Trop Anim Health Prod (2013) 45:641–648
DOI 10.1007/s11250-012-0271-5

REGULAR ARTICLES

Characterization of non-O157 shiga toxin-producing *Escherichia coli* isolates from healthy fat-tailed sheep in southeastern of Iran

Reza Ghanbarpour · Mojtaba Kiani

Accepted: 18 September 2012 / Published online: 27 September 2012
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Abstract The objectives of this study were to determine the presence and prevalence of non-O157 shiga toxin-producing *Escherichia coli* (STEC) isolates from faeces of healthy fat-tailed sheep and detection of phylogenetic background and antibiotic resistance profile of isolates. One hundred ninety-two *E. coli* isolates were recovered from obtained rectal swabs and were confirmed by biochemical tests. Antibiotic resistance profiles of isolates were detected and phylogenetic background of isolates was determined according to the presence of the *chuA*, *yjaA* and TspE4.C2 genetic markers. The isolates were examined to determine stx_1, stx_2 and *eae* genes. Non-O157 STEC isolates were identified by using O157 specific antiserum. Forty-three isolates (22.40 %) were positive for one of the stx_1, stx_2 and *eae* genes, whereas 10.42 % were positive for stx_1, 19.38 % for *eae* and 2.60 % for stx_2 gene. None of the positive isolates belonged to O157 serogroup. Twenty isolates possessed stx_1 were distributed in A (six isolates), B1 (13) and D (one) phylogroups, whereas stx_2 positive isolates fell into A (three isolates) and B1 (two) phylogenetic groups. Eighteen isolates contained *eae* gene belonged to A (five isolates), B1 (seven) and D (six) phylogroups. The maximum and minimum resistance rates were recorded against to penicillin and co-trimoxazole respectively. The positive isolates for stx_1, stx_2 and *eae* genes showed several antibiotic resistance patterns, whereas belonged to A, B1 and D phylogroups. In conclusion, faeces of healthy sheep could be considered as the important sources of non-O157 STEC and also multidrug-resistant *E. coli* isolates.

Keywords Non-O157 · Shiga toxin · *Escherichia coli* · Sheep

R. Ghanbarpour (✉) · M. Kiani
Microbiology Department of Faculty of Veterinary Medicine,
Shahid Bahonar University,
76169-14111, Kerman, Iran
e-mail: ghanbar@mail.uk.ac.ir

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) or verotoxin-producing *E. coli* (VTEC) and particularly *E. coli* O157:H7, have emerged as the important zoonotic food-borne pathogens and confirming the risk to public health (Fernández et al. 2012; Paris et al. 2010). The most common STEC serotype associated with human disease is O157, but there is a growing recognition of over 100 non-O157 serotypes that also may result in human illness. Some of these non-O157 STEC strains cause outbreaks of diarrhoea and severe disease such as haemolytic uremic syndrome (HUS) and haemorrhagic colitis (Coombes et al. 2011; Nguyen and Sperandio 2012). Sources of human infection with STEC strains and outbreaks occur through ingestion of contaminated food or water including: undercooked meat, unpasteurised milk and dairy products, vegetables or water and contact with animal carriers or the environment (Pacheco and Sperandio 2012; Sanchez et al. 2010). Apparently, healthy cattle have been reported to be a major reservoir of STEC (VTEC); however, several studies suggest that other wild and domesticated ruminants may be important in contributing to human clinical cases (Ferens and Hovde 2011). The presence of *E. coli* O157:H7 has been reported in sheep and lamb products and/or faecal samples internationally (Kumar et al. 2012; Momtaz et al. 2012). On the other hand, cattle and sheep have been shown to carry non-O157 STEC strains, which mostly belong to O26, O111, O103, O121, O45 and O145 serogroups (Bai et al. 2012; Evans et al. 2008).

Two main virulence factors of STEC are Shiga toxins (Stx) 1 and 2 encoded by stx_1 and stx_2 genes respectively, which inhibit protein synthesis in mammalian cells. Several additional virulence factors, among many others, are a plasmid-encoded enterohemolysin and, in strains lacking intimin, an autoagglutinating adhesin (Saa) which could be involved in adhesion (Alonso et al. 2012; Andrade et al.

2012). The *eae* virulence gene, which encodes an outer membrane protein, intimin involved in the intimate attachment of the *E. coli* O157:H7 to the intestinal epithelium and induces attaching and effacing (AE) lesions (Bandyopadhyay et al. 2011).

The emergence of antibiotic resistance in both commensal and pathogenic strains has become an important public health issue (Saei et al. 2010). Development and persistence of antibiotic resistance in commensal and non-pathogenic bacteria is one of worldwide concerns, because they are thought to act as a reservoir of resistance genes capable of transferring genes to foodborne and other zoonotic pathogens (Zhang et al. 2009).

According to phylogenetic studies, *E. coli* strains can be assigned to one of the four main phylogenetic groups, A, B1, B2 and D, which can be divided into seven subgroups (Carlos et al. 2010). A study on molecular characterization of STEC isolates from ruminant and donkey raw milk samples and traditional dairy products in Iran showed that samples from sheep were positive for O157 and non O157 STEC strains (Momtaz et al. 2012). According to FAOSTAT (2010) report on livestock numbers of Iran, sheep are by far the most numerous farm animal, followed by goats, cattle, donkeys, horses, water buffalo and mules. The objectives of this study were (1) to determine the presence and prevalence of O157 and non-O157 STEC isolates from faeces of healthy sheep in southeastern of Iran; (2) to investigate the phylogenetic distribution and antibiotic resistance profile of the shiga toxin and intimin genes possessed isolates.

Materials and methods

Bacterial isolates

Rectal swab samples were obtained from 192 apparently healthy fat-tailed sheep between June 2010 and February 2011. The sampled animals were originated from eight flocks in Kerman province (southeastern), Iran. The flocks were in traditional husbandry conditions and there was not any history of previous antibiotic therapy. Each of the sampled flocks included nearly 80–120 sheep, which 20–25 sheep were chosen for sampling from each flock. One hundred and sixty-eight of the sampled sheep were 2–3 years old and 24 animals were 4 years old. Among the 192 sampled sheep, 153 were female and 39 were male. Swab samples were streaked onto Mac Conkey agar (Biolife Laboratories, Milan, Italy) and the plates were incubated at 37 °C for 24 h. After overnight incubation, suggestive of *E. coli* colonies were further streaked onto eosin methylene blue and incubated overnight at 37 °C again. Green metallic sheen colonies indicative of *E. coli* were subjected to biochemical tests, which included indole, methyl red, Voges–Proskauer and Simmons citrate tests for *E. coli* identification. The confirmed *E. coli* isolates were stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30 % sterile glycerol at −80 °C. From each sample, one confirmed isolate were chosen for serology and antibiotic susceptibility tests and PCR assays.

Reference strains of Sakaï (*eae*+, stx_1+ and stx_2+) and ECOR62 (*chuA*+, *yjaA*+ and TspE4 C2+) were used as positive controls. Non-pathogenic *E. coli* strain MG1655 was used as a negative control for target genes.

Antimicrobial susceptibility test

Antibiotic resistance profile of isolates against eight selected antibacterial agents was determined by disc diffusion method according to Clinical and Laboratory Standards Institute's guidelines (CLSI 2004). The following antimicrobial discs (Padtan-Teb, Tehran, Iran) were used in disc diffusion assay: co-trimoxazole (SXT; 25 μg), cephalexin (CN; 30 μg), ciprofloxacin (CP; 5 μg), enrofloxacin (NFX; 5 μg), kanamycin (K; 30 μg), oxytetracycline (T; 30 μg), penicillin (P; 10 μg) and streptomycin (S; 10 μg).

PCR assays

Freshly grown over night cultures of *E. coli* isolates and reference strains were used for DNA extraction by boiling. Two multiplex PCR reactions were used to screen each *E. coli* isolate for the presence of stx_1, stx_2, *eae, chuA*, *yjaA* and *TspE4 C2* genes. Specific primers (TAG Copenhagen, Denmark) used for amplification of the genes are presented in Table 1. DNA extracts of isolates were tested by PCR assay for the presence of the stx_1, stx_2 and *eae* genes as described by China et al. (1996). According to the presence and/or absence of the three genetic markers (*chuA*, *yjaA* and TspE4.C2) four main phylogenetic groups (A, B1, B2 and D) and seven phylogenetic subgroups (A_0, A_1, B1, $B2_2$, $B2_3$, D_1 and D_2) were determined (Clermont et al. 2000; Gordon et al. 2008).

O157 serogroup determination

O serogroup of stx_1, stx_2 and *eae*-positive *E. coli* isolates were examined for the presence and/or absence of O157 serogroup by using of specific O157 antiserum according to the manufacturer's recommendations. (Mast Diagnostics, Mast Group Ltd., Merseyside, England).

Results

In bacteriological examinations, 192 *E. coli* were isolated from same number of recta swab samples. Analysis of PCR

Table 1 Oligonucleotide primers used in this study

Gene	Primer Sequence (5′–3′)		Product size (bp)
stx_1	AGAGCGATGTTACGGTTTG	TTGCCCCCAGAGTGGATG	388
stx_2	TGGGTTTTTCTTCGGTATC	GACATTCTGGTTGACTCTCTT	807
eaeA	AGGCTTCGTCACAGTTG	CCATCGTCACCAGAGGA	570
chuA	GACGAACCAACGGTCAGGAT	TGCCGCCAGTACCAAAGACA	279
yjaA	TGAAGTGTCAGGAGACGCTG	ATGGAGAATGCGTTCCTCAAC	211
TspE4C2	GAGTAATGTCGGGGCATTCA	CGCGCCAACAAAGTATTACG	152

results for determination of phylogenetic groups showed that the *E. coli* isolates belonged to three main groups A (42.71 %), B1 (48.44 %) and D (8.85 %). None of the isolates belonged to B2 group or its subgroups. Phylogenetic subgroups classification revealed that 93 isolates fell in to B1 group, whereas 99 isolates (51.56 %) fell into four phylogenetic subgroups: 34 isolates (17.71 %) into A_0, 48 isolates (25.00 %) into A_1, 12 isolates (6.25 %) into D_1 and 5 isolates (2.60 %) into D_2 (Table 2).

Multiplex PCR tests results showed that 43 isolates (22.40 %) were positive for one of the stx_1, stx_2 and *eae* genes. According to the results all of the positive isolates had only one of the stx_1, stx_2 and *eae* genes and there is not any combination of the examined genes in the positive isolates. Out of positive *E. coli* isolates, 20 (10.42 %) were positive for stx_1, 18 (9.38 %) for *eae* and 5 isolates (2.60 %) for stx_2 gene.

According to serological tests none of the Stx- and intimin-positive isolates belonged to O157 serogroup. Twenty isolates possessed stx_1 were distributed in A (six isolates), B1 (13 isolates) and D (one isolate) phylogroups, whereas belonged to phylogenetic subgroups A_0, A_1, B1 and D_1 (Table 2). The stx_2-positive isolates fell into A (three isolates) and B1 (two isolates) phylogenetic groups. Eighteen isolates contained *eae* gene belonged to A (five isolates), B1 (seven isolates) and D (six isolates) phylogroups, which fell into A_1, B1, D_1 and D_2 phylogenetic subgroups (Table 2).

Antibiogram of isolates against eight antibiotics showed that all of the 192 isolates were resistant against two or more examined antibacterial agents. The most prevalent resistance were recorded against to penicillin (98.44 %), cephalexin (94.79 %) and oxytetracycline (91.15 %). The minimum resistance rates were against co-trimoxazole (51.04 %) and ciprofloxacin (61.98 %), respectively (Table 3). The resistant isolates were distributed in all of the detected phylogenetic groups and subgroups, whereas their prevalence in each phylogroup differed in relation to tested antibiotics (Table 3).

Results of antibiotic susceptibility tests showed that 192 *E. coli* isolates could be classified in 16 different groups according to antibiotic resistance patterns. Fifty-five isolates (28.65 %) were resistant to all of the tested antibiotic, which were the most prevalent antibiotic resistance pattern followed by CN, K, NFX, P, S, T and CN, CP, K, NFX, P, S, T patterns were found in 14.58 and 14.06 % of isolates, respectively. Prevalence of 16 detected antibiotic resistance patterns in each phylogenetic group and subgroups are presented in Table 4.

The 43 positive isolates for one of the stx_1, stx_2 and *eae* genes showed several antibiotic resistance patterns which were distributed in three phylogenetic groups A, B1 and D. Distribution of positive *E. coli* isolates in phylogenetic groups according to multi-drug resistance patterns are presented in Table 5.

Discussion

E. coli O157 STEC strains known to cause sporadic cases and outbreaks of potentially life-threatening illness in

Table 2 Distribution of negative and positive isolates for virulence genes (*eae*, stx_1 and stx_2) in detected phylogroups/subgroups

Phylogroup	A no. (%)		B1 no. (%)	D no. (%)		Total no. (%)
Phylosubgroup	A_0 (34)	A_1 (48)	B1 (93)	D_1 (12)	D_2 (5)	
Virulence gene						
stx_1	4 (20.00)	2 (10.00)	13 (65.00)	1 (5.00)	–	20 (10.42)
stx_2	2 (40.00)	1 (20.00)	2 (40.00)	–	–	5 (2.60)
eae	–	5 (27.78)	7 (38.89)	5 (27.78)	1 (5.56)	18 (9.38)
Positive isolates	6 (13.95)	8 (18.60)	22 (51.16)	6 (13.95)	1 (2.333)	43 (22.40)
Negative isolates	28 (18.79)	40 (26.85)	71 (47.65)	6 (4.02)	4 (2.68)	149 (77.60)
Total phylosubgroup	34 (17.71)	48 (25.00)	93 (48.44)	12 (6.25)	5 (2.60)	192 (100.00)
Total phylogroup	82 (42.71)		93 (48.44)	17 (8.85)		192 (100.00)

Table 3 Number and percent of antibiotic resistant isolates in relation to detected phylogroups/subgroups in 192 *E. coli* isolates

Phylogroup	A (82)		B1 (93)	D (17)		Total no. (%)
Phylosubgroup	A_0 (34)	A_1 (48)	B1 (93)	D_1 (12)	D_2 (5)	
Antibiotic						
CN no. (%)	31 (17.03)	46 (25.27)	89 (49.90)	11 (6.04)	5 (2.75)	182 (94.79)
CP	23 (19.33)	25 (21.00)	65 (54.62)	4 (3.36)	2 (1.68)	119 (61.98)
K	27 (17.20)	43 (27.39)	74 (47.13)	9 (5.73)	4 (2.55)	157 (81.77)
NFX	25 (14.45)	47 (30.92)	69 (45.39)	8 (5.26)	3 (1.97)	152 (79.17)
P	33 (17.46)	48 (25.40)	92 (48.68)	12 (6.35)	4 (2.17)	189 (98.44)
S	25 (16.34)	35 (22.88)	78 (50.98)	11 (7.19)	4 (2.61)	153 (79.69)
STX	20 (20.41)	26 (26.53)	45 (45.92)	4 (4.08)	3 (3.06)	98 (51.04)
T	30 (17.14)	46 (26.29)	85 (48.57)	10 (5.71)	4 (2.29)	175 (91.15)

CN cephalexin, *CP* ciprofloxacin, *K* kanamycin, *NFX* enrofloxacin, *P* penicillin, *S* streptomycin, *SXT* co-trimoxazole, *T* oxytetracycline

human. Non-O157 STEC isolates are also associated with HUS, whereas retrospective reports have estimated that 37–50 % of STEC infections per year are caused by non-O157 STEC organisms (Schaffzin et al. 2012). In general, human infections with STEC strains occurs after consumption of contaminated food or contact with an infected animal or human, therefore identification of the sources of infection is an important step towards decreasing the prevalence of this pathogen and thus decreasing the risk of infection of humans (Ayala et al. 2010; Pedersen et al. 2006). Cattle and sheep are thought to be the major reservoir of STEC and often carry STEC in their intestinal flora and serve as source of food contamination (Martin and Beutin 2011). Although there are several reports of isolation and characterization of STEC from cattle, there is limited information about the prevalence of STEC in small ruminants (Bhat et al. 2008; Coombes et al. 2011). In the present study, 25 isolates (13.02 %) were positive for Stx1 (20 isolates) and Stx2 (five isolates) coding genes, which do not belonged to O157 serogroup. Therefore, these isolates were considered as non-O157 STEC isolates. In Spain, STEC O157:H7 strains were isolated from one percent of animals in six flocks, whereas non-O157 STEC strains were isolated from 35 % of lambs in 33 flocks (Rey et al. 2003). Another study on *E. coli* isolates from healthy cattle, sheep and swine herds in northern Spain revealed that 8.7 and 50.8 % of ovine isolates were positive for O157:H7 and non-O157 STEC serotypes, respectively (Oporto et al. 2008). Blanco et al. (2003)

Table 4 Detected antibiotic resistance patterns and phylogenetic phylogroups/subgroups in 192 *E. coli* isolates form sheep

Phylogenetic groups	A (82)		B1 (93)	D (17)		Total no. (%)
Phylogenetic subgroups Antibiotic resistance pattern	A_0 (34)	A_1 (48)	B1 (93)	D_1 (12)	D_2 (5)	
CN, CP, K, NFX, P, S, STX, T	10	17	26	2	–	55 (28.65)
CN, CP, K, NFX, P, STX, T	2	3	–	–	–	5 (2.60)
CN, CP, K, NFX, P, S, T	3	4	19	–	1	27 (14.06)
CN, K, NFX, P, STX, T	4	6	13	–	1	24 (12.50)
CN, CP, K, P, S, STX, T	4	–	5	–	–	9 (4.69)
CN, K,NFX, P, S, STX	–	–	2	–	–	2 (1.04)
CN, CP, P, S, STX, T	–	–	–	1	1	2 (1.04)
CN, K, NFX, P, S, T	3	13	6	5	1	28 (14.58)
CN, K, P, S, STX, T	–	–	–	1	–	1 (0.52)
CN, K, NFX, S, T	–	–	1	–	–	1 (0.52)
CN, CP, P, S, T	4	1	15	1	–	21 (10.94)
CN, NFX, P, T	–	2	–	–	–	2 (1.04)
CN, K, P, S	–	–	2	1	–	3 (1.56)
CN, K, S	1	–	–	–	1	2 (1.04)
NFX, P	3	2	2	1	–	8 (4.17)
P, S	–	–	2	–	–	2 (1.04)
Total	34	48	93	12	5	192 (100.00)

CN cephalexin, *CP* ciprofloxacin, *K* kanamycin, *NFX* enrofloxacin, *P* penicillin, *S* streptomycin, *SXT* co-trimoxazole, *T* oxytetracycline

Table 5 Phylogenetic groups and multi-drug resistance patterns of positive *E. coli* isolates for *eae*, stx_1 and stx_2 genes

Gene (no. of positive isolates)	Antibiotic resistance pattern	Phylogenetic group				Total
		A	B1	B2	D	
stx_1 (20)	CN, CP, K, NFX, P, S, STX, T	–	5	–	–	5
	CN, CP, K, NFX, P, S, T	2	4	–	–	6
	CN, K, NFX, P, S, T	2	3	–	–	5
	CN, K, NFX, S, T	–	1	–	–	1
	CN, CP, P, S, T	1	–	–	–	1
	CN, K, P, S	–	–	–	1	1
	NFX, P	1	–	–	–	1
stx_2 (5)	CN, CP, K, P, S, STX, T	1	1	–	–	2
	CN, K,NFX, P, S, STX	–	1	–	–	1
	CN, NFX, P, T	1	–	–	–	1
	NFX, P	1	–	–	–	1
eae (18)	CN, CP, K, NFX, P, S, STX, T	2	2	–	1	5
	CN, CP, K, NFX, P, S, T	1	–	–	1	2
	CN, K, NFX, P, S, T	1	3	–	3	7
	CN, K, P, S, STX, T	–	–	–	1	1
	CN, CP, P, S, T	1	1	–	–	2
	P, S	–	1	–	–	1
Total		14	22	–	7	43

CN cephalexin, *CP* ciprofloxacin, *K* kanamycin, *NFX* enrofloxacin, *P* penicillin, *S* streptomycin, *SXT* co-trimoxazole, *T* oxytetracycline

have isolated STEC O157:H7 strains from 0.4 % faecal swabs of healthy lambs in 93 flocks and non-O157 STEC strains were isolated from 36 % lambs in 63 flocks. In India, a study on *E. coli* isolates from lambs with and without diarrhoea indicated that 9.6 and 24.1 % of isolates belonged to STEC pathotype, respectively (Wani et al. 2009). Contamination of meat and raw milk by the main pathogenic O157 and non-O157 (O26, O103, O111 and O145) STEC O-serogroups representing a major public health concern (Perelle et al. 2007). Similarly, non-O157 STEC pathotype were isolated from Spanish raw ewe's milk cheese, whereas the isolates belonged to O14 and nontypeable groups (Caro and García-Armesto 2007). Evans et al. (2008) have recovered O26 vtx_1^+ and/or vt_2^+ *E. coli* isolates from 4 % of faecal samples of healthy sheep, whereas all of the positive isolates carried *eaeA* gene. PCR analysis of carcass, faeces and fleece samples from Irish lambs showed that both the vt_1 and vt_2 genes were carried by *E. coli* O157:H7 isolates (Lenahan et al. 2007).

In this study, any combination of examined virulence genes was detected. In contrast to these results, various combinations of stx_1, stx_2 and *eae* genes in non-O157 STEC isolates from sheep were reported previously (Evans et al. 2008; Momtaz et al. 2012). In non-O157 STEC strains from lamb 55 % of strains carried the stx_1 gene, 3 % possessed the stx_2 gene and 42 % carried both the stx_1 and the stx_2 genes (Rey et al. 2003). According to the results, 18 isolates 9.38 % possessed *eae* gene. Enteropathogenic *E. coli* (EPEC) strains are defined as intimin-containing diarrhoeagenic *E. coli* isolates that possess the ability to form AE lesions on intestinal cells and that do not possess genes coding for shiga toxins (Alonso et al. 2012; Bhat et al. 2008). STEC and EPEC isolates are commonly recovered from the faeces of food-producing animals and pose threats to health of humans and livestock (Wani et al. 2009). Vettorato et al. (2009) showed that *eae*-positive isolates from faeces of Brazilian sheep belonged to non-O157 serogroups O128, O145, O153 and O178.

Regarding to the results, all of the stx_1 and stx_2-positive isolates were negative for intimin coding gene. The absence of the *eae* gene in the VTEC strains could indicate that these strains are less virulent for humans that the classical *eae*-positive enterohaemorrhagic *E. coli* types (Cortes et al. 2005). Wani et al. (2009) have detected *eae* gene in 46.2 and 36.8 % of STEC isolates from lambs with and without diarrhoea, respectively.

In the current study, phylogenetic background of *E. coli* isolates were detected, which were distributed in A (42.71 %), B1 (48.44 %) and D (8.85 %) groups. Phylogenetic group determination of faecal *E. coli* isolates from human and animals indicated that strains from group B1 were present in all hosts analysed but were more prevalent in sheep, goat and cow samples (Carlos et al. 2010). Similar to the results of this study, none of the faecal isolates from domesticated animals in South Korea belonged to B2 group (Unno et al. 2009). Johnson et al. (2005) reported that conversion to antibiotic resistance occurs more readily in non-B2 phylogenetic groups of *E. coli* strains. This link

between strain phylogeny and antibiotic resistance could explain why in farm animals subjected to antibiotic pressure, A and B1 strains are selected and B2 strains counter selected (Escobar-Paramo et al. 2006). Examination of 29 *E. coli* from sheep revealed that the isolates fell into three B1 (20 isolates), D (five) and A (four) phylogroup (Carlos et al. 2010). The possible influence of geographic conditions, dietary factors, use of antibiotics and/or host genetic factors on the distribution of phylogenetic groups has been reported (Unno et al. 2009).

Phylogenetic analysis of stx_1- and stx_2-positive isolates showed that the isolates belonged to B1 (15 isolates), A (nine) and D (one isolate) groups. In the USA, 2 % of *E. coli* isolates from diverse human and animal sources belonged to STEC strains. On the other hand, the majority of the STEC strains, which were initially isolated from the ruminants sheep, goats and deer, carried the stx_{1c} and/or stx_{2d}, *ehxA* and *saa* genes and belonged to *E. coli* phylogenetic group B1 (Ishii et al. 2007). Phylotyping of enterohaemorrhagic (EHEC) and intimin-positive STEC isolates from animals mostly segregated in B1 (38.7 %) and A (35.5 %) phylogenetic groups (Tramuta et al. 2008). Phylogenetic study on human diarrheagenic *E. coli* isolates showed that EHEC strains were distributed in phylogroups A and B1 (Gordon et al. 2008).

In the current study, prevalence of antibiotic resistance was detected and phylogenetic background of multi-drug resistant isolates was determined. In comparison to the results of current study, different prevalences of antibiotic-resistant isolates have been reported in ovine isolates from Great Britain, Greece and New Zealand (Enne et al. 2008; Solomakos et al. 2009; Yu et al. 2010). Due to the enormous exploitation of antibiotics in the field of veterinary medicine, problems associated with the presence of antibiotic-resistant bacteria have reached epidemic proportions in recent years (Saei et al. 2010). It is believed that retail foods, and especially meat and meat products, may be an important vehicle for community-wide dissemination of antimicrobial-resistant *E. coli* and extraintestinal pathogenic *E. coli* (Sunde and Norstrom 2006). On the other hand, a study on the effect of antibiotic (tetracycline or streptomycin)-supplemented feed on faecal enterohaemorrhagic *E. coli* O157:H7 population in lambs showed reduction in total number of *E. coli* O157:H7 shed in the faeces (Lema and Nahashon 2006).

In conclusion, in this part of world, faeces of healthy sheep could be considered as the important sources of non-O157 STEC pathotype and also multidrug-resistant *E. coli* isolates. Regarding the presence of the isolates which were positive only for *eae* gene, the researches on initial screening of *eae*-positive bacteria and *eae* gene subtyping from ovine sources would help elucidate the role and importance of these isolates. According to the high prevalence of multidrug resistance, it is important to monitor the resistance to antibiotics not only in pathogenic isolates but also in commensal bacteria of sheep origin.

Acknowledgments This work was supported by a grant from the Shahid Bahonar University of Kerman.

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