Clostridium difficile Toxin Testing in a Pediatric Population

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Revised Abstract

Background: Diagnosis of *C. difficile* infection (CDI) relies on clinical symptoms and is supported by laboratory testing. Common testing for CDI in the laboratory include a single or multi-step algorithm encompassing molecular and/or enzyme immunoassays (EIA). Most appropriate testing for CDI in children remains unclear. The aim of this study was to determine which algorithmic approach best informs CDI in a pediatric institution.

Results: A total of 180 samples from pediatric inpatients were tested by PCR and EIA. 8 (4.4%) were PCR+/Tox+, 25 (13.9%) were PCR+/Tox-, and 94 (74.4%) were PCR-/Tox-. False positive rate for toxin A/B EIA (PCR-/Tox+) was 7.2% (8). Documentation of diarrhea/loose stool by clinicians was 84.8% (n=27) for PCR+ patients and 67.9% (n=91) for PCR- patients. Laxative use was found in none of the PCR+/Tox+ patients, two (8.0%) PCR+/Tox- patients, and 17 (12.7%) PCR-/Tox- patients. All PCR+ patients were negative for other diarrhea etiological agents. No deaths were reported and all PCR+ patients were treated with oral or intravenous (IV) metronidazole or vancomycin within 24hrs of results. Within the PCR+/Tox- patient population, 10 (40%) were immunocompromised and one (4.0%) patient had Crohn's disease. Age, duration of diarrhea, use of antibiotics or laxatives, WBC count, creatinine level, and comorbidity did not predict a positive C. difficile toxin result.

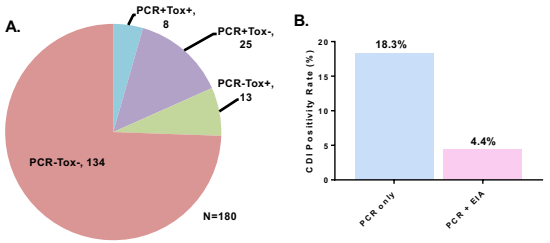
Conclusion: The use of a reverse two-step algorithm (PCR + EIA), in which a negative EIA is interpreted as colonization, would have lowered the CDI rate by 13.9%. However, 25 CDI patients would have been missed, including 11 immunocompromised patients at risk for developing severe/fulminant CDI. In our hands, a low specificity using toxin A/B EIA was determined which could impact institutions using a two-step algorithm (GDH + toxin). Along with clinical presentation, a single step molecular algorithm is most appropriate in diagnosing CDI in our pediatric patients.

Methods

- The clinical microbiology laboratory at Children's Hospital Los Angeles (CHLA) solely performs tcdB PCR (PCR, Simplexa *C. difficile* Universal Direct) for the detection of *C. difficile* in stool.
- Formed stools are rejected from *C. difficile* testing and is verified visually and physically using Bristol stool chart.
- From May 2018-January 2019, EIA for toxin A/B production (Tox, Premier Toxin A+B) was performed on stools ≤ 48 h old after *C. difficile* PCR.
- Qualified stools for EIA were held at 4ºC according to the manufacturer's protocol.
- PCR-Tox+ were sent to a reference laboratory for *C. difficile* cytotoxicity assay, when available.
- Clinical data including diarrhea/loose stool documentation, laxative use, prior antibiotic use, immune status, and treatment was collected two weeks after PCR results were finalized.

Results

Figure 1. CDI rates based upon PCR and EIA testing



A) Number of stools that tested as PCR+Tox+, PCR+Tox-, PCR-Tox+, and PCR-Tox-. Due to limited stool volume, only 9 of the PCR-Tox+ stools were confirmed to be negative by *C. difficile* cytotoxicity assay performed by a reference laboratory. B) *C. difficile* positivity rates if using a single-step algorithm (PCR+) or reverse two-step algorithm (PCR+Tox+). Of the 180 qualified stools tested from May 2018 – April 2019, 18.3% would be reported positive by a single-step algorithm and 4.4% by the reverse two-step algorithm. CDI, *Clostridium difficile* infection.

Results

Table 1. Diarrhea and loose stool documentation among pediatric patients

Variables	PCR Positive		PCR Negative
	PCR+/Tox+ (n = 8)	PCR+/Tox- (n = 25)	PCR-/Tox- (n = 134)
Diarrhea documented	62.5% (5)	60.0% (15)	42.5% (57)
Loose stool only documented	12.5%(1)	28.0% (7)	27.4% (34)
Diarrhea or loose stool are not documented	12.5% (1)	4.0% (1)	20.1% (27)
Denies diarrhea or loose stool	12.5% (1)	8.0% (2)	12.9% (16)
Stool Count Documented			
≥3 loose stools	25.0% (2)	24.0% (6)	14.9% (20)
Laxatives given ≤ 48hrs prior to stool collection	0.0% (0)	8.0% (2)	12.7% (17)

Documentation of unexplained and new-onset ≥ 3 unformed stools in 24 hours prior to stool collection among patients tested. Laxative usage up to 48 hours prior to stool collection was recorded. There were no institutional pre-screening restrictions for *C. difficile* stool testing based upon diarrhea/loose stool symptoms or prior laxative usage.

Table 2. WBC and creatinine levels do not predict *C. difficile* positivity

Variables	PCR Positive		PCR Negative
	PCR+/Tox+ (n = 8)	PCR+/Tox- (n = 25)	PCR-/Tox- (n = 134)
< 24 months	12.5% (1)	4.0% (1)	4.6% (6)
C. difficile positive within previous 90 days by PCR	37.5% (3)	16.0% (4)	5.2% (7)
Immunocompromised	37.5 %(3)	40.0% (10)	32.1% (43)
Inflammatory bowl disease	0% (0)	4.0% (1)	13.4% (18)
WBC Count ≥15,000/µl 24hrs prior to stool collection	25.0% (2)	12.0% (3)	6.7% (9)
Creatinine ≥ 1.5mg/dl 24hrs prior to stool collection	0.0% (0)	0.0% (0)	3.0% (4)

Additional patient history was recorded from retrospective chart review. Among the pediatric population, infants < 24 months of age have a high prevalence of asymptomatic carriage of toxigenic *C. difficile*. In the adult population, elevated white blood cell counts (WBC) and creatinine levels have been associated with CDI.

Table 3. Prior antibiotic treatment patterns among tested pediatric patients

Variables	PCR Positive		
	PCR+/Tox+ (n = 8)	PCR+/Tox- (n = 25)	All PCR + (n = 33)
Cephalosporin or carbapenem ≤ 14 days prior to stool collection	62.5% (5)	24.0% (6)	33.3% (11)
Quinolone ≤ 14 days prior to stool collection	12.5% (1)	4.0% (1)	6.1% (2)
Macrolide ≤ 14 days prior to stool collection	0.0% (0)	8.0% (2)	6.1% (2)
Clindamycin ≤ 14 days prior to stool collection	0.0% (0)	4.0% (1)	3.0% (1)
Total # of patients at increased risk for CDI due to antibiotics	75.0% (6)	40.0% (10)	48.4% (16)

Exposure to cephalosporins, carbapenems, quinolones, clindamycin, and macrolides have been found to the highest risk for developing CDI.

Results

Table 4. De-escalation and escalation of therapy for CDI and outcomes

Variables	PCR Positive		PCR Negative
	PCR+/Tox+ (n = 8)	PCR+/Tox- (n = 25)	PCR-/Tox- (n = 134)
Metronidazole (PO or IV) or vancomycin (IV) day of stool collection	37.5% (3)	0.0% (0)	20.1% (27)
De-escalation within 24hrs after negative PCR result	N/A	N/A	70.4% (19)
Treatment with metronidazole (PO or IV) or vancomycin (PO) after PCR +	100% (8)	96.0% (24)	N/A
Death within 30 Days	0.0 (0)	0.0 (0)	N/A

Infectious Disease Society of America (ID) recommends metronidazole (PO or IV) or vancomycin (PO) as treatment for *C. difficile* infection in the pediatric population.

Table 5. Characterization of high risk patients that would have been negative by a reverse-two step algorithm

Variables	Patients at high risk for developing fulminant CDI
	PCR+/Tox- (n = 11)
Diarrhea documented	45.4 (5)
Loose stool only documented	54.6 (6)
Stool Count Documented	
>3 loose stools	12.0 (3)
Laxatives given ≤ 48hrs prior to stool collection	0 (0)
Metronidazole (PO or IV) or vancomycin (IV) day of stool collection	0 (0)
WBC count ≥15,000/µl 24hrs prior to stool collection	18.1 (2)
WBC count ≤100/µl 24hrs prior to stool collection	81.8 (9)
Negative Rota EIA, Bacterial and Parasite PCR	100 (11)

High risk patients is defined as those that are immunocompromised or have inflammatory bowel disease (Cohn's disease).

Results

- A total of 13 PCR-/Tox+ results were identified and suspected to be false positives.
- 100% of PCR-/Tox+ specimens available for testing (n=9) by *C. difficile* cytotoxicity assay were negative, confirming likely false positive toxin A/B results.
- Documentation of diarrhea/loose stool was 84.8% (n=27) for PCR+ patients and 67.9% (n=91) for PCR- patients.
- All PCR+ patients were negative for other diarrhea etiological agents.
- PCR+ patients were treated with oral or intravenous (IV) metronidazole or vancomycin within 24hrs of results and no deaths were reported.
- Within the PCR+/Tox- patient population, 10 (40%) were immunocompromised and one (4.0%) patient had Crohn's disease.

Conclusion

- Poor diarrhea/loose stool documentation resulted in significant inappropriate patient testing
- A reverse two-step algorithm (PCR + EIA) would have lowered the CDI rate by 13.9%
- A single step algorithm (PCR+) correctly identified 11 patients who are at risk for developing severe/fulminant CDI.
- Low specificity of the toxin A/B EIA can impact institutions using a two-step algorithm (GDH + toxin).
- Clinical presentation along with a single step molecular algorithm is most appropriate in diagnosing CDI in our pediatric population.