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Original Research Article

Prevalence of STEC O157 in paediatric diarrhoeas and evaluating efficacy of SMAC and O157 antigen detection in the diagnosis of O157 E.coli

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Abstract: Shiga toxin producing Escherichia coli (STEC) is an important group of E. coli that can cause severe diarrhoea and many food borne outbreaks worldwide. STEC is known to be associated with large scale outbreaks affecting hundreds of people reported over the past two decades. In Indian scenario, the incidence of diarrheagenic E.coli (DEC) is largely unknown. Most persons with diarrhoea-associated HUS have an O157 STEC infection. Prompt and accurate diagnosis of STEC O157 infection is important because appropriate treatment, early in the course of infection might decrease renal damage and improve patient outcome. This prospective cross sectional study was taken up to know the prevalence of E.coli O157 in paediatric diarrhoea cases, to assess the role of Sorbitol MacConkey agar (SMAC) in the preliminary identification of O157 E.coli and also to evaluate O157 antigen detection in confirmation of non-sorbitol fermenting E.coli as O157 E.coli. 100 diarrhoea stool specimens from paediatric group of less than five years were processed and inoculated on Sorbitol MacConkey agar. All non-sorbitol fermenting E.coli strains were tested for O157 antigen using rapid immunocard kit. 13% of E.coli isolates produced non sorbitol fermenting colonies on Sorbitol MacConkey agar (SMAC), of these 8 isolates (61.5%) was positive for E.coli O157 by antigen detection test. The magnitude of the E.coli O157 diarrhoea in children is to be considered due to complications and high drug resistance and regular screening for this isolate is recommended, utilising Sorbitol MacConkey agar (SMAC) as a routine media.
Keywords: Diarrhoea, Shiga toxin producing Escherichia coli (STEC), Diarrheagenic E.coli (DEC), Escherichia coli O157, Sorbitol MacConkey agar (SMAC), O157 antigen detection.

INTRODUCTION:

Shiga toxin producing Escherichia coli (STEC) is an important group of E. coli that can cause severe diarrhoea and many food borne outbreaks worldwide. The serotype of a STEC is based on the O antigen determined by the polysaccharide portion of cell wall lipopolysaccharide. They are also referred as Verocytotoxin-producing E. coli (VTEC) or Entero haemorrhagic E. coli (EHEC), as they can cause haemorrhagic colitis through the production of cytotoxins. The most important serotype is E. coli O157:H7 [1]. STEC infection also causes haemolytic-uremic syndrome (HUS), a life-threatening condition characterized by haemolytic anaemia, thrombocytopenia, and renal failure. E. coli O157 infection and HUS are largely paediatric illnesses, although they can occur at any age [2]. STEC infection is transmitted by faecal-oral route through contaminated food and water. The incubation period for STEC is 2 to 10 days, usually three to four days. Though STEC infection may be asymptomatic, it typically begins with watery diarrhoea associated with abdominal pain, and occasionally with nausea and vomiting. Fever is not a prominent symptom. The watery diarrhoea may or may not progress to bloody diarrhoea.

Between 2001 and 2009, 71.2% STEC illness notifications were reported in Australia, of these 58% were found to be O157 strains. In US 96,534 cases of STEC O157 are reported each year [3]. 8% of O157 STEC infection develop haemolytic uremic syndrome (HUS), 2% to 15% of HUS reported in children below 5 years and the case fatality rate of HUS was 3% to 5% [4].

In Indian scenario, the incidence of diarrheagenic E.coli (DEC) is largely unknown. Very few laboratories can identify these organisms to the serotype level. Mrudul Lanjewar from Mumbai in 2010

isolated 68.25% DEC strains. The STEC encountered in their study was 37.21% in DEC [5].

Most persons with diarrhoea-associated HUS have an O157 STEC infection. Prompt and accurate diagnosis of STECO157 infection is important because appropriate treatment, early in the course of infection might decrease renal damage and improve patient outcome.

Hence the present study was taken up to know the prevalence of E.coliO157 in paediatric diarrhoea cases, to assess the role of Sorbitol MacConkey agar (SMAC) in the preliminary screening of O157 E.coli and to evaluate O157 antigen detection in confirmation of non-sorbitol fermenting E.coli as O157 E.coli.

MATERIAL AND METHODS:

This was a prospective cross sectional study carried over a period of 6 months, from June – November 2015 at clinical Microbiology Laboratory, Guntur Medical College hospital, Guntur. Study was approved by institution ethics committee. Informed consent was obtained from parents/guardians.

All children aged less than 5 years suffering from acute diarrhoea admitted in the paediatric ward were included in the study. Children above 5 years, children with dysentery, chronic diarrhoeas and children with prior antibiotic usage were excluded. A total of 100 stool samples were collected from hospitalized children with acute diarrhoea. Prior instructions were given to parents /guardians about collection of stool specimens. Approximately 5 – 10 ml of stool samples was collected in sterile containers and was transported within 2 hours to the clinical Microbiology lab. According to the laboratory protocol all the stool samples were examined macroscopically for visible worms, blood, mucus and microscopic examination was done by wet film for presence of pus cells, RBC, ova & cysts. Then culture processing for common enteric pathogens was done by enriching in Selenite F broth. The Procedure for isolation of E.coli O157 was, all the stool samples were inoculated on Sorbitol MacConkey agar (SMAC), which is a partial selective media for detection of E.coli O157. After 24 hours incubation at 37^0C, non-sorbitol fermenting colonies were provisionally identified as E.coli O157 [6]. All such non-sorbitol fermenting E.coli strains were tested for O157 antigen using rapid immunocard kit (Immunocard stat E.coli O157 plus, Meridian biosciences) whose sensitivity and specificity was 100%.

RESULTS AND DISCUSSION:

Among the total stool specimens processed, enteric pathogens detected, other than E.coli was only one Shigella species (1%). E.coli had grown on Sorbitol MacConkey agar (SMAC) from all the 100 stool samples (100%). Out of these E.coli isolated, Sorbitol fermenting (SF) were 87 (87%) and non-sorbitol fermenting were (NSF) 13 (13%). Among non-sorbitol fermenting E.coli (n=13), which were subjected to O157 antigen detection test, 08 (61.5%) strains were positive for O157and 5 (38.5%) strains were negative (Figure I).

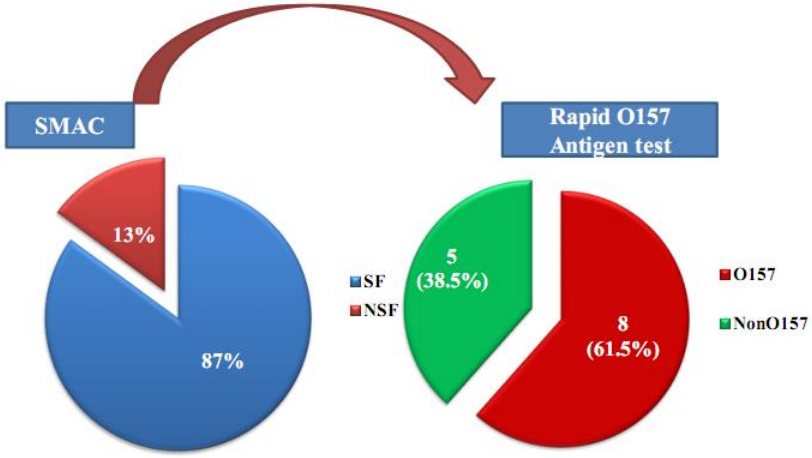

Fig-1: Showing Shiga toxin producing Escherichia coli (STEC) on Sorbitol MacConkey agar (SMAC) and confirmation of same by rapid O157 Antigen test

Most outbreaks of Shiga toxin producing Escherichia coli (STEC) infections and most cases of HUS have been caused by O157. Estimated approximately 11 million children under age of 5 die because of E.coli gastroenteritis [7]. Sorbitol MacConkey agar (SMAC), which contains 1% sorbitol instead of 1% lactose, utilizes the inability of most strains of E.coli O157 to ferment sorbitol to improve detection. Early in the course of the disease, within 1 to 2 days of the onset of diarrhoea, Sorbitol MacConkey

agar (SMAC) culture detects organisms from most patients with infections caused by sorbitol-negative E.coli O157. As the disease progresses, the detection rate falls to 33% [8].

In the present study the prevalence of E.coli O157 in paediatric diarrhoea was 8 %, similar results of 9% O157 E.coli were reported by Helge Karch *et al.;* in 2000 [9]. But Sheetal verma *et al.;* in 2013 from Lucknow, did a 10 year epidemiological survey across India and observed 0.5% positivity for E.coli O157 [7].

Looking into the efficacy of SMAC in detection of O157 from stool specimens , the present study showed 13 % of E.coli are non-sorbitol fermenting (NSF) on Sorbitol MacConkey agar (SMAC), but it was reported as low as 3.2% and 3 % by Shaban R *et al.;* in 2013 from Libya [10] and Jennifer R Stapp *et al.;* in 2000 [11] respectively. Mrudul Lanjewar reported that 25% of Shiga toxin producing Escherichia coli (STEC) did not ferment sorbitol [5]. Sorbitol MacConkey agar, though advantageous for its inability to ferment sorbitol, detects only 50 to 60% of STEC O157. Still Sorbitol MacConkey agar (SMAC) may be useful for provisional identification of O157 E.coli, and confirming these strains using antigen detection test as E.coli O157, which was a cost effective in resource poor settings.

On evaluating the role of O157 antigen detection in the confirmation of O157 E.coli from non-sorbitol fermenting E.coli on SMAC, present study reported that out of 13 non sorbitol fermenting (NSF) E.coli 8 (61.5%) were detected as positive for O157 antigen by Immunocard test. Similar study was carried by Witold A Ferens *et al.;* in 2011 reported that among 139 NSF 14.6% were O157 antigen positive [12]. Whereas Abdolvahab Alborzi *et al.;* in 2008 reported none of 22 non sorbitol fermenting (NSF) E.coli were positive for O157 antigen.[13] These differences may be due to variations in the existing E.coli O157 strains in different geographical locations. Social and economic factors also play a role in predisposing infections in developing countries. Also under diagnosis and under reporting, as many labs do not look for this isolate, might be another reason. As the data from India is limited, our study helps to obtain the information regarding prevalence of STEC O157 from patients with diarrhoea.

CONCLUSION:

To conclude the magnitude of the E.coli O157 diarrhoea in children is to be considered due to complications and high drug resistance. Regular screening for this isolate is recommended, utilising Sorbitol MacConkey agar (SMAC) as a routine media for identification of Shiga toxin producing Escherichia coli (STEC) from stool samples.

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