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Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat

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ABSTRACT

To assess the presences of *Escherichia coli*, its serogroups, virulence factors and antibiotic resistance properties in ruminant's meat, a total of 820 raw meat samples were collected and then evaluated using culture, PCR and disk diffusion methods. Totally, 238 (29.02%) samples were positive for presence of *Escherichia coli*. All of the isolates had more than one virulence gene including *Stx1*, *Stx2*, *eaeA* and *ehly*. All investigated serogroups were found in beef and sheep and all except O145, O121 and O128 were found in goat. The O91, O113, O111, O103, O26 and O157 serogroups were found in camel. Totally, *aadA1–blaSHV* combination was the most predominant antibiotic resistance gene. The highest resistance of STEC strains was seen against penicillin while resistance to nitrofurantoin and ciprofloxacin was minimal. These findings showed that health care and meat inspection should be reconsidered in Iranian slaughterhouses and butchers.

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1. Introduction

Meat and meat products have an important role in the nutrition of Iranian people. Animals are raised as complete foods. Besides, many products, such as burger, sausage and salami are produced from the meats of animal species. Every day millions of people use the meat and meat products. Therefore, hygienic quality of meat is very important for public health as consumption of poor quality meat may cause several infections and illnesses.

All humans and animals carry *Escherichia coli* (*E. coli*) bacteria in their intestines — they are Gram-negative, rod-shaped, flagellated, non-sporulating and facultative anaerobic bacteria which belongs to the *Enterobacteriaceae* family and are usually harmless. However, there are several types of *E. coli* strains that may cause gastrointestinal illness in humans. Among all of them, vero-toxin-producing or Shiga-toxin producing *E. coli* (VTEC or STEC) have recently emerged as important food-borne pathogens, especially O157, O26, O103, O111, O145, O45, O91, O113, O121 and O128 serogroups (Momtaz, Farzan, Rahimi, Safarpoor Dehkordi, & Souod, 2012). STEC are found in a wide variety of animal species, including cattle, sheep, goats, pigs, water buffalos, and wild ruminants (Caprioli, Morabito, Brugère, & Oswald, 2005). Ruminants are the most important reservoir of the zoonotic STEC which are transmitted to humans through their products like meat. Several studies in various countries including the USA (Hancock et al., 1994), the European countries (Caprioli et al., 2005), Japan (Kobayashi et al., 2001), Korea (Jo et al., 2004), Italy (Caprioli et al., 2005), Brazil (Irino et al., 2005) and Botswana (Magwira, Gashe, & Collison, 2005) showed the high outbreak of STEC strains in animals. The prevalence of STEC strains in beef cattle was 10%–20% in the USA (Hancock et al., 1994) and as high as 17% in Italy (Caprioli et al., 2005).

The Shiga toxins produced by various serogroups of *E. coli* may cause anything from uncomplicated diarrhea to hemorrhagic colitis, which can progress into hemolytic uremic syndrome (HUS), composed of micro-angiopathic hemolytic anemia, thrombocytopenia and severe acute renal failure requiring intensive care (ECDC/EFSA, 2011). The pathogenic capacity of STEC resides in a number of virulence factors, including Shiga toxins (*Stx1* and *Stx2*), protein intimin (*eae*) and enterohemolysin (*Ehly*) (Law, 2000).

It seems that in the majority of cases, if the treatment was not sufficient, diseases caused by *E. coli*, in addition to weakening body immunity and increasing susceptibility to other diseases, can lead to death. Treatment of diseases caused by this bacterium often requires antimicrobial therapy; however antibiotic-resistant strains of bacteria cause more severe diseases for longer periods of time than their antibiotic-susceptible counterparts. Previous study showed that antibiotic resistance in *E. coli* is increasing these days (Li, Wang, & Li, 2011). Therefore, identification of antibiotic resistance genes of bacteria seems to be very essential in the reduction of treatment costs.

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Totally, the epidemiology and prevalence of serogroups, virulence factors and antimicrobial resistance properties of STEC strains isolated from ruminant meat samples in Iran are essentially unknown. Therefore, this research was carried out in order to study the serogroups, virulence factors and antibiotic resistance properties of STEC strains isolated from beef, sheep, goat and camel meat samples.

2. Materials and methods

2.1. Sample collection, preparation and E. coli identification

Overall 820 raw meat samples were collected: beef (n = 340), sheep (n = 220), goat (n = 168) and camel (n = 92) raw meat samples were purchased from farm butchers from several geographic regions in Iran, from January 2012 to August 2012 (Fig. 1). The carcasses in which the meat samples were collected from for this study were clinically healthy and the meat samples showed normal physical characteristics. Before collecting meat samples, the external surfaces were disinfected with 70% alcohol to minimize surface contamination. Separate 10-g femur muscle samples were collected using sterile scissors and tissue forceps. Samples were collected under sterile hygienic conditions and were immediately transported to the Food Microbiology Laboratory of the Islamic Azad University, Shahrekord branch, in Iran at 4 °C in a cooler with ice packs. Meat samples were homogenized in 3 mL of sterile phosphate-buffered saline (PBS) (pH 7.2) by using a mortar and pestle.

3 mL of each homogenized sample was blended with 225 mL of nutrient broth (Merck, Germany) for 2 min at normal speed, using a Stomacher lab blender and incubated at 37 °C for 24 h. A 1 mL sample of the nutrient broth culture was mixed with 9 mL of MacConkey broth (Merck, Germany) and further incubated at 37 °C for 24 h. One loop of each tube was streaked on MacConkey agar (Merck, Germany). One colony from each plate with typical *E. coli* morphology was selected and examined by biochemical tests, including hydrogen sulfide, citrate, urease and indol.

2.2. DNA isolation

Bacterial strains were grown overnight in trypticase soy agar (TSA — Merck, Germany) at 37 °C. One colony was suspended in 100 μL of sterile distilled water. After boiling the suspension for 13 min; this was followed by freezing and subsequently centrifuged at 14,000 rpm for 15 min to pelletize the cell debris (Reischl et al., 2002). The supernatant was used as a template for amplification reaction.

2.3. Polymerase chain reaction and electrophoresis

PCR assays for detection of STEC serogroups, virulence factors and antibiotic resistance genes in *E. coli* isolated from raw beef, sheep, goat and camel meat samples were performed using specific primers (Table 1).

All conditions including volumes and temperatures of the reactions are based on the method described by Momtaz et al. (2012). All PCR reactions were performed in a thermocycler (Eppendrof Mastercycler 5330; Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany), and PCR products were visualized by electrophoresis in 1.5% agarose gel, strained with ethidium bromide, and examined under ultraviolet illumination. Strains of *E. coli* O157:K88ac:H19, CAPM 5933 and *E. coli* O159:H20, CAPM 6006 were used as positive controls.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by the Kirby–Bauer disk diffusion method using Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). After incubating the inoculated plate aerobically at 37 °C for 18–24 h in an aerobic atmosphere, the susceptibility of the *E. coli* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). *E. coli* ATCC

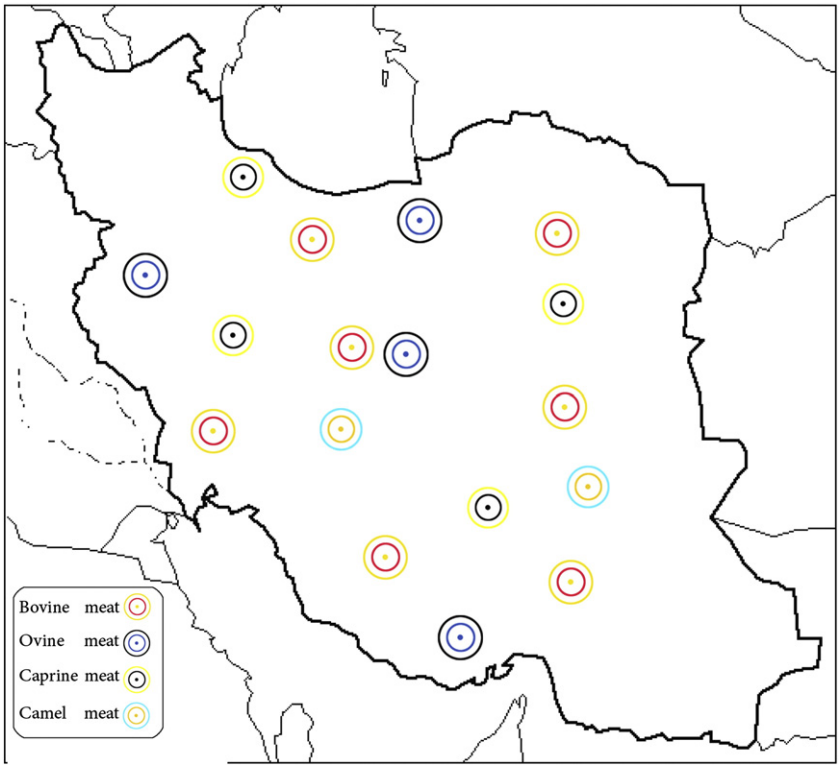

Fig. 1. Places of ruminant meat sampling in Iran.

Table 1
Primers used for detection of virulence genes, serogroups and antimicrobial resistant genes in Shiga toxin-producing *Escherichia coli* isolated from ruminant's meat.

Target gene	Primers name	Primer sequences (5′–3′)	Product size (bp)	Reference
Shiga toxin 1 (*Stx1*)	Stx1f	AAATCGCCATTCGTTGACTACTTCT	366	Brian et al. (1992)
	Stx1r	TGCCATTCTGGCAACTCGCGATGCA		
Shiga toxin 2 (*Stx2*)	Stx2f	CGATCGTCACTCACTGGTTTCATCA	282	Brian et al. (1992)
	Stx2r	GGATATTCTCCCCACTCTGACACC		
Enteropathogenic attachment & effacement (*eaeA*)	EAE1	TGCGGCACAACAGGCGGCGA	629	Heuvelink, van de Kar, Meis, Monnens, & Melchers (1995)
	EAE2	CGGTCGCCGCACCAGGATTC		
Hemolysin (*ehly*)	Hly F	CAATGCAGATGCAGATACCG	432	Idress, Mussarat, Badshah, Qamar, & Bokhari (2010)
	Hly R	CAGAGATGTCGTTGCAGCAG		
wzx	O26-F	CAG AAT GGT TAT GCT ACT GT	423	Possé, De Zutter, Heyndrickx, & Herman (2007)
	O26-R	CTT ACA TTT GTT TTC GGC ATC		
wzx	O103-F	TTGGAGCGTTAACTGGACCT	321	Possé et al. (2007)
	O103-R	GCTCCCGAGCACGTATAAG		
wzx	O111-F	TAG AGA AAT TAT CAA GTT AGT TCC	406	Possé et al. (2007)
	O111-R	ATA GTT ATG AAC ATC TTG TTT AGC		
wzx	O145-F	CCATCAACAGATTTAGGAGTG	609	Possé et al. (2007)
	O145-R	TTTCTACCGCGAATCTATC		
wzx	O157-F	CGG ACA TCC ATG TGA TAT GG	259	Possé et al. (2007)
	O157-R	TTG CCT ATG TAC AGC TAA TCC		
wzx1	O45-F	CCG GGT TTC GAT TTG TGA AGG TTG	527	DebRoy, Fratamico, Roberts, Davis, & Liu (2005)
	O45-R	CAC AAC AGC CAC TAC TAG GCA GAA		
gnd	O91-F	GCTGACCTTCATGATCTGTTGA	291	Perelle, Dilasser, Grout, & Fach (2002)
	O91-R	TAATTTAACCCGTAGAATCGCTGC		
wzx	O113-F	GGGTTAGATGGAGCGCTATTGAGA	771	DebRoy et al. (2004)
	O113-R	AGGTCACCCTCTGAATTATGGCAG		
wzx	O121-F	TGGCTAGTGGCATTCTGATG	322	Fratamico, Briggs, Needle, Chen, & DebRoy (2003)
	O121-R	TGATACTTTAGCCGCCCTTG		
galF	O128-F	GCTTTCTGCCGATATTTGGC	289	Shao, Li, Jia, Lu, & Wang (2003)
	O128-R	CCGACGGACTGATGCCGGTGATT		
aadA1	Streptomycin	(F) TATCCAGCTAAGCGCGAACT	447	Randall et al. (2004)
		(R) ATTTGCCGACTACCTTGGTC		
tetA	Tetracycline	(F) GGTTCACTCGAACGACGTCA	577	Randall et al. (2004)
		(R) CTGTCCGACAAGTTGCATGA		
tetB	Tetracycline	(F) CCTCAGCTTCTCAACGCGTG	634	Randall et al. (2004)
		(R) GCACCTTGCTGATGACTCTT		
dfrA1	Trimethoprim	(F) GGAGTGCCAAAGGTGAACAGC	367	Toro et al. (2005)
		(R) GAGGCGAAGTCTTGGGTAAAAAC		
qnr	Fluoroquinolone	(F) GGGTATGGATATTATTGATAAAG	670	Mammeri, Van De Loo, Poirel, Martinez-Martinez, & Nordmann (2005)
		(R) CTAATCCGGCAGCACTATTTA		
aac(3)-*IV*	Gentamicin	(F) CTTCAGGATGGCAAGTTGGT	286	Van, Chin, Chapman, Tran, & Coloe (2008)
		(R) TCATCTCGTTCTCCGCTCAT		
sul1	Sulfonamide	(F) TTCGGCATTCTGAATCTCAC	822	Van et al. (2008)
		(R) ATGATCTAACCCTCGGTCTC		
blaSHV	Cephalothin	(F) TCGCCTGTGTATTATCTCCC	768	Van et al. (2008)
		(R) CGCAGATAAATCACCACAATG		
CITM	Ampicillin	(F) TGGCCAGAACTGACAGGCAAA	462	Van et al. (2008)
		(R) TTTCTCCTGAACGTGGCTGGC		
cat1	Chloramphenicol	(F) AGTTGCTCAATGTACCTATAACC	547	Van et al. (2008)
		(R) TTGTAATTCATTAAGCATTCTGCC		
cmlA	Chloramphenicol	(F) CCGCCACGGTGTTGTTGTTATC	698	Van et al. (2008)
		(R) CACCTTGCCTGCCCATCATTAG		

25922 was used as quality control organisms in antimicrobial susceptibility determination.

2.5. Statistical analysis

The data were analyzed by using SPSS software (version 17. SPSS Inc., USA) and *P* value was calculated using Chi-square and Fisher's exact tests to find any significant relationship. *P* value less than 0.05 was considered statistically significant.

3. Results

In the current study, all *E. coli* colonies were tested by applying PCR method in order to detect 16S rRNA gene of bacterium (Sabat, Rose, Hickey, & Harkin, 2000). According to the data, out of 820 animal meat samples, 238 samples (29.02%) were positive for presence of *E. coli*. Totally, out of these 238 *E. coli* positive samples, 153 samples (64.28%) were STEC. Our results showed that sheep meat (35.45%) had the highest and camel meat (19.56%) had the lowest rate of *E. coli* in raw meat samples.

Table 3 shows the distribution of virulence factors in *E. coli* subtypes isolated from ruminant meat samples. It shows that in all animal species the AEEC subtype has the highest prevalence than the EHEC serotype. Besides, in all meat samples, the *Stx1* virulence gene had the highest while *Stx2* virulence gene had the lowest prevalence of AEEC strains. In addition, in all meat samples, all EHEC positive strains had all *Stx1*, *eaeA* and *ehly* virulence genes (the prevalence rate was 100%) (Table 2).

By applying specific primers for the detection of STEC serogroups in various samples, it was indicated that out of 238 positive samples for STEC serogroups, 47 (30.71%), 25 (16.33%), 12 (7.84%), 21 (13.72%), 12 (7.84%), 8 (5.23%), 13 (8.49%), 9 (5.88%), 3 (1.96%) and 3 (1.96%) samples were positive for incidences of O157, O26, O103, O111, O145, O45, O91, O113, O121 and O128 serogroups, respectively (Table 3). Results showed that in all species the O157 serogroup had the highest prevalence.

Table 2
Distribution of virulence factors in *Escherichia coli* subtypes isolated from ruminant's meat.

Meat	Subtype	No. of positive samples	Virulence gene
Beef (340)	Non detected	34 (33.66%)	–
	EHEC	21 (20.79%)	*Stx1*, *eaeA*, *ehly*: 21 (100%)
	AEEC	46 (45.54%)	*Stx1*: 42 (91.30%)
			Stx2: 3 (6.52%)
			eaeA: 41 (89.13%)
			Stx1, *eaeA*: 34 (73.91%)
			Stx2, *eaeA*: 9 (19.56%)
			Stx1, *Stx2*, *eaeA*: 3 (6.52%)
	Total	101 (29.70%)	
Sheep (220)	Non detected	29 (37.17%)	–
	EHEC	14 (17.94%)	*Stx1*, *eaeA*, *ehly*: 14 (100%)
	AEEC	35 (44.87%)	*Stx1*: 31 (88.57%)
			Stx2: 4 (11.42%)
			eaeA: 29 (82.85%)
			Stx1, *eaeA*: 26 (74.28%)
			Stx2, *eaeA*: 5 (14.28%)
			Stx1, *Stx2*, *eaeA*: 4 (11.42%)
	Total	78 (35.45%)	
Goat (168)	Non detected	16 (39.02%)	–
	EHEC	9 (21.95%)	*Stx1*, *eaeA*, *ehly*: 9 (100%)
	AEEC	16 (39.02%)	*Stx1*: 13 (81.25%)
			Stx2: 2 (12.50%)
			eaeA: 12 (75.00%)
			Stx1, *eaeA*: 7 (43.75%)
			Stx2, *eaeA*: 7 (43.75%)
			Stx1, *Stx2*, *eaeA*: 2
	Total	41 (24.40%)	(12.50%)
Camel (92)	Non detected	6 (33.33%)	–
	EHEC	3 (16.66%)	*Stx1*, *eaeA*, *ehly*: 3 (100%)
	AEEC	9 (50.00%)	*Stx1*: 7 (77.77%)
			Stx2: 1 (11.11%)
			eaeA: 6 (66.66%)
			Stx1, *eaeA*: 4 (44.44%)
			Stx2, *eaeA*: 4 (44.44%)
			Stx1, *Stx2*, *eaeA*: 1 (11.11%)
	Total	18 (19.56%)	

Table 4 shows the distribution of antimicrobial resistance genes in STEC strains isolated from ruminant and camel meat samples. Based on this table, *blaSHV* (70.14%) and *cmlA* (11.94%) in beef meat, *blaSHV* (75.51%) and both *cat1* (6.12%) and *cmlA* (6.12%) in sheep meat, *blaSHV* (72%) and *cat1* (4%) in goat meat and finally *blaSHV* (66.66%) and both *cat1* (0.0%) and *cmlA* (0.0%) in camel meat had the highest and lowest prevalences of antibiotic resistance genes in STEC strains.

The results of disk diffusion method showed that resistance to penicillin was the highest, followed by tetracycline while the STEC strains had the lowest resistance to ciprofloxacin and nitrofurantoin (Table 5). Besides, the STEC strains which were isolated from bovine meat samples had the highest resistance to antibiotics while the STEC strains which were isolated from camel meat samples had the lowest resistance to antibiotics.

4. Discussion

The present study showed that the ruminant meats have a relatively high potential for causing human infection. This potential occurs usually as human food poisonings. Our results showed that these contaminated meats can cause human infection. The meat samples of our investigation are sources of O157 and non O157 strains of *E. coli* and it is in agreement with previous studies (Fairbrother & Nadeau, 2006; Lekowska-Kochaniak, Czajkowska, & Popowski, 2002).

The ovine meat (35.45%) was the most contaminated while the camel meat (19.56%) was the less contaminated in our investigation. Significant differences ($P < 0.05$) were seen for the presence of *E. coli* between sheep and camel meat samples. Similar studies in Italy (Franco et al., 2009), Egypt (Abdul-Raouf, Ammar, & Beuchat, 1996), Australia (Phillips, Jordan, Morris, Jenson, & Sumner, 2006) and Germany (Beutin, Geier, Steinrück, Zimmermann, & Scheutz, 1993), have shown the higher incidence of STEC strains in sheep meat. It seems that the higher water activity of sheep meat is the main cause for its higher contamination. The water activity influences different chemical reactions in meat as well as the survival and the resistance of micro-organisms like *E. coli*. Mutton is softer than other meats. Therefore, due to its softness, has higher free water. Therefore, it has a higher capacity for proliferation and survival of *E. coli*.

Beef is the most frequently consumed meat in the world. Our results showed that 29.70% of beef samples were contaminated with STEC strains. Similar results which have been reported from the USA, India, New Zealand and Belgium showed that the contamination of beef with STEC strains was 1.8% to 50% (Brooks et al., 2001; Khan, Yamasaki, et al., 2002; Piérard, Stevens, Moriau, Lior, & Lauwers, 1997; Samadpour et al., 2002) which was in agreement with our findings. Besides beef, the presences of STEC strains have been reported previously in sheep, goat and camel meats (Barlow, Gobius, & Desmarchelier, 2006; Miko et al., 2009; Ojo et al., 2010; Rahimi, Kazemeini, & Salajegheh, 2012). Many different incidences of STEC meat contamination have been reported from various sites in the world (Ojo et al., 2010; Rahimi et al., 2012). The different prevalence reports of STEC strains in meat which were reported previously from various sites in the world depend on method of sampling, types of samples, age of samples (fresh or old), method of experience, geographic zone, customs and even animal nutrition.

Our results showed that *Stx1*, *Stx2*, *eaeA* and *ehly* are prevalent virulence genes in the STEC strains isolated from animal meat samples. Results revealed significant differences ($P < 0.05$) between the presence of *Stx1* and *eaeA* with *Stx2* virulence gene in all animal meat samples. Besides, the presences of AEEC and EHEC subtypes had significant differences in beef, sheep and camel meat samples

Table 3
Incidence of Shiga toxin-producing *Escherichia coli* serogroups isolated from ruminant's meat.

Meat (STEC positive)	O157	O26	O103	O111	O145	O45	O91	O113	O121	O128
Beef (67)	21	11	3	7	8	3	9	3	1	1
Sheep (49)	14	7	4	9	4	3	1	3	2	2
Goat (25)	9	5	3	4	–	2	1	1	–	–
Camel (12)	3	2	2	1	–	–	2	2	–	–
Total (153)	47 (30.71%) [A*,B, c**, d]	25 (16.33%) [e, f]	12 (7.84%)	21 (13.72%)	12 (7.84%)	8 (5.22%) [c]	13 (8.49%)	9 (5.88%) [d]	3 (1.96%) [A, e]	3 (1.96%) [B, f]

* Similar capital letters in horizontal rows have significant differences of about $P < 0.01$.
** Similar small letters in horizontal rows have significant differences of about $P < 0.05$.

Table 4
Distribution of antimicrobial resistance genes in Shiga toxin-producing *Escherichia coli* isolated from ruminant's meat.

Meat (STEC positive)	Virulence genes (%)										
	aadA1	*tetA*	*tetB*	*dfrA1*	*qnr*	*aac(3)-IV*	*sul1*	*blaSHV*	*CITM*	*cat1*	*cmlA*
Beef (67)	33 (49.25%)	39 (58.20%)	20 (29.85%)	32 (43.28%)	21 (31.34%)	43 (64.17%)	20 (29.85%)	47 (70.14%)	31 (46.26%)	9 (13.43%)	8 (11.94%)
Sheep (49)	21 (42.85%)	22 (44.89%)	21 (42.85%)	18 (36.73%)	15 (30.61%)	21 (42.85%)	13 (26.53%)	37 (75.51%)	21 (42.85%)	3 (6.12%)	3 (6.12%)
Goat (25)	13 (52%)	15 (60%)	8 (32%)	9 (36%)	10 (40%)	10 (40%)	8 (32%)	18 (72%)	10 (40%)	1 (4%)	2 (8%)
Camel (12)	4 (33.33%)	5 (41.66%)	6 (50%) [d, e]	2 (16.66%) [c**]	4 (33.33%)	3 (25%)	3 (25%)	8 (66.66%) [A, B, c]	4 (33.33%)	0 (0%) [A*, d]	0 (0%) [B, e]

* Similar capital letters in horizontal rows have significant differences of about $P < 0.01$.
** Similar small letters in horizontal rows have significant differences of about $P < 0.05$.

($P < 0.05$). The multiple presences of virulence genes have been reported in our study and other investigations (Karch, Bielaszewska, Bitzan, & Schmidt, 1999; Momtaz et al., 2012; Osek & Gallien, 2002). Our results showed that the *Stx* genes are the most important virulence genes in animal meat samples. These results were consistent with previous reports (Barlow et al., 2006; Khan, Das, et al., 2002; Osés, Rantsiou, Cocolin, Jaime, & Rovira, 2010) but our results were entirely in contrast with other investigations (Pradel et al., 2001; Schmidt, Geitz, Tarr, Frosch, & Karch, 1999). The *Stx1* and *Stx2* genes were found to be associated mainly with uncomplicated diarrhea or asymptomatic excretion (Beutin, Krause, Zimmermann, Kaulfuss, & Gleier, 2004; Friedrich et al., 2003). Also, these virulence genes are associated with the high virulence of STEC and with HC and HUS (Bielaszewska, Friedrich, Aldick, Schurk-Bulgrin, & Karch, 2006; Friedrich et al., 2003). The *eae* and *hly* genes have been found in over 90% of STEC illnesses, including HUS (Brooks et al., 2005; Jelacic et al., 2003). There is evidence that strains containing *Stx2* and *eae* are associated with severe clinical illness caused by both *E. coli* O157 and non-O157 STEC (Friedrich et al., 2002; Beutin et al., 2004). Therefore, consumption of this contaminated meat can cause various diseases such as diarrhea, HC and HUS in humans. One interesting finding of our study was that the *Stx1*, *Stx2*, *eae* and *hly* virulence genes of the STEC strains had higher incidence in beef and mutton. This finding shows that the beef and mutton have a higher potential for causing human infection.

This study showed that the STEC meat contamination has been associated with the presences of some major serogroups such as O157, O26 and O111. Statistical analyses were significant ($P < 0.01$) between the presence of O157 with O121 and O128 serogroups and also were significant ($P < 0.05$) between the presence of O157 with O45 and O113 STEC serogroups isolated from animal meat samples. Besides, statistical analyses were significant ($P < 0.05$) between the presence of O26 with O121 and O128 serogroups. Our results showed the multiple presences of STEC serogroups isolated from animal meat samples and it is in agreement with previous studies (Beutin et al., 1993; Blanco et al., 2003; Momtaz et al., 2012). Another interesting finding of our study was that beef and sheep meats have the highest incidence of O-serogroups of STEC strains. Hajian, Rahimi, and Mommtaz (2011) showed the high incidence of *E. coli* O157 in beef minced meat (11.1%), followed by beef meat (8.9%), goat meat (1.7%), and camel meat (1.3%) which was in agreement with our investigation. Ojo et al. (2010) showed that all seven investigated STEC serogroups were found in cattle, all except O145 were found in sheep, O157, O26 and O111 were found in goats and three O157, O111 and O128 in pigs which was in agreement with our results. The high presences of O157, O26 and O111 in meat have been reported previously by Hussein (2007).

Our results revealed that all STEC strains isolated from animal meat samples had more than 6 antibiotic resistance genes. The multiple presences of antibiotic resistance genes in STEC strains isolated from foods have been reported previously, too (Momtaz et al., 2012; Mora et al., 2005).

β-Lactam antibiotics such as ampicillin, cephalothin and penicillin are the most useful clinical drugs for treatment of bacterial infections because they combine safety with high potency against Gram-negative bacteria, such as members of the family *Enterobacteriaceae*, including *E. coli*. However, veterinary prescription of gentamicin, tetracycline, ampicillin, streptomycin, chloramphenicol and trimethoprim is abundant in Iran. Totally, *blaSHV*, *aac(3)IV*, *tetA*, *CITM*, *aadA1* and *dfrA1* had the highest incidence of antibiotic resistance genes of STEC strains in all meat samples. A new finding of our study was that the incidence of *blaSHV* was at the maximum while the incidences of *cmlA* and *cat1* antibiotic resistance genes were at the minimum. Statistical analysis showed significant differences about $P < 0.01$ between the presences of *blaSHV* with *cat1* and *cmlA* and $P < 0.05$ between *blaSHV* with *dfrA1* resistance genes. Also, $P < 0.05$ was seen between presences of *tetB* with *cat1* and *cmlA* antibiotic resistance genes. Therefore, the genes encoding gentamicin, tetracycline, ampicillin, streptomycin and trimethoprim, were the most common antibiotic resistance genes. Similar studies have been reported previously by Srinivasan, Nguyen, Headrick, Murinda, and Oliver (2007), Li et al. (2011) and Rao, Gill, Ravi Kumar, and Sandeep Ghatak (2011).

The results of disk diffusion method confirmed the results of PCR technique for detection of antibiotic resistance genes. The disk diffusion method showed that the STEC isolates had the highest resistance to penicillin, tetracycline, gentamicin and streptomycin and it was in agreement with the frequency of antibiotic resistance genes. The STEC strains which were isolated from meat samples had the lowest resistance to nitrofurantoin and ciprofloxacin. Our results indicated the significant differences between the resistances of all O-serogroups to penicillin with nitrofurantoin, chloramphenicol and ciprofloxacin ($P < 0.01$) and also $P < 0.05$ between resistance to penicillin and trimethoprim, gentamycin, sulfamethoxazol and streptomycin antibiotics. In addition, resistance to all antibiotics in STEC strains isolated from animals had significant differences between beef and camel ($P < 0.05$).

One of the most exciting findings was that the chloramphenicol, nitrofurantoin and ciprofloxacin were the most effective antibiotics for treatment of the cases of *E. coli* infection in all bovine, ovine, caprine and camel species. Both chloramphenicol and nitrofurantoin are prohibited antibiotics. Therefore, we recommended prescription of ciprofloxacin for treatment of *E. coli* infections in all animals. Ojo et al. (2010) showed the low incidence of STEC resistance against ciprofloxacin. Also, the multiple drug resistance of *E. coli* strains has been reported in our study and many other previous investigations (Meng, Zhao, Doyle, & Joseph, 1998; Randall, Cooles, Osborn, Piddock, & Woodward, 2004; Toro et al., 2005).

Table 5
Antimicrobial resistance properties in Shiga toxin-producing *Escherichia coli* isolated from ruminant's meat.

Meat (STEC positive)	Antimicrobial resistance properties (%)										
	TE30*	S10	C30	SXT	GM10	NFX5	CF30	CIP5	TMP5	F/M300	AM10	P10
Beef (67)	54 (80.59%)	28 (41.79%)	11 (16.41%)	18 (26.86%)	37 (55.22%)	13 (19.40%)	23 (34.32%)	4 (5.97%)	27 (40.29%)	4 (5.97%)	20 (29.85%)	67 (100%)
Sheep (49)	39 (79.59%)	18 (36.73%)	3 (6.12%)	12 (24.48%)	17 (34.69%)	8 (16.32%)	16 (32.65%)	6 (12.24%)	14 (28.57%)	3 (6.12%)	26 (53.06%)	49 (100%)
Goat (25)	20 (80%)	10 (40%)	2 (8%)	6 (24%)	8 (32%)	6 (24%)	8 (32%)	2 (8%)	6 (24%)	2 (8%)	15 (60%)	25 (100%)
Camel (12)	9 (75%)	2 (16.66%) ^{g}	0 (0%) ^{C}	2 (16.66%) ^{f}	2 (16.66%) ^{e***}	3 (25%)	5 (41.66%)	0 (0%) ^{B}	2 (16.66%) ^{d}	0 (0%) ^{A**}	3 (25%)	12 (100%) $^{A, B, C, d, e, f, g}$

* In this table: TE30 = tetracycline (30 μg/disk); S10 = streptomycin (10 μg/disk); C30 = chloramphenicol (30 μg/disk); SXT = sulfamethoxazol (25 μg/disk); GM10 = gentamycin (10 μg/disk); NFX5 = enrofloxacin (5 μg/disk); CF30 = cephalothin (30 μg/disk); CIP5 = ciprofloxacin (5 μg/disk); TMP5 = trimethoprim (5 μg/disk); F/M300 = nitrofurantoin (300 μg/disk); AM10 = ampicillin (10 μ/disk); and P10 = penicillin (10 μ/disk).
** Similar capital letters in horizontal rows have significant differences of about $P < 0.01$.
*** Similar small letters in horizontal rows have significant differences of about $P < 0.05$.

5. Conclusion

Despite previous studies, our results showed that the STEC strains in Iran have different virulence and resistance profile patterns. These differences in prevalence of STEC serogroups, virulence genes, antibiotic resistance genes and pattern of resistance showed that the properties of STEC strains are closely dependent on geographic region and even the weather or climate of each region. It seems that the epidemiology and prevalence of STEC strains isolated from animal meat are different in Iran. Probably because customs, animal food diets, levels of public health, slaughterhouse sanitary conditions, methods of sampling and even methods of diagnosis have great roles in the prevalence of serogroups, virulence genes, antibiotic resistance genes and pattern of resistance of STEC strains isolated from meat samples.

Totally, the animal meat samples are a potential reservoir for antimicrobial resistance genes and play important roles in the ecology of antimicrobial resistance of bacterial populations. This study demonstrated that meats, mainly of sheep, cattle, goat and camel were contaminated with diverse STEC strains. We conclude that the high prevalence of serogroups, virulence factors and antimicrobial resistance detected in our study is a source of concern, and cautious use of antibiotics in animals is highly recommended. Besides, the presence of atypical STEC strains in meat is also of concern due to their potential to cause human infections.

Our results revealed that (i) using raw meat without cooking, using unsanitary methods in slaughterhouses and even butchers are the main resources for growth, proliferation and survival of *E. coli* and cause several disorders for humans. Therefore, vaccinating animals (if necessary), improving methods of meat preparation, checking slaughterhouses in order to detect *E. coli*, observing hygiene during euthanization, boiling and cooking meat, keeping meats in cool and dry places, away from sunlight and finally preventing contamination of meats with extrinsic factors like insects and dust are the best ways to prevent *E. coli* infections. (ii) Chloramphenicol and nitrofurantoin are prohibited antibiotics and the low antibiotic resistance to them in our study indicated why veterinarians don't prescribe them. (iii) We recommend PCR method as an accurate, safe and fast diagnostic for detection of pathogens in animal meat samples. (iv) Due to antibiotic resistance especially in *E. coli*, veterinarians should pay more attention in prescribing antibiotics. (v) *E. coli* virulence genes especially *Stx1*, *Stx2*, *eae* and *ehly* are well distributed in animal meat in Iran. (vi) In order to prevent antibiotic resistance in bacteria, we should apply antibiotics more cautiously in animals, detect resistance genes and finally use different antibiotics periodically.

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