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Original Article

Characterization of Shiga Toxin-Producing *Escherichia coli* Isolated from Humans between 2011 and 2014

Revasiye Gulesen[1], Belkis Levent[1], Tulin Demir[1,2]*, Mesut Akgeyik[1], and Sibel Kuran[1]

[1]*Public Health Institution of Turkey. National Reference Laboratory, Ankara; and* [2]*Ahi Evran University, Research and Training Hospital, Microbiology Department, Kirsehir, Turkey*

SUMMARY: Although *E. coli* O157:H7 is the major serotype among Shiga toxin-producing *Escherichia coli* (STEC) strains, non-O157 serotypes have caused numerous outbreaks worldwide. We aimed to evaluate the distribution of serogroups, serotypes, virulence genes, and antimicrobial resistance of STEC strains recovered from stool samples. A total of 395 stool samples characterized by watery/bloody diarrhea and/or symptoms of hemolytic-uremic syndrome were included in this study. Strains compatible with *E. coli*, based on biochemical tests, were tested for the presence of Shiga toxin by ELISA. Toxigenic strains were tested by serotyping and serogrouping. Virulence genes, *stx1*, *stx2*, *aggR*, *hlyA*, and *eae* were detected by polymerase chain reaction. Overall, 26 (6.6%) stool culture samples tested positive for STEC. Shiga toxin was positive in 28 (7.1%) patient isolates based on ELISA and PCR. Two isolates could not be serotyped. STEC strains were distributed into 10 serogroups and 14 serotypes. Of the serotyped strains, 92.3% were non-O157, with the major distribution in O104:H4 and O26:HNM. All were negative for extended-spectrum β-lactamase enzyme and 62.5% were resistant to at least 1 drug. This study demonstrated the wide distribution of non-O157 STEC strains from our patient group. Further studies should be performed to better understand STEC characteristics on a larger scale.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) can cause a variety of human illnesses such as diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome (HUS), and related acute encephalopathy (1–9). Infections are generally transmitted by contaminated food and water, animal exposure, and human-to-human transmission. More severe clinical symptoms occur in children and elderly patients (2,7,10–12).

The pathogenesis of the infection is related to several virulence factors, such as *stx1* and *stx2*, encoded by genes located on bacteriophages. The *eae* gene, encoding intimin, promotes adhesion to the enterocyte, and *hlyA*, a plasmid-based gene encoding hemolysine, enhances infectivity of the strain (1,2,6,10,13,14). Most strains carry *stx2*, and about two-thirds carry *stx1* (15). The cytotoxicity of *stx2* is stronger than that of *stx1* (7,15). Over 200 non-O157 STEC serotypes have been identified, and half of these serotypes are associated with human illness (11,14). Although, the largest outbreaks are due to the O157:H7 serotype, non-O157 serogroups such as O26, O111, O145 have caused numerous outbreaks of food-borne disease and HUS with increasing frequency, particularly in Europe (1,6,8,10–12,16,17).

O157 isolates are more easily detected on culture media compared to non-O157 strains, as O157:H7 does not ferment sorbitol and is β-glucuronidase-negative. These differences make it easy to identify O157:H7 strains in clinical samples and food products. Identification of non-O157 STEC stains is often limited to a small number of laboratories.

International HUS cases of O157 and non-O157 STEC strains have been reported, especially in Europe among travelers returning from Turkey (18–22). STEC infection is a category IV notifiable infectious disease in Turkey, according to the Law Concerning the Prevention of Infectious Diseases, and all STEC cases must be reported by the physician who made the diagnosis. Our institution conducts EHEC isolation, serotyping, and verotoxin typing for all suspected cases.

In this study, we aimed to determine the prevalence of STEC O157:H7 and non-O157 in the stool samples of sporadic human cases with bloody diarrhea with or without HUS, between 2011 and 2014 in Turkey, and establish the frequencies of serotypes, virulence genes, and antimicrobial resistance rates.

MATERIALS AND METHODS

Study design and samples: Stool samples of patients suspected to have STEC infection, characterized by bloody diarrhea with or without HUS, were sent to the Public Health Center, National Reference Laboratory for Enteric Pathogens, Ankara, during the study period (June 2011–May 2014). Samples were inoculated onto 5% sheep blood agar, *Salmonella-Shigella* agar, eosine-methylene blue, xylose-lysine-deoxycholate, thiosulfate-citrate-bile-salt-sucrose, sorbitol MacConkey without cefixime tellurite, and Butzler agar for the recovery of *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* O1 and O139, STEC, *Campylobacter* spp., and *Aeromonas*

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*Corresponding Author: Mailing address: Public Health Institution of Turkey. National Reference Laboratory. Refik Saydam Campus, Saglik Mah. Adnan Saygun Str. No: 55 E Block, Sihhiye/ Ankara/ Turkey. Tel: +90 312 565 55 06, Fax: +90 312 565 54 55, E-mail: drtulin@yahoo.com

spp., and incubated for 18–24 hours at 37°C, except for Butzler agar, which was incubated for 48 hours at 42°C. Conventional biochemical tests were performed for identification of the microorganisms.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion tests according to the Clinical and Laboratory Standards Institute guidelines (23), using ampicillin (AMP; 10 μg), chloramphenicol (C; 30 μg), streptomycin (S; 10 μg), tetracycline (TE; 30 μg), sulphonamide (S3; 300 μg), co-trimoxasole (SXT; 1.25/23.75 μg), ciprofloxacin (CIP; 5 μg), nalidixic acid (NA; 30 μg), cefuroxime (CXM; 30 μg), cefotaxime (CTX; 30 μg), ceftriaxone (CRO; 30 μg), ceftizoxime (ZOX; 30 μg), cefpodoxime (CPD; 10 μg), cefoperazone (CFP; 75 μg), ceftazidime (CAZ; 30 μg), trimethoprim (W; 5 μg), imipenem (IMP; 10 μg), and meropenem (MEM; 10 μg) (Oxoid, Hampshire, England). *E. coli* ATCC 25922 was used as the quality control strain for each test.

Shiga toxin ELISA: *E. coli* strains were tested for the presence of Shiga toxin by ELISA (Remel ProSpecT™ Shigatoxin *E. coli* microplate ELISA kit; Remel, Inc., Lexena, KS, USA).

Serotyping: Toxigenic strains were tested for serotyping and serogrouping, using polyvalent and monovalent *E. coli* antisera (Statens Serum Institut, Copenhagen, Denmark).

DNA extraction and detection of virulence genes: DNA extraction and PCR for *stx1*, *stx2*, and *eae* genes were performed according to the PCR protocol recommended by the WHO (24). *hlyA* gene detection was performed according to the recommendations of Toma et al. 2004 (25). Additionally, O104:H4 strains were tested for the presence of the *aggR* gene locus (2).

Table 1. Culture, serotyping, ELISA, and PCR results of STEC strains

Strain	Serotype	ELISA	PCR			
			stx1	*stx2*	*eae*	*hlyA*
EC 1	O104:H4	+	−	+	−	−
EC 2	O104:H4	+	−	+	−	−
EC 3	O104:H4	+	−	+	−	−
EC 4	O104:H4	+	−	+	−	−
EC 5	O104:H4	+	−	+	−	−
EC 6	O104:H4	+	−	+	−	−
EC 7	O104:H4	+	−	+	−	−
EC 8	O26:NM	+	−	+	+	+
EC 9	O26:NM	+	−	+	+	+
EC 10	O26:NM	+	+	−	+	+
EC 11	O26:NM	+	−	+	+	+
EC 12	O145:NM	+	−	+	+	+
EC 13	O145:NM	+	+	−	+	−
EC 14	O145:NM	+	+	−	+	+
EC 15	O157:H7	+	−	+	+	+
EC 16	O157:H7	+	+	+	+	+
EC 17	O26: H6	+	+	+	+	+
EC 18	O26: H12	+	−	+	+	+
EC 19	O103: H2	+	−	+	+	+
EC 20	O103: H2	+	−	+	+	+
EC 21	O174:H21	+	−	+	−	+
EC 22	O181:H4	+	+	−	−	−
EC 23	O45:H2	+	+	+	−	−
EC 24	ONT:H5	+	+	−	−	−
EC 25	ONT:NM	+	−	+	−	−
EC 26	O111	+	+	+	−	−
EC 27	NI	+	−	+	−	−
EC 28	NG	+	+	−	+	+

NG, no growth; NI, not identified.

RESULTS

A total of 395 stool samples were tested for gastrointestinal bacterial pathogens in the study period of June 2011–May 2014. Of these samples, one of each bacteria (*S. flexneri* tip 2a, *Salmonella* enterica serotype Enteritidis, enteropathogenic *E. coli*, and *C. jejuni*) was recovered and 26 (6.6%) were defined as STEC by stool culture. Shiga toxin was positive in 28 (7.1%) patient samples by ELISA. Strains were distributed into 10 serogroups and 14 serotypes. Two of the strains were O157:H7 (n = 2; 7.7%) and 92.3% of the serotyped strains were non-O157, with the predominant serotypes being O104:H4 (n = 7), O26:HNM (n = 4), and O145:HNM (n = 3). Evaluation of virulence genes of STEC strains revealed that 18 (64.3%) were positive for *stx2* only, 6 (21.4%) were positive for *stx1* only, and 4 (14.2%) were positive for both; 50% of strains were positive for the *hlyA* and *eae* gene loci. Culture testing, ELISA, and PCR results of STEC strains are shown in Table 1. Median age of patients with STEC positive isolates was 5 years (9 months–76 years) and 42% of these were below 2 years of age.

The first O104:H4 case was recovered in September 2011 in our laboratory, 2 months after the O104:H4 outbreak (26) occurred in Germany in 2011. We isolated 3 additional O104:H4 strains in 2011, (1 in October and 2 in December), 1 in 2012, and 2 in 2013, displaying the same genotype, including; *stx1*(−), *stx2a*(+), *eae*(−), *hly*(−), *aggR*(+). Additionally, these strains were ESBL-negative and were found to be resistant to AMP, S, S3, SXT, W, TE, and NA and were susceptible to carbapenems. Thus, these isolates displayed similar characteristics to the 2011 German outbreak strains.

The most common serogroups were O104 (n = 7) and O26 (n = 6) during the study period of 2011–2014. Whereas O157 and O104 were common in 2011, O104, O26, and O145 were the predominant serogroups in 2013. Distribution of *E. coli* serotypes by year and by seasonal distribution of the strains isolated in 2011 and 2012–2014 are shown in Fig. 1 and Fig. 2.

Evaluation of antimicrobial susceptibility testing was performed for the 24 isolates. All STEC strains were ESBL-negative and susceptible to carbapenem. The common resistance pattern was AMP-TE-S-S3 and 62.5% of the strains were resistant to at least 1 drug. Resistance rates for AMP, TE/S, S3, SXT/W, NA/CTX/CRO, and CXM were 58.3%, 41.6%, 37.5%, 33.3%, 12.5%, and 8.3%, respectively. All O157 and O103 strains were susceptible to all antimicrobials tested. Of the 7 O104:H4 strains, 2 were intermediately susceptible to NA and 6 were resistant to AMP-TE-SXT-S-S3. Antimicrobial resistance rates of STEC strains by serogroup are shown on Fig. 3.

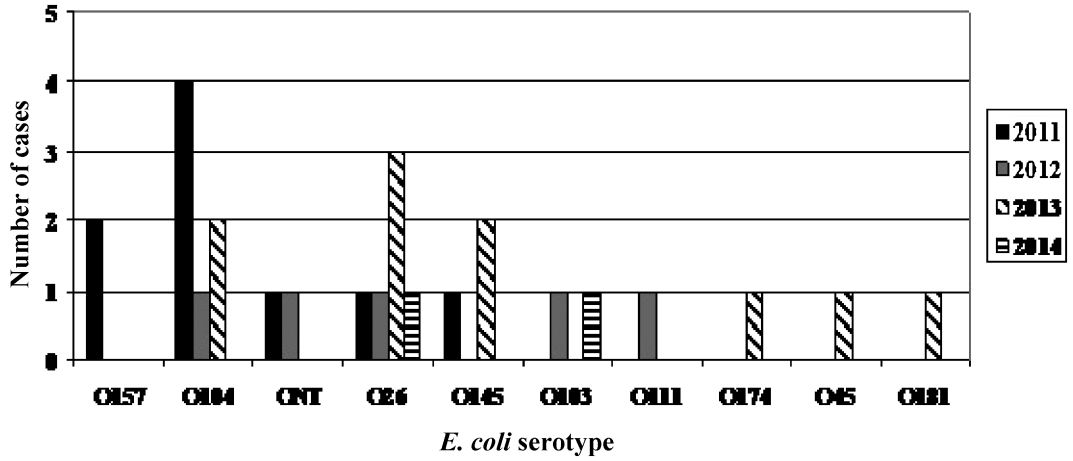

Fig. 1. Distribution of *E. coli* serotypes isolated during the study period between 2011–2014.

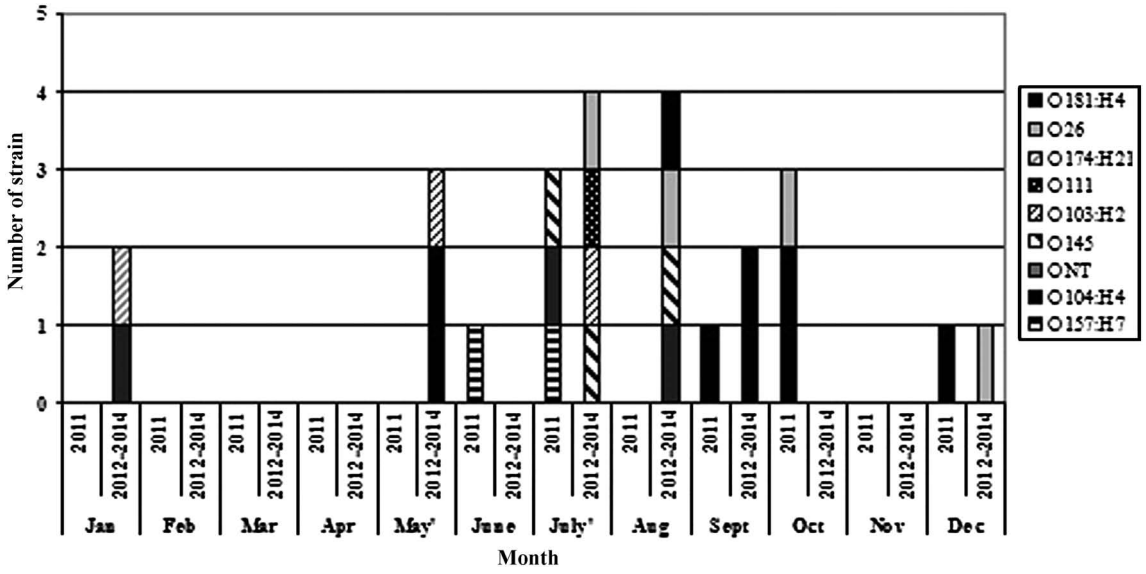

Fig. 2. Seasonal distribution of *E. coli* serotypes isolated in 2011 and 2012–2014.

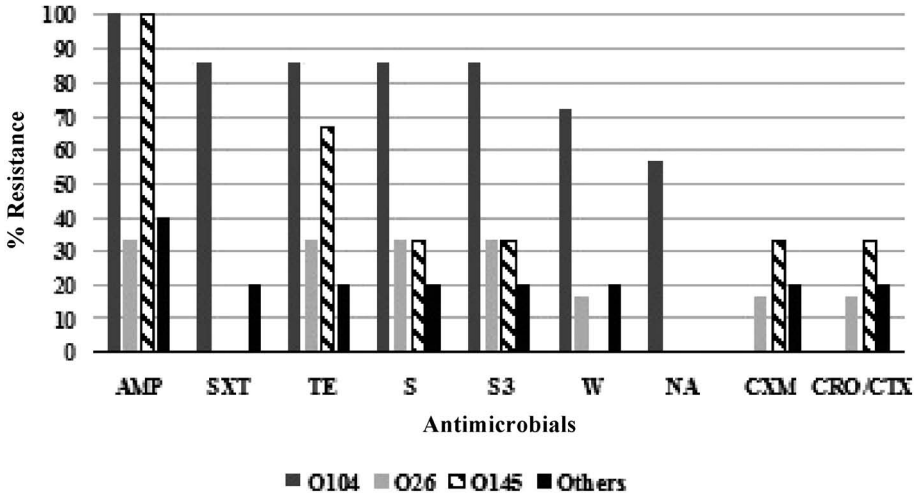

Fig. 3. Distribution of antimicrobial susceptibilities of STEC strains by serotypes. AMP, ampicillin; SXT, co-trimoxasole; TE, tetracycline; S, streptomycin; S3, sulphonamide; W, trimethoprim; NA, nalidixic acid; CXM, cefuroxime; CRO, ceftriaxone; and CTX, cefotaxime.

DISCUSSION

STEC infections pose an important public health problem especially among children under 5 years and the elderly, due to the development of HUS. The prevalence of STEC infections is unclear, owing to under-reporting and under-diagnosis based on insufficient diagnostic capacity in most laboratories (1,13,27). In this study, we evaluated the prevalence of STEC infections, the distribution of serotypes, and antimicrobial susceptibility rates of strains recovered from humans in Turkey.

STEC isolation rate varied based on country and the diagnostic capacity of the laboratories. Whereas infection prevalence was as high as 14–39.6% in India and Iran due to geographic characteristics, environmental conditions, animal population in the area, and contamination of food and water sources (6,28), low rates were observed (0.87–2.5%) in Bangladesh and Spain (29,30). Data is scarce in Turkey with the reported prevalence being 0–1.2% for O157:H7 strains (31,32). In this study, STEC prevalence was 6.6%, which is higher in comparison to previous rates reported in Turkey (31,32).

It has been reported that non-O157 serotypes are increasing in prevalence in both developing and developed countries; recent studies have suggested that non-O157 serotypes are more common than O157, and cause up to 40–80% of STEC infections (6–8,10,13,16). According to data from Foodnet 2012 (33) in the USA, the common non-O157 serogroups were O26 (25%), O103 (21.5%), and O111 (13.3%). Additionally, several studies showed that O26:H11 is the common serotype among human related STEC strains (1,2,7,8,12,27). In our study, non-O157 serotypes represented 92.3% of all *E. coli* strains, and were the major cause of STEC-related infections; 27% of non-O157 strains were O104:H4. The second most common non-O157 serotype was O26 (23%), similar to previous reports. During the study period, the first O104:H4 strain was detected in September 2011 in our laboratory, which was shortly after the occurrence of the O104:H4 outbreak in Germany (26). Moreover, we detected 6 additional O104:H4 strains between 2011 and 2013 with the same genetic characteristics, specifically *stx1*(−), *stx2a*(+), *eae*(−), *hly*(−), *aggR*(+), and antimicrobial resistance to AMP, S, S3, SXT, W, TE, NA. Although our O104:H4 isolates seemed to be related to the 2011 Germany outbreak strains, 1 major difference was ESBL-negativity in our strains. The major limitation of this study was the lack of molecular epidemiological data that could confirm and determine genetic relatedness between our strains and outbreak strains.

Molecular studies have shown that 2–20% of stool samples isolates tested for STEC were positive for any of the virulence gene, 2–83% were positive for *stx2* only, 14–89% for *stx1* only, 7–97% for *eae*, and 7–100% for *hlyA* (1,2,7,8,15,16,27,29,30,34). In this study, all strains had at least 1 virulence gene, with the distribution being 64.2% for *stx2* only, 21.4% for *stx1* only, 14.2% for both *stx1* and *stx2*, and 50% for *eae* and *hlyA*.

Antimicrobial treatment is not recommended for STEC infections, but monitoring resistance has an important role in assessing infections from an epidemiological standpoint (35). High resistance rates, up to 32% for S3, 32–54% for S, and 20–86.8% for TE was reported in some studies among non-O157 strains (1,6,9,34). However, a low resistance rate of 2.9% was also detected for TE and S (15). Similar results were observed for TE, S3, and S resistance in our study. Resistance to third-generation cephalosporins was not detected in several studies (1,21,22), similar to results of our report. Whereas AMP and CN resistance rates have been reported to be widely variable, specifically 0–100% (1,6,9,15,34) and 0–62.2% (1,6,15), respectively, low resistance rates (up to 8%) for SXT have been reported (9,15,34). In our study, resistance to at least 1 drug was 62.5%, and we detected high resistance rates for AMP, TE, S3, S, and SXT. High resistance rates found in our study for AMP and SXT could be explained by the excessive use of these drugs in the treatment of *E. coli*-related infections in our country. Multidrug resistance was observed especially among the O104:H4 serotype and resistance rates were higher among non-O157 strains.

In this study, most STEC strains were non-O157 and the common serotype was 0104:H4. To our knowledge, these are the first isolates of O104:H4 reported in Turkey, to date. Compared to results of this study, *stx2* gene involvement and antimicrobial resistance profiles of O104 cases were similar to those of previous reports (21,22) of STEC cases occurring in patients visiting Turkey before infection, suggesting that the STEC serogroup O104 circulates in these area.

In conclusion, children with acute bloody diarrhea should be tested for STEC. The increase in non-O157 serotypes indicates the necessity for more comprehensive studies concerning STEC strains recovered from humans, animals, and food.

Conflict of interest None to declare.

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