Case Report

The importance of testing whole stool for Shiga toxin: a clinical and microbiological perspective

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Abstract

This case report emphasizes the importance of testing whole stool for the presence of Shiga toxin; especially in light of clinical suspicion, or whenever a grossly bloody stool specimen is received in the lab. Since organisms other than Escherichia coli O157:H7 can elaborate Shiga toxin, a negative stool culture for the usual enteric pathogens does not rule out the possibility of a Shiga toxin-mediated pathology in the appropriate clinical setting. The presence of this toxin in stool influences a physician's approach towards the patient's antimicrobial management.

Introduction

A new food-borne zoonosis was described in 1982, when patients in two separate outbreaks in the United States presented with symptoms of haemorrhagic colitis and hemolytic-uremic syndrome (HUS). A Shiga-toxin producing Escherichia coli was isolated in their stool, and was subsequently serotyped as O157:H7.[1]

Strains of E.coli are classified according to their O and H antigens. To date, more than 200 different O serogroups of E.coli have demonstrated the ability to produce Shiga toxin, and over half of these have been associated with disease in humans. The Shiga toxin-producing E.coli (STEC) family includes subtypes that can produce either one, or both strains of the Shiga toxin.[2] The toxins elaborated by STEC, both O157 and non-O157, have the potential to bind to peripheral blood leukocytes and endothelial cells, resulting in a pro-thrombotic state that may precede HUS or lead to the development of haemorrhagic colitis.

Disease caused by O157 and non-O157 strains of E.coli can range from mild diarrhoea to haemorrhagic colitis or life-threatening HUS. Since initiation of antibiotic therapy can lead to increased toxin production and more severe disease, a timely diagnosis of STEC infection may prevent unnecessary antibiotic therapy in patients, and hence prevent progression to severe systemic disease of HUS. The following case report illustrates this point.

Case Report

A two-year-old Hispanic girl presented to the emergency room at Children's Hospital at Montefiore in Bronx, NY, with a three-day history of foul-smelling watery diarrhea. Parents reported 3 to 4 stools per day, with mucous and streaks of blood. She had no fever, vomiting or abdominal pain. There was no history of exposure to pets or travel, and her immunizations were up to date.

On examination, she was a happy toddler with stable vital signs. Her physical examination was normal, she was well-hydrated, the abdomen was soft, no organomegaly, and there was no rash or peri-anal excoriation. The following tests were ordered in the emergency room: stool culture, enterotoxin assay and stool for occult blood, ova and parasites.

The stool sample was plated to solid media including BBL Blood Agar Plate (BAP), BBL Hektoen, BBL MacConkey, and BBL Campy-BAP, and additionally to BBL Selenite and BBL Campylobacter-Thioglycolate broths (Becton, Dickinson and Company, Franklin Lakes, NJ) for stool culture as per lab protocol. Subsequent biochemical test were preformed after non-lactose fermenting, gram-negative colonies were observed on the MacConkey agar.

BBL Inoculated Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), and Motility Indole Ornithine (MOI) media (Becton, Dickinson and Company, Franklin Lakes, NJ) excluded Shigella, Salmonella, Campylobacter and Yersinia as potential enteric pathogens. Ova and parasites and occult blood tests on the stool were negative.

Simultaneous testing for Shiga toxin using Premier EHEC (Meridian Bioscience, Inc. Cincinnati, OH) was positive. In the light of this finding, the original stool sample was plated to BBL Sorbitol MacConkey Agar (SMAC)(Becton, Dickinson and Company, Franklin Lakes, NJ) and non-sorbitol fermenting colonies were subsequently identified. The non-sorbitol fermenting colonies were used for Escherichia coli (E. coli) O157:H7 serology identification and were found to be negative using the RIM E. coli O157:H7 Latex Test (Remel, Lenexa, KS). With the suspicion for Shiga toxin-producing, non-0157:H7 E. coli, the stool specimen was sent to the New York City Department of Health (NYC-DOH) for further identification. The NYC-DOH identified a sorbitol fermenting E. coli using SMAC and BBL CHROMagar O157 (Becton, Dickinson and Company, Franklin Lakes, NJ). Subsequently, a panel containing 43 E. coli O somatic antibodies (Denka Seiken, Japan available through REMEL, Lenexa, KS) was run, and all were found to be negative.

These results confirmed the presence of a Shiga toxin- positive non-O157: H7 E. coli. The sample was sent to the Centers for Disease Control and Prevention, Atlanta (CDC), where it was later identified as a Shiga toxin 1, urea positive, Escherichia coli O undetermined: H8.

In lieu of these findings, antimicrobial therapy was withheld from the patient. At follow up one week later, her diarrhoea and bloody stools had resolved. Stool was re-tested twice for Shiga toxin, and found to be negative. Her haematocrit, platelets and serum creatinine remained stable throughout the illness.

Discussion

The microbiology laboratory at Montefiore Medical Center routinely tests stool for the presence of Shiga toxin if there is a high index of suspicion for STEC infection, or if grossly bloody stool samples are sent to the lab. Studies have shown that STEC is more likely to be isolated from visibly bloody stool specimens, than those without visible blood.[3] Isolation of Shiga toxin from stool is a risk factor for the development of haemolytic uraemic syndrome (HUS), a triad of thrombocytopenia, acute renal failure and microangiopathic haemolytic anaemia. HUS is the most common cause of acute renal failure in early childhood[4], and is associated with enteric infections caused by Shiga-toxin producing organisms like E.coli O157:H7, O26:H11 and Shigella dysenteriae. E. coli O157:H7 is the most important cause of HUS in Western Europe and the US.[5]

Some studies, have demonstrated an increase in the occurrence of HUS in patients with STEC who are treated with antibiotics during the phase of bloody diarrhoea[6]; this is caused by increased elaboration of Shiga toxin triggered by antibiotics. Since bloody diarrhoea is a concerning clinical finding in all patients, especially children, clinicians may be compelled to initiate early empiric antimicrobial therapy in patients presenting with bloody diarrhoea. However, according to the available data and current recommendations, antibiotics should be withheld in patients suspected of having an infection caused by STEC. In our patient, isolation of Shiga toxin from stool allowed us to make rational decisions about withholding antibiotic therapy and monitoring the patient closely for development of HUS.

Conclusion

Strains of E.coli O157:H7 are easier to detect on stool culture than non-O157 strains, since the former do not metabolize sorbitol and produce colourless colonies on MacConkey agar. Most non-O157 STEC, enterotoxogenic and enteropathogenic E. coli, and gut commensals metabolize sorbitol and produce pink colonies on MacConkey medium. Hence, it is easier to identify O157 strains of STEC than the non-O157 strains simply by visualizing colonies on MacConkey agar. Given the serodiversity of non-O157 strains, testing stool for the elaboration of Shiga toxin is the most direct way of testing for non- O157 STEC. Two enzyme immunoassays (EIA) are currently in use in the US for direct detection of Shiga toxin.[2]

Laboratories that routinely test grossly bloody stool for Shiga toxin production despite a negative stool culture in the setting of bloody diarrhoea allow clinicians to make important decisions regarding patient management and withholding antimicrobial treatment. Paediatricians need to be aware that HUS can result from toxins elaborated by many organisms other than E.coli, and antibiotic therapy should be withheld in children presenting with bloody diarrhoea, pending laboratory detection of Shiga toxin in stool.

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