DETECTION OF *Enterohaemorrhagic Escherichia coli* *(E. coli O157:H7)* AND ITS DRUG RESISTANCE PATTERN

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ABSTRACT

Enterohaemorrhagic *Escherichia coli* (EHEC), is an emerging pathogen causing haemorrhagic colitis and Haemolytic Uraemic Syndrome (HUS). The most common EHEC is *E. coli* O157:H7. Human may acquire EHEC infection primarily from consumption of contaminated food and drinking water or direct contact with feces of infected person. The treatment of this pathogenic *E. coli* infection is increasingly becoming difficult because of the multidrug resistance exhibited by the organism. In India, few research reports are suggesting that this enteropathogen may pose a serious public health problem. In present study an attempt was made to isolate EHEC from diarrheagenic stool sample of patients in Surat (India) and determination of their antimicrobial resistance pattern. Isolation and identification of *E. coli* was carried out from stool samples of patients with intestinal infection by standard procedure. *E. coli* isolates were inoculated for the identification of EHEC (*E. coli O157:H7*). The isolates were then further processed for the determination of antibiogram. 13.91% isolates from 115 *E. coli* isolates were identified as EHEC. The least resistance was detected against Gentamicin (25%). Very high resistance was detected against Ampicillin (100%), Tetracycline (93.75%), Cefturoxime and Co-Trimoxazole (75%), Ceftriaxone, Chloramphenicol and Ciprofloxacin (68.75%). Our study findings indicate high frequency of EHEC in diarrheagenic stool sample. Multiple drug resistance was observed. Hence, routine screening of diarrheagenic stool sample for EHEC and their resistance pattern may be useful. However, further characterization of these isolates from large population of diarrheagenic individual and healthy control is necessary to know their role as emerging pathogens.

Key Words: *Enterohaemorrhagic Escherichia coli* (EHEC), *E. coli* O157:H7, Drug resistance, Pathogen, Haemolytic uraemic Syndrome (HUS)

INTRODUCTION

*Escherichia coli* (*E. coli*) are ubiquitous intestinal bacterial flora of animals and humans. Despite this benign relationship pathogenic strains or clones of *E. coli* exist. *E. coli* causes human infections ranging from gastrointestinal infections, as well as extra-intestinal illness, these diseases include both well recognized diarrheal syndromes, urinary tract infections; neonatal meningitis and less frequently encountered conditions viz. wound infections, septicemia and Gram negative pneumonia. *E. coli* is also a major cause of nosocomial infections. During the past twenty years, a clonal group of *E. coli* has emerged or been recognized that possess a unique disease-causing virulence factor armament, asymptomatically colonize food-producing ruminants, and are capable of causing significant morbidity and mortality in humans. The emergence of or recognition of Shiga-toxigenic *Escherichia coli* (STEC) as a cause of diarrhea, Hemorrhagic Colitis (HC), Hemolytic Uremic Syndrome (HUS), and Thrombotic Thrombocytopenic Purpura (TTP) in humans is a significant public health concern worldwide. STEC isolated from humans with specific clinical signs are called *Enterohemorrhagic E. coli* (EHEC). Transmission of EHEC to humans from animal reservoirs typically occurs by fecal contamination of food or water, direct or indirect contact with
animals, or by person-to-person contact. Disease in animals is less commonly recognized, although many domestic and food animals are colonized by EHEC. Healthy cattle and other ruminant species appear to be reservoirs from which EHEC that are pathogenic to humans originate. Within E. coli, a large number of EHEC serotypes have been documented in humans, ruminant food animals, other domestic animals, wild animals, and invertebrates. E. coli is classically differentiated based on the modified Kauffman serotyping scheme. Numerous O (somatic), H (flagellar), and K (capsular) surface antigens have been described. The “serotype” is defined by the O:H antigen combination. Multiple EHEC strains from over 100 serotypes are potentially able to cause human STEC infection, with serotype O157:H7 being the most notable. Infections with EHEC O157:H7 are thought to be twice as common as non-O157 serotypes and thought by some to result in greater hospitalization rates and case fatality rates. Attention focused on EHEC O157:H7 as an "emerging infectious disease" is warranted. It has been reported as the leading cause of HUS in the United States, Canada, and Europe and was estimated to cause 85–95% of HUS cases in North America. The number of outbreaks reported has increased since recognition of EHEC O157:H7 as a cause of food borne illness. The number of sporadic cases attributed to the O157:H7 serotype increased as well.

AIMS AND OBJECTIVES

Present study has been designed to isolate EHEC from diarrheagenic stool sample of patients in Surat (India), which will enable to understand their spread in population under study and determination of their antimicrobial resistance pattern.

MATERIAL AND METHODS

Specimen collection

This study was carried out on all patients who were present with diarrhea. Stool specimens (whole stools, swabs prepared from whole stools or rectal swabs with visible fecal staining) were collected. Samples were collected as per the guideline mentioned by Isenberg and WHO Manual. Among all the isolates, E. coli were selected and studied further. The clinicodemographic details were noted to correlate the results for retrospective studies. Isolation and presumptive identification of E. coli

The isolates were identified from their cultural, biochemical and serological test described in Bergey’s Manual of Systematic Bacteriology (2001) and Jean F. MacFaddin. Initially the Samples were streaked on MacConkey agar plates for selective isolation and differentiation and their colonial characteristics were noted. The typical colonies suspected to be of E. coli were then selected and further streaked on the Eosin Methylene Blue agar plate and biochemical test Indole production Test (I), Methyl Red (M), Vogus Proscure test (Vi) and Citrate Utilization test (C) for confirmation. The colonies showing Metallic sheen and in biochemical test IMViC-+, +, _, _ were identified that these isolates were E. coli. Unlike other E. coli, nearly all isolates of E. coli O157:H7 ferment D-sorbitol slowly, or not at all. Sorbitol-MacConkey (SMAC) agar was developed to take advantage of this characteristic by substituting the carbohydrate sorbitol for lactose in MacConkey agar and is the medium of choice for isolation of E. coli O157:H7. Unlike approximately 92% of E. coli, E. coli O157:H7 strains that produce Shiga-like toxins lack the enzyme β-glucuronidase and are MUG negative. For this reason the MUG assay used in conjunction with testing for sorbitol fermentation. In MUG test of E. coli O157 strains agar medium containing the substrate 4-methylumbelliferyl-B-D-glucuronide (MUG) was incorporated the enzyme β-glucuronidase cleaved substrate MUG and, a fluorescent product 4-Methyl-umbelliferone is produced that is detectable with long-wave ultraviolet light. β-D-glucuronidase-producing organisms shows fluorescent, however EHEC (E. coli O157:H7) (in contrast to commensal E. coli strains) does not synthesize this enzyme and thus when its colonies are exposed to long wave UV light, no fluorescence is observed. Hence Special group of serovars (E. coli O157:H7) were identified using highly selective medium, MUG-Sorbitol MacConkey agar plates from stool samples.
Susceptibility testing

The determinations of antibiogram for the collected strain were then carried out using modified Kerbev- Bauer Disc-Agar diffusion technique recommended by Clinical Laboratory Standard Institute (CLSI).17 The antibiotics were selected as per the CLSI guidelines. The commercially available (Hi Media Laboratories Pvt. Limited) antibiotics disc and their concentrations (µg) used in this study were Ampicillin (A) 25 µg/disc, Ceftriaxone (CI) 30 µg/disc, Cefuroxime (CU) 30 µg/disc, Chloramphenicol (C) 25 µg/disc, Ciprofloxacin (CF) 10 µg/disc, Co Trimoxazole (Co) 25 µg/disc, Gentamicin (G) 30 µg/disc, Tetracycline (T) 25 µg/disc. E. coli ATCC 25922 was used as a control strain.

Table 1: Growth and colonial characteristic of isolates on specialized media

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<thead>
<tr>
<th>Medium</th>
<th>Growth characteristic</th>
<th>Growth under UV</th>
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<tbody>
<tr>
<td>MUG Sorbitol Mac Conkey Agar(MUG-SMAC)</td>
<td>E. coli O157:H7 colonies were pale colourless sorbitol non fermentor.</td>
<td>No fluorescence was observed under long wave UV light (MUG test negative )</td>
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</table>

(0.6% to 2.4%) of E. coli O157:H7 serovars for all diarrheal cases in USA. Khan A et al; Calcutta; 200219,20 have also reported very low incidence of EHEC or STEC in humans. 2 out of 150 diarrheagenic children admitted to the hospital were also found positive for STEC by Chattopadhyay UK, Gupta S. and Dutta S.21 When compared to these studies, the recovery of EHEC O157:H7 is higher in our study. STEC, an emerging zoonosis, has been associated clinically with a wide range of presentation extending from a symptomatic infection to severe bloody diarrhoea or haemorrhagic colitis, which can lead to life-threatening sequel like haemolytic uraemic syndrome.21 The etiological role of STEC in causing individual infections as well as outbreaks in the developed countries has been identified, while reports from developing countries like India and Bangladesh are sparse22-24 (Fig. 1).

Fig. 1: Colonial and growth characteristic on MUG Sorbitol MacConkeys Agar plate
CONCLUSION

Therefore emphasis should be given to screen the pediatric patients suffering from diarrhea for the presence of EHEC as a part of surveillance system. This will enable to reveal the actual magnitude of the problem caused by EHEC and also give early warning regarding any outbreaks in future. The progressive increase in antibiotic resistance among enteric pathogens, particularly in developing countries, is becoming a special concern. Of greatest immediate concern is the need for an effective, inexpensive antimicrobial agent that can be used safely for treatment of children with diarrhea, especially in developing countries such as India.

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