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Prevalence and antimicrobial susceptibility of *Escherichia coli* O157:H7 on beef cattle slaughtered in Amman abattoir

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ABSTRACT

Cattle are the main asymptomatic reservoir of *Escherichia coli* O157:H7 which can cause illness to human. The objectives of the study were to measure the prevalence of *E. coli* O157:H7 on cattle slaughtered in Amman abattoir, detect virulence factors in the isolates, determine antibacterial resistance of the isolates, and know how the isolates are different or similar when compared to characterized isolates from developed countries. A total of 540 samples (feces, hide, and carcass) were tested for *E. coli* O157:H7 using the method of ISO 16654:(E). Conventional and multiplex PCR assays were used for serotype confirmation and virulence factor detection, respectively. Fifty *E. coli* O157:H7 isolates were identified and virulence factors *eaeA* and *hlyA* were present in all of the isolates. 60%, 12%, and 22% of the isolates harbored stx_1, stx_2, and stx_1 and stx_2, respectively. The prevalence rates of enterotoxigenic *E. coli* O157:H7 (n = 47) were 8.3%, 10%, and 7.8% in feces, hides and carcasses, respectively. The antimicrobial profiles of the isolates showed an extensive resistance to erythromycin, neomycin and vancomycin and high sensitivity to ampicillin, ciprofloxacin, gentamicin, kanamycin and tetracycline.

1. Introduction

Escherichia coli O157:H7 is foodborne pathogen that can cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) to human (Gooding & Choudary, 1997). The virulence factor associated with these illnesses is the Shiga-like toxin which is encoded by stx_1 and stx_2 genes of the pathogen (Nataro & Kaper, 1998; O'Brien & Holmes, 1987). *E. coli* O157:H7 strains also harbor a virulence factor called intimin for attachment to the host intestinal epithelial cells encoded by the *eaeA* gene (Boerling, McEwen, Wilson, Johnson, & Gyles, 1999) and may have additional virulence factors such as the enterohemolysin encoded by *ehxA* gene (Schmidt, Beutin, & Karch, 1995).

Cattle are considered the main asymptomatic reservoir of *E. coli* O157:H7 and play a vital role in human infection (Chapman, Siddons, Cerdan Malo, & Harkin, 1997; Nataro & Kaper, 1998). The organism is carried in the gastrointestinal tract and is shed in the feces (Bach, McCallister, Mears, & Schwartzkopf-Genswein, 2004), thus, this pathogen can be found on cattle hides (Arthur et al., 2004; Elder et al., 2000). Hide contamination may occur from direct or indirect fecal contamination during production and in lairage environments (Arthur et al., 2010). *E. coli* O157:H7 can be transmitted to carcass surfaces when gut contents or fecal materials get in contact with meat surfaces during the processes of skinning and evisceration (Dickson & Anderson, 1992). Contaminated undercooked ground beef has been the most frequently identified vehicle of *E. coli* O157:H7 in the foodborne outbreaks (Barret et al., 1994).

Earlier studies have reported the prevalence rate of *E. coli* O157:H7 in cattle fecal samples, and on hide and carcasses in the range of 2.4 to 24% (Alam & Zurek, 2006; Alonso et al., 2007; Jacob, Almes, Shi, Sargeant, & Nagaraja, 2011; McEvoy et al., 2003), 11 to 76 % (Arthur et al., 2004; Elder et al., 2000), and 0.2 to 26.7 % (Alonso et al., 2007; Barkocy-Gallagher et al., 2003; FSIS, 1994; McEvoy et al., 2003; Vanderlinde, Shay, & Murray, 1998), respectively. In a local study in Jordan; the prevalence of *E. coli* O157:H7 in calves' fecal samples was 7.9% (Abu-Ashour, 2003).

Antimicrobials are widely used in cattle for disease prevention and as growth promotion (Mora et al., 2005). There are some signals indicating that antimicrobial resistance of *E. coli* O157:H7 is rising (Threlfall, Ward, Frost, & Willshaw, 2000). In Jordan, two studies were reported on the antimicrobial susceptibility of *E. coli* O157:H7 isolated from sheep and goat. The first study showed that the isolated pathogen was highly sensitive to gentamicin and co-trimoxazole and highly resistant to tetracycline and ampicillin (Tarawneh, Al-Tawarah, Abdel-Ghani, Al-Majali, & Khleifat, 2008). The second study revealed that all lamb isolates were resistant against one or more of the tested antibiotics. One isolate was resistant to chloramphenicol, and sulphonamide, another isolate was resistant to ampicillin, chloramphenicol, sulphonamide, and trimethoprim-sulfamethoxazole (co-trimoxazole) and five isolates

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were resistant to ampicillin, chloramphenicol, streptomycin, and sulphonamide. One isolate showed a multi resistance pattern against ampicillin, chloramphenicol, ceftazidime, aztreonam, gentamicin, cephalothin, streptomycin, sulphonamide, tetracycline, trimethoprim and sulfamethoxazole (Novotna et al., 2005). Harakeh et al. (2005) reported that 100% and 63.5% of tested *E. coli* O157:H7 from meat-based fast food in Lebanon were resistant to erythromycin and vancomycin, respectively.

Because of lack of information on the prevalence of *E. coli* O157:H7 in dressed beef cattle in Jordan, this work was conducted to determine: i) the prevalence of *E. coli* O157:H7 on hides and carcasses and in feces of cattle slaughtered in Amman abattoir to determine the relation between the presence of *E. coli* O157:H7 on beef meat and specific handling practices, ii) the presence of different virulence factors in isolated *E. coli* O157:H7, and iii) and the antibacterial resistance profiles of the isolates. The study also aimed in keeping up with isolate characteristics for any global changes in antimicrobial susceptibility patterns, isolate distribution and similarities and prevalence of common virulence factors.

2. Materials and methods

2.1. Reference bacterial strains

The reference bacterial strains used in this study were *E. coli* O157:H7 (ATCC 43895) as positive control and non-toxigenic *E. coli* O157:H7 (NCTC 12900) as a negative control.

2.2. Sample collection

A total of 540 samples representing 180 feces, 180 hide, and 180 carcass samples were collected from calves slaughtered at Amman slaughter house during the period from January to May 2010 to investigate the presence of *E. coli* O157:H7. Amman slaughterhouse, a manually operated gravity rail system, is a public abattoir that is located in Amman City with a design capacity of 25–30 head per h and with slaughtering average of 80–100 calves per day. Most slaughtered animals were imported calves (Australia, Romania, Uruguay and Brazil) that were reared for 3–6 months in farms located 80–100 km eastern to Amman before being delivered for slaughtering. Each of the above 180 samples represents a composite sample from three different animals. The fecal sample (100 g) was collected from colon contents of slaughtered animals; longitudinal incisions were made aseptically in the colon to obtain a representative sample of the fecal content. Hide was sampled by cutting a piece representing an area of 10×10 cm (100 cm^2) from the hide from each of three different locations (back, belly, and thigh). The meat was sampled by slicing a thin meat slices (thickness 1–1.5 cm) from external surfaces from each of three different locations (leg, flank and neck) of the post-washed carcass The samples were then transferred to the laboratory in sterile containers under aseptic conditions using an ice box. Samples were processed within 2–3 h after collection.

2.3. Isolation of E. coli O157:H7 using conventional bacteriological methods

2.3.1. Pre-enrichment, selective enrichment and immunomagnetic separation

The method described by ISO 16654: (E) (2001) was followed for the isolation of *E. coli* O157:H7. Twenty five grams of each sample (hide, meat and feces) was placed in Stomacher bag (Seward, UK) containing 225 ml of modified tryptone soy broth (m-TSB) (Oxoid, Ltd., Basingstoke, UK) supplemented with 20 mg/l novobiocine (Oxoid). After stomacher homogenization for 2 min at 320 rpm, the samples were incubated for 6 h at 41.5 °C. After that, 1 ml from the pre-enriched homogenate was added to 20 μl magnetic beads coated with specific antibody against O157 (Dynabeads anti *E. coli* O157, Prod. Dynal, Norway) in a 1.5 ml Eppendorf tube. Afterward, immunomagnetic separation (IMS) was performed according to the manufacturer's instructions.

2.3.2. Isolation of E. coli O157:H7

Fifty microliter quantities of the suspension obtained after performing the IMS were plated on Cefixime Tellurite-Sorbitol MacConkey agar (CT-SMAC) (Oxoid) and CHROMagar (Dynal Prod.). The plates were incubated at 37 °C for 22 h for CT-SMAC agar and for 24 h for CHROMagar. Five typical colorless colonies (sorbitol negative) from CT-SMAC or mauve colonies from CHROMagar were plated on tryptone soy agar + yeast extract (TSA-YE) (Oxoid), and incubated at 37 °C for 24 h, for purification and running confirmatory tests as described below.

2.3.3. Confirmatory tests for E. coli O157 and E. coli O157:H7

The pinpoint indole assay was conducted on the suspected isolates. One typical colony was picked up and placed in one drop of indole reagent (Oxoid) using filter paper. Mauve color reaction indicated positive results. Indole-positive colonies were streaked onto Sorbitol MacConkey agar with BCIG (SMAC- BCIG), (Oxoid) and incubated at 37 °C for overnight. Colonies that did not show fluorescence on the SMAC- BCIG agar when illuminated with UV light (366 nm) were recognized as the distinctive characteristics of *E. coli* O157 species.

All sorbitol-negative, indole-positive, BCIG-negative isolates were sub-cultured on TSA-YE and incubated for 24 h at 37 °C. The obtained isolates were inspected by latex agglutination using the *E. coli* O157 antiserum (Oxoid) followed by H7 antisera (Remel Inc, KS, USA). Colonies that showed a positive agglutination reaction with O157 antiserum were considered as *E. coli* O157, whereas colonies that showed positive agglutination reaction with the H7 antiserum were considered as *E. coli* O157:H7.

2.4. Molecular confirmation and virulence factor detection of E. coli O157:H7

E. coli O157:H7 reference strain and *E. coli* O157:H7 isolates were cultured on TSA-YE at 37 °C for 24 h, and then sub-cultured into soya broth (SB) (Oxoid) for additional 24 h. Thereafter, 1 ml of the suspension was transferred into 1.5 ml microcentrifuge tubes to extract the DNA. The genomic DNA was isolated from the reference strain and bacterial isolates using Promega Wizard® Genomic DNA Purification Kit (Promega, USA) as per manufacturer's instructions.

The extracted DNAs were used for serotype (O157 and H7) confirmation (presence of $rfbE_{O157}$ and *fliCh7* genes) and virulence factor (stx_1, stx_2, *eaeA* and *hlyA*) detection in *E. coli* O157:H7 isolates. The primers (Thermo Electron, MA, USA) used in this analysis are presented in Table 1. The methods described by Desmarchelier et al. (1998) and Fratamico, Bagi, and Pepe (2000) were followed in conventional PCR assay for serotype confirmation and the methods described by Fratamico et al. (2000) and Sarimehmetoglu et al. (2009) were followed in multiplex PCR assay for virulence factor detection in the *E. coli* O157:

Table 1
Primers used for serotype confirmation and virulence factor determination of *Escherichia coli* O157:H7 isolates.

Primer	Sequence (5′–3′)	Target gene	PCR product (bp)
SLT1-F	TGTAACTGGAAAGGTGGAGTATACA	stx_1	210
SLT1-R	GCTATTCTGAGTCAACGAAAAATAAC		
SLTII-F	GTTTTTCTTCGGTATCCTATTCC	stx_2	484
SLTII-R	GATGCATCTCTGGTCATTGTATTAC		
AE22	ATTACCATCCACACAGACGGT	*eaeA*	397
AE20-2	ACAGCGTGGTTGGATCAACCT		
MFS1-F	ACGATGTGGTTTATTCTGGA	*hlyA*	166
MFS1-R	CTTCACGTCACCATACATAT		
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	*flich7*	625
FLICH7-R	CAACGGTGACTTTATCGCCATTCC		
O157-AF[a]	AAGATTGCGCTGAAGCCTTTG	*rfbE*	497
O157-AR[a]	CATTGGCATCGTGTGGACAG		

[a] Sarimehmetoglu et al. (2009) and Desmarchelier et al. (1998).

H7 isolates. Thermal cycling was conducted in Veriti Thermal cycler (Applied Biosystems, USA) and PCR products were visualized with UV transilluminator and photographed with the gel documentation system (Gel Doc 2000, BIO-RAD, USA).

2.5. Susceptibility to antimicrobial agents-microbroth dilution method

E. coli O157:H7 isolates in addition to the reference bacterial strain *E. coli* O157:H7 (ATCC 43895) were maintained on TSA-YE. Active cultures were prepared by growing the isolates and the reference strain in 10 ml quantities of Mueller–Hinton broth (MHB) (Oxoid). The densities of bacterial growth in MHB were standardized at ca. 1.5×10^8 CFU/ml and were adjusted according to McFarland standard solution. To determine the minimal inhibitory concentration (MIC) of antimicrobials against tested strains in MHB at 37 °C, the microbroth dilution method described by Lalitha (2008) was followed. The MIC values of the isolates and the reference strain were compared to breakpoint values from international standards or published researches (BSAC, 2008; Fitzgerald et al., 2003; NCCLS, 1999; Soussy, Cluzel, Courvalin, & the Comite de Antibiogramme de la Societe Francaise be Microbiologie, 1994).

2.5.1. Statistical analysis

The differences among numbers of contaminated samples (carcasses, hides and feces) with enterotoxigenic *E. coli* O157:H7 were tested by Chi-square test using SAS program, version 8.1 (SAS Institute Inc., Cary, NC). Significance level is considered at ≤ 0.05.

3. Results

3.1. Isolation of E. coli O157:H7 by conventional bacteriological method

The number of samples with typical *E. coli* O157:H7 characteristics on CT-SMAC or CHROMagar was 63 samples (22 feces samples, 17 hide samples, and 24 carcass samples). All of the presumptively identified *E. coli* O157:H7 isolates from the tested samples were positive for spot indole test and presence of O157 antigen, whereas, 55 of them (87%) were positive for presence of H7 antigen using latex agglutination antisera (Table 2).

3.2. Serotype confirmation of E. coli O157:H7 using conventional PCR

All of the presumptive isolates (63 isolates) were subjected to PCR analyses for serotype confirmation. The results showed that 51 isolates (80.9%) harbored $rfbE_{O157}$ gene (O157) and only 50 isolates (79%) contained both $rfbE_{O157}$ and *Flich7* genes (O157:H7). Accordingly, only one isolate out of the 51 of the confirmed serotype O157 did not harbor the H7 gene. To compare latex agglutination data with PCR results, 51 out of the 63, O157 latex positive isolates were confirmed as *E. coli* O157 (83%), whereas 50 out of the 55 latex H7 positive were confirmed as *E. coli* O157:H7 (91%). The overall prevalence of PCR confirmed *E. coli* O157:H7 in feces and on hide and carcass samples was 17 (9.4%), 19 (10.6%), and 14 (7.8%), respectively (Table 2).

3.3. Detection of virulence factors in the confirmed E. coli O157:H7 using multiplex PCR

Multiplex PCR results revealed that all of the 50 confirmed *E. coli* O157:H7 isolates harbored both intimin and enterohemolysin (*eaeA* and *hlyA*) virulent genes while 47 (94%) of the isolates revealed the presence of stx_1 and/or stx_2 virulence genes; 30 (60%), 6 (12%), and 11 (22%) isolates harbored stx_1, stx_2, and both genes, respectively. The 3 isolates (6%) that did not have stxs genes were isolated from hides (1 isolate) and feces (2 isolates). The 47 molecularly confirmed enterotoxigenic *E. coli* O157:H7 were distributed between 15 isolates from feces (8.3%), 18 isolates for hides (10%) and 14 isolates from carcasses (7.8%) (Table 3). There were no significant differences ($P > 0.05$) among the contamination level of enterotoxigenic *E. coli* O157:H7 on carcasses and hides and in feces. The stx_1 gene was of high prevalence among hides isolates (68%, n = 13) followed by carcass isolates (64%, n = 9) and finally feces isolates (47%, n = 8). Again and in the same meaning the stx_2 gene was the highest one (15.8%, n = 3) among hide isolates followed by feces (11.8%, n = 2) and the carcass (7%, n = 1) isolates. On the other hand, both stx_1 and stx_2 genes were of high prevalence in feces isolates (29.4%, n = 5) followed by carcass (28.5%, n = 4) and hide isolates (10.5%, n = 2).

3.4. Distribution of toxigenic E. coli O157:H7 in feces and on hide and carcass samples

Out of the 14 positive carcasses-animal for *E. coli* O157:H7, 2 composite samples were only carcass positive; 5 composite samples were positive for both carcasses and hides; 2 composite samples were positive for carcasses and feces and finally 5 composite samples were positive for carcass, hide and feces simultaneously. In the same meaning out of 18 positive hide-animal, 6 composite samples were only hide positive, 5 composite samples were positive for hides and carcasses; 2 composite samples were positive for hides and feces and the other 5 composite samples were positive for hides, feces and carcasses simultaneously. On the other hand out of the 15 positive feces-animal, 6 composite samples were only positive for feces samples; 2 composite samples were positive for both feces and hides; 2 composite samples were positive for feces and carcasses and finally 5 composite samples were positive for feces, hides and carcasses simultaneously (Table 4).

3.5. Susceptibility to antimicrobial agents

None of the 40 *E. coli* O157:H7 isolates were resistant to ampicillin, ciprofloxacin or tetracycline, whereas all were resistant to erythromycin, doxycycline, vancomycin and, neomycin. 50% of the isolates were resistant to three antibiotics namely erythromycin, vancomycin and neomycin. 10 out of 40 (25%) of the isolates were multi-resistant to four antibiotics namely erythromycin, vancomycin, doxycycline and neomycin, and another 10 (25%) were multi-resistant to other four different patterns (erythromycin, vancomycin, neomycin and streptomycin). On the other hand, 2 out of 40 (5%) isolates were multi-resistant to five antibiotics namely erythromycin, vancomycin, doxycycline, neomycin and streptomycin (Table 5).

Table 2
Presumptive and confirmed *Escherichia coli* O157 and *Escherichia coli* O157:H7 isolates (%).

Sample	No. of samples	Presumptive *E. coli* O157:H7 on selective media	Latex agglutination		Serotype confirmation by PCR	
			E. coli O157	*E. coli* O157:H7	*E. coli* O157	*E. coli* O157:H7
Carcasses	180	17 (9.4)	17 (9.4)	14 (7.2)	14 (7.8)	14 (7.8)
Hides	180	24 (13.3)	24 (13.3)	21 (11.7)	19 (10.6)	19 (10.6)
Feces	180	22 (12.2)	22 (12.2)	20 (11.1)	18 (10.0)	17 (9.4)
Total	540	63	63	55	51	50

Table 3
Virulence genes detected in *Escherichia coli* O157:H7 isolates by multiplex PCR.

Source of isolates	Number of isolates	Virulence gene profiles (%)					
		eaeA	*hlyA*	*stx*			
				stx_1	stx_2	$stx_1 + stx_2$	No *stx*
Carcasses	14	14 (100)	14 (100)	9 (64.3)	1 (7.1)	4 (28.6)	0 (0)
Hides	19	19 (100)	19 (100)	13 (68.4)	3 (15.8)	2 (10.5)	1 (5.3)
Feces	17	17 (100)	17 (100)	8 (47)	2 (11.8)	5 (29.4)	2 (11.8)
Total	50	50 (100)	50 (100)	30 (60)	6 (12)	11 (22)	3 (6)

4. Discussion

The prevalence rates of *E. coli* O157:H7 (9.4%, 10.6% and 7.8%) or toxigenic *E. coli* O157:H7 (8.3%, 10% and 7.8%) in feces and on hide and carcass samples are within the rates reported in literature. The prevalence rate of *E. coli* O157:H7 in feces samples has been reported in the range of 2.4 to 24% (Alam & Zurek, 2006; Alonso et al., 2007; Jacob et al., 2011; McEvoy et al., 2003). The prevalence rate of *E. coli* O157:H7 on hide and carcass samples has been reported in the range of 11 to 76 % (Arthur et al., 2004; Elder et al., 2000) and 0.2 to 26.7 % (Alonso et al., 2007; Barkocy-Gallagher et al., 2003; FSIS, 1994; McEvoy et al., 2003; Vanderlinde et al., 1998), respectively. The low prevalence rates of *E. coli* O157:H7 reported in this study compared with those reported in the previously mentioned studies may be related to differences in methodology of sampling. However, the prevalence of *E. coli* O157:H7 on beef carcasses was higher in the current study than that in a number of published studies. In the United States, the prevalence of *E. coli* O157:H7 on beef carcasses after dressing was determined to be 0.2% (FSIS, 1994). Two studies that were conducted in the United Kingdom have reported that 0.47% and 1.4% of carcasses were contaminated with *E. coli* O157:H7 (Chapman, Cerdán Malo, Ellin, Ashton, & Harkin, 2001; Richards et al., 1998). But, a study in northern Italy found that 12 out of 100 (12%) carcasses sampled were contaminated with *E. coli* O157:H7 (Bonardi, Maggi, Pizzin, Morabito, & Caprioli, 2001). The higher isolation rate (7.8%) observed for *E. coli* O157:H7 on carcasses in this study might be related to the absence of antimicrobial interventions through dressing processes at abattoirs level or due to cross contamination during the slaughter process which in overall reflect the general unhygienic conditions in employees, utensils and environmental sanitation of the slaughter house under study.

The contamination levels of feces, hides and carcasses are closely related. This seems to be quite logical as the main source of contamination is the fecal material which found its way to animal external surface due to poor hygienic conditions during slaughtering process of the animals. Contamination of carcasses with *E. coli* O157:H7 can occur when gut contents, fecal matter or contaminated hides come in contact with meat surfaces. These observations come in close agreement with other findings in that a significant correlation does exist between the prevalence of *E. coli* O157 in feces and on hides and carcasses (Bonardi et al., 2001; Elder et al., 2000). The distribution of *E. coli* O157:H7 among hides and carcasses depends mainly on level of hygienic precautions taken during slaughtering to avoid cross contamination during hide removal, evisceration and carcass dressing. This could be shown when six animals revealed the presence of *E. coli* O157:H7 from intestinal contents but not from either hide or carcasses (Table 4). Therefore, the main sources of possible carcass contamination in abattoir are both of hide and fecal contents. However, possible cross contamination from utensils and employers is not to be neglected. This might be apparent from following observation recorded in this study, where in two cases the pathogen was recovered from carcasses only, but not from hides or feces. Pathogenicity of *E. coli* O 157:H7 is related to many virulence factors like Shiga toxins, intimin and enterohemolysin (Nataro & Kaper, 1998). In the present study, a total of 50 PCR identified isolates from 540 animals were tested for virulence factor by multiplex PCR procedure. Out of these, 47 toxigenic isolates (harbored stx_1 and/or stx_2 genes) were confirmed. 30 (64%) isolates harbored stx1; 6 (13%) isolates harbored stx_2; and 11(23%) isolates harbored both toxins. The stx_2 producing *E. coli* O157:H7 strains are more virulent than stx_1 or stx_1 and stx_2 producing strains (Donnenberg, 2002), and being more often linked with human disease problems (Boerling et al., 1999).

Similar to our study, non- pathogenic *E. coli* O 157:H7 strains (without toxins factors) have also been identified from feces or beef carcass (Guyon et al., 2001; Rogerie et al., 2001).

Cagney et al. (2004) studied the prevalence of *E. coli* O157:H7 in ground beef and beef burger (n = 1533) from supermarkets and butcher shops. In all of the 43 isolates, *eaeA*, *hlyA* and *fliCh7* genes were detected and in 41 isolates stx_1 and stx_2 genes were determined. The presence of the two (*eaeA* and *hlyA*) virulence factors comes in agreement with the isolates identified in our study, though *fliCh7* genes were only present in 91% of our isolates. The toxigenicity percentage among all isolates (95%) was in close agreement with the 94% reported in our study. In another study on ground beef in Turkey, Sarimehmetoglu et al. (2009) identified 2 *E. coli* O157:H7 isolates. Both isolates have *eaeA*, *hlyA* and *flicH7* genes, one of the isolates has stx_1 and the other one has stx_2 only. Whereas, Vernozy-Rozand et al. (2002) recovered 4 *E. coli* O157:H7 from the 3450 ground beef samples in France and all the strains had both stx_1 and stx_2. Therefore, it appears that the distribution of stxs in isolates might be different from food to other or from different geographical locations.

Antimicrobial resistance may arise either spontaneously by selective pressure or due to antimicrobial misuse by humans or overuse in feeding or treatment of beef cattle by farmers (Schroeder et al., 2002). Resistance development also might be related to exchange of resistance factors between related bacteria (Tenover, 2006). The resistance of *E. coli* O157:H7 to both erythromycin and vancomycin also comes in agreement with the results of Harakeh et al. (2005). The results of the present study indicated that *E. coli* O157:H7 were susceptible to gentamicin and ciprofloxacin, which comes in agreement with a local Jordanian study on isolates from sheep and goat (Tarawneh et al., 2008) and other studies in the United States on isolates from human, cattle, swine and food (Harakeh et al., 2005; Schroeder et al., 2002). In the present study all *E. coli* O157:H7 tested were also susceptible to tetracycline which again comes in parallel with the results of Dontorou et al. (2003) on three isolates obtained from ewes' milk, fresh sausages and swine intestines in Greece. Whereas this observation contradicts Tarawneh et al.'s (2008) report on local Jordanian isolates from sheep and goat. Ali, Farooqui, Khan, Khan, and Kazmi (2010) showed that 15 out of 51

Table 4
Distribution of toxigenic *Escherichia coli* O157:H7 in feces and on hides and carcasses of the tested animals.

Sample type	Carcass (C) (14)	Hides (H) (18)	Feces (F) (15)
No of isolates for each location	C = 2	H = 6	F = 6
	C + H = 5	H + C = 5	F + H = 2
	C + F = 2	H + F = 2	F + C = 2
	C + H + F = 5	H + F + C = 5	F + H + C = 5

Table 5
Antimicrobial minimum inhibitory concentration (MIC) values for *Escherichia coli* O157:H7 (ATCC 43895) and 40 selected toxigenic *Escherichia coli* O157:H7 isolates.

Total no. of isolates	Ery[a] (≤0.5–≥8) [b]	Gen (≤2–>4)	Amp (≤8–≥16)	Cip (≤1–≥4)	Kan (≤8–≥16)	Tet (≤4–≥16)	Dox (<1–≥1)	Str (≤16–≥64)	Van (≤4–≥16)	Neo (–≥16)
E. coli O157:H7 (ATCC 43895)	32	0.25	4	0.03	0.5	1	1	0.5	64	128
2	32	0.25	2	0.25	2	2	1	8	>512	>512
1	32	0.25	4	0.25	1	0.5	1	2	>512	>512
1	32	0.5	4	0.015	4	0.5	1	64	>512	>512
4	32	1	4	0.015	8	0.5	1	128	>512	>512
1	32	0.5	8	0.25	8	0.5	2	2	>512	>512
1	32	1	8	0.25	8	0.5	1	2	>512	>512
3	64	2	4	0.015	8	1	2	64	>512	>512
1	32	4	2	0.015	8	1	1	64	>512	>512
2	32	2	2	0.25	8	0.5	2	32	>512	>512
1	32	4	8	0.25	8	1	2	8	>512	>512
2	64	8	4	0.015	16	1	2	16	>512	>512
3	64	1	8	0.25	16	1	2	8	>512	>512
1	32	1	4	0.5	4	1	1	4	>512	>512
4	32	0.25	4	0.25	1	0.5	1	2	>512	>512
2	32	0.125	4	0.125	8	1	1	1	>512	>512
1	32	0.25	8	0.5	1	1	1	2	>512	>512
3	32	1	4	0.015	8	1	1	64	>512	>512
2	32	0.5	4	0.25	2	0.5	1	2	>512	>512
1	32	0.5	4	0.5	2	1	1	2	>512	>512
4	32	2	4	0.5	8	1	2	8	>512	>512
Resistance %	100%	5%	0%	0%	5%	0%	100%	12.5%	100%	100%

[a] Ery: erythromycin, Gen: gentamicin, Amp: ampicillin, Cip: ciprofloxacin, Kan: kanamycin, Tet: tetracycline, Dox: doxycycline, Str: streptomycin, Van: vancomycin, Neo: neomycin.
[b] Breakpoints: (BSAC, 2008, 2012; Fitzgerald et al., 2003; NCCLS, 1999; Soussy et al., 1994).

(29%) *E. coli* O157:H7 meat isolates in Pakistan were resistant to doxycycline, which is quite highly lower than the 100% resistance reported in the present study. The resistance patterns for our tested isolates for kanamycin, streptomycin and ampicillin come on the same line with a study in Japan where 15 isolates (16.9%) of enterohemorrhagic *E. coli* O157:H7 isolates obtained from diarrhea patients, were resistant to ampicillin, kanamycin or streptomycin (Miwa et al., 2002). Our observation for ampicillin susceptibility disagreed with other local studies on *E. coli* O157:H7 isolates from sheep and goat (Tarawneh et al., 2008). Once more, all tested *E. coli* O157:H7 were resistant to neomycin, which also comes in accordance with Nizza et al. (2010), as 100% of tested isolates from buffalo calves with diarrhea in Italy were resistant to neomycin.

Multiple antimicrobial resistances in *E. coli* may arise from the spread of genetic materials including integrons, transposons, and plasmids (Zhao et al., 2001). The increasing developing multi-drug resistant bacteria is signaling a serious alarm from treatment point of view or the possible transforming of resistance genes to other related pathogens (Okeke, Lamikanra, & Edelman, 1999). Multiple antimicrobial resistance is a common phenomenon among *E. coli* O157:H7 isolates drawn from different sources. Schroeder et al. (2002) in the USA, showed that 17%, 7.5%, 8%, 5%, 2% and 0.1% of the *E. coli* O157 isolates were resistant to one, two, three, four, five and six drugs, respectively. Also, Zhao et al. (2001) studied multiple resistance of isolates recovered from human, animals and food in the USA. They found that out of the twenty nine tested *E. coli* O157:H7, four isolates showed multiple resistance to five antimicrobials: tetracycline ampicillin, streptomycin, kanamycin, and sulfamethoxazole. Two isolates were from cattle, and two isolates were from human and ground beef.

It is essential to keep up with isolate characteristics for any global changes in isolate distribution and similarities and prevalence of common virulence factors. Also, it is essential to track the resistance pattern recorded globally to follow changes in antimicrobial sensitivity patterns that may require a reassessment of zoonotic control strategy. Monitoring of antimicrobial resistance in *E. coli* O157:H7 isolates is valuable for epidemiological uses and for monitoring the increase of antimicrobial resistance among different microbial species. Continuous surveillance of emerging antimicrobial resistance among zoonotic foodborne pathogens, including *E. coli* O157:H7 is required to ensure global one inspiration public health.

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