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PATHOLOGY ◆ LABORATORY MEDICINE ◆ LABORATORY MANAGEMENT

Multiplex PCR test for detection of enteropathogens in an infant

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAPTODAY *readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. Case report No. 10, which begins here, comes from Diatherix Laboratories. If you would like to submit a case report, please send email to the AMP at* amp@amp.org. *For more information about the AMP and all previously published case reports, visit* www.amp.org.

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Clostridium difficile is an anaerobic spore-forming, Gram-positive bacteria transmitted by the fecal-oral route. The virulence of *Clostridium difficile* is primarily conferred from two toxins, A and B. Disruption of the normal gut flora, typically from intake of antimicrobials, allows *Clostridium difficile* to proliferate, causing a broad spectrum of clinical symptoms from asymptomatic colonization to colitis, a spectrum of diarrhea severity, and a protracted course of disease. The incidence of *Clostridium difficile* infection (CDI) among hospitalized children has increased dramatically in the past decade. Here, we report on a case of *Clostridium difficile* and *Salmonella enterica* co-infection in a seven-month-old patient with previous antibiotic treatment for sinusitis using Augmentin. The presence of *Salmonella enterica* detected in this patient may have acted synergistically or compounded the symptoms of the infection.

Introduction. Infectious diarrheal diseases are the second leading cause of mortality and morbidity, and age-specific rates are highest for children under age three. Although CDI predominantly affects adult

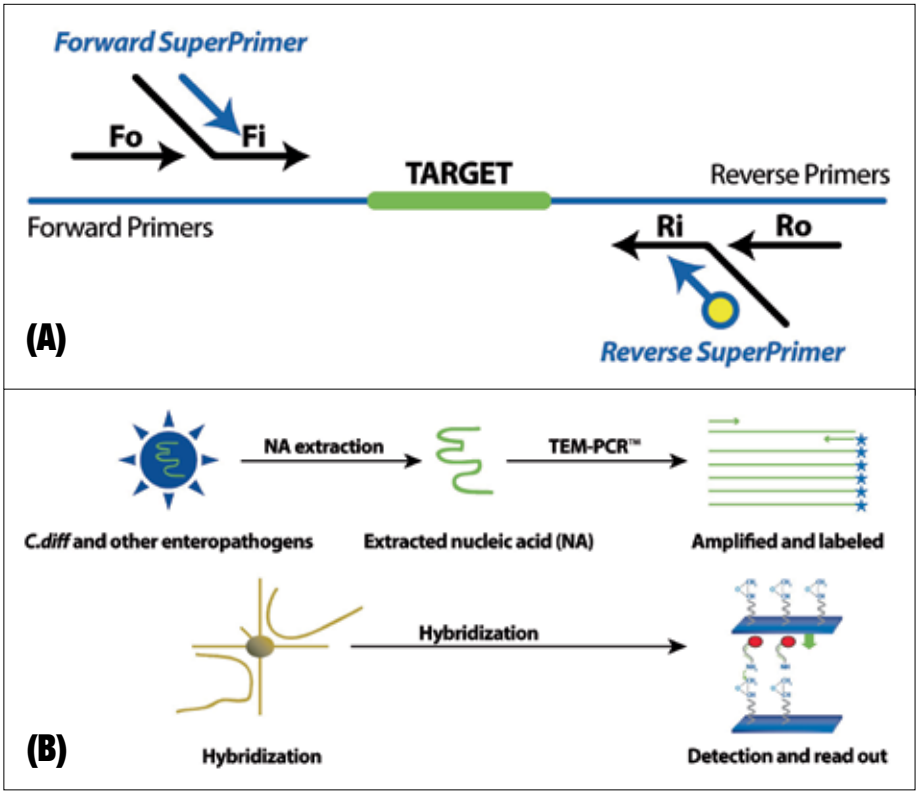

Fig. 1. Schematic of target-enriched multiplex PCR (A) and workflow of sample processing on multiplex gastrointestinal panel (B).

and elderly populations,[1] a recently published retrospective cohort study suggested that pediatric CDI is associated with increased mortality, longer hospitalization, and higher patient care costs.[2] The pediatric population, previously considered at low risk for CDI, has had an increased incidence of CDI-associated hospital admissions. Although testing of infants is not recommended, recent data have shown that 26 percent of children hospitalized with CDI were infants younger than one year and five percent were neonates. Recognized risk factors for children with detected CDI include extensive antimicrobial therapy, gastrointestinal surgeries, frequent hospitalizations, and impaired immunity. Breastfed infants have a lower carriage rate of *Clostridium difficile* compared with formula-fed infants. This pediatric population group may be asymptomatic for *Clostridium difficile* infection in the face of a positive test when the colonic wall receptor site for toxin may be nonfunctional or immature.[3]

Although the conventional stool culture is the well-established diagnostic tool for enteric pathogens, its effectiveness is very low, with reporting times ranging from two to four days, and it may be less sensitive than molecular methods.[4,5] Furthermore, widespread use of antibiotics can adversely affect the growth of potential pathogens in bacterial culture, rendering false-positive or false-negative results. PCR amplification has emerged as a useful tool to detect pathogen DNA and RNA rapidly (four to six hours) with higher sensitivity and specificity. Multiplex PCR-based tests for the detection of enteric pathogens in a single stool specimen are well suited to clinical purposes.

The gastrointestinal panel developed by Diatherix Laboratories (Huntsville, Ala.) provides simultaneous detection of the following pathogens: *Clostridium difficile* toxin B gene, *Campylobacter jejuni, Escherichia coli* strain O157, *Listeria monocytogenes, Salmonella enterica, Shigella flexneri, Shigella sonnei, Vibrio parahaemolyticus, Giardia lamblia, Cryptosporidium parvum,* adenovirus 40, and adenovirus 41. Target-enriched multiplex PCR (TEM-PCR) is the core molecular technology used for this panel. Nested gene-specific primers at extremely low concentrations are used to amplify targets during the first few cycles of PCR of the target-enrichment step. This is followed by exponential amplification using universal SuperPrimers. The Reverse SuperPrimer is labeled with biotin for subsequent detection of amplicons (**Fig. 1A**). The concentration of Forward and Reverse SuperPrimers facilitates asymmetric PCR producing biotin-labeled PCR products (**Fig. 1B**). These PCR products are hybridized to a complementary target-specific probe covalently coupled to a glass microarray (Microarrays Inc.) and detected with Streptavidin-labeled Phycoerythrin conjugate. Fluorescent signal corresponding to hybridized PCR products is detected on FLAIR reader (Sensovation, Germany), and results are reported as positive or negative for pathogens detected in the gastrointestinal panel.

Case. A seven-month-old female presented with a three-day history of diarrhea. The patient's mother reported that the child was passing four to five bloody stools per day and experiencing low-grade fevers. The child's oral intake remained normal and no abdominal pain was reported. The patient had been seen two weeks prior to the current visit with purulent nasal drainage and was treated with a seven-day course of Augmentin for sinusitis. Past medical history was unremarkable, and the patient was on no long-term medications. Physical exam revealed a well-nourished child in no apparent distress. Tympanic temperature was 99.2°F and heart rate was 124. Oral mucous membranes were moist and skin turgor was normal. The abdominal exam revealed normal active bowel sounds and no tenderness or mass.

A rectal swab stool specimen was obtained and submitted for laboratory testing with Diatherix's gastrointestinal panel. Nucleic acid extractions, multiplex PCR amplification, and positive/negative signal detection were performed at the CLIA-certified Diatherix Laboratories. The results were reported to the physician's office within seven hours of the sample having been received for laboratory testing. The patient's rectal swab sample was positive for both *C. difficile* toxin B and *Salmonella enterica*. The patient was treated preferentially with a seven-day course of ampicillin for *Salmonella enterica*. Diarrhea subsequently resolved without recurrence.

Discussion. The significance of *Clostridium difficile* as a cause of gastroenteritis in the pediatric population has been a subject of debate for decades. Studies that have documented early colonization of the bowel flora in neonates and the apparent lack of symptoms in the face of positive cultures for the organism and the presence of toxin[6] have cast doubt on the significance of *Clostridium difficile* as a cause of disease in this patient group. However, the emergence of a more virulent strain of *Clostridium difficile* that produces a binary toxin (B1/NAP1/O27) has led to a significant rise in the prevalence of the organism in hospitalized children who do not have comorbid factors.[7] As a result, there has been a shift in the spectrum and prevalence of the disease in the pediatric population over the past decade.

Clinical presentation does not always provide direction for the diagnosis of gastroenteritis whether

in the adult or pediatric patient. Early stages of *Clostridium difficile* infection are usually accompanied by mild diarrhea (five to 10 watery stools a day), low-grade fever, and mild abdominal cramping and tenderness. In the more severe forms of the disease, fever (usually 102° to 104°F), severe diarrhea (more than 10 watery stools a day) with blood, and marked abdominal pain and tenderness are present. These symptoms are not unique, however, as there is overlap with other intestinal pathogens that have both toxin and invasive components.

This case also exhibits co-detection of *Clostridium difficile* toxin B in association with a second pathogen, *Salmonella enterica*. In the new era of molecular diagnostics and specifically multiplex PCR in which multiple pathogens can be detected in a single specimen, clinicians are faced with a new level of information. The traditional paradigm wherein one pathogen causes clinical infection may be under challenge. Co-pathogens may work synergistically to cause clinical disease. Future molecular diagnostic testing will need to include, in addition to pathogen detection, other features that assist in determining pathogenesis and clinical illness.

We have presented this case report to draw attention to the fact that the spectrum and etiology of gastroenteritis in the pediatric population may be changing and molecular diagnostic tests are necessary to uncover complex etiology behind appropriate diagnosis and treatment of the pediatric population. □

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AMP CAP TODAY case report

Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereviews *and will be published next month in* CAP TODAY.

1. What is the clinical presentation of gastroenteritis in the neonatal pediatric population?

a) Onset of diarrhea with or without vomiting
b) Low-grade fever
c) Abdominal cramping
d) All of the above

2. Recent data have shown that 26 percent of children hospitalized with *Clostridium difficile* infection were infants younger than one year. What are the risk factors associated with CDI in the pediatric population?

a) Extended use of antibiotics
b) Gastrointestinal tract surgeries
c) Breastfeeding
d) Impaired immunity

3. What is the average turnaround time for molecular tests versus microbiological culture of stool?

a) Four to six hours versus two to four days
b) 24 hours versus two to four days
c) None of the above