator of Clostridium difficile in pediatric patients]. *Med Dosw Mikrobiol*. 2015;67(1):1–8
24. Boone JH, DiPersio JR, Tan MJ, et al. Elevated lactoferrin is associated with moderate to severe Clostridium difficile disease, stool toxin, and 027 infection. *Eur J Clin Microbiol Infect Dis*. 2013; 32(12):1517–1523
25. Chhabra P, Payne DC, Szilagyi PG, et al. Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008-2009. *J Infect Dis*. 2013; 208(5):790–800
26. Payne DC, Sulemana I, Parashar UD; New Vaccine Surveillance Network. Evaluation of effectiveness of mixed rotavirus vaccine course for rotavirus gastroenteritis. *JAMA Pediatr*. 2016; 170(7):708–710
27. Hassan F, Kanwar N, Harrison CJ, et al. Viral etiology of acute gastroenteritis in <2-year-old US children in the post-rotavirus vaccine era. *J Pediatric Infect Dis Soc*. 2019;8(5):414–421
28. Nicholson MR, Van Horn GT, Tang YW, et al. Using multiplex molecular testing to determine the etiology of acute gastroenteritis in children. *J Pediatr*. 2016;176:50.e2–56.e2
29. González-Del Vecchio M, Álvarez-Uria A, Marin M, et al. Clinical significance of Clostridium difficile in children less than 2 years old: a case-control study. *Pediatr Infect Dis J*. 2016;35(3):281–285
30. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. *Clin Infect Dis*. 2006;43(7): 807–813
31. Furuya-Kanamori L, Marquess J, Yakob L, et al. Asymptomatic Clostridium difficile colonization: epidemiology and clinical implications. *BMC Infect Dis*. 2015;15:516
32. Martin J. The contribution of strains and hosts to outcomes in Clostridium difficile infection. *Infect Dis Clin North Am*. 2015;29(1):51–61
33. Loo VG, Bourgault AM, Poirier L, et al. Host and pathogen factors for Clostridium difficile infection and

colonization. *N Engl J Med*. 2011;365(18):1693–1703
34. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RL, Donskey CJ. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic Clostridium difficile strains among long-term care facility residents. *Clin Infect Dis*. 2007;45(8):992–998
35. Chilton CH, Crowther GS, Śpiewak K, et al. Potential of lactoferrin to prevent antibiotic-induced Clostridium difficile infection. *J Antimicrob Chemother*. 2016;71(4):975–985

Detection of *Clostridioides difficile* by Real-time PCR in Young Children Does Not Predict Disease

Barbara A. Pahud, Ferdaus Hassan, Christopher J. Harrison, Natasha B. Halasa, James D. Chappell, Janet A. Englund, Eileen J. Klein, Peter G. Szilagyi, Geoffrey A. Weinberg, Ashley K. Sherman, Christopher Polage, Mary E. Wikswo, L. Clifford McDonald, Daniel C. Payne and Rangaraj Selvarangan

Hospital Pediatrics 2020;10;555
DOI: 10.1542/hpeds.2020-0012 originally published online June 1, 2020;

Hospital Pediatrics®

AN OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Detection of *Clostridioides difficile* by Real-time PCR in Young Children Does Not Predict Disease

Barbara A. Pahud, Ferdaus Hassan, Christopher J. Harrison, Natasha B. Halasa, James D. Chappell, Janet A. Englund, Eileen J. Klein, Peter G. Szilagyi, Geoffrey A. Weinberg, Ashley K. Sherman, Christopher Polage, Mary E. Wikswo, L. Clifford McDonald, Daniel C. Payne and Rangaraj Selvarangan

Hospital Pediatrics 2020;10;555
DOI: 10.1542/hpeds.2020-0012 originally published online June 1, 2020;