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Virulence genes and antibiotic resistance profile of shiga toxin producing *Escherichia coli* and enterotoxigenic *Escherichia coli* from diarrhoeic cattles and human handlers in Asom (India)

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Shiga toxin producing *Escherichia coli* (STEC) and enterotoxigenic *E. coli* (ETEC), a serologically diverse group of zoonotic pathogens, have emerged as one of the most virulent groups of bacteria associated with cases of food borne disease in humans. STEC are associated with a disease spectrum ranging from diarrhea and hemorrhagic colitis (HC) to the potentially fatal hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Rahal *et al.* 2015) whereas, ETEC is the major cause of travelers' diarrhea and a major cause of diarrheal disease in developing countries, especially for children less than 5 years old (Cai *et al.* 2016). In India, there is a paucity of information on STEC and EPEC, since, it has not been identified as a significant etiological agent among diarrhoeic calves and humans, therefore, the present study was undertaken on isolation and characterization of STEC and EPEC from diarrhoeic calves and humans in Asom, India.

Samples (100) originating from 80 diarrhoeic calves and 20 humans faeces handling the cattle were collected from different parts of Asom. All the samples were processed using standard bacteriological techniques for isolation and identification of *E. coli* followed by phenotypic characterization based on standard morphological and biochemical properties (Quinn *et al.* 2004). *E. coli* isolates obtained in this study were serotyped based on their somatic (O) antigens at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India. The isolates confirmed by conventional tests from overnight cultures were suspended in 200 µl sterile distilled water and boiled at 100ºC for 10 min. After boiling, the cell suspensions were cooled in an ice bath for 10 min and the lysates were centrifuged again at 6000 rpm for 10 min and the supernatant was used as template DNA. Multiplex PCR based screening of stx_1, stx_2, *elt, est and hly* virulence genes among the isolates was carried out as described by Rahman (2002). Amplified products were analysed by agarose gel (2%) electrophoresis and documented. *In vitro* antimicrobial susceptibility pattern of the STEC isolates obtained in the present study were determined by disc diffusion method (Bauer *et al.* 1966) using 12 commonly used antimicrobial agents, ampicillin (10 mcg), cloxacillin (5 mcg), chloramphenicol (25 mcg), co-trimoxazole (25 mcg), ciprofloxacin (30 mcg), enrofloxacin (10 mcg), furazolidone (50 mcg), gentamicin (10 mcg), nitrofurantoin (300 mcg), nalidixic acid (30 mcg), norfloxacin (10 mcg) and tetracycline (30 mcg).

Out of the 134 presumptive *E. coli* isolates picked from 80 diarrhoeic calves, 31 isolates could be serotyped into 23 different serogroups and 19 strains of *E. coli* from human beings, 13 strains could be serotyped into 10 different serogroups. The frequently encountered serogroups from cattle was O157 and from humans, O131 respectively.

STEC serotype O157 has the highest prevalence in the world in recent years and this bacterium can be transmitted to humans through contaminated food, water, direct contact with animals and human-to-human transmission, and can cause serious diseases (Oloyede *et al*. 2016). Several new serogroups of *E. coli* are also being established as potential pathogens for human and animals therefore, their clinical significance in calves diarrhoea needs to be explored by further studies. In Multiplex PCR based screening of virulence genes showed that out of 31 isolates from diarrhoeic calves, 14 (45.16%) and in humans handling the cattles, 5 (26.32%) (Table 1) were carried at least one virulence gene, based on which eight and five different virulence gene profiles were identified. The virulence gene profile of the STEC and ETEC isolates showed that the prevalence of stx_2 gene (16.13%) in cattle and in human *stx2* gene (15.79%) were higher than stx_1 gene which was in agreement to the findings of earlier workers (Tahamtan *et al*. 2010) as the stx_2 gene is considered to be the most important virulence factor which in mice was shown to be 400 fold more toxic than stx_1 and it also induces feto-placental re-absorption, intrauterine hematoma, fibrin

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Table 1. Distribution of virulence genes in *E.coli* from diarrhoeic calves and humans in Assam

Virulence	Calves (n= 31)	Total	Humans (n=13)	Total
*stx*1 only	1	1 (3.23%)	1	1 (5.26%)
*stx*2 only	5	5 (16.13%)	3	3(15.79%)
*stx*1 and *stx*2	1	1 (3.23%)	-	
*stx*2 and *hly*	2	2 (6.45%)	-	
hly only	1	1 (3.23%)	-	
*stx*1, *stx*2 and *hly*	2	2 (6.45%)	1	1(5.26%)
elt	1	1 (3.23%)	-	
est	1	1(3.23%)	-	
Total	14	14 (45.16%)	5	5 (26.32%)

deposition and neutrophil infiltration (Kataria *et al.* 2014). Thus isolation of STEC isolates with high frequency of stx_2-positivity from cattle and humans is a serious concern as strains expressing stx_2 are more likely to be associated with the development of HUS. One (3.23%) isolate carried *est* virulence gene and 1 (3.23%) isolate carried *elt* virulence gene (ETEC) but no virulence genes could be detected in humans which was in contrast to Islam *et al.* (2015). This variation in prevalence rate may be associated with different factors such as species, season of the study, geographical location, environment and hygienic conditions in the farm. Majority of *E. coli* strains were exhibited high level of resistance to furazolidone (98%), cloxacillin (91%), enrofloxacin (90%), ampicillin (90%), tetracycline (88%), nitrofurantoin (78%). The best antimicrobial agents in terms of sensitivity were gentamicin (100%), ciprofloxacin (96%), chloramphenicol (88.31%), norfloxacin (83.77%), Co-trimoxazole (68.18%) and nalidixic acid (60.40%). Multiple drug resistance in EPEC and STEC strains may result from spread of genetic elements, such as plasmids, prophages or transposons, allowing horizontal transfer within and between bacterial species, predominantly in environments such as the gut micro-biome (Amaechi *et al.* 2015). Since similar type of antimicrobial agents are also options for treating enteric infection in humans, therefore, getting a large number of cattle isolates resistant to them is a matter of concern. This finding suggests that use of antibiotic for disease prevention and treatment in animals and humans may be responsible for the resistant phenotype in STEC and ETEC isolates.

SUMMARY

The present study was undertaken to isolate and characterize STEC and ETEC from diarrhoeic calves and humans in Assam, India. Out of the 134 presumptive *Escherichia coli* isolates picked from 80 diarrhoeic calves, 31 isolates could be serotyped into 23 different serogroups and 19 strains of *E. coli* from human beings, 13 strains could be serotyped into 10 different serogroups. O157 from cattle and O131 from humans were the frequently encountered serogroups. The virulence gene profile of the STEC and ETEC isolates showed that the prevalence of stx_2 gene and the emergence of multi drug resistant STEC and ETEC isolates in high proportion indicates that they may act as an important reservoir posing a possible threat to public health and may complicate future therapeutic options.

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