Indian J Med Res 140, December 2014, pp 778-784

Characterization of Shiga-toxigenic *Escherichia coli* isolated from cases of diarrhoea & haemolytic uremic syndrome in north India

Ajay Kumar, Neelam Taneja, Bhavneet Bharti* & Meera Sharma

*Department of Medical Microbiology & *Advanced Pediatric Centre, Postgraduate Institute of Medical Education & Research, Chandigarh, India*

Received May 6, 2013

Background & objectives: **Shiga toxin producing *Escherichia coli* (STEC) is an important zoonotic foodborne pathogen, capable of causing haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). As data from India on human infections caused by STEC are limited, this study was carried out for hospital based surveillance for STEC as a causative agent of diarrhoea, bloody diarrhoea and HUS at a tertiary care centre and to study the virulence gene profile and strain relatedness by multi locus variable tandem repeat analysis (MLVA).**

Methods: **A total of 600 stool samples were studied. Stool samples of every fifth patient presenting with non-bloody diarrhoea, all cases of bloody diarrhoea and diarrhoea associated HUS (D+HUS) were collected from October 2009 to September 2011. Stool samples were cultured for STEC and characterization of STEC was done by serogrouping, virulence genes analysis, and MLVA typing.**

Results: **STEC were isolated as a sole pathogen from 11 stool samples [5 of 290 (1.7%) non-blood diarrhoea and 5 of 300 (1.6%) blood diarrhoea cases]. STEC was also isolated from one fatal case of HUS who was an eight month old child. Only six of 11 isolates were positive for *stx*2 gene, whereas *stx*1 was present in all 11 isolates. Only one isolate was positive for *eae.* Other adhesion genes present were *iha* in five isolates, followed by *toxB* and *efa*1 in two each and *saa* gene in one, isolate. Among the plasmid encoded genes, *esp*P, *hly* and *etp*D were each present in one isolate each. In the MLVA typing, diverse profiles were obtained except two untypeable isolates from different patients shared the same MLVA profile. Both these isolates were not epidemiologically linked.**

Interpretation & conclusions: **This study demonstrated that STEC could be a causative agent of diarrhoea, bloody diarrhoea and sporadic HUS. However, further work needs to be done to study and explore the prevalence of these organisms in the food chain in this region.**

Key words Bloody diarrhoea - haemolytic uremic syndrome - human - Shiga-toxigenic *Escherichia coli*

Shiga-toxigenic *Escherichia coli* (STEC) is the most common food-borne zoonotic pathogen implicated in causing gastrointestinal illnesses, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). Though O157:H7 is the most common serotype implicated in human illness, other serotypes are also emerging. The Centers for Disease Control and Prevention (CDC) data show six non-O157 serotypes [O111:H8 or Non-

motile (NM); O103:H2, H11, H25; O26:H11 or NM; O45:H2 or NM; O121:H19 or H7; and O145:NM] to be responsible for 71 per cent of non-O157 STEC illnesses in the USA[1]. STEC infections are a cause of concern particularly for elderly and paediatric patients because of the higher risk of HUS in these populations[2]. CDC has updated guidelines in October 2009 for the detection of STEC in acute community-acquired diarrhoea, and recommended tests for detection for shiga toxins or *stx* genes in addition to conventional culture[3] (*http://www.cdc.gov/mmwr /preview/mmwrhtml/rr5812a1.html*).

In contrast to the developed world, little is known about STEC epidemiology in humans in India, though multiple reservoirs for STEC have been described, including healthy and diarrhoeagenic cattle, calves, goats and yaks[4-7]. STEC were isolated from 1.4 and 0.6 per cent of human bloody and watery diarrhoea samples in a study from Kolkata[5]. A study from Vellore has reported 2.3 per cent isolation in preschool-aged children admitted to the hospital with diarrhoea and an equivalent (2%) asymptomatic population[8]. However, from India no outbreaks of human illness are reported and no data are available supporting the role of STEC in HUS. Therefore, we conducted a hospital based surveillance for these pathogens in human cases of diarrhoea and HUS presenting to a tertiary case centre in north India. The virulence profile and the genetic inter-relatedness of these organisms were also studied using multi locus variable tandem repeat analysis (MLVA).

Material & Methods

Sample collection: Stool samples (n=600) were collected from every fifth patient presenting with non-bloody diarrhoea (290 of 1438), all cases of bloody diarrhoea and diarrhoea associated HUS (D+HUS) (n=310) in patients from October 2009 to September 2011 in Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. The samples were submitted to the Enteric Laboratory of department of Medical Microbiology at the PGIMER, Chandigarh. A total of 1270 cases were excluded from the study. Acute non-bloody diarrhoea was defined as the sudden onset of diarrhoea with frequent loose stool >3 times/day without the presence of visible blood. Bloody diarrhoea was defined as the presence of visible blood in loose stools, and diarrhoea associated HUS was defined as the occurrence of haemolytic anaemia, acute renal failure and thrombocytopenia after an episode of bloody diarrhoea. Patients presenting with diarrhoea at the time of OPD of both pediatric and adult patients (Medicine and Gastroenterology OPDs) visit /admission or developing diarrhoea within 48 h of admission were included while patients who developed diarrhoea after 48 h of admission to the hospital were excluded. The study protocol was approved by the Institute Ethics committee. Written informed consent was obtained from patients.

Culture of stool samples: About 5 ml of fresh stool was collected in a wide mouth container and a small portion of the stool specimen was transferred to Cary-Blair medium (Difco, USA). The stool sample was grossly examined for presence of blood and mucus. All stool samples were plated onto the MacConkey agar (Hi-Media Ltd., Mumbai, India), xylose lysine deoxycholate agar (XLD) (Hi-Media Ltd., Mumbai, India), thiosulphate citrate bile salt sucrose agar (TCBS) (Difco, USA), blood agar (Difco, USA) and inoculated into selenite F (Hi-Media Ltd., Mumbai, India) and alkaline peptone water (Hi Media Ltd., Mumbai, India) for isolation of *Salmonella, Shigella, Vibrio cholerae* and *Aeromonas*. A multiplex PCR assay was used to characterize the biochemically confirmed *E. coli* for enteroaggregative (EAEC), enterotoxigenic (ETEC) and enteropathogenic *E. coli* (EPEC)[9].

Processing of samples for STEC: For STEC screening, enrichment of stool specimens was performed by inoculating. 5 ml of stool samples into 3 ml of enriched culture (EC) medium (Difco, USA) and incubated overnight at 37˚C with constant shaking. DNA was extracted from one ml of the broth culture by boiling method[10]. Supernatant was used in the PCR for the detection of *stx1* and *stx2* genes by using primers and PCR conditions as described previously[11]. Broth cultures positive for either *stx*1 and/or *stx*2 by PCR, were serially diluted in 10 mM phosphate-buffered saline (PBS) (*p*H 7.0) and 100 µl volume of each dilution was spread on Sorbitol MacConkey agar (SMAC) (Difco, USA), in duplicate. Isolated presumptive *E. coli* colonies including non-sorbitol fermenting colonies were picked up randomly from dilution plates and subjected to biochemical analysis for confirmation.The *E.coli* colonies were further spot- inoculated on master Luria agar plates. After division into rows and columns, colonies from each row and each column were pooled into fresh EC broths. After overnight incubation at 37˚C, DNA was extracted by boiling method. Duplex PCR, using primers for *stx*1 and *stx*2 was performed. When positive results were obtained in one column and one row, the shared colony was considered as STEC positive. A *stx*-positive colony was preserved in brain

heart infusion broth (BHI, Difco, USA) containing 15 per cent glycerol at -80°C, till further use.

Phenotypic characterization

Serogrouping: On the basis of O antigen, isolates were serogrouped by the National Salmonella and Escherichia Centre at the Central Research Institute at Kasauli, India.

Analysis of virulence genes: PCR assays were carried out targeting the STEC virulence genes *stx*1*, stx*2, *eae, hly, katP, espP* and adhesion genes *iha, saa, efa1* and *toxB* as described previously[12-17]. The following standard strains of STEC were used as controls in the virulence gene detection: EDL933, VT3 and Wani 1. EDL933 was gifted from Pasteur Institute, Paris, France. VT3 strain was obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata and Wani 1, was obtained from Dr Shakil Wani, S.K University of Agriculture Sciences and Technology of Kashmir, Srinagar. All three belonged to serogroup O157 and were characterized for *stx1* and *stx2* as well as various other virulence genes.

Antimicrobial drug susceptibility testing: Isolates were tested for antimicrobial drug resistance by Kirby Bauer disc diffusion method[18] for nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (5µg), amikacin (30 µg), ceftriaxone (30 µg), cefoperazone (30 µg), gentamicin (30 µg), co-trimoxazole (25 µg), azithromycin (30 µg), and ciprofloxacin (5 µg) (Oxoid Limited, Hampshire, UK) according to CLSI (Clinical and Laboratory Standards Institute) guidelines[19]. *E.coli* ATCC 25922 was used as the control strain.

MLVA typing: MLVA typing was performed by using five VNTR loci CVN001, CVN004, CVN007, CVN014 and CVN015 as described previously[20]. These loci were chosen on the basis of reproducibility and discriminatory indices and PCR was performed as described[20]. EDL933 was used as the control strain. After amplification, products were separated on a 6 per cent denaturing polyacrylamide gel (PAGE) by using SequiGen gel apparatus 38 × 50 × 0.4 cm (BioRad Laboratories Inc., USA). After electrophoresis, gels were fixed overnight in 10 per cent acetic acid and then silver stained using the Promega silver staining kit (Promega, USA). On the basis of amplicon size, allele numbers were assigned to each locus[20] and allele string was made for each isolate with an order: CVN001-CVN004-CVN007-CVN014-CVN015.
The dendrogram was constructed by using UPGMA clustering method with START vs. 2.0.5 software[21]. Minimum spanning tree (MST) was constructed by using the online software (*http://pubmlst.org/analysis.*).

Results

Table I shows the age and sex distribution along with results of stool culture. Of the 600 cases, 56 per cent were males (mean age 4.7 yr; range from 2 months to 60 yr) and 44 per cent were females (mean age 5.2 yr; range from 40 days to 67 yr). From 600 stool samples (290 from patients with acute non-bloody diarrhoea, 300 from bloody diarrhoea and 10 from HUS) screened by PCR, STEC were isolated as a sole pathogen from 11 (1.8%) samples. Of these 11 isolates, five isolates each were obtained each from acute non-bloody diarrhoea (1.7%) and bloody diarrhoea (1.6%) cases and one was isolated from a case of HUS.

Table II shows details of 11 patients from whom STEC were isolated. Of these, eight were admitted to

Table I. Age, sex and type of disease distribution of human patients whose stool samples grew STEC

Age group (yr)	Acute non-bloody diarrhoea		Bloody diarrhoea and HUS		Other organisms isolated
	Male	Female	Male	Female	
<2 (n=182)	30	42	74	36	STEC (2.1%), *Shigella flexneri* (5.4%), *Shigella dysenteriae* (1.09%), Enteroaggregative *E. coli* (8.2%)
2-5 (214)	58	26	74	56	STEC (0.9%), *Shigella flexneri* (5.6%), Enterotoxigenic *E.coli* (7%), Enteropathogenic *E. coli* (3.7%)
> 5 (204)	70	64	40	30	STEC (2.4%), *Salmonella* (1.04%), *Aeromonas* (0.9%)
Total (n=600)	158	132	188	122	

HUS, haemolytic uremia syndrome; STEC, shiga-toxigenic *E. coli*

the hospital while the remaining three were out-door patients. Their age ranged from <2 months to 60 yr. All patients were admitted to different wards of the hospital. Stool sample of only one HUS case, an eight month old male infant, was positive for STEC. HUS developed after a week of bloody diarrhoea. The patient had the typical triad of acute renal failure (urea 164 mg/dl, creatinine 7.9 mg/dl), anaemia (6.4 g/dl) and thrombocytopenia (platelet count 99000/µl). There was an epidemiological evidence of contact with animals and the case was fatal[22]. The STEC isolate was positive for *stx*1, *stx*2, *eae* and *iha*. Thus enterohaemorrhagic *E.coli* (EHEC) which is a subset of STEC (defined as *stx*1$^+$/*stx*2$^+$ and *eae*$^+$) was isolated from stool sample of only one patient.

Characterization of virulence genes: The *stx*1 gene was the most common virulence gene, present in 11 (100%) isolates followed by *stx*2 in six (54.5%) isolates. The *eae* gene was present in HUS isolate only. Among the plasmid encoded genes, *esp*P, *hly* and *etp*D were each present in one (9%) isolate. The most common adhesion gene was *iha*, present in five (45.4%) isolates, followed by *toxB* and *efa*1 each in two (18%) isolates and *saa* gene in one (9%), isolate (Table II).

Serogrouping: Isolates belonged to serogroup O85 (2 isolates), O95 (one isolate) and O102 (one isolate). Six isolates were found to be untypable with somatic (O) antiserum and one was found to be rough.

Antimicrobial drug susceptibility testing & MLVA: All isolates were found to be sensitive to all the antimicrobials tested. In the dendrogram analysis, 11 MLVA genotypes were observed (Fig. 1); 10 genotypes were represented by one isolate each and one genotype was shared by two isolates (9,10). Two main clades were identified (clade I and clade II). Clade I included four isolates (including EDL933) and clade II included eight isolates. In the MST analysis, HUS and two other isolates were found to be closely related to EDL933 control STEC strain (Fig. 2).

Discussion

This study was carried out in a tertiary care centre of north India. The geographic region around this centre is a major milk producing area of India and animal rearing is one of the main sources of income. In the present study, STEC were isolated from five cases non-bloody diarrhoea (1.7%) and five bloody diarrhoea (1.6%) cases. These rates were similar to those reported from the Netherlands (1.7% from cases of bloody diarrhoea)[23] and higher than those reported from Alberta, Canada (1.4% in stool samples suspected for viral gastroenteritis)[2]. Although STEC reservoirs exist and are clearly present in the food chain and environment in India, yet, the human illness appears to be infrequent. In a study from Kolkata, STEC were isolated from 1.4 and 0.6 per cent of human bloody and watery diarrhoea samples[5]. Other pathogens like *Salmonella*, *Shigella flexneri, S. dysentriae*, *Aeromonas*, EPEC, ETEC and EAEC were also isolated from the stool samples in our study. Isolation rate for STEC as a causative agent in our hospital-based surveillance for community acquired diarrhoea was slightly higher than *Salmonella* (1.04%), indicating that these organisms should also be routinely looked for in the diagnostic microbiology laboratories, especially in areas where animals are reared.

In the present study, all STEC were sensitive to all 10 antimicrobials tested. Khan *et al*[24] demonstrated antimicrobial resistance in 49.2 per cent of the STEC isolated from cow stool, beef and human stool samples collected from Kolkata, and resistance was commonly observed to ampicillin, tetracycline, and streptomycin and less frequently to cephalothin, co-trimoxazole, nalidixic acid, and neomycin. A high level of antimicrobial resistance has been reported in other pathotypes of diarrhoeagenic *E. coli* (enteroaggregative *E coli*, enterotoxigenic *E. coli* and enteropathogenic *E. coli*) from our region[9]. The antimicrobial sensitivity of STEC may be due to less antimicrobial pressure on these bacteria.

It has been shown that a large spectrum of serologically different STEC types can infect humans[25]. The serogroups found in this study have also been reported from animal stool and food samples from India[4,6,7]. The serogroup O157 was conspicuously absent.

The *stx*1 gene was present in all the isolates whereas *stx*2 was present in six of 11 isolates. Strains carrying *eae* and *stx*2 are considered to be more pathogenic to humans[26]. The low prevalence of *eae* positivity indicates the absence of LEE (locus for enterocyte attaching and effacing lesions) or presence of *eae* variants/other adhesins in these isolates. Though many studies have shown that LEE is essential for host colonization and virulence[27,28], others have demonstrated that some STEC isolates without LEE, such as STEC O113:H21,O1:H7, NT:H7, O76:H7, O128:H2, O91:H, O113:H21, O116:H21, O130:H11 and O5:H$^-$ are associated with sporadic cases and outbreaks of HUS[29-31]. Other

Table II. Demographic details of STEC positive acute non-bloody and bloody diarrhoea cases and virulence factors harboured

STEC strain number	Age (yr)/Sex	Clinical characteristics	Ward/OPD	Serogroups	Virulence genes										
					stx1	*stx2*	*eae*	*hly*	*etpD*	*katP*	*espP*	*iha*	*saa*	*ToxB*	*efa*
H1	19 /M	Acute non-bloody diarrhoea	GEC	O102	+	+	-	-	-	-	-	+	+	+	-
H2	40 /M	Acute non-bloody diarrhoea	OPD	O95	+	+	-	-	-	-	-	-	-	-	-
H3	60 /M	Acute non-bloody diarrhoea	CCU	UT	+	-	-	-	-	-	-	+	-	-	+
H4	9 /M	Bloody diarrhoea	APC-2B	UT	+	-	-	-	+	-	-	-	-	-	-
H5	20/M	Acute non-bloody diarrhoea	EMW	UT	+	+	-	+	-	-	-	-	-	+	-
H6	<2 months/F	Bloody diarrhoea	APC-2B	O85	+	-	-	-	-	-	-	+	-	-	-
H7	5 years/M	Bloody diarrhoea	APC-OPD	O85	+	-	-	-	-	-	-	+	-	-	-
H8	9 months/F	Bloody diarrhoea	ChOPD	ROUGH	+	+	-	-	-	-	+	-	-	-	-
H9	27 months/F	Bloody diarrhoea	APC-2B	UT	+	+	-	-	-	-	-	-	-	-	+
H10	2 years/M	Acute non-bloody diarrhoea	APC-2B	UT	+	-	-	-	-	-	-	-	-	-	-
H11	8 months/M	HUS	APC	UT	+	+	+	-	-	-	-	+	-	-	-

APC, Advance Paediatric Centre; ChOPD, child out door patients; CCU, critical care unit; GEC, Gastoenterology centre; EMW, emergency ward; OPD, out door patients; HUS, haemolytic uremic syndrome; UT, untypeable

The isolates belonged to serogroup O85 (2 isolates), O95 (1 isolate) and O102 (1 isolate). Six isolates were found to be untypable with somatic (O) antiserum and one was found to be rough

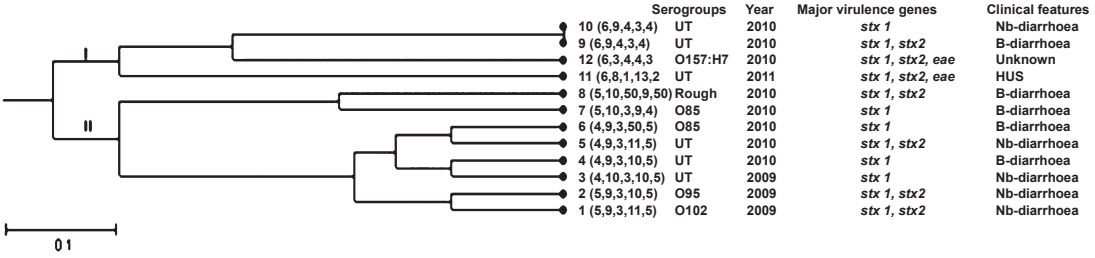

Fig. 1. Dendrogram based on MLVA profiles of 11 non-O157 STEC isolates. Dendrogram based on allelic profiles of MLVA for 11 isolates including reference strain 12 (EDL933) was constructed by using START v2. Nb, non-bloody diarrhoea; B, bloody.

adhesins described for STEC include *efa*I (EHEC factor for adherence), chromosomal *Iha* (iron-regulated gene A homologue)[32], STEC autoagglutinating adhesin (Saa)[33], and plasmid-encoded *ToxB*[34]. In our study, the most common adhesion gene was *iha*, followed by *toxB* and *efa*1; *saa* gene was present in one isolate only. Other toxins such as *espP, etpD, hly* and *katP* present on the large plasmid may also contribute to the severity of STEC illness. These genes were found to be variably present in our isolates. Though, the interplay between these genes is not well understood, but these factors provide additional traits to increase the virulence in STEC.

Diverse MLVA profiles were obtained from patients except for two showing the same MLVA profile. Both of these isolates were not epidemiologically linked. Small number of HUS cases was the main limitation of our study. To conclude, this study demonstrates the presence of STEC isolates as causative agents of diarrhoea and bloody diarrhoea and sporadic HUS. Further work needs to be done to study the epidemiological and evolutionary relationships between STEC present in the environment, cattle and/or the food chain, and STEC ultimately acquired by humans.

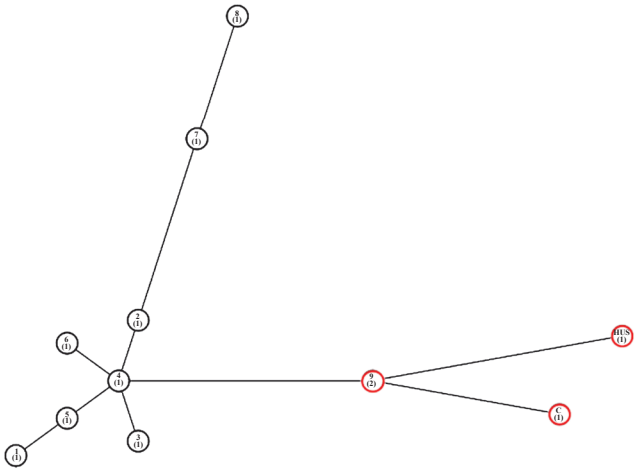

Fig. 2. Minimum spanning tree (MST). MST based on the MLVA profiles of STEC isolates was constructed. Profile 9 in red circle represents the two isolates (H9, H10) sharing the profile. C-EDL933 control.

Acknowledgment

Authors acknowledge the Indian Council of Medical Research (ICMR), New Delhi, India for financial support and the Director, National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, India for serogrouping of the isolates.

References

1. Bosilevac JM and Koohmaraie M. Prevalence and characterization of non-O157 shiga toxin-producing *Escherichia coli* isolates from commercial ground beef in the United States. *Appl Environ Microbiol* 2011; *77* : 2103-12.
2. Couturier MR, Lee B, Zelyasand N, Chui L. Shiga-toxigenic *Escherichia coli* detection in stool samples screened for viral gastroenteritis in Alberta, Canada. *J Clin Microbiol* 2011; *49* : 574-8.
3. Available from: *http://www.cdc.gov/mmwr /preview/ mmwrhtml/rr5812a1.html.* accessed on October 12, 2012.
4. Bandyopadhyay S, Lodh C, Rahaman H, Bhattacharya D, Bera AK, Ahmed FA, *et al*. Characterization of shiga toxin producing (STEC) and enteropathogenic *Escherichia coli* (EPEC) in raw yak (Poephagus grunniens) milk and milk products. *Res Vet Sci* 2012; *93* : 604-10.
5. Khan A, Yamasaki S, Sato T, Ramamurthy T, Pal A, Datta S, *et al*, Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India. *Emerg Infect Dis* 2002; *8* : 54-62.
6. Wani SA, Hussain I, Fayaz I, Mirand MA, Nishikawa Y. Subtype analysis of *stx1*, *stx2* and *eae* genes in Shiga toxin-producing *Escherichia coli* (STEC) and typical and atypical enteropathogenic *E. coli* (EPEC) from lambs in India. *Vet J* 2009; *182* : 489-90.
7. Wani SA, Samanta I, Munshi ZH, Bhatand MA, Nishikawa Y. Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* in healthy goats in India: occurrence and virulence properties. *J Appl Microbiol* 2006; *100* : 108-13.

8. Rajendran P, Rajan DP, Kangand G, Thorpe CM. Shiga toxin-producing *Escherichia coli* infection in South India. *J Med Microbiol* 2009; *58* : 1525-6.
9. Aranda KRS, Fagundes-Neto U, Scaletsky ICA. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic *Escherichia coli* and *Shigella* spp. *J Clin Microbiol* 2004; *42* : 5849-53.
10. Queipo-Ortuño MI, De Dios Colmenero J, Macias M, Bravo MJ, Morata P. Preparation of bacterial DNA template by boiling and effect of immunoglobulin Gas an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clin Vaccine Immunol* 2008; *15* : 293-6.
11. Kumar A, Taneja N, Kumarand Y, Sharma M. Detection of Shiga toxin variants among Shiga toxin-forming *Escherichia coli* isolates from animal stool, meat and human stool samples in India. *J Appl Microbiol* 2012; *113* : 1208-16.
12. Paton AW, Paton JC. Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *stx1*, *stx2*, *eae*, *ehxA*, and *saa*. *J Clin Microbiol* 2002; *40* : 271-4.
13. Paton AW and Paton JC, Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfbO111*, and rfbO157. *J Clin Microbiol* 1998; *36* : 598-602.
14. Schmidt H, Henkeland B, Karch H. A gene cluster closely related to type II secretion pathway operons of Gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. *FEMS Microbiol Lett* 1997; *148* : 265-72.
15. Brunder W, Schmidtand H, Karch H. EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Mol Microbiol* 1997; *24* : 767-78.
16. Tarr CL, Large TM, Moeller CL, Lacher DW, Tarr PI, Acheson DW, et al. Molecular characterization of a serotype O121:H19 clone, a distinct Shiga toxin-producing clone of pathogenic *Escherichia coli*. *Infect Immun* 2002; *70* : 6853-59.
17. Brunder W, Schmidtand H, Karch H, Kat P, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology* 1996; *142* : 3305-15.
18. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; *45* : 493-6.
19. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*; 18th Informational Supplement. M100-S18. Wayne, PA: CLSI; 2008.
20. Lindstedt BA, Brandal LT, Aas L, Vardundand T, Kapperud G. Study of polymorphic variable-number of tandem repeats loci in the ECOR collection and in a set of pathogenic *Escherichia coli* and *Shigella* isolates for use in a genotyping assay. *J Microbiol Methods* 2007; *69* : 197-205.
21. Joley KA, Feil EJ, Chanand MS, Maiden MC. Sequence type analysis and recombinational tests (START). *Bioinformatics* 2001; *17* : 1230-31.
22. Kumar A, Taneja N, Singhi S, Shahand R, Sharma M. Haemolytic uraemic syndrome in India due to Shiga toxigenic *Escherichia coli. J Med Microbiol* 2013; *62* : 157-60.
23. van Duynhoven YT, Friesema IH, Schuurman T, Roovers A, van Zwet AA, Sabbe LJ, *et al*. Prevalence, characterisation and clinical profiles of Shiga toxin-producing *Escherichia coli* in The Netherlands. *Clin Microbiol Infect* 2008; *14* : 437-45.
24. Khan A, Das SC, Ramamurthy T, Sikdar A, Khanam J, Yamasaki S, *et al*. Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. *J Clin Microbiol* 2002; *40* : 2009-15.
25. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnsonand RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol* 1999; *37* : 497-503.
26. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia* coli O157:H7 infections. *J Infect Dis* 1989; *160* : 994-8.
27. Marches O, Nougayrede JP, Boullier S, Mainil J, Charlier G, Raymond I, *et al*. Role of tir and intimin in the virulence of rabbit enteropathogenic *Escherichia coli* serotype O103:H2. *Infect Immun* 2000; *68* : 2171-82.
28. McKee ML, Melton-Celsa, AR, Moxley RA, Francis DH, O'Brien AD. Enterohemorrhagic *Escherichia coli* O157:H7 requires intimin to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells. *Infect Immun* 1995; *63* : 3739-44.
29. Bonnet R, Souweine B, Gauthier G, Rich C, Livrelli V, Sirot J, *et al*. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic-uremic syndrome in adults. *J Clin Microbiol* 1998; *36* : 1777-80.
30. Elliott EJ, Robins-Browne RM, O'Loughlin EV, Bennett-Wood V, Bourke J, Henning P, *et al*. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Arch Dis Childhood* 2001; *85* : 125-31.
31. Newton HJ, Sloan J, Bulach DM, Seemann T, Allison CC, Tauschek M, *et al*. Shiga toxin-producing *Escherichia coli* strains negative for locus of enterocyte effacement. *Emerg Infect Dis* 2009; *15* : 372-80.
32. Tarr PI, Bilge SS, Vary JC Jr, Jelacic S, Habeeb RL, Ward TR, *et al*. *Iha*: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. *Infect Immun* 2000; *68* : 1400-7.
33. Paton AW, Srimanote P, Woodrowand MC, Paton JC. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. *Infect Immun* 2001; *69* : 6999-7009.
34. Tatsuno I, Horie M, Abe H, Miki T, Makino K, Shinagawa H, *et al*. ToxB gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. *Infect Immun* 2001; *69* : 6660-9.

Reprint requests: Dr Neelam Taneja, Additional Professor, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research. Chandigarh 160 012, India
e-mail: drneelampgi@yahoo.com