Kafkas Univ Vet Fak Derg
19 (3): 461-467, 2013
DOI: 10.9775/kvfd.2012.8040

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Isolation, Virulence Genes and Antimicrobial Susceptibilities of Shiga Toxin-Producing *Escherichia coli* O157 from Slaughtered Cattle in Abattoirs and Ground Beef Sold in Elazığ

Hakan KALENDER *

* Department of Food Engineering, Faculty of Engineering, Firat University, TR 23119 Elazığ - TURKEY

Makale Kodu (Article Code): KVFD-2012-8040

Summary

Shiga Toxin-Producing *Escherichia coli* O157 (STEC O157) is a foodborne pathogen. Contaminated meat and meat products have an important role in human STEC O157 outbreaks. The aims of this study were to investigate the presence of STEC O157 in slaughtered cattle in abattoirs and ground beef sold in Elazığ, and to determine virulence genes and antimicrobial resistance patterns of STEC O157 isolates. A total of 540 rectal swab samples were collected immediately after slaughter. In addition, 100 ground beef samples were obtained from the butcher shops. Selective enrichment, immunomagnetic separation and plating on Sorbitol MacConkey Agar with cefixime and tellurite (CT-SMAC Agar) were used for the culture. Presence of genes encoding shiga toxin 1 and 2 (*stx1* and *stx2*), H7 flagella (*fliCh7*), enterohemolysin (*hlyA*), intimin (*eae*) and O157 (*rfbE*) in the isolates was detected by Polymerase Chain Reaction (PCR). In the PCR analysis of rectal swab samples., 34 of 82 sorbitol negative isolates were positive for *E. coli* O157. 22 (64.7%) of *E. coli* O157 isolates belonged to *E. coli* O157:H7. STEC O157 was detected in 18 (3.3%) of rectal swab samples. STEC O157:H7 was isolated from 2 (2%) of ground beef samples. All STEC O157 isolates contained *hlyA* and *eae* genes. All STEC O157 isolates obtained from both rectal swab and ground beef samples were resistant to four or more antimicrobials. All STEC O157 isolates were resistant to penicillin, clindamycin, tiamulin and tilmicosin. Two STEC isolates were resistance to ampicillin. Six STEC O157 isolates were resistance to chlortetracycline and sulphadimethoxine. One STEC O157 isolate was resistant to enrofloxacin, florfenicol and oxytetracycline.

Keywords: *E. coli O157, Cattle, Ground beef, Virulence genes, Antibiotic resistance*

Elazığ'da Mezbahalarda Kesilen Sığırlardan ve Piyasada Satılan Kıymalardan Shiga Toksin Üreten *Escherichia coli* O157'nin İzolasyonu, Virulens Genleri ve Antibiyotiklere Duyarlılıkları

Özet

Shiga toxin üreten *Escherichia coli* O157 (STEC O157) gıda kaynaklı enfeksiyonlara yol açan bir patojendir. Kontamine et ve et ürünleri insanlarda salgınların görülmesinde önemli bir rol oynamaktadır. Bu çalışma Elazığ'da mezbahalarda kesilen sığırlardan ve piyasada satılan kıymalardan STEC O157'nin izolasyonu, izolatların virulens genlerinin ve antibiyotiklere duyarlılıklarının belirlenmesi amacıyla yapılmıştır. Kesimden sonra 540 rektal swap örneği ve kasaplardan 100 kıyma örneği toplanmıştır. Etken izolasyonu için selektif zenginleştirme ve immunomagnetik separasyondan sonra sefiksim ve tellürit suplementi içeren Sorbitol MacConkey Agar kullanıldı. Polimeraz zincir reaksiyonu(PCR) ile izolatlarda shiga toxin 1 ve 2 (*stx1* and *stx2*), H7 flagella (*fliCh7*), enterohemolysin (*hlyA*), intimin (*eae*) ve O157 (*rfbE*) genlerinin varlığı araştırıldı. Rektal sıvap örneklerinin PCR testinde, 82 sorbitol negatif izolatın 34'ü *Escherichia coli* O157 yönünden pozitif bulundu. *Escherichia coli* O157 izolatlarının 22'si (%64.7) *Escherichia coli* O157:H7 olarak tiplendirildi. Rektal swap örneklerinin 18'inde (%3.3) STEC O157 saptandı. Kıyma örneklerinin %2'sinden STEC O157 izole edildi. STEC O157 izolatlarının tümünde *hlyA* and *eaeA* genleri tesbit edildi. Rektal sıvap ve kıyma örneklerinden elde edilen tüm STEC O157 izolatları penisilin, klindamisin, tiamulin ve tilmikosin'e dirençli bulundu. Altı STEC O157 izolatı klortetrasiklin ve sülfadimetoksin'e, iki STEC O157 izolatı ampisilin'e ve bir STEC O157 izolatı enrofloksasin, florfenikol ve oksitetrasiklin'e dirençli bulundu.

Anahtar sözcükler: *E. coli O157, Sığır, Kıyma, Virulens genler, Antibiyotik direnci*

İletişim (Correspondence)
+90 424 2370000
hkalender@firat.edu.tr

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) strains are the most important emerged group of foodborne pathogens worldwide. *E. coli* O157:H7 is considered a highly pathogenic serotype responsible for severe human diseases [1]. Cattle are the main reservoir of STEC and shed the bacteria through their feces spreading these pathogens among cattle herds and the environment [2]. Most infections caused by *E. coli* O157:H7 result from the consumption of food and water contaminated with animal feces [3]. Elderly and pediatric patients are at an increased risk of developing *E. coli* O157:H7 associated conditions such as hemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura and death [4,5].

The main virulence factor of STEC is the production of shiga toxins encoded by *stx*1 and *stx*2 genes. Additional virulence factors have also been described including intimin (encoded by the *eaeA* gene) and EHEC hemolysin (encoded by EHEC *hlyA* gene) [1,6]. The widespread use of antibiotics in food animals has resulted in an increase in resistant strains of bacteria. Development of resistance in zoonotic bacteria constitutes a public health risk. Antibiotic resistance strains of STEC have been reported in many countries [7-9]. The presence of *E. coli* O157 in cattle [10-13], ground beef [14-17] and red meat samples [18] has previously been reported in Turkey.

This study was carried out to determine the occurrence of virulence genes and antimicrobial resistance patterns of STEC O157 isolates from slaughtered cattle and ground beef samples in Elazığ.

MATERIAL and METHODS

Sampling

Rectal swab samples were collected immediately at slaughter during the period of December 2011 to June 2012 from 540 cattle at two abattoirs (named A and B) located in Elazığ. The abattoirs were visited once weekly. At each visit, 20 rectal swab samples were taken. The animals sampled were randomly selected. The swabs were placed in a modified tryptone soya broth (mTSB) (LAB165; Lab M) supplemented with novobiocin in 10 ml tubes and then transported immediately to the laboratory. A total of 100 ground beef samples were obtained from the butcher shops. The ground beef samples were brought into the laboratory within sterile containers preserved in ice cold packs.

Isolation of STEC O157

Aproximately 25 g of ground beef samples taken under aseptic conditions were homogenized within 225 ml of mTSB. The mTSB medium containing rectal swab and ground beef samples was incubated at 41.5°C for 24 h. Then immunomagnetic separation (IMS) was performed according to the manufacturer's instructions (Captive O157, Lancashire, UK). The IMS samples were plated onto Sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT-SMAC Agar) (LAB161; Lab M). The agar plates were incubated at 37°C for 24 h. Sorbitol negative colonies on CT-SMAC Agar were considered presumptive *E. coli* O157. Presumptive *E. coli* O 157 colonies were confirmed by amplification of the gene encoding O157 somatic antigen (*rfbE*) by PCR [19].

Detection of Virulence Genes, O157 and Flagellar H7 Gene by PCR

Cultures were grown overnight at 37°C on nutrient agar. A small amount of the culture was resuspended in 200 µl of distilled water, heated to 99°C for 15 min, and centrifuged for 2 min at 12.000 x g. The resulting supernatant was used as a template for PCR. Shiga toxin genes *stx*1 and *stx*2 were detected by multiplex PCR. Single gene PCR was used to determine the presence of genes encoding H7 flagella *(fliCh7)*, enterohemolysin *(hlyA)*, intimin *(eae)* and O157 (rfbE). The primers used in this study are listed in *Table 1*. Reaction contents for each PCR (11-µl total reaction volume) consisted of 3 µl of template DNA, 0.5 µM of primers, 0.18 mM concentration of each deoxyribo-

Table 1. *The primers used in the study*
Tablo 1. *Çalışmada kullanılan primerler*

Target Gene	Sequence of Primers (5'-3')	Size (bp) of PCR Product	Reference
*stx*1	F: ACA CTG GAT GAT CTC AGT GG R: CTG AAT CCC CCT CCA TTA TG	582	Paton and Paton [21]
*stx*2	F: GGC ACT GTC TGA AAC TGC TCC R: TCG CCA GTT ATC TGA CAT TCT G	255	Paton and Paton [21]
eae	F: GTG GCG AAT ACT GGC GAG ACT R: CCC CAT TCT TTT TCA CCG TCG	890	Gannon et al.[22]
$rfbE_{O157}$	F: AAC GGT TGC TCT TCA TTT AG R: GAG ACC ATC CAA TAA GTG TG	678	Nagano et al.[23]
fliCh7	F: TAC CAC CAA ATC TAC TGC TG R: TAC CAC CTT TAT CAT CCA CA	560	Nagano et al.[23]
hlyA	F: AGC CGG AAC AGT TCT CTC AG R: CCA GCA TAA CAG CCG ATG T	525	Fratamico et al.[24]

nucleotide, 4 mM $MgCl_2$, 0.4 U of *Taq* DNA polymerase, 50 mM Tris (pH 8.3), 250 µg/ml Bovine Serum Albumin (BSA), 2% sucrose, and 0.1 mM cresol red. The PCR was performed using rapid-cycle DNA amplification method. The reactions consisted of 30 cycles of template denaturation 94°C, primer anneling at 54°C, and primer extension at 74°C for 30 s. Amplified products were electrophoresed in 1% agarose gels at 200 V for 30 min. The gels were stained with ethidium bromide and were visualized under ultraviolet light. Positive samples were identified based on the presence of bands of the expected sizes compared with results with a positive control strain *(E. coli* ATCC 43895) [7,20].

Antimicrobial Susceptibility Testing

A total of 20 STEC O157 isolates were examined for antimicrobial susceptibility. Minimum inhibitory concentrations (MIC) were measured using the Sensititre Susceptibility System. The following antimicrobial agents were used: ampicillin, ceftiofur, chlortetracycline, clindamycin, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulphadimethoxine, tiamulin, tilmicosin, trimethoprim + sulphamethoxazole, tulathromycin and tylosin. All plates were inoculated following the guidelines recommended by the Clinical and Laboratory Standarts Institute (CLSI), and CLSI breakpoints for interpretation of MIC results [25].

RESULTS

A total of 82 sorbitol-negative isolates were obtained from rectal swab samples from 540 slaughtered cattle. Of these isolates, 34 were positive for *E. coli* O157. 22 (64.7%) of *E. coli* O157 isolates belonged to *E. coli* O157:H7. STEC O157 was detected in 18 (3.3%) of rectal swab samples. *Stx*2 gene was only detected alone in 14 STEC O157 isolates. Four STEC O157 isolates were positive for both *stx*1 and *stx*2 genes. PCR products of *stx*1 (582 bp) and *stx*2 (255 bp) were shown in *Fig. 1*.

All STEC O157 isolates from rectal swab samples were positive for both *eae* and *hlyA* genes. STEC O157 was detected in 2.59% (7/270) and 4.07% (11/270) of rectal swab samples collected from the abattoir A and the abattoir B, respectively. All of the STEC O157 isolates from rectal swab samples were susceptible to ceftiofur, danofloxacin, gentamicin, neomycin, spectinomycin, trimethoprim-sulphamethoxazole, tulathromycin and tylosin. But all of them were resitant to penicillin, clindamycin, tiamulin and tilmicosin. Two STEC O157 isolates were resistance to ampicillin. Six STEC O157 isolates were resistant to chlortetracycline and sulphadimethoxine. One STEC O157 isolate was resistant to enrofloxacin, florfenicol and oxytetracycline *(Table 2)*.

Two sorbitol-negative isolates from ground beef samples were positive for STEC O157:H7. These isolates were positive for *stx*2, *eae* and *hlyA* genes, but none were positive for *stx*1. All of the two STEC O157 isolates from ground beef samples were resistance to penicillin, clindamycin, tiamulin and tilmicosin *(Table 3)*.

DISCUSSION

STEC O157 has been recognized as a growing public health all around the world. Although it is isolated from many animal species, it was reported that STEC O157 is largely hosted in cattle intestines without showing any symptoms [6]. It was reported that cattle beef, milk and the products obtained from them play an important role in the development of human STEC infections [1].

In the studies conducted in several countries for determining STEC O157 prevalence in cattle faecal samples,

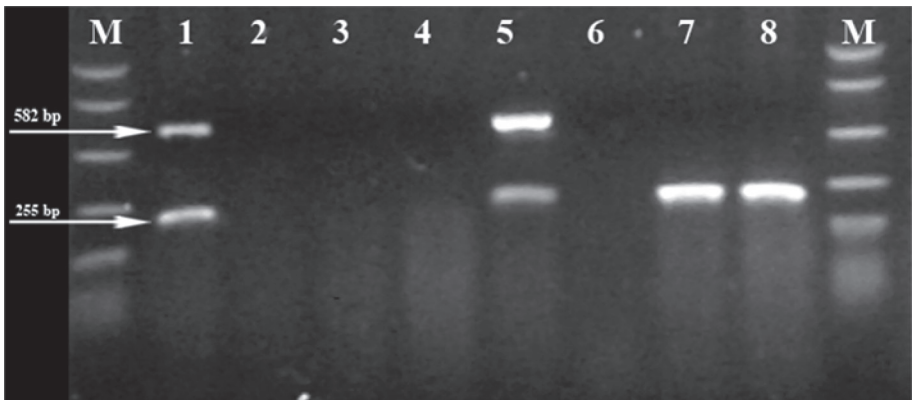

Fig 1. Agarose gel electrophoresis of PCR products of *E. coli* O157 isolates by multiplex PCR

M: Marker (50, 150, 300, 500, 750, 1.000 bp) Lane 1: Positive control, Lane 2: Negative control, Lane 3, 4, 6: Negative samples, Lane 5: Positive sample for *stx*1 and *stx2*, Lane 7, 8: Positive samples for *stx*2

Şekil 1. Multiplex PCR ile *E. coli* O157 izolatlarından elde edilen ürünlerinin agaroz jel elektroforezi

M: Marker (50, 150, 300, 500, 750, 1.000 bp) 1: Pozitif kontrol, 2: Negatif kontrol, 3, 4, 6: Negatif örnekler 5: *Stx*1 ve *stx*2 pozitif örnek, 7, 8: *Stx*2 pozitif örnekler

Table 2. *Virulence genes and antimicrobial resistance of E. coli O157 isolates from cattle*
Tablo 2. *Sığırlardan izole edilen E. coli O157 izolatlarının virulens genleri ve antibiyotik direnci*

Abattoir	Isolate No.	*O157 rfbE*	*fliCh7*	*Stx1*	*Stx2*	*eaeA*	*hlyA*	Resistance[a] Pattern
A	1	+	-	-	-	ND	ND	ND
A	2	+	-	-	-	ND	ND	ND
B	3	+	-	-	-	ND	ND	ND
A	4	+	-	-	-	ND	ND	ND
B	5	+	+	+	+	+	+	CLI, PEN, SUL,TIL
A	6	+	-	-	-	ND	ND	ND
A	7	+	-	-	-	ND	ND	ND
A	8	+	-	-	-	ND	ND	ND
A	9	+	-	-	-	ND	ND	ND
B	10	+	+	+	+	+	+	CHL,CLI, PEN, SUL, TIA,TIL
B	11	+	+	+	+	+	+	CHL,CLI, PEN, SUL,TIA,TIL
B	12	+	+	+	+	+	+	CHL,CLI, PEN, SUL, TIA,TIL
B	13	+	-	-	-	ND	ND	ND
B	14	+	-	-	-	ND	ND	ND
A	15	+	-	-	-	ND	ND	ND
B	16	+	+	-	+	+	+	AMP, CLI, ENF, PEN, SUL,TIA, TIL, TRM
B	17	+	+	-	-	ND	ND	ND
B	18	+	+	-	-	ND	ND	ND
B	19	+	+	-	+	+	+	CHL, CLI, PEN, TIA, TIL
B	20	+	+	-	+	+	+	CHL, CLI, PEN, SUL,TIA,TIL
B	21	+	+	-	+	+	+	AMP, CHL, CLI, FLO, OXY, PEN, SUL, TIA, TIL
A	22	+	-	-	-	ND	ND	ND
B	23	+	+	-	+	+	+	CLI, PEN, TIA, TIL
B	24	+	+	-	-	ND	ND	ND
B	25	+	+	-	-	ND	ND	ND
A	26	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	27	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	28	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	29	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	30	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	31	+	+	-	+	+	+	CLI, PEN, TIA, TIL
B	32	+	+	-	+	+	+	CLI, PEN, TIA, TIL
A	33	+	+	-	+	+	+	CLI, PEN, TIA, TIL
B	34	+	+	-	+	+	+	CLI, PEN, TIA, TIL

[a] ***AMP,*** *ampicillin;* ***CHL,*** *chlortetracycline;* ***CLI,*** *clindamycin;* ***ENF,*** *enrofloxacin;* ***FLO,*** *florfenicol;* ***OXY,*** *oxytetracycline;* ***PEN,*** *penicillin;* ***SUL,*** *sulphadimethoxine;* ***TIA,*** *tiamulin;* ***TIL,*** *tilmicosin;* ***TRM,*** *trimethoprim+sulphamethoxazole,* ***ND,*** *not done*

Table 3. *Virulence genes and antimicrobial resistance of E. coli O157 isolates from ground beef*
Tablo 3. *Kıymalardan izole edilen E. coli O157 izolatlarının virulens genleri ve antibiyotik direnci*

Isolate No.	*O157 rfbE*	*fliCh7*	*Stx1*	*Stx2*	*eaeA*	*hlyA*	Resistance[a] Pattern
1	+	+	-	+	+	+	CLI, PEN, TIA, TIL
2	+	+	-	+	+	+	CLI, PEN, TIA, TIL

[a] ***CLI,*** *clindamycin;* ***PEN,*** *penicillin;* ***TIA,*** *tiamulin;* ***TIL,*** *tilmicosin*

prevalence rates varying by the countries were obtained. It was reported that the worldwide prevalence is between 0.3 and 27.3% for beef cattle and between 0.2 and 48% for dairy cattle [6,26]. Hancock *et al.*[27] detected STEC O157 in 1.8% of cattle faeces in the USA, while Islam *et al.*[28] reported detection of the bacterium in 7.2% of the cattle slaughtered in slaughter houses in Bangladesh, Manna *et al.*[29] in 2% of cattle slaughtered in slaughter houses in India, Sasaki *et al.*[30] in 8.9% of beef cattle in Japan, and Zhou *et al.*[31] in 1.7% of cattle faeces in China. In studies conducted in several regions of Turkey, it is reported that *E. coli* O157 was detected in cattle faeces with rates varying between 0.6% and 25% [10-13]. However, in some of these studies the presence of shiga toxin producing *E. coli* O157 was examined. In the study by Inat and Siriken [13] conducted in Samsun city, the authors reported they had isolated STEC O157 from 18% of the rectal swab samples taken from 100 slaughtered cattle. In the study conducted by Aslantas *et al.*[11] in Hatay city, it was reported that in 11% of 565 cattle faecal samples, STEC O157 was detected. In another study carried out in Turkey, STEC O157 was isolated from 1.2% of cattle faecal samples of 251 cattle [12]. In this presented study, STEC O157 was detected in 3.3% of 540 cattle rectal swab samples. In comparison with the studies conducted in Turkey and in other countries, this rate is higher than the findings of some researchers [12,27,29,31] and lower than the findings of others [11,13,28,30]. This may result from the differences in seasons, ages of animals, method of breeding and geographic differences. It was reported that also sampling method and isolation technique affect prevalence [28,32]. It is reported that IMS method is one of the most sensitive methods for STEC O157 isolation from faeces and food samples [12,13]. Also in the present study the IMS method was employed for isolation. However, samples were collected in winter and spring months. It was reported that in ruminants the prevalence of *Escherichia coli* O157:H7 is the highest in summer months and decreases in winter months [6,26].

Pathogenic STEC strains produce toxins that cause human illnesses and can produce other virulence factors that may increase the severity of illnesses. These factors include intimin and enterohemolysin, which are encoded by the *eae* and *hlyA* genes, respectively [6]. In the present study, *eae* and *hlyA* genes were detected as well as shiga toxin genes (4 *stx*1 and *stx*2, 14 only *stx*2) in 18 of the total 34 *E. coli* O157 isolates obtained from rectal swab samples. *Stx*2-producing strains are often more related to HUS than *stx*1-producing strains [4,6,33]. In this study, the predominant stx type found was *stx*2, in aggrement with previous studies [2,28,32]. The presence of a combination *eae, stx* and *hlyA* genes is generally regarded as a highly virulent genetic mix [34]. Results of the presented study show that seemingly healthy cattle contain *E. coli* O157 strains that are highly pathogenic for humans. *E. coli* O157 was isolated from diarrheal human faeces in Turkey and the suspected source of contamination was reported to be foodstuff [35,36]. However, there is a need for a comprehensive study that examines the relation of human *E. coli* O157 infections in Turkey with foodstuffs. In the studies conducted in Turkey on faecal samples of cattle, Aslantas *et al.*[11] reported that 74 of a total of 77 *E. coli* O157 isolates contained *hly*A, while 72 of them contained *eae,* 62 contained *stx*2 and 3 contained both *stx*1 and *stx*2 genes. In the study by Kuyucuoglu *et al.*[37], it was reported that all of 5 *E. coli* O157:H7 isolates were positive for *hly*A, while 2 of them were positive for *eae* gene. In another study conducted by Ongor *et al.*[12], it was determined that 2 of a total of 4 *E. coli* O157 isolates contained *eae, stx*1 and *stx*2, while one contained *eae* and another contained *eae* and *stx*2 genes.

In studies conducted in several countries with the purpose of determining *E. coli* O157 in ground beef samples it was reported that in Italy Conedera *et al.*[38] isolated STEC O157 from 0.43% of 931 samples, in the Netherlands Heuvelink *et al.*[39] from 1.1% of 571 samples and in Peru Mora *et al.*[40] isolated *E. coli* O157 from 23% of 102 samples. In a study conducted in Argentina, *E. coli* O157 was isolated from 3.8% of a total of 160 samples [41]. In England, *E. coli* O157 was detected in 0.35% of 1979 samples [42]. In the studies carried out in Turkey, Alisarli and Akman [14] reported that they isolated *E. coli* O157 from 4.6% of 150 ground beef samples, Sarimehmetoglu *et al.*[15] reported 7.6% isolation from 255 ground beef samples in Ankara city, Cadirci *et al.*[17] reported 1% of isolation from 100 ground beef samples in Samsun city and Aksu *et al.*[16] reported that they isolated *E. coli* O157 from 6% of 50 ground beef samples in Istanbul city. In the presented study, STEC O157 was isolated from 2% of 100 ground samples. In many countries, low rates similar to the results of the present study were obtained. When compared with the other studies conducted in Turkey, the rate determined with the present study is lower than the rates found in some studies [14-16]. However, Cadirci *et al.*[17] reported a rate (1%) close to the rate determined in this study. The differences in isolation rates from ground beef samples may occur due to the differences in sampling method, isolation method, season and geography. Also inadequate hygienic implementations at butcher shops and slaughter houses can affect isolation rate. Sarimehmetoglu *et al.*[15] reported that one of the total 19 *E. coli* O157 strains isolated from ground beef contained *stx*1, *stx*2, *eae*, *hlyA* and *fliCh7* genes, while the genes of *stx*1, *eae, hlyA* and *fliCh7* genes were found in all other strains. In the present study, *stx*1, *stx*2, *eae, hlyA* and *fliCh7* genes were detected in all ground beef isolates.

Antimicrobial resistance in animal STEC isolates may be spread to humans through the food chain. Strains of STEC are commonly found in the ruminant gastrointestinal tract and can serve as indicator organisms for the development of antibiotic resistance [7,8]. In the present study, all STEC O157 isolates were found to be resistant to penicillin, clindamycin, tiamulin and tilmicosin. Howewer, all the isolates were susceptible to ceftiofur, danofloxacin, gentamicin, neomycin,

spectinomycin, trimethoprim-sulphamethoxazole, tulatromycin and tylosin. Six (33.3%) of the isolates were resistant to chlortetracycline and sulphadimethoxine. Cephalosporins and fluoroquinolones often are the drugs of choice for treatment of infections in humans. Although no resistance against ceftiofur was found in the isolates in this study, one isolate was found to be resistant against enrofloxacin. In Japan, resistance to dihydrostreptomycin in 241 STEC O157 isolates from beef cattle was detected most frequently (9.5%), followed by resistance to oxytetracycline (7.9%) and ampicillin (5.4%) [9]. In aggrement with our results, In the USA, all *E. coli* O157:H7 isolates from cattle were found to be susceptible to ceftiofur, gentamicin and trimethoprim-sulphamethoxazole [7]. It was reported that 100% of the 6 STEC O157 strains isolated from ground beef in the Czech Republic were resistant against ampicilline, cephazolin and tetracycline, while 83% were resistant against chloramphenicol and colistin, and 50% were resistant against cefuroxime and cefoxitine [43]. There are very limited numbers of studies in Turkey concerning the determination of the resistances of the STEC O157 strains isolated from cattle faeces and ground beef samples. In a study conducted by Aksoy *et al.*[44], it was reported that all of the 4 STEC O157 strains isolated from cattle were resistant against all antibiotics. Sasaki *et al.*[9] determined that the antibiotics resistance of STEC O157 isolates that contain both *stx*1 and *stx*2 was higher than the resistance of the isolates that contain only *stx*2. However, no significant relation could be found in this study between antibiotics resistance and type of stx.

In conclusion, this study showed cattle are an important reservoir of STEC O157 in Turkey. Cross contamination of carcasses may occur during the slaughter of cattle. This constitutes a serious hazard to human health as it may lead to outbreaks of human STEC O157 infections. Appropriate hygienic measures in food industries including abattoirs may be implemented to reduce the risk of STEC O157 infection. Consumers should take proper care for prevention of the organism such as cold temperature and cooking before consumption. More studies should be carried out to understand a genetic relationship between food, animal and human isolates.

Acknowledgements

The author wishes to thank Professor Bhushan M. Jayarao and Dr. Choby Debroy (The Pennsylvania State University, USA) for their help.

REFERENCES

1. Paton JC, Paton AW: Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. *Clin Microbiol Rew,* 11, 450-479, 1998.

2. Polifroni R, Etcheverria AI, Sanz ME, Cepeda RE, Krüger A, Lucchesi PMA, Fernandez D, Parma AE, Padola NL: Molecular characterization of shigatoxin-producing *Escherichia coli* isolated from the environment of a dairy farm. *Curr Microbiol,* 65, 337-343, 2012.

3. Ateba CN, Bezuidenhout CC: Characterization of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *Int J Food Microbiol,* 128, 181-188, 2008.

4. Murinda SE, Nguyen LT, Landers TL, Draughon FA, Mathew AG, Hogan JS, Smith KL, Hancock DD, Oliver SP: Comparison of *Escherichia coli* isolates from humans, food, and farm and companion animals for presence of shiga toxin-producing *E. coli* virulence markers. *Foodborne Pathog Dis,* 1, 181-184, 2004.

5. Hiroi M, Takahashi N, Harada T, Kawamori F, Iada N, Kanda T, Sugiyama K, Ohashi N, Hara-Kudo Y, Masuda T: Serotype, shiga toxin (stx) type, and antimicrobial resistance of stx-producing *Escherichia coli* isolated from humans in Shizuoka Prefecture, Japan (2003-2007). *Jpn J Infect Dis,* 65, 198-202, 2012.

6. Hussein HS, Bollinger LM: Prevalence of shiga toxin-producing *Escherichia coli* in beef cattle. *J Food Protect,* 68, 2224-2241, 2005.

7. Schroder CM, Zhao C, DebRoy C, Torcolini J, Zhao S, White DG, Wagner DD, McDermott PF, Walker RD, Meng J: Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine and food. *Appl Environ Microbiol,* 68, 576-581, 2002.

8. Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, Gonzales EA, Bernardez MI, Blanco J: Antimicrobial resistance of shigatoxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *Res Microb,* 156, 793-806, 2005.

9. Sasaki Y, Usui M, Murakami M, Haruna M, Kojima A, Asai T, Yamada Y: Antimicrobial resistance in shiga toxin-producing *Escherichia coli* O157 and O26 isolates from beef cattle. *Jpn J Infect,* 65, 117-121, 2012.

10. Yılmaz A, Gün H, Yılmaz H: Frequency of *Escherichia coli* O157:H7 in Turkish cattle. *J Food Protect,* 65, 1637-1640, 2002.

11. Aslantas O, Erdogan S, Cantekin Z, Gulactı I, Evrendilek GA: Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from Turkish cattle. *Int J Food Microbiol,* 106, 338-342, 2006.

12. Ongor H, Kalin R, Cetinkaya B: Investigations of *Escherichia coli* O157 and some virulence genes in samples of meat and faeces clinically healthy cattle in Turkey. *Vet Rec,* 161, 392-394, 2007.

13. Inat G, Siriken B: Detection of *Escherichia coli* O157 and *Escherichia coli* O157:H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun province, Turkey. *J Vet Sci,* 11, 321-326, 2010.

14. Alişarlı M, Akman HN: Parekende satılan kıymaların *Escherichia coli* O157 yönünden incelenmesi. *YYÜ Vet Fak Derg,* 15, 65-69, 2004.

15. Sarımehmetoğlu B, Aksoy MH, Ayaz ND, Ayaz Y, Kuplulu O, Kaplan YZ: Detection of *Escherichia coli* O157:H7 in ground beef using immunomagnetic separation and multiplex PCR. *Food Control,* 20, 357-361, 2009.

16. Aksu H, Arun ÖÖ, Aydın A, Uğur M: *E. coli* O157:H7'nin hayvansal kökenli gıda maddelerinde varlığı. *Pendik Vet Mikrobiyol Derg,* 30, 77-81, 1999.

17. Cadirci O, Siriken B, Kevenk TO: The prevalence of *Escherichia coli* O157 and O157:H7 in ground beef and raw meatball by immunomagnetic separation and the detection of virulence genes using multiplex PCR. *Meat Sci,* 84, 553-556, 2010.

18. Temelli S, Eyigör A, Anar Ş: Prevalence of *Escherichia coli* O 157 in red meat and meat products determined by VIDAS ECPT and lightcycler PCR. *Turk J Vet Anim Sci,* 36, 305-310, 2012.

19. Oporto B, Esteban I, Aduriz G, Juste RA, Hurtado A: *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli* in healthy cattle, sheep, and swine herds in Northern Spain. *Zoonose Public Health,* 55, 73-81, 2008.

20. DebRoy C, Roberts E: Screening petting zoo animals for the presence of potentially pathogenic Escherichia coli. *J Vet Diagn Invest,* 18, 597-600, 2006.

21. Paton AW, Paton JC: Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*1, *stx*2, *eae*, enterohemorrhagic *E. coli hlyA, rfb* O111 and *rfb* O157. *J Clin Microbiol,* 36, 598-602, 1998.

22. Gannon VPJ, Rashed M, King RK, Thomas EJG: Detection and characterization of the *eae* gene of shiga-like toxin producing *Escherichia coli* using polymerase chain reaction. *J Clin Microbiol,* 31, 1268-1274, 1993.

23. Nagano I, Kunishima M, Itoh Y: Detection of verotoxin producing *Escherichia coli* O157:H7 by multiplex polymerase chain reaction. *Microbiol Immunol,* 42, 371-376, 1998.

24. Fratamico PM, Debroy C, Liu Y: The DNA sequence of the *Escherichia coli* O22 O-antigen gene cluster and detection of pathogenic strains belonging to *E. coli* serogroups O22 and O91 by multiplex PCR assays targeting virulence genes and genes in the respective O-antigen gene clusters. *Food Anal Methods*, 2, 169-179, 2009.

25. NCCLS: MO7-06 Methods for Diluation Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Second ed., Approved Standart M7-A2. Wayne, PA, NCCLS.

26. Hussein HS, Sakuma T: Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J Dairy Sci,* 88, 450-465,2005.

27. Hancock DD, Rice DH, Thomas LA, Dargatz DA, Besser TE: Epidemiology of *Escherichia coli* O157 in feedlot cattle. *J Food Protect,* 60, 462-465, 1997.

28. Islam MA, Mondol AS, de Boer E, Beumer RR, Zwietering MH, Talukder KA, Heuvelink AE: Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Appl Environ Microbiol,* 74, 5414-5421, 2008.

29. Manna SK, Brahmane MP, Manna C, Batabyal K, Das R: Occurrence, virulence characteristics and antimicrobial resistance of *Escherichia coli* O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. *Lett Appl Microbiol,* 43, 405-409, 2006.

30. Sasaki Y, Tsujiyama Y, Kusukawa M, Murakami M, Katayama S, Yamada Y: Prevalence and characterization of shiga toxin producing *Escherichia coli* O157 and O26 in beef farms. *Vet Microbiol,* 150, 140-145, 2011.

31. Zhou Z, Nishikawa Y, Zhu P, Hong S, Hase A, Cheasty T, Smith HR, Zheng M, Haruki K: Isolation and characterization of shiga toxin-producing *Escherichia coli* O157:H7 from beef, pork and cattle fecal samples in Changchun, China. *J Vet Med Sci,* 64, 1041-1044, 2002.

32. Ojo OE, Ajuwape ATP, Otesile EB, Owoade AA, Oyekunle MA, Adetosoye AI: Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. *Int J Food Microbiol,* 142, 214-221, 2010.

33. Etcheverria AI, Padola NL, Sanz ME, Polifroni R, Krüger A, Passucci J, Radriguez EM: Occurrence of shiga toxin-producing *E. coli* (STEC) on carcasses and retail beef cuts in the marketing chain of beef in Argentina. *Meat Sci,* 86, 418-421, 2010.

34. Possé B, De Zutter L, Heyndrickx M, Herman L: Metabolic and genetic profiling of clinical O157 and non-O157 Shiga-toxin-producing *Escherichia coli. Res Microbiol,* 158, 591-599, 2007.

35. Erdoğan H, Levent B, Erdoğan A, Güleşen R, Arslan H: Gastroenteritli olgularda verotoksijenik *Escherichia coli* O157:H7 insidansının araştırılması. *Mikrobiyol Bul,* 45, 519-525, 2011.

36. Yeniiz E, Öncül O, Çavuşlu Ş: İshalli hastaların dışkılarında *Escherichia coli* O157:H7 varlığının araştırılması. *J Med Sci,* 29, 1398-1405, 2009.

37. Kuyucuoglu Y, Seker E, Sareyyupoglu B, Gurler Z. Detection of enterohemolysin and intimin genes in *Escherichia coli* O157:H7 strains isolated from calves and cattle in Afyonkarahisar-Turkey. *Kafkas Univ Vet Fak Derg,* 17, 663-666, 2011.

38. Conedera G, Dalvit P, Martini M, Galiero G, Gramaglia M, Goffredo E, Loffredo G, Morabito S, Ottavian, D, Paterlini F, pezzotti G, Pisanu M, Semprini P, Caprioli A: Verocytotoxin-producing *Escherichia coli* O157 in minced beef and dairy products in Italy. *Int J Food Microbiol,* 96, 67-73, 2004.

39. Heuvelink AF, Zwartkruis-Nahuis JT, Beumer RR, De Boer E: Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in The Netherlands. *J Food Protect,* 62, 1115-1122, 1999.

40. Mora A, Leon SL, Blanco M, Blanco JE Lopez C, Dahbi G, Echelta A, Gonzales EA, Blanco J: Phage types virulence genes and PFGE profiles of shiga toxin-producing *Escherichia coli* O157:H7 isolated from raw beef meat, soft cheese and vegetables in Lima (Peru). *Int J Food Microbiol,* 114, 204-210, 2007.

41. Chinen I, Tanaro JD, Miliwebsky E, Lound LH, Chillemi G, Ledri S, Baschkier A, Scarpin M, Manfredi E, Rivas M: Isolation and characterization of *Escherichia coli* O157:H7 from retail meats in Argentina. *J Food Protect,* 64, 1346-1351, 2001.

42. Chapman PA, Cerdan Malo AT, Ellin M, Ashton R, Harkin MA: *E coli* O157 in cattle, and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int J Food Microbiol,* 64, 139-150, 2001.

43. Lukasova J, Abraham B, Cupakova S: Occurence of *Escherichia coli* O157 in raw material and food in Czech Republic. *J Vet Med B,* 51, 77-81, 2004.

44. Aksoy A, Yıldırım M, Kacmaz B, Apan TZ, Gocmen JS: Verotoxin production in strains of *Escherichia coli* isolated from cattle and sheep, and their resistance to antibiotics. *Turk J Vet Anim Sci,* 31, 225-231, 2007.