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CURRENT SCENARIO OF ESCHERICHIA COLI AND ITS SEROTYPE "O157:H7" IN INDIAN SUBCONTINENT

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Abstract: Shiga toxin producing *Escherichia coli* (STEC) is a newly emerged pathogen has gained immense attention across the globe. It causes large scale epidemics and thousands of sporadic cases of diarrheal illness every year. STEC infections have been reported across the world and causes mild illness to severe bloody diarrhoea and even life-threatening complications such as HUS in humans. The serotype O157:H7 has recently become a public-health problem of serious concern as it targets infants and young children. The review here describes the epidemiology of STEC in Indian subcontinent.

Keywords: STEC, Diarrhoea, PCR, *E. coli, O157:H7*

I. INTRODUCTION

Escherichia coli (E. coli) were first isolated by Escherich in 1885 from stools of infants with enteritis and it belongs to the tribe Escherichiae and family Enterobacteriaceae.It is a predominant normal flora of the intestinal tracts of humans and animals. Mostly, the E. coli strains are harmless commensals except few pathogenic ones. E. coli is a diverse species both phenotypically and genetically and is identified on the basis of 'O', 'H' and 'K' antigens, which together constitutes the serotype. The serological typing is determined by somatic antigens (O: O1-O173) and flagella antigen (H: H1-H56).The categorization is based on virulence, pathogenicity and serogroups.

According to World health organization (WHO) Report, approximately 11 million children under the age of five die because of E. coli-mediated gastroenteritis [1]. There are wide differences in the prevalence of different categories of diarrheagenic E. coli (DEC). These groups include Enteropathogenic E. coli (EPEC), Enteroinvasive E. coli (EIEC), Enterotoxigenic E. coli (ETEC), Enteroaggregative E. coli (EAggEC), Diffuse-adhering E. coli (DAEC) and Verotoxin-producing E. coli (VTEC) or Shiga -toxin producing E. coli (STEC) [includes Enterohaemorrhagic E. coli (EHEC)]. The incidence of DEC is largely unknown in India as very few laboratories can identify these organisms. ETEC and EPEC are the major bacterial enteric pathogens amongst DEC but the newer increasingly reported DEC in the recent years are the Shiga-like toxin producing E. coli (STEC) and entero aggregative E. coli (EAggEC)[2,3]

The STEC is a diverse family having more than 200 serotypes and around 160 of these have been recovered from humans. Epidemiologically majority of serious infections in humans indicates occur with O157: H7 serogroup. The other important serotypes are O26: H11, O111: H-, O145: H-, O45: H2 and O4: H-. Various studies have reported significant morbidity and mortality associated with outbreaks of gastrointestinal disease caused by STEC and have highlighted its threat to human health [4-6].

In India, there is little information on the prevalence of STEC across the country. There are no major reports which have identified it as a significant etiologic agent of diarrhoea for humans in India. The few cytotoxic strains of E. coli (O157 and non-O157 serogroups) reported from human patients with diarrhoea in India have been uncharacterized with uncertain origin [7]. There are not many reports of isolation of STEC from various animal species in India.

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II. STEC IN ANIMAL SOURCES

The STEC from non-diarrhoeic animal sources in India was first isolated in 1999. In faecal samples from asymptomatic 67 cattle STEC was detected in 10.5% faecal samples by multiplex PCR and culture on SMAC agar. The eight strains recovered were ONT: H2, O82: H8, O28ac: H21, O146: H1, O149: HNT, ONT: H19, O88: HN. [8]. The sea food was suspected as vehicle of transmission) when 2/60 fish samples and 2/48 clam samples were positive for *stx* and *hlyA* genes by PCR. STEC strains belonged to non-O157 serogroups from fresh fish, shellfish and meat sold in open markets [9]. 249 bovine and 60 ovine *E. coli* strains from faecal samples of 391 calves and 101 lambs were subjected to multiplex PCR for detection of *stx1*, *stx2*, *eaeA* and EHEC *hlyA* genes. STEC strains belonging to different serogroups were detected in 9.73% of calves and 6% of lambs studied. *E.coli* O157 was isolated from both bovine and ovine faecal samples [10]. The necropsy samples (liver, lung and intestine) collected from the pigs that died of edematous disease were characterized to confirm the virulence by PCR and molecular typing by randomly amplified polymorphic DNA-PCR (RAPD-PCR). The 3 *E. coli* strains showed the presence of the *stx2* and *eae* genes and 2/3 isolates were genetically similar [11].

The direct PCR assays in fishes and shrimps samples targeted virulence markers of EHEC (*eaeA* gene, *hlyA* gene, and *stx* gene). 1 shrimp sample was positive for all these virulence markers and seven typical *E. coli* O157:H7 isolates were recovered from the marker-positive shrimp sample [12]. 105 *E. coli* isolates obtained from stool samples of 190 healthy *Bos frontalis* belonged to 55 serogroups. 21.90% *E. coli* isolates belonging to 14 serogroups detected eiher *Stx1, stx2*, *eaeA* or *hlyA* genes using multiplex PCR. *E. coli* O157:H7 was not isolated [13].

In faecal samples from 165 buffaloes in West Bengal, 363 isolates of *E. coli* were obtained. 6·61% had at least one gene characteristic for STEC which belonged to 13 different serogroups. Out of the 23 *stx1* positive strains 30·43% were having *stx1C* subtype. 20 isolates having *stx2* gene, 25% had *stx2C* and 10% had *stx2d* gene. The isolated STEC strains were resistant most frequently to erythromycin (95·83%), cephalothin (62·5%), amikacin (54·17%), kanamycin (45·83%) and gentamicin (41·67%) [14].

III. STEC IN ANIMAL, FOOD AND HUMAN STUDIES

876 samples were screened for the presence of STEC by conventional and PCR methods. 12, 1 and 4 STEC strains from animal, human and food samples respectively were recovered. In 6.02% diarrhoeic animals, 3.12% diarrhoeic handler and 1.78% raw beef samples showed STEC. There was no significant difference in the antibiotic profile of the isolates. STEC strains were uniformly sensitive to common antibiotics except tetracycline, cephalexin, dicloxacillin, erythromycin and lincomycin [15].

The study on strains of STEC to assess antibiotic resistance, virulence gene and other molecular profiles from human stool samples, cow stool samples and beef samples was done over a period of two years in Kolkata. In 49.2% of the STEC strains there was resistance to one or more drug. The 44.5% of strains had *stx1* and *stx2*, 19% of strains had *stx2* and *hlyA* and 6.4% had *eae gene*. RAPD and PFGE found no similarity in the profiles between strains of STEC isolated from cows and humans [16].

The prevalence of STEC in hospitalized patients with diarrhoea, as well as in healthy cattle and raw beef samples were studied using multiplex PCR targeting s*tx1* and s*tx2* genes. STEC was detected in 50% of food samples, 18% of cattle samples, and 1.4% of bloody and 0.6% of watery faecal samples from hospitalized patients. *hly*A, *Kat*P, *esp*P and *etp*D were also detected. Antimicrobial susceptibility profile of 63 STEC isolates from 19 human faecal samples, 40 cattle samples and 4 food samples in Kolkata were assessed. The strains were resistant to ampicillin (25.4%), tetracycline (23.8%), streptomycin (14.3%), cephalothin (1.1%), cotrimoxazole (9.5%), nalidixic acid (6.4%) and neomycin (3.2%). 14 strains showed resistance to multiple antibiotics but no common pattern was observed among the strains [17].

STEC was detected in 1.3% of children suffering with diarrhoea but PCR was found to have higher sensitivity than hybridization technique [18]. The bead-ELISA, cytotoxic assay, PCR and colony hybridization were used to detect *stx1* and *stx2* genes to characterize STEC strains from seafood and beef in Mangalore. Shiga toxins were found in 4 strains from seafood and 6 from beef. The STEC strains were negative for *eae* gene but 2 STEC strains isolated from seafood had auto-agglutinating adhesion gene [19].

The stool samples were collected to find the etiological agent from 158 children with and 99 children without diarrhoea in a hospital situated in South India by culture, microscopy, enzyme immunoassays and molecular methods (six PCR-based assays). 2.5% (4/158) cases were positive by ELISA for EHEC and there was an increase by 1.9% with PCR. [20]. In 326 *E. coli* isolates that were obtained from 326 faecal samples, 7.97% samples had either *eaeA* or *lt* gene. The ELISA for Shiga toxin(s) was negative in all the samples [21].

The 10 year epidemiological survey of *E. coli* O157 across India from humans, food, animals and the environment conducted in 5678 human samples and 11,415 non human samples. There were only 30 (0.5%) human samples which were positive for *E. coli* O157. The *E. coli* O157 was seen in meat 0.9%, (13/1376), milk and its

products 1.8%, (10/553), seafood 8.4%, (16/190) and water 1.6%, (8/486). The isolates obtained were widely distributed among both domestic & wild animals [22].

The diarrheagenic stool and meat samples were screened for STEC by culture methods and PCR. 1 sample was positive for *stx1, stx2, rfb* O157 and EHEC *hlyA* and belonged to O157 serogroup out of 40 *eae* positive *E. coli* isolates from meat sample. 2 were positive for EHEC *hlyA* and belonging to serogroup O8 and 1 was positive for *bfp* gene and found to be of O6 serogroup out of the 110 *eae* positive *E. coli* isolated from stool samples [23]. The duplex assay using two specific molecular beacons by real-time PCR was used to target *rfb* gene of *E. coli* O157:H7 and the *hly* gene of *L. monocytogenes*. In 60 market samples, 3 samples (raw milk, kulfi, and paneer) were positive for *E. coli* O157:H7, while 1 sample (raw milk) was positive for *L. Monocytogenes* [24].

The *uidA* gene was targeted by multiplex PCR assay to detect *E. coli* and *Shigella sp.*, and *ipaH* and stx_1 genes to detect *Shigella sp.* and STEC. In 100 naturally milk samples, *E. coli were detected in all the samples* and VTEC in 15 samples. The *Shigella* sp. was not encountered in any samples [25]. The study conducted to determine the prevalence of DEC in dysentery cases with special reference to STEC, during a two-year period, 1066 stool samples were collected. Out of the 100 *E. coli* strains isolated in pure culture, 43% were found to be DEC, giving an isolation rate of 4.03%. Results of serotyping showed 37.21% STEC were more common in children. *E. coli* O157 was not found and it was seen that 25% of STEC did not ferment sorbitol. The DEC strains showed maximum *in vitro* sensitivity to amikacin (83.72%) and all strains were resistant to nalidixic acid. As complications, about 16.67% of children developed HUS and 10.53% of patients developed acute renal failure [26].

The STEC and EPEC were detected and characterized from an outbreak of acute diarrhea in poultry birds in Mizoram. *E. coli* was isolated and identified from various organs and sites from 19 poultry birds which had died. It was identified by standard bacteriological culture, biochemical and serotyping. The multiplex PCR assay used to target genes like *stx 1, stx 2, eaeA* and *hlyA*. In 42 *E. coli* isolates, 24 belonged O64, O89 and O91 and 18 were untypable. The 14 (33.33%) had at least 1 virulence gene, 10 (23.81%) were STEC and 4 (9.52%) were EPEC. Out of 10 STEC isolates, *stx2* was in one case, *stx 2* and *hlyA* in one, *stx1* , *stx2* and *hlyA* in four, *stx 1*, *eaeA* and *hlyA* genes in two and *stx 1* and *eaeA* in two cases [27].

A surveillance study conducted in semi-urban and rural areas of North India found STEC isolates (80 animal faeces, 39 food, and 21 human faeces). The variants found were *stx2c* (25·1%), *stx1d* (13%), *stx1c* (10·7%) and *stx2d* (9·2%); and 38% human isolates had *stx* variants [28].

In another study conducted in Manglore, 200 children with diarrhoea were characterized foe DEC using 2 multiplex PCR assays. The targets were *eae* and *bfpA* for EPEC, *hlyA* for EHEC, *elt* and *stla* for ETEC, CVD432 for EAEC and *ial* for EIEC. In 52/200 (26%) diarrheal children DEC were found and in 8/100 (8%) controls DEC strains were found. EAEC was found in 13% cases, EPEC in 16% cases, ETEC in 7 (3.5%) and EIEC in 3 (1.5%) of the diarrheal cases. The EHEC was not isolated in the present study [29].

IV. CONCLUSION

In India various studies conducted have found either no or rare occurrence of STEC in humans in India. But the frequent isolation of STEC strains from non human sources like animals, food and other products along with the identification of multidrug resistance and virulence genes across the Indian subcontinent poses a serious threat of the outbreaks that can occur in the future.

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