IRANIAN
JOURNAL
OF
VETERINARY
RESEARCH

Original Article

Virulence factors, serogroups, and antibiotic resistance of Shiga-toxin producing *Escherichia coli* from raw beef, chicken meat, and vegetables in Southwest Iran

Kholdi, S.[1]; Motamedifar, M.[2, 3]; Fani, F.[4]; Mohebi, S.[1] and Bazargani, A.[2*]**

[1]Ph.D. Student in Bacteriology, Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran; [2]Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran; [3]Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran; [4]Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence: A. Bazargani, Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. E-mail: abdolahbazargani@yahoo.com

**Co-correspondence:* M. Motamedifar, Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran. E-mail: motamedm@sums.ac.ir

Abstract

Background: Shiga-toxin-producing *Escherichia coli* (STEC) is an important food-borne pathogen causing human diseases with severe symptoms. Although the O157 serotype has been mostly isolated from human specimens, the increasing incidence rates of non-O157 serogroups have attracted special attention in recent years. **Aims:** Evaluation of the epidemiology and identification of different characteristics of STEC isolates from raw beef, chicken meat, and vegetable samples in Shiraz, Southwest Iran. **Methods:** Two hundred beef and chicken meat samples from different parts of carcasses and four hundred vegetable samples (carrots, lettuce, cucumber, and leafy greens) were randomly taken; STEC were isolated and confirmed using standard microbiological methods. Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method. Polymerase chain reaction (PCR) was used for the identification of O-serogroups, virulence, and antibiotic resistance genes. **Results:** 52% of beef, 8% of chicken, and 7.2% of vegetable samples were STEC-positive. Further, the highest frequency of virulence factors belonged to the co-existence of *stx1* and *stx2*. O157 serogroup was only detected in beef (3.8%) and lettuce (16.6%) isolates, while the rates of the non-O157 serogroups were relatively high (up to 44.2%). The highest resistance rate in the STEC isolates of different samples belonged to nalidixic acid (62.5%), tetracycline (55.7%), and ampicillin (48%). **Conclusion:** Paying more attention to non-O157 serogroups in future studies is recommended due to the relatively high prevalence of theses STEC serogroups in our study. Besides, the high level of resistance to some antibiotics observed in this study needs to be addressed.

Key words: ESBL, Foodborne pathogen, O-serogroups, STEC, Virulence genes

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC), known as verotoxin-producing *E. coli* (VTEC) as well, has been emerged as one of the most important food-borne pathogens (Noguera *et al*., 2011). This pathogen usually causes diseases with severe clinical outcomes, including bloody and non-bloody diarrhea (BD), haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and Hemorrhagic colitis (HC) (Kohansal and Asad, 2018; Ranjbar *et al*., 2019). The STEC strains with a zoonotic nature are transmitted to humans in several ways such as person-to-person or by animal contact. Common factors playing an important role in pathogen transmission include consumption of meat-related products like chicken, burger, sausage, salami, or vegetables, or water contaminated by faeces of the carriers or cross-contamination by improper food handling (Ateba and Mbewe, 2011; Ercoli *et al*., 2016).

Of all the mentioned sources, cattle that can be asymptomatic vectors are known as the principal reservoir with a pivotal role in the transmission of STEC strains to humans (Ferens and Hovde, 2011).

Vegetables may also act as vehicles for the transmission of STEC from different sources such as manure-contaminated soil and water. This highlights their role in human infections (Ozpınar *et al*., 2013).

Enterohemorrhagic *E. coli* (EHEC) is the most important STEC pathotype in which O157 serotype has been mostly isolated from human specimens worldwide. Although O157 serogroup remains the prevalent STEC, recent studies show that non-O157 serogroups are currently considered because of their increasing

incidence rates and their ability to cause mild to severe diseases. However, they may sometimes cause no morbidity in humans. Of about 150 non-O157 STEC serogroups, six serogroups including O26, O45, O103, O111, O121, and O145 have been shown to account for 70% of non-O157 STEC infections (Conrad *et al*., 2016; Ranjbar *et al*., 2017b).

Several virulence factors have been attributed to STEC from which *Stx1* and *Stx2* are known as the most important toxins usually carried by prophages integrated into the *E. coli* genome (Leotta *et al*., 2008). The cytopathic effect of these toxins on the intestinal epithelial cells is involved in dysentery. Despite distinct immunological characteristics, these two cytotoxins share 55 to 60% of amino acid sequences.

Intimin, as another virulence factor, is an outer membrane protein, which has been identified as an effective factor in the pathogenicity of STEC strains. This factor is encoded by the *eae* gene, located on a chromosomal pathogenicity island called the locus of enterocyte effacement (LEE) (Franz *et al*., 2015). Attachment of *E. coli* to the epithelial cells by intimin is attributed to this locus activating signal transduction pathways of the host cells; subsequently, it results in attaching-and-effacing intestinal lesions characterized by cytoskeletal changes such as polymerized F-actin accumulation (Cepeda-Molero *et al*., 2017).

Enterohaemolysin encoded by *hly* gene is another virulence-associated factor of these strains as a pore-forming cytolysin which contributes to bacterial invasion into the intestinal epithelial cells (Melton-Celsa and Toxin, 2014). Despite its low outbreak, STEC is widely considered as an important bacterial pathogen all over the world because of its low infectious dose, its ability to survive in a variety of foods and its sever clinical manifestation in spite of harsh condition of the gastrointestinal tract for growth. Therefore, WHO commonly insists on its continuous monitoring (Etcheverria and Padola, 2013).

The antimicrobial resistance of bacterial pathogens is an important issue in the treatment of infectious diseases. The strong correlation between the presence of extended spectrum beta-lactamase (ESBL) producing bacteria in meat products and prevalence of infections in humans may lead to the assumption that antibacterial resistance may be transmitted to bacterial agents in the human population by contaminated food of animal origin. Strains of STEC are of food-borne ESBL-producer pathogens in which bla$_{CTX}$, bla$_{TEM}$, and bla$_{SHV}$ genes are studied more frequently (Minh *et al*., 2016).

As meat, meat products, and fresh vegetables are major foods, and can be contaminated with STEC isolates, and since there is no recent surveillance study about STEC in these sources in our area, we aimed to explore the epidemiology, prevalence of serogroups, virulence factors, antimicrobial resistance, and genotypic detection of some beta-lactamases of STEC isolated from beef, chicken meat, and fresh vegetable samples in Shiraz, Southwest Iran. This study determined an up-to-date prevalence of STEC in food resources, strain variability, and drug resistance, simultaneously.

Materials and Methods

Sample collection, preparation, and *E. coli* identification

Overall, 200 random meat samples (100 samples of each raw beef and chicken meat), and 400 vegetables samples (100 samples from each carrots, lettuce, cucumber, and leafy greens) were collected. The raw beef samples were collected from two main abattoirs of Shiraz, the biggest city in the southwest of Iran; however, the chicken (4-week-old broiler chickens) and vegetable samples were collected from distinct municipal daily markets. The sampling period was from October 2018 to September 2019. After immediate transfer to the laboratory in cool boxes, meat samples were taken from disinfected different parts of carcasses using sterile swabs. Swab or 25 g of each homogenized vegetable sample was then transferred to 225 ml modified tryptic soy broth (Merck, Germany) supplemented with novobiocin (2 mg/L); they were then incubated at 37°C for 24 h.

To isolate bacteria, all the enriched samples were sub-cultured onto Sorbitol MacConkey agar (SMAC) supplemented with cefixime (50 ng/ml) and potassium tellurite (2.5 mg/ml); which were then incubated at 37°C. After 24 h, sorbitol-positive and sorbitol-negative colonies were isolated and cultured onto Eosin Methylene Blue agar for evaluation of lactose fermentation. Eventually, for the final verification of bacteria, different chemical media such as citrate, triple sugar iron (TSI) agar, and sulfide, indole, motility medium (SIM) and standard microbiological methods were used (Koochakzadeh *et al*., 2014).

Antimicrobial susceptibility testing (AST)

The Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) was applied for AST following the Clinical and Laboratory Standards Institute (CLSI) guidelines (Raeispour and Ranjbar, 2018; Dehkordi *et al*., 2020). After incubation of the plates for 18-24 h at 37°C, the susceptibility of the isolates to ampicillin (AMP)(10 μg), tetracycline (TET) (30 μg), amoxicillin (AM) (25 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), chloramphenicol (C) (30 μg), imipenem (IPM) (30 μg), gentamicin (GM) (10 μg), meropenem (MRP) (30 μg), nalidixic acid (NA) (30 μg), and ciprofloxacin (10 μg/disk) (Mast Diagnostics, Merseyside, UK) was measured. The interpretation of the results was done based on the interpretive criteria provided by CLSI (Wayne, 2017). *Escherichia coli* ATCC 25922 strain was used as the quality control organism for the AST (Nasrolahei *et al*., 2014; Momtaz *et al*., 2013b).

Phenotypic tests for recognition of ESBLs

Double-disk synergy test (DDST) was used as a phenotypic test for ESBLs detection in which cefotaxime

(30 µg) and ceftazidime (30 µg) were used alone and in combination with clavulanic acid (10 µg) (MAST Co., UK). Negative and positive control strains were *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603, respectively. *Escherichia coli* ATCC 35218 carrying bla_{TEM} and *K. pneumonia* ATCC 700603 harboring bla_{TEM}, bla_{SHV}, and bla_{CTX-M} were used as positive controls, and *E. coli* ATCC 25922 was used as a negative control. More than a 5 mm increase in the zone diameter for Ceftazidime-Clavulanic acid was considered as positive ESBL production (Mandakini *et al*., 2015).

DNA extraction

The isolated bacteria were cultured in trypticase soy agar (TS-Merck, Germany) and incubated at 37°C overnight. Then, a loopful of colonies was added into a 1.5-ml Eppendorf tube containing 100 µL of sterile distilled water. After a 10 s of vortex, the tube was put in a hot plate for 15 min and then chilled on ice for 10 min. In the next step, the tube was centrifuged at ~15500 × g (13000 rpm) for 15 min to remove the debris. The supernatant containing DNA was transferred to a fresh tube and stored at -20°C for the next step (Ahmed and Dablool, 2017).

Polymerase chain reaction (PCR) and agarose gel electrophoresis

Polymerase chain reaction assay was applied for identification of virulence factors, O-serogroups, and antibiotic resistance genes by the use of the primers, as represented in Table 1 (Momtaz *et al*., 2013a; Memariani *et al*., 2015). To perform PCR reactions, we used a total volume of 50 µL, including 5 µL PCR buffer (Thermo Scientific, Maxima Hot Start Taq DNA polymerase, EP0602), 2.5 mM of MgCl$_2$ (Thermo Scientific, Maxima Hot Start Taq DNA polymerase, EP0602), 0.4 ngdNTP (Fermentas), 15 pmol primers (Bioneer, South Korea), 2.5 IU of Taq DNA polymerase (Fermentas), and 2 µL of the template. Amplification reactions were carried out based on primer-specific programs using a DNA thermo-cycler (Eppendorf, Singapore). Electrophoresis in 1.5% agarose gel was done for analyzing the amplified samples and the safe stain was used for staining. A molecular weight marker with 100 bp increments (100 bp DNA ladder, Fermentas) was used as a size standard. Strains of *E. coli* O157:K88ac:H19, CAPM 5933 and *E. coli* O159:H20, CAPM 6006 were used as positive controls (Mohammadi-Sardo *et al*., 2017).

Statistical analysis

The results are presented as descriptive statistics in terms of relative frequency as percentages using SPSS™ software, version 21.0 (IBM Co., Armonk, NY, USA).

Results

In this study, we collected a total of 200 meat samples consisting of 100 samples of beef and 100 samples of chicken meat. Of them, 52% and 8% of beef and chicken samples were found to be positive for STEC, respectively. While in vegetables, 6% of carrots, 12% of lettuce, 3% of cucumbers, and 8% of leafy greens (totally 7.2% of vegetable samples) were STEC-positive.

In molecular testing of virulence genes, we found that the highest frequency belonged to the co-existence of

Table 1: The primers used for detection of virulence factors, O-serogroups, and antibiotic resistance genes

Primer	Sequence (5´-3´)	Annealing Temp.	PCR product (bp)
stx1	F- CGCTGAATGTCATTCGCTCTGCT	55	366
	R- CGTGGTATAGCTACTGTCACC		
stx2	F- CCTCGGTATCCTATTCCCGG	56	282
	R- CTGCTGTGACAGTGACAAAACGC		
eae	F- CTGAACCAGATCGTAACGGC	55	629
	R- TGATAAGCTGCAGTCGAATCC		
hly	F- CAATGCAGATGCAGATACCG	57	432
	R- CAGAGATGTCGTTGCAGCAG		
O26	F- CAATGGGCGGAAATTTTAGA	53	155
	R- ATAATTTTCTCTGCCGTCGC		
O45	F- TGCAGTAACCTGCACGGGCG	62	238
	R- AGCAGGCACAACAGCCACTACT		
O111	F- TGTTTCTTCGATGTTGCGAG	55	438
	R- GCAAGGGACATAAGAAGCCA		
O145	F- TTCATTGTTTTGCTTGCTCG	53	750
	R- GGCAAGCTTTGGAAATGAAA		
O157	F- TCGAGGTACCTGAATCTTTCCTTCTGT	63	894
	R- ACCAGTCTTGGTGCTGCTCTGACA		
O103	F- TTGGAGCGTTAACTGGACCT	57	321
	R- GCTCCCGAGCACGTATAAAG		
bla CTXM	F- GGTTAAAAAATCACTGCGTC	54	863
	R- TTGGTGACGATTTTAGCCGC		
bla TEM	F- ATGAGTATTCAACATTTCCGC	53	856
	R- CAATGCTTAATCAGTGAGG		
bla SHV	F- AAGATCCACTATCGCCAGCAG	56	230
	R- ATTCAGTTCCGTTTCCCAGCGG		

stx1 and *stx2*, but the presence of *hly* accompanied with *stx1* and *stx2* showed the least frequency in all sample sources. The *stx* genes alone or in combination with other genes were also detected in various frequencies, as presented in Figs. 1 and 2.
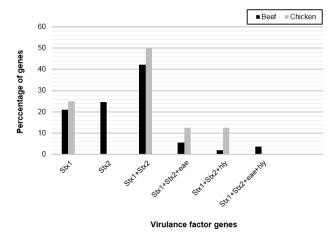

Fig. 1: The percentages of virulence genes in STEC isolates from beef and chicken meat sample
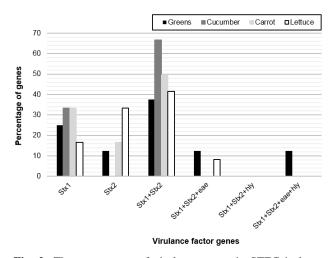

Fig. 2: The percentages of virulence genes in STEC isolates from vegeteble samples

Detection of O157 and non-O157 serogroups of STECs in all samples was done by PCR assay. Only 2 (3.8%) beef STEC isolates and 2 (16.6%) lettuce STEC isolates were detected to be positive for O157 serogroup, while this serogroup was not detected in other sources. Amongst five non-O157 serogroups evaluated in this study, O26 and O103 were the most and the least prevalent serogroups in beef samples, respectively.

Regarding the chicken samples, O26, O111, and O103 serogroups were detected equally, while the samples were not positive for the two other non-O157 serogroups. However, in the vegetable samples, the distribution of the mentioned non-O157 serogroups showed various patterns in different sources. Table 2 shows these results in detail.

In the AST, the beef samples showed the highest resistance to tetracycline (55.7%) and ampicillin (40.3%), while in the chicken samples, the highest

resistance rate belonged to nalidixic acid (62.5%) followed by tetracycline with 50%. Furthermore, the isolates in both groups were mildly resistant to ceftazidime (7.6% and 12.5% in beef and chicken samples, respectively). Although a few beef samples were resistant to imipenem and meropenem (7.6% and 9.6%, respectively), all isolates of the chicken source were sensitive to these two antibiotics (Figs. 3 and 4). In the vegetable isolates, the highest resistance rate was detected against ampicillin and/or amoxicillin in all sources, while all isolates showed the highest sensitivity to imipenem and ceftazidime.
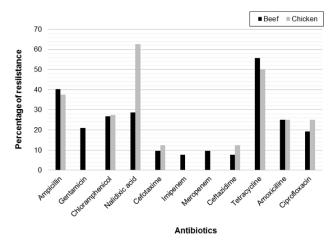

Fig. 3: Antibiotic resistance pattern of STEC isolates of beef and chicken meat samples
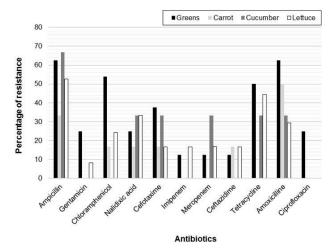

Fig. 4: Antibiotic resistance pattern of STEC isolates from vegeteble samples

By application of phenotypic combined disk assay, we found that 22 (42.3%) beef isolates, 3 (37.5%) chicken isolates, and 5 (17.2%) vegetable isolates were ESBL-producers. On the other hand, in the genotypic analysis, PCR, *CTX* (5.7%), *TEM* (30.6%), and *SHV* (19.3%) ESBL genes were detected in beef samples, while in the chicken samples only *TEM* (28%) and *SHV* (2.5%) were identified. In the vegetable isolates, *SHV* and *CTX* were detected equally (12.5%) in the isolates of leafy greens samples, *TEM* and *SHV* were detected, with 33.3% and 25% for the isolates of carrot; those for

Table 2: The distribution (number and percentage) of O-serogroups of the studied STEC isolates

Samples	Serogroups					
	O157	O26	O45	O103	O111	O145
Beef (n=52)	2 (3.8%)	23 (44.2%)	5 (9.6%)	4 (7.6%)	8 (15.3%)	6 (11.5%)
Chicken (n=8)	0 (0%)	2 (25%)	0 (0%)	2 (25%)	2 (25%)	0 (0%)
Carrot (n=6)	0 (0%)	0 (0%)	2 (33.3%)	1 (16.6%)	0 (0%)	0 (0%)
Cucumber (n=3)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33.3%)
Leafy greens (n=8)	0 (0%)	3 (37.5%)	0 (0%)	1 (12.5%)	1 (12.5%)	0 (0%)
Lettuce (n=12)	2 (16.6%)	2 (16.6%)	1 (8.3%)	2 (16.6%)	1 (8.3%)	0 (0%)

STEC: Shiga toxin-producing *Escherichia coli*

lettuce samples were 16.6% and 8.3%, respectively, while in the cucumber isolates, *CTX* (33.3%) was the only detected ESBL gene.

Discussion

Shiga-toxin producing *E. coli*, as a food-borne pathogen, is a serious threat to human health globally (Majowicz *et al*., 2014). Ruminant and poultry meat products along with vegetables with remarkable contributions to the human diet are known to be one of the main sources of STEC (Dulo, 2014). Thus, paying attention to the hygienic quality of meat and vegetables for the prevention of different infections and illnesses is of great importance for public health. Therefore, identification of the sources of infection is an important step towards decreasing the prevalence of this pathogen and thus decreasing the risk of probable infection in humans (Ojo *et al*., 2010).

Our findings showed that 52% of beef samples and 8% of chicken samples were STEC-positive, while the vegetable samples showed the lowest frequency with 7.2%. The presence of STEC in meat products reported in previous studies from different countries varied from 1.8% to 50%. In this regard, our results also supported the previous reports. However, some researches such as those conducted by Ojo *et al*. (2010) from Nigeria and Momtaz *et al*. (2013a) from Iran have shown higher prevalence rates of STEC in meat samples than the mentioned range. Regarding the vegetable samples, a review of 606 outbreaks associated with leafy greens over a 39-year period in the United States showed that the STEC was the cause of 18% of the outbreaks (Herman *et al*., 2015).

Further, we focused on virulence factors of STEC, especially *stx1*, *stx2*, *eae*, and *hly* genes in the current study. Our results showed that the prevalence of *stx2* outnumbered the *stx1* in beef samples as has been previously reported; however, the co-existence of these two genes in our samples was higher than each gene alone in both beef and chicken isolates. In line with a previous report by Zahraei Salehi *et al*. (2006), the association of each of *eae* and *hly* with both *stx1* and *stx2* was low in our study.

In the vegetables, the higher frequency of *stx2* than *stx1* was observed only in the carrot samples, while co-existence of these two genes was high in all samples. The presence of *hly* and *eae* genes was very low and only in lettuce and leafy greens samples. Our finding is consistent with the study of Bardasi *et al*. (2015) from Italy and contrary to that for Bonyadian *et al*. (2017) in Iran.

The *stx* genes have been previously shown to be the most important virulence factors of the STEC isolated from animal meat. The higher frequency of *stx2* (25%) detected in our samples compared to *stx1* (21%) is of importance because of the stronger association of *stx2* with clinical disorders such as HUS reported by (Tzipori *et al*., 2004). Furthermore, Osek *et al*. (2002) reported that more toxicity of Shiga toxins might be due to the simultaneous carriage of *stx1* and *stx2* in some strains. Thus, our results regarding the higher incidence of *stx1* and *stx2* together (42.3% and 50% in beef and chicken samples, respectively) support the previous findings.

O157:H7 and non-O157 STEC, primarily found in cattle as the main source, usually cause food-borne diseases in humans (Ferens and Hovde, 2011). Many studies all around the world have evaluated the presence of O157 serogroup of STEC in meat products because of its important role in outbreaks and serious infections (Panel *et al*., 2020). However, the challenges in detection of non-O157 serogroups, such as the unavailability of routine reliable user-friendly detection methods, have sometimes led to the ignorance of these organisms. Evidence shows that sporadic cases or outbreaks resulting from non-O157 STEC strains have been increasingly detected during recent years. About 150 non-O157 serotypes are known to be responsible for various diseases like bloody diarrhea, HUS, and sometimes death. The following six strains have been identified as the major foodborne pathogens: O26, O45, O103, O111, O121, and O145. Because of the high incidence of infections caused by these serogroups, about 64% annually, by notable addressing of their prevalence, seem to be of great importance (Coombes *et al*., 2011; Fan *et al*., 2019). In this study, by PCR, we tested our samples for the presence of five most important non-O157 serogroups, including O26, O45, O103, O111, and O145, as described by Balamurugan *et al*. (2017).

The low prevalence rate of O157 serogroup in cattle samples in our study was in accordance with the previous reports by Hessain *et al*. (2015). Also, its detection in lettuce samples in our study was in line with the findings of Özpınar *et al*. (2013). The O157 serogroup can survive for a relatively long time in the vegetables, for example, 15 days in the lettuce (Bonyadian *et al*., 2017). Therefore, detection of this serogroup in lettuce samples in our study is important because vegetables make a

major part of a healthy human diet.

As it has been previously shown in some studies such as that for Ranjbar *et al.* (2017a), O26-positive samples outnumbered the other non-O157 serogroups and its prevalence was also considerably higher than that for O157-positive samples. The importance of this finding can be attributed to the observed association of non-O157 *E. coli*, especially O26 and O111 serotypes with HC and HUS, as well as the contribution of the expression of *stx2* in non-O157 serogroups to the severity of human disease (Boerlin *et al.*, 1999). However, contrary to our results, Momtaz *et al.* (2013a) and Ranjbar *et al.* (2018) from Iran, and Etcheverria and Padola (2013) from Spain found a higher prevalence of O157 than other serogroups in their samples.

Antimicrobial resistance is of great importance because of the possibility of transfer of resistant genes from bacteria which have infected animal to the human through the consumption of contaminated products. It leads to the inefficiency of antibacterial treatments in humans and consequently increases the cost of health care (Lavilla *et al.*, 2008; Manyi-Loh *et al.*, 2018).

High prevalence of antibiotic resistance in bacteria can be attributed to the widespread and indiscriminate uses of antimicrobial agents in veterinary medicine for several purposes, including treatment and prevention of diseases as well as growth promotion (Iweriebor *et al.*, 2015).

In this study, 11 antibiotics were examined for antimicrobial resistance. Our results showed that the highest resistance rates in our meat samples belonged to tetracycline, nalidixic acid, and ampicillin. These findings were in agreement with the previous reports by Hemmatinezhad *et al.* (2015), and Mashak (2018) from Iran and also support the previous findings from other countries (Iweriebor *et al.*, 2015). However, in the vegetable isolates, the highest resistance rate was against ampicillin and amoxicillin. The results of previous studies showed variability of antibiotic resistance in vegetable isolates (Hassan *et al.*, 2011; Schwaiger *et al.*, 2011).

Furthermore, resistance to chloramphenicol as a forbidden antibiotic for use in food producing animals (Attari *et al.*, 2014) was also found to be relatively high (26.9% in beef and 27.5% in chicken samples) in our study. Considering the contraindication of tetracycline and chloramphenicol in veterinary treatment, our findings suggest the probable unlicensed prescription of these drugs for food animals. The congruency of our observations in both samples of beef and chicken promotes this assumption (Mooljuntee *et al.*, 2010).

The presence of ESBL producers in healthy dairy cattle and retail meat was described for the first time by American researchers (Geser *et al.*, 2012). Increased resistance to beta-lactam antimicrobials has been reported for different bacteria such as *Enterobacteriaceae*, especially *E. coli* which is a source of contamination in meat products (Stuart *et al.*, 2012; Minh *et al.*, 2016). Accordingly, the presence and prevalence of *SHV*, *TEM*, and *CTX* genes as

representatives of major families of ESBL, were considered in this study. We observed that more samples carried STEC harboring *TEM* gene (about 30%) rather than *CTX* and *SHV* genes. The higher rate of *TEM* gene than the two others in our study is comparable to those reported by Iweriebor *et al.* (2015) and Alegría *et al.* (2020). On the contrary, the highest prevalence of ESBL genes was found to belong to *CTX* in the study by Dutta *et al.* (2013). However, the discrepancies found between our results and the previous reports may result from different facts such as sources of samples, sampling methods, detection methods, and geographical region of sampling.

In conclusion, given the importance of STECs in food-borne diseases, the prevalence of both O157 and non-O157 serogroups was evaluated in this study, showing that the non-O157 serogroups outnumbered the other one. Based on this finding, considering the non-O157 serogroups in future epidemiological studies is recommended. As it has been previously shown, different virulence and resistance profile patterns observed in distinct epidemiological studies may result from several issues, including geographical region and other factors such as the nutrition of animal food, the sanitary conditions of slaughterhouses, and meat-products processing, as well as different colonization capacities in different vegetables. Because contaminated animal meat and vegetables with bacteria contribute to the transmission of antimicrobial resistance to humans, the high level of resistance to some antibiotics observed in this study can be a concern for public health in our region. As the vegetables are commonly consumed rawly and the pathogens are more likely to transmit to the human in this way, proper disinfection of vegetables or cooking them before consumption is highly recommended. Meat is also suggested to be cooked well to kill any pathogen. Furthermore, the antimicrobial resistance pattern of STEC isolates detected in this study can help clinicians to choose more effective antibiotics for treatment of such bacterial infections in the future.

Conflict of interest

The authors report no conflicts of interest in this work.

References

Ahmed, OB and Dablool, A (2017). Quality improvement of the DNA extracted by boiling method in gram negative bacteria. Int. J. Bioassays. 6: 5347-5349.

Alegría, Á; Arias-Temprano, M; Fernández-Natal, I; Rodríguez-Calleja, JM; García-López, ML and Santos, JA (2020). Molecular diversity of ESBL-producing *Escherichia coli* from foods of animal origin and human patients. Int. J. Environ. Res. Public Health. 17: 1312-1321.

Ateba, CN and Mbewe, M (2011). Detection of *Escherichia coli* O157: H7 virulence genes in isolates from beef, pork, water, human and animal species in the northwest province, South Africa: public health implications. Res. Microbiol.,

162: 240-248.

Attari, VE; Abbasi, MM; Abedimanesh, N; Ostadrahimi, A and Gorbani, A (2014). Investigation of enrofloxacin and chloramphenicol residues in broiler chickens carcasses collected from local markets of Tabriz, Northwestern Iran. Health Promot. Perspect., 4: 151-157.

Balamurugan, S; Ahmed, R; Gao, A and Strange, P (2017). Comparison of the fate of the top six non-O157 shiga-toxin producing *Escherichia coli* (STEC) and *E. coli* O157: H7 during the manufacture of dry fermented sausages. Int. J. Food Microbiol., 259: 14-21.

Bardasi, L; Taddei, R; Nocera, L; Ricchi, M and Merialdi, G (2015). Shiga toxin-producing *Escherichia coli* in meat and vegetable products in Emilia Romagna Region, years 2012-2013. Ital. J. Food Saf., 4: 33-37.

Boerlin, P; McEwen, SA; Boerlin-Petzold, F; Wilson, JB; Johnson, RP and Gyles, CL (1999). Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. J. Clin. Microbiol., 37: 497-503.

Bonyadian, M; Moshtaghi, H and Mohamadtaghipour, L (2017). Antibiotic resistance of Verotoxigenic *Escherichia coli* isolated from vegetables. BJM., 5: 9-16.

Cepeda-Molero, M; Berger, CN; Walsham, AD; Ellis, SJ; Wemyss-Holden, S; Schüller, S; Frankel, G and Fernández, LÁ (2017). Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. PLoS Pathogens. 13: 257-263.

Conrad, C; Stanford, K; McAllister, T; Thomas, J and Reuter, T (2016). Shiga toxin-producing *Escherichia coli* and current trends in diagnostics. Anim. Front., 6: 37-43.

Coombes, BK; Gilmour, MW and Goodman, CD (2011). The evolution of virulence in non-O157 Shiga toxin-producing *Escherichia coli*. Front. Microbiol., 2: 90-94.

Dehkordi, FS; Tavakoli-Far, B; Jafariaskari, S; Momtaz, H; Esmaeilzadeh, S; Ranjbar, R and Rabiei, M (2020). Uropathogenic *Escherichia coli* in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infect., 38: 1-11.

Dulo, F (2014). Prevalence and antimicrobial resistance profile of *Escherichia coli* O157: H7 in goat slaughtered in dire dawa municipal abattoir as well as food safety knowledge, attitude and hygiene practice assessment among slaughter staff, Ethiopia. MSc Thesis, Addis Ababa, Ethiopia: Addis Ababa University.

Dutta, T; Warjri, I; Roychoudhury, P; Lalzampuia, H; Samanta, I; Joardar, S; Bandyopadhyay, S and Chandra, R (2013). Extended-spectrum-β-lactamase-producing *Escherichia coli* isolate possessing the Shiga toxin gene (*stx 1*) belonging to the O64 serogroup associated with human disease in India. J. Clin. Microbiol., 51: 2008-2009.

Ercoli, L; Farneti, S; Zicavo, A; Mencaroni, G; Blasi, G; Striano, G and Scuota, S (2016). Prevalence and characteristics of verotoxigenic *Escherichia coli* strains isolated from pigs and pork products in Umbria and Marche regions of Italy. Int. J. Food Microbiol., 232: 7-14.

Etcheverria, AI and Padola, NL (2013). Shiga toxin-producing *Escherichia coli*: factors involved in virulence and cattle colonization. Virulence. 4: 366-372.

Fan, R; Shao, K; Yang, X; Bai, X; Fu, S; Sun, H; Xu, Y; Wang, H; Li, Q and Hu, B (2019). High prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in beef cattle detected by combining four selective agars. BMC Microbiol., 19: 213-219.

Ferens, WA and Hovde, CJ (2011). *Escherichia coli* O157: H7: animal reservoir and sources of human infection. Foodborne Pathog. Dis., 8: 465-487.

Franz, E; van Hoek, AH; Wuite, M; van der Wal, FJ; de Boer, AG; Bouw, E and Aarts, HJ (2015). Molecular hazard identification of non-O157 Shiga toxin-producing *Escherichia coli* (STEC). PLoS One, 10: 511-516.

Geser, N; Stephan, R and Hächler, H (2012). Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet. Res., 8: 21-26.

Hassan, SA; Altalhi, AD; Gherbawy, YA and El-Deeb, BA (2011). Bacterial load of fresh vegetables and their resistance to the currently used antibiotics in Saudi Arabia. Foodborne Pathog. Dis., 8: 1011-1018.

Herman, K; Hall, A and Gould, L (2015). Outbreaks attributed to fresh leafy vegetables, United States, 1973-2012. Epidemiol. Infect., 143: 3011-3021.

Hessain, AM; Al-Arfaj, AA; Zakri, AM; El-Jakee, JK; Al-Zogibi, OG; Hemeg, HA and Ibrahim, IM (2015). Molecular characterization of *Escherichia coli* O157: H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. Saudi J. Biol. Sci., 22: 725-729.

Iweriebor, BC; Iwu, CJ; Obi, LC; Nwodo, UU and Okoh, AI (2015). Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in feces of dairy cattle farms in Eastern Cape of South Africa. BMC Microbiol., 15: 1-9.

Kohansal, M and Asad, AG (2018). Molecular analysis of Shiga toxin-producing *Escherichia coli* O157: H7 and non-O157 strains isolated from calves. Onderstepoort J. Vet. Res., 85: 1-7.

Koochakzadeh, A; Badouei, MA; Mazandarani, E and Madadgar, O (2014). Survey on O157: H7 enterohemorrhagic *Escherichia coli* (EHEC) in cattle in Golestan province, Iran. Iran J. Microbiol., 6: 276-282.

Lavilla, S; Gonzalez-Lopez, J; Sabate, M; Garcia-Fernandez, A; Larrosa, M; Bartolome, R; Carattoli, A and Prats, G (2008). Prevalence of *qnr* genes among extended-spectrum β-lactamase-producing enterobacterial isolates in Barcelona, Spain. J. Antimicrob. Chemother., 61: 291-295.

Leotta, GA; Miliwebsky, ES; Chinen, I; Espinosa, EM; Azzopardi, K; Tennant, SM; Robins-Browne, RM and Rivas, M (2008). Characterisation of Shiga toxin-producing *Escherichia coli* O157 strains isolated from humans in Argentina, Australia and New Zealand. BMC Microbiol., 8: 46-51.

Majowicz, SE; Scallan, E; Jones-Bitton, A; Sargeant, JM; Stapleton, J; Angulo, FJ; Yeung, DH and Kirk, MD (2014). Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog. Dis., 11: 447-455.

Mandakini, R; Dutta, TK; Chingtham, S; Roychoudhury, P; Samanta, I; Joardar, SN; Pachauau, AR and Chandra, R (2015). ESBL-producing Shiga-toxigenic *E. coli* (STEC) associated with piglet diarrhoea in India. Trop. Anim. Health Prod., 47: 377-381.

Manyi-Loh, C; Mamphweli, S; Meyer, E and Okoh, A (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules. 23: 795-801.

Mashak, Z (2018). Virulence genes and phenotypic evaluation of the antibiotic resistance of vero toxin producing *Escherichia coli* recovered from milk, meat, and

vegetables. Jundishapur J. Microbiol., 11: 1-8.

Mathusa, EC; Chen, Y; Enache, E and Hontz, L (2010). Non-O157 Shiga toxin-producing *Escherichia coli* in foods. J. Food Prot., 73: 1721-1736.

Melton-Celsa, AR (2014). Shiga toxin(Stx) classification, structure, and function. Microbiol. Spectr., 54: 333-337.

Memariani, M; Peerayeh, SN; Salehi, TZ and Mostafavi, SKS (2015). Occurrence of SHV, TEM and CTX-M β-lactamase genes among enteropathogenic *Escherichia coli* strains isolated from children with diarrhea. Jundishapur J. Microbiol., 8: 1-8.

Minh, DH; Minh, SH; Honjoh, KI and Miyamoto, T (2016). Isolation and bio-control of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* contamination in raw chicken meat by using lytic bacteriophages. LWT., 71: 339-346.

Mohammadi-Sardo, MR; Salehi, S; Mirbaha, S and Abdollahi, A (2017). Shiga toxigenic *Escherichia coli* antimicrobial resistance properties in diabetic and nondiabetic pediatric patients; a case-control study. Int. J. Pediatr., 5: 5999-6008.

Momtaz, H; Dehkordi, FS; Rahimi, E; Ezadi, H and Arab, R (2013a). Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat. Meat Sci., 95: 381-388.

Momtaz, H; Karimian, A; Madani, M; Dehkordi, FS; Ranjbar, R; Sarshar, M and Souod, N (2013b). Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann. Clin. Microbiol. Antimicrob., 12: 1-12.

Mooljuntee, S; Chansiripornchai, P and Chansiripornchai, N (2010). Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. Wetchasan Sattawaphaet., 40: 311-315.

Nasrolahei, M; Zahedi, B; Bahador, A; Saghi, H; Kholdi, S; Jalalvand, N and Esmaeili, D (2014). Distribution of bla OXA-23, IS Aba, Aminoglycosides resistant genes among burned & ICU patients in Tehran and Sari, Iran. Ann. Clin. Microbiol. Antimicrob., 13: 1-4.

Noguera, P; Posthuma-Trumpie, G; Van Tuil, M; Van der Wal, F; De Boer, A; Moers, A and Van Amerongen, A (2011). Carbon nanoparticles in lateral flow methods to detect genes encoding virulence factors of Shiga toxin-producing *Escherichia coli*. Anal. Bioanal. Chem., 399: 831-838.

Ojo, O; Ajuwape, A; Otesile, E; Owoade, A; Oyekunle, M and Adetosoye, A (2010). 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.

Osek, J and Gallien, P (2002). Molecular analysis of *Escherichia coli* O157 strains isolated from cattle and pigs by the use of PCR and pulsed-field gel electrophoresis methods. Vet. Med. (Praha)., 47: 149-158.

Ozpınar, H; Turan, B; Tekiner, IH; Tezmen, G; Gökçe, I

and Akıneden, O (2013). Evaluation of pathogenic *Escherichia coli* occurrence in vegetable samples from district bazaars in Istanbul using real-time PCR. Lett. Appl. Microbiol., 57: 362-367.

Panel, EB; Koutsoumanis, K; Allende, A; Alvarez-Ordóñez, A; Bover-Cid, S; Chemaly, M; Davies, R; De Cesare, A; Herman, L and Hilbert, F (2020). Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. EFSA J., 18: 1-8.

Raeispour, M and Ranjbar, R (2018). Antibiotic resistance, virulence factors and genotyping of Uropathogenic *Escherichia coli* strains. Antimicrob. Resist. Infect. Control., 7: 1-9.

Ranjbar, R; Dehkordi, FS; Shahreza, MHS and Rahimi, E (2018). Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products. Antimicrob. Resist. Infect. Control., 7: 53-59.

Ranjbar, R; Masoudimanesh, M; Dehkordi, FS; Jonaidi-Jafari, N and Rahimi, E (2017a). Shiga (Vero)-toxin producing *Escherichia coli* isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. Antimicrob. Resist. Infect. Control., 6: 4-8.

Ranjbar, R; Seif, A and Safarpoor Dehkordi, F (2019). Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic *Escherichia coli* recovered from hospital food. Jundishapur J. Microbiol., 12: 1-8.

Ranjbar, R; Sheikhshahrokh, A and Jonaidi Jafari, N (2017b). Shiga (vero) toxin producing *Escherichia coli* in various types of food stuffs; virulence factors, O-serogroups and antimicrobial resistance properties. J. Food Saf., 37: 1-7.

Schwaiger, K; Helmke, K; Hölzel, CS and Bauer, J (2011). Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. supermarket). Int. J. Food Microbiol., 148: 191-196.

Stuart, JC; van den Munckhof, T; Voets, G; Scharringa, J; Fluit, A and Leverstein-Van Hall, M (2012). Comparison of ESBL contamination in organic and conventional retail chicken meat. Int. J. Food Microbiol., 154: 212-214.

Tzipori, S; Sheoran, A; Akiyoshi, D; Donohue-Rolfe, A and Trachtman, H (2004). Antibody therapy in the management of Shiga toxin-induced hemolytic uremic syndrome. Clin. Microbiol. Rev., 17: 926-941.

Zahraei Salehi, T; Safarchhi, A and Rabbani Khorrasgani, M (2006). Identification of virulence genes in isolated *Escherichia coli* from diarrheic calves and lambs by multiplex polymerase chain reaction. Pak. J. Biol. Sci., 9: 191-196.