Evaluation and Assessment of Clinical Relevance of Antigen and PCR Tests for Detection of Clostridium difficile Infection in Children

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ABSTRACT (updated)

Introduction. Clostridium difficile (Cd) is the leading cause of antibiotic and healthcare-associated diarrhea. Rapid methods are available for detection of cad and/or toxin A and B. The aims of this study were to (1) compare the performance of antigen and PCR tests to detect Cd infection in children and (2) to study the clinical relevance of Cd test results in detection of Cd associated disease in children.

Methods. A total of 200 frozen stool specimens were evaluated by Cd-IF Quick Chek Complete assay (QCC), BD GeneOhm Cdiff Test B Assay (BD), and ProGastro Cd Assay (PG) and toxigenic culture (TC). The results of these tests and historical results from Premier C. difficile toxin A/B assay (EIA) were compared to the TC-gold standard. Clinical data were abstracted to determine the clinical relevance of PCR results.

Results. Detection of toxigenic Cd (true positives) in 200 stool specimens by different methods are as follows: TC = 48, EIA: 29, QCC = 34, BD43 and PG48. The sensitivity, specificity, positive predictive value, negative predictive value and positive likelihood ratio for these tests were as follows: EIA: 98%, 92%, 89%, 96%, 100%; QCC: 70%, 97%, 94%, 89% and 91%; BD: 89%, 96%, 96%, 89% and 96%; PG: 100%, 93%, 82% and 100% PCR assays (BD and PG) detected 11 additional positives compared to TC. The discrepancy analysis on these additional PCR positives by amplifying alternate target of tcdC gene and Agilent Bioanalyzer analysis confirmed 7 true positives. Out of 7 PCR true positives, only 3 patients appeared to have Cd disease based on retrospective patient chart review and TC consultation.

Conclusions. PCR tests were superior to antigen tests, EIA and in detecting Cd PG PCR assay was the best performing assay. Retrospective review of clinical data indicated that only 3/7 patients (detected by PCR only) had Cd disease. A prospective study may be helpful in identifying true clinical relevance of PCR detection. Considering our study results, using QCC-GDH as a prescreening tool prior to PCR confirmation may be appropriate because of good negative predictive value. Cost analysis also showed that implementing QCC-GDH prescreening may reduce the test cost by 36% compared to direct PCR testing.

INTRODUCTION

Clostridium difficile (Cd) is a spore-forming, Gram-positive, and a common nosocomial pathogen that causes antibiotic-associated diarrhea. In the United States, increased incidence of Cd infection (CDI) from 3.7 cases per 1000 discharges in 2001 and the severity increased from 0.15 to 0.60 cases per 1000 discharges (Muto et al., 2005). Recent studies suggest that the epidemiology of Cd in children is also changing since an underlying gastrointestinal problem was noted in 15% of children who tested positive for CDI (Blumberg et al., 2007) and an overall increase in pediatric Cd-related hospitalization has been reported (Zilberberg et al., 2010).

Our lab has developed a flow enzyme immunoassays (EIA) or enzyme-linked immunosorbent assay (ELISA) targeting Cd-specific glutamate dehydrogenase (GDH) and/or toxins A and B are widely used due to their ease of use and rapid test results. A gold standard in generating reliable results is the limiting factor of these assays (Alcala et al., 2008). GDH-based two-step algorithm was rapidly proposed to screen all stool specimens and only a few are then selected for toxigenic culture. A number of reports followed by confirmation of toxigenic culture by cell culture cytotoxicity assay or PCR tests (Gilligan, 2008 and Sharaf et al., 2010). Direct testing of stool specimens using PCR was also suggested (Duong et al., 2010). However, very little information available on the performance of these test results from pediatric patients is currently available. Also, the clinical relevance of PCR test results is poorly understood.

In this study we evaluated the performance characteristics of TestChlb Cd Diff Quik Chek Complete EIA assay (QCC), GenProbe ProGastro Cdiff Test (PG) and BD GeneOhm Cdiff Test (BD) using frozen stool specimens from children. The results were compared with our present laboratory test for Clostridium difficile toxin A/B (EIA). The aims of this study were to (1) compare the performance of antigen and PCR tests to detect Cd infection in children (2) to study the clinical relevance of PCR test results in detecting Cd disease.

MATERIALS AND METHODS

Clinical specimens. A total of 200 frozen stool specimens (50 positive and 150 negative) previously characterized by Premier C. difficile toxin A/B assay were evaluated retrospectively. C. difficile Quik Chek Complete (QCC), the QCC (Test) (GenProbe), Blackburg, VA lateral-flow assay was performed as per the manufacturer’s instructions to detect GDH common antigen and toxins A and B in the same assay.

Toxigenic culture (TC). A 200 µl of liquid stool or 180 – 200 mg of soft/firmed stool specimens was mixed with 200 µl of absolute ethanol and incubated for 30 min at room temperature. A 200 µl of this mixture was plated with pre-reduced cyclohexane/cadmium-ferrous agar. The remaining 200 µl of stool/ethanol mixture was inoculated into 5 ml of pre-reduced Brain Heart Infusion (BHI) broth supplemented with C Diff Supplemental. The plates were held at 5°C in the dark to allow for growth of toxigenic C. difficile. Three colonies per specimen were isolated and tested to toxin production by cell culture cytotoxicity neutralization (CCCN) at TestChlb (Blackburg, VA).

Real-time PCR assays. BD GeneOhm Cdiff assay (BD) and ProGastro Cd Assay (PG) were performed as the manufacturer’s instructions. For each BD and PG run, C. difficile ATCC 9629 was used as a run control after mixing 50.000 CFU/ml into Cd negative stool specimen.

Clinical chart review. Electronic medical records of patients positive only by PCR testing (n=27) were reviewed and consulted with an ID physician to analyze the clinical relevance of PCR results. Data analysis. The equivalency analyses were performed using the y’ test and Fisher’s exact test. A p-value of >0.05 by Fisher’s exact test indicates that the categories are statistically equivalent. The y’ test gives an asymptotic probability for the same data.