Original Article

Molecular Detection and Antimicrobial Resistance Patterns of Shiga Toxigenic *Escherichia coli* Isolated from Bovine Subclinical Mastitis Milk Samples in Kurdistan, Iran

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ABSTRACT

Bovine subclinical mastitis is regarded as a devastating disease due to the economic costs imposed on dairy husbandry. Moreover, it is a hazard in the public sector in the cases of zoonotic bacteria because of the potential role of unpasteurized milk and dairy products to propagate the infectious agent to the human food chain. The present study aimed to evaluate the frequency, virulence content, and antimicrobial resistance profile of Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from bovine subclinical mastitis in Kurdistan Province, West of Iran. A total of 400 bovine subclinical mastitis milk samples recognized in the California Mastitis Test were collected aseptically and analyzed for the presence of *E. coli* phenotypically and molecularly. The isolates were genotypically screened for *stx1*, *stx2*, and *eae* genes. Furthermore, O157:H7 STEC strain was searched among the isolates in a duplex polymerase chain reaction. The antimicrobial resistance scheme of the isolates was determined using the agar disk diffusion method. In general, 173 (43.25%) *E. coli* isolates were detected among which 39 (22.54%) isolates were STEC. The frequency of STEC virulence genotypes was *stx2* (25 isolates, 64.10%), *stx2+eae* (6 isolates, 15.38%), *stx1+stx2* (6 isolates, 15.38%), and *stx1+stx2+eae* (2 isolates, 5.12%). In addition, three O157: H7 strains were identified with the genetic content of *stx1+stx2+eae* (2 isolates) and *stx1+stx2* (1 isolate). The most prevalent antimicrobial resistance was observed against streptomycin, tetracycline, and ampicillin. Gentamycin, amoxicillin-clavulanic acid, and trimethoprim-sulfadiazine were the most effective antibiotics against O157 strains, whereas gentamycin, ciprofloxacin, and nitrofurantoin were effective against non-O157 strains. The results revealed the significant role of STEC in bovine subclinical mastitis in the studied region. In addition, the distribution of O157:H7 strain and high prevalence of multidrug resistance among the isolates is a matter of concern. Therefore, there is a potential threat of human infection following the consumption of contaminated milk with STEC in Kurdistan Province, Iran.

Keywords: Bovine, Drug resistance, Mastitis, Shige toxin-producing *Escherichia coli* (STEC), Virulence

Détection Moléculaire et Modèles de Résistance aux Antimicrobiens de *Escherichia coli* Productrices de Shiga Toxines Isolés d'Échantillons de Lait de Mammite Subclinique de Boviné au Kurdistan, Iran

Résumé: La mammite subclinique du boviné est considérée comme une maladie dévastatrice en raison des coûts économiques imposés à l'industrie laitière. De plus, c'est un danger dans le secteur public dans le cas des bactéries zoonotiques en raison du rôle potentiel du lait et des produits laitiers non pasteurisés dans la propagation des agents infectieux dans la chaîne alimentaire humaine. Cette étude visait à évaluer la fréquence, la virulence et le profil de résistance aux antimicrobiens des souches d'*Escherichia coli* productrices de

Shiga-toxines (STEC) qui ont été isolées de la mammite subclinique des bovinés dans la province du Kurdistan, à l'ouest de l'Iran. Un total de 400 échantillons de lait de mammite subclinique de boviné, identifiée par le test de mammite de Californie, a été prélevé aseptiquement et analysé pour la présence d'*E. Coli* phénotypiquement et moléculairement. Les isolats ont été sélectionnés génotypiquement pour les gènes *stx1*, *stx2* et *eae*. En outre, la présence de la souche O157: H7 STEC a été explorée parmi les isolats par le biais d'une réaction en chaîne par polymérase duplex. Le profil de résistance aux antimicrobiens des isolats a été déterminé en utilisant la méthode de diffusion sur disque d'agar.Au total, 173 isolats d'*E. Coli* (43,25%) ont été détectés, dont 39 (22,54%) étaient des STEC. La fréquence des génotypes de virulence STEC était *stx2* (25 isolats, 64,10%), *stx2+eae* (6 isolats, 15,38%), *stx1+stx2* (6 isolats, 15,38%) et *stx1+stx2+eae* (2 isolats, 5,12 %). En outre, trois souches O157:H7 ont été identifiées avec le contenu génétique correspondant au *stx1+stx2+eae* (2 isolats) et *stx1+stx2* (1 isolat). La résistance antimicrobienne la plus répandue a été observée contre la streptomycine, la tétracycline et l'ampicilline. La gentamycine, l'amoxicilline-acide clavulanique et la triméthoprime sulfadiazine étaient les antibiotiques les plus efficaces contre les souches O157, tandis que la gentamycine, la ciprofloxacine et la nitrofurantoïne étaient efficaces contre les souches non-O157. Les résultats ont révélé le rôle important des STEC dans la mammite subclinique du boviné dans la région étudiée. De plus, la distribution de la souche O157:H7 et la forte prévalence de la résistance aux médicaments multiples parmi les isolats est un sujet de préoccupation. Par conséquent, il existe une menace potentielle d'infection humaine suite à la consommation de lait contaminé par STEC dans la province iranienne du Kurdistan.

Mots-clés: Boviné, Résistance aux médicaments, Mammite, *Escherichia coli* productrices de Shiga-toxines (STEC), Virulence

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), one of the most contentious pathogenic strains of *E. coli*, resides in the gut tract of cattle as the principal reservoir of the bacterium. Pathogeneses of the STEC strains is moderated through the liberation of two toxins, namely Stx1 and/or Stx2, which are encoded by *stx1* and *stx2* genes, respectively. In addition, *eae* harboring strains may produce an additional virulence factor (i.e., intimin), which impairs the intestinal epithelium through attaching and effacing lesions (Mahanti et al., 2013). Although several serotypes are pertinent to severe illnesses in humans, O157: H7 is the most notorious STEC serotype (Momtaz et al., 2012b). The proclivity of the STEC strains to produce infections in cattle and humans is delineated in several studies (Güler and Gündüz, 2007; Dormanesh et al., 2014; Tavakoli and Pourtaghi, 2017; Zafarane et al., 2017). Mastitis is the most economically important disease of dairy cattle. Contamination with feces may

lead to the colonization of *E. coli*, including STEC strains in mammary glands. This is especially highlighted during the early lactation as a consequence of dearth in neutrophil number and function (Güler and Gündüz, 2007). Foodborne infections with STEC through the consumption of contaminated unpasteurized milk and milk products or undercooked meat in humans may trigger a wide spectrum of clinical manifestations fluctuating from mild to severe gastrointestinal symptoms to life-threatening ones, such as haemolytic uraemic (HUC) and haemorrhagic colitis (HC) syndromes (Mahanti et al., 2013). Prophylactic or growth promotion usage of antibiotics in animal husbandry is a common feature in Iran. This may impose a selective pressure to develop resistance genes among not only pathogenic but also commensal bacteria. Potential propagation and dissemination of resistance genes/bacteria from animals to humans is a matter of public health hazard (Iweriebor et al., 2015). Despite the controversies about the prescription of antibiotics in STEC infections in humans, it is

substantiated that the application of certain antimicrobial agents in the early stages of the infection may diminish the progress of disease toward HC and HUC (Dormanesh et al., 2014). Although milk plays a potential role and acts as a vehicle for transferring the infection to humans in Kurdistan Province, West of Iran, where unpasteurized milk and dairy products are consumed pervasively, there is a dearth of research on the role of STEC in subclinical bovine mastitis. Therefore, this study aimed to evaluate the frequency of STEC strains focusing on the prevalence of genes (i.e., *stx1*, *stx2*, and *eae*), O157: H7 serotype, and antimicrobial resistance profile of the isolates recovered from subclinical bovine mastitis in the mentioned area.

MATERIAL AND METHODS

Sample Collection and *E. coli* Isolation. A total of 400 subclinical bovine mastitis milk samples were collected equally in four seasons from all over Kurdistan Province, West of Iran, from September 2016 to 2017 and included in this cross-sectional study. The subclinical mastitis was determined using the California Mastitis Test. Approximately, five mL of milk was collected in sterile glass bottles following the disinfection of the udder. The samples were chilled until their delivery to the laboratory within a maximum of five h. The initial cultivation of the samples was performed on Mac Conkey (MC) agar (Quelab, Canada). After the incubation period at 37 °C for 24-36 h, a pink colony from each plate suspicious *to E. coli* was subcultured on MC agar and Eosin-Methylene blue (EMB) agar (Quelab, Canada). Biochemical identification of the isolates as *E. coli* was determined based on IMViC, Urea, and TSI reactions. The isolates were cultured in Luria-Bertani broth (Merck, Germany), and after adding 15% glycerol, they were stored at -20 °C until further molecular analysis. Moreover, to confirm the role of *E. coli* as the sole cause of mastitis, milk samples in which *E. coli* was isolated were screened for other bacterial agents.

DNA Extraction and Molecular Identification of *E. coli*. The genomic repertoire of each *E. coli* isolate was extracted from an overnight culture of the bacterium in Tryptic soy broth(Merck, Germany) using the boiling method (Guler et al., 2008). Molecular conformation of *E. coli* isolates was performed using species-specific primers (Riffon et al., 2001) (Table 1). The optimized thermal condition included initial denaturation at 94 °C for 4 min followed by 35 cycles of 94 °C for 45 sec, 57 °C for 1 min, 72 °C for 2 min, and a final expansion at 72 °C for 10 min. Polymerase chain reaction (PCR) was prepared in a final volume of 25 µl comprising 12.5 µlready-to use 2X PCR master mix (CinnaGen, Iran), 50 ng of the template DNA, and 0.7 µM of each primer. It should be noted that *E. coli* O157:H7 (ATCC 43895) was used as a positive control.

Molecular Detection of *stx* and *eae* Virulence Genes. The prevalence of *stx* genes was evaluated in a duplex PCR reaction using the primer pairs described by Cebula et al. (1995) (Table 1). The PCR master mix included 25 µl ready-to-use 2X PCR master mix (CinnaGen, Iran), 0.8 µM of each *stx1* and *stx2* primers, and 50 ng of the DNA template. *E. coli* isolates, in which an amplicon for *stx* genes was produced, were additionally examined for *eae* gene (Batchelor et al., 1999). The reaction mixture of *eae* amplification was the same as the species-specific PCR reaction. The PCR condition for both reactions was based on the method proposed by Osek (2003) including initial denaturation at 94 °C for 4 min followed by 30 cycles at 94, 53, and 72 °C for 1 min, and a final expansion at 72 °C for 5 min(Osek, 2003). It is worth mentioning that *E. Coli* ATCC 43895 strain was used as a positive control.

Molecular Detection of *rfb*O157 and *flic*H7 Genes. To categorize the *stx*/*eae* harboring isolates to O157 or non-O157 serotypes and screen *flic*H7 gene, a duplex PCR assay was applied based on the method presented by Pan et al. (2002) (Table 1). The PCR master mix contained the same volumes as to the duplex *stx* PCR

reaction. The positive control was the same as the preceding assays. The PCR thermal condition was 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 63 °C for 30 sec, and elongation at 72 °C for 80 sec (Pan et al., 2002). All PCR products were electrophoresed on 1.2% agarose gel stained with DNA SYBR safe (Invitrogen, USA) and visualized under ultraviolet light.

Antimicrobial Susceptibility Testing. Antimicrobial resistance patterns of the isolates were determined using the standard agar disk diffusion method under the Clinical and Laboratory Standards Institute recommendations (CLSI, 2013). The quality control organism used in the antibiogram test was *E. coli* ATCC 25922. The antibiotics that were tested included chloramphenicol (CHL: 30 µg), gentamycin (GEN: 5 µg), nitrofurantoin (NIT: 300 µg), ciprofloxacin (CIP: 5 µg), streptomycin (STR: 10 µg), lincomycin (LIN: 15 µg), tetracycline (TET: 30 µg), trimethoprim-sulfadiazine (SXT: 5+300 µg), amoxicillin-clavulanic acid (AUG: 20+10 µg), nalidixic acid (NAL: 30 µg), ampicillin (AMP: 10 µg), and ceftriaxone (CTR: 30 µg). The antibiotic discs were purchased from Patan Teb Co., Iran.

Statistical Analysis. The correlation between the frequency of *E. coli* and STEC isolates with season was analyzed in SPSS software (version 21.0) using the Chi-square test. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 173 (43.25%) *E. coli* strains were isolated and identified from the samples based on phenotypic and genotypic methods (Figure 1). Moreover, molecular screening of s*tx1*, *stx2*, and *eae* genes revealed that 39 isolates (22.54%) harbored at least one of the mentioned virulence genes. Duplex PCR for *stx1* and *stx2* genes manifested that 31 (79.48%) and eight (20.51%) isolates possessed *stx2* and *stx1+stx2* genes, respectively. The *stx1* gene was not detected in any isolates alone. The gene *eae* was detected in eight isolates (20.51%), of which six and two were in *stx2*

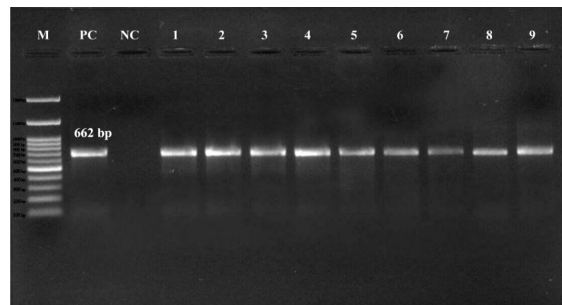and *stx1+stx2* harboring isolates, respectively (Figure 2).

Figure 1. Agarose gel electrophoresis of PCR products with *E. coli* species-specific primers (662 bp). M: 100 bp DNA Ladder (CinnaGen, Iran), PC: positive control (*E. coli* O157:H7 ATCC 43895). NC: negative control. Lanes 1-9: field samples.
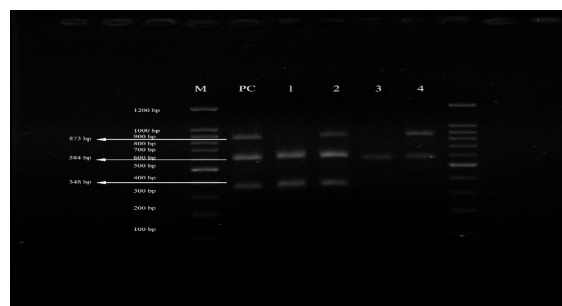

Figure 2. Agarose gel electrophoresis representing diverse genetic profiles among STEC isolated from subclinical bovine mastitis milk samples. M: 100 bp DNA Ladder (CinnaGen, Iran), PC: positive control (*E. coli* O157:H7 ATCC 43895: the amplicon sizes of 837 bp, 584 bp, and 348 bp allocate for *eae*, *stx2*, and *stx1* genes, respectively). Lane 1: profile *stx1+stx2* (six isolates); Lane 2: profile *stx1+stx2+eae* (two isolates); Lane 3: profile *stx2* (25 isolates); Lane 4: profile *stx2+eae* (six isolates).
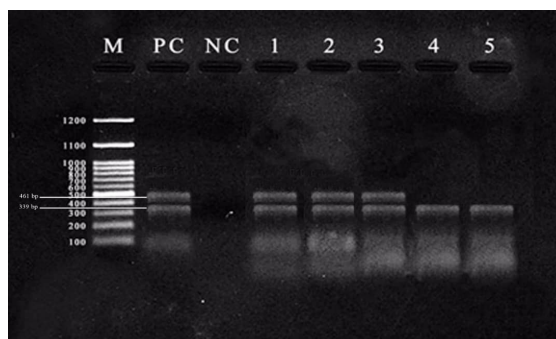

Figure 3. Agarose gel electrophoresis of PCR products with *rfb*O157 (339 bp) and *flic*H7 (461 bp) primers. M: 100 bp DNA Ladder (CinnaGen, Iran), PC: positive control (*E. coli* O157:H7 ATCC 43895). NC: negative control. Lanes 1-3: O157:H7 field samples (3 isolates). Lanes 4-5: O157: H⁻ field samples (2 isolates).

Table 1. Primer characteristics used in the present study

Virulence Gene	Sequence (5'→3')	Amplicon size (bp)	Reference
species-specific	GCTTGACACTGAACATTGAG GCACTTATCTCTTCCGCATT	662	(Riffon et al., 2001)
eae	GGGATC GAT TACCGT CAT TTTATCAGCCTTAAT CTC	837	(Batchelor et al., 1999)
stx1	CAG TTAATGTCGTGGCGAAGG CAC CAG ACAATGTAACCGCTG	348	(Cebula et al., 1995)
stx2	ATC CTA TTCCCGGGAGTTTAC G GCGTCATCG TAT ACA CAG GAG C	584	(Cebula et al., 1995)
rfb	AGCCGATGTCGATGCAATT CATGATTCCAAGCCTTGTGC	339	(Pan et al., 2002)
flic	ACCATCGGTGGAAGCCAG GAAGCATACCCGGCAACAG	461	(Pan et al., 2002)

Table 2. Prevalence of O157 and non-O157 STEC regarding diverse genotypes isolated from subclinical mastitis milk samples

Genetic profile	No. of isolates		Percent of isolates in each group		Percent of isolates in total	
	O157 serotype	non-O157 serotype	O157 serotype	non-O157 serotype	O157 serotype	non-O157 serotype
stx2	-	25	0%	100%	0%	64.10%
stx2+eae	2	4	33.33%	66.66%	5.12%	10.25%
Stx1+stx2	1*	5	16.66%	83.33%	2.56%	12.82%
Stx1+stx2+eae	2*	-	100%	0%	5.12%	0%

Table 3. The frequency of *E. coli* and STEC strains isolates from subclinical milk samples in various seasons

Season	No. of samples	No. of *E. coli* isolates	No. of STEC isolates		
			non-O157 serotype	O157 serotype	
				H7	Non-H7
Autumn	100	52	12	2	0
Winter	100	66	14	1	1
Spring	100	37	5	0	1
Summer	100	18	3	0	0
Total	400	173	34	3	2
P-value	-	0.059	0.051		

The virulence gene profile of the STEC strains was in four diverse combinations, of which *stx2* genotype had the highest frequency (64.10%) (Table 2). Similarly, five (12.82%) out of 39 STEC isolates possessed the *rfb*O157 gene, among which three of them carried the *flic*H7 gene (Figure 3). Furthermore, the other 34 (81.17%) isolates belonged to non-O157 serotypes. The genotypes of O157 and non-O157 STEC isolates are indicated in Table 2. In addition, no other bacteria were isolated from the milk samples contaminated with *E. coli*. Table 3 represents the frequency of *E. coli* and STEC isolates regarding the season. All of the *E. coli*

isolates were resistant to at least one antimicrobial agent; however, no isolate was resistant to all of them. Multidrug resistance, resistance to at least three classes of antibiotics, was detected in 31 (79.48%) isolates. Moreover, no common resistance pattern was represented among the isolates. All of the non-O157 STEC serotypes were resistant to STR, TET, and AMP. Resistance to CTR (85.29%), LIN (85.29%), and CHL (79.41%) was also observed. NAL (23.52%), SXT (32.35%), NIT (32.35%), CIR (44.11%) and GEN (50%) were the most effective antibiotics against the non-O157 strains, respectively. All five O157 STEC

isolates were resistant to AMP and STR but susceptible to TET (60%). Resistance to NIT (20%), NAL (20%), CTR (20%), SXT (40%), AUG (40%), and GEN (40%) was observed in less frequency. In the same vein, no significant relationship was revealed between the frequency of *E. coli* with the season (Table 3). In addition, there was no statistical association between the frequency of STEC and season (Table 3).

DISCUSSION

The insidious economic costs imposed on dairy husbandry by mastitis are intensive worldwide. This is more emphasized in the cases of subclinical mastitis because of the lack of obvious symptoms (Momtaz et al., 2012a). *Escherichia coli* is incriminated as a cause of bovine clinical and subclinical mastitis. The STEC strains are not only isolated frequently from mastitis cases but also severe human illnesses (Momtaz et al., 2012b; Dormanesh et al., 2014; Tavakoli and Pourtaghi, 2017; Zafarane et al., 2017). Consumption of unpasteurized milk and dairy products are very common and popular in Kurdistan Province, West of Iran. This underlines the potential role of milk as a vehicle for transferring the bacterium to the human food chain. In addition to cattle feces, other various sources of STEC contamination include the contaminated environment, water, equipment, infected workers or unhygienic handling, and marketing of milk constitute. A beneficial advantage of the direct sampling from the udder of lactating cows is to prevent extraneous contamination of the samples. This ensures the direct origin of presented bacteria from mammary glands. Accordingly, the probable sources of bacterial contamination in milk are identified promptly (Momtaz et al., 2012b). The overall prevalence of STEC isolated in the present study was 22.54%. In the other studies undertaken in different districts of Iran, the distribution values of STEC in bovine mastitis milk samples were 64.38%, 21.6% (Tavakoli and Pourtaghi, 2017), and 2.5% (Zafarane et al., 2017). In line with the results of the present study, the prevalence of STEC investigated in mastitis milk samples in Brazil was 22% (Lira et al.,

2004). Lower and higher frequencies of STEC in mastitis milk samples were reported from Brazil (9.5%) (Rangel and Marin, 2009) and Ogun State, Nigeria (32%) (Ivbade et al., 2014), respectively. On the other hand, no STEC isolate was detected from mastitis milk samples in Konya, Turkey (Güler and Gündüz, 2007). The fluctuation in the frequency of STEC in bovine mastitis milk samples in different parts of Iran and the world may be related to geographical and seasonal variables, sampling period, hygienic and management status, the herds and sample sizes, and the breed of cows (Momtaz et al., 2012a; Zafarane et al., 2017). Additionally, in some internal studies, the rates of raw milk contamination with STEC were determined at 0.76% in Mashhad, Northeast of Iran (Brenjchi et al., 2011), 17.47% in Kermanshah, West of Iran (Mohammadi et al., 2013), 35.28% in Shahrekord, Center of Iran (Momtaz et al., 2012a), and 33.33% in Tehran, Capital of Iran (Mashak, 2018). Generally, based on the preceding reports, unpasteurized milk may be a potential vector for STEC in Iran. Our results represented a higher prevalence of *stx2*, compared to *stx1* which coincides with the results obtained from STEC strains isolated from bovine mastitis milk samples in Tehran (Zafarane et al., 2017). In addition, 100% of the *E. coli* strains isolated from clinical and subclinical buffalo mastitis milk samples in Egypt were harboring *stx2* (Lamey et al., 2013). However, this is in contrast with the higher distribution of *stx1* reported by Momtaz et al. (2012b), Tavakoli and Pourtaghi (2017), and Mashak (2018) from Iran (Momtaz et al., 2012b; Tavakoli and Pourtaghi, 2017; Mashak, 2018). It is clarified that *stx2* is more associated with human HUS and HC syndromes. The simultaneous existence of *stx1* and *stx2* genes was detected in 20.51% of the STEC isolates in the present study, compared to 13.88%, 22.2%, and 7.7% reported from Shahrekord, Tehran, and Alborz Province, Iran, respectively (Momtaz et al., 2012b; Tavakoli and Pourtaghi, 2017; Zafarane et al., 2017). Moreover, our results were in contrast with those reported *stx1* as the predominant *stx* gene harbored by STEC strains isolated from cattle (Kobori

et al., 2004). Differences in sampling time are verified as a plausible explanation for the variation in the frequencies of *stx* genes among different studies, and it is proved that the distribution of *stx* genes has fluctuated at different times. The proportion of *eae* gene among the STEC isolates in the present study was 20.51%. This value is lower than the frequency of *eae* detected from STEC strains isolated from bovine mastitis milk samples in other internal studies (Tavakoli and Pourtaghi, 2017;Zafarane et al., 2017). Although the mode of action of *eae* gene in bovine mastitis is unknown, intimin is assumed as a complementary virulence factor for human illnesses with STEC through the induction of attaching and effacing lesions in gut mucosa (Mahanti et al., 2013). The distribution of O157 serotype was 12.82% (5 isolates), among which 60% (3 isolates) of them were O157:H7. Higher (15.06%) and lower (11.11%) frequencies of O157 serotype were reported from STEC isolated from mastitis milk samples in Shahrekord and Tehran, Iran, respectively (Momtaz et al., 2012b; Zafarane et al., 2017). To the best of our knowledge, no data is available about the frequency of O157:H7 serotype involved in bovine mastitis in internal studies. Brenjchi et al. (2011) reported a 6.15% frequency of STEC in raw bulk tank milk samples delivered to Pegah Pasteurization Factory of Mashhad, among which only one isolate (12.5%) was O157:H7 (Brenjchi et al., 2011). The prevalence of STECO 157:H7 serotype in raw milk in the Ogun State of Nigeria was 2% (Ivbade et al., 2014), whereas no O157 serotype was detected from raw milk in Kermanshah, Iran (Mohammadi et al., 2013). Although the origin of STECO157:H7 isolates was the infected udder in the present study, the contaminated skin, feces, and environment could be the sources of infection in raw milk. Therefore, in addition to the control and treatment of mastitis, the promotion of hygienic conditions and the application of sanitary measures in farms is vital to reduce the rate of raw milk contamination with STEC strains (Zafarane et al., 2017; Mashak, 2018).

Unfortunately, indiscriminative use of antibiotics is a common feature, and exertion of rigid control over the misuse of antimicrobial drugs, particularly in animal husbandry, is not acceptable in Iran. Therefore, screening of new emerging antimicrobial resistance against pathogenic bacteria with a focus on the zoonotic pathogens is of great concern (Momtaz et al., 2012b). A total of 100% of the examined isolates in the present study depicted antimicrobial resistance to one or several examined antimicrobial agents. The highest resistance levels were exhibited to streptomycin, ampicillin, and tetracycline, which was in agreement with the results obtained from the previous studies. In contrast, a discrete level of resistance to the preceding antibiotics has been reported by Lanz et al. (2003) in Switzerland (Lanz et al., 2003). In other investigations undertaken in Iran, resistance to penicillin, tetracycline, lincomycin, ampicillin, tetracycline, trimethoprim-sulphamethoxazole, and chloramphenicol (Zafarane et al., 2017), as well as penicillin, tylosin, oxytetracycline, erythromycin, ampicillin, streptomycin, and neomycin (Tavakoli and Pourtaghi, 2017) was the highest among STEC strains isolated from bovine with mastitis. Briefly, based on the available data, prescription of tetracycline and beta-lactamases may not be effective against *E. coli*-originated bovine mastitis in Iran (Zafarane et al., 2017). A high proportion of multidrug resistance (79.48%) was identified in the present study. The rates of multidrug resistance among the STEC isolated from bovine mastitis in Tehran and Alborz Provinces, Iran, were 100% and 77.77%, respectively (Tavakoli and Pourtaghi, 2017; Zafarane et al., 2017), whereas no STEC isolates with multidrug resistance were reported from raw milk samples in Kermanshah, Iran (Mohammadi et al., 2013). In addition, multidrug resistance has been reported in other studies (Mora et al., 2005; Rangel and Marin, 2009). The existence of high rates of multidrug resistance among STEC strains represents a negative prognosis for the treatment of life-threatening infections caused by STEC in human. Moreover, the propagation of resistance genes from *E.*

coli strains to other pathogenic or commensal strains is the other aspect of the hazard in the public and dairy sector (Dormanesh et al., 2014).

It can be concluded that the wide distribution of multidrug resistance STEC, as well as harboring *stx1*, *stx2*, and *eae* genes in mastitis bovine milk in western Iran, makes milk a potential vector for zoonotic STEC transmission from cattle to humans. This should be taken into account to apply adequate control and prevention procedures and establish long-term strategies to guarantee the safety of food from dairy cattle. Furthermore, continued surveillance of *E. coli* isolates should be conducted on dairy cows to detect subclinical mastitis. Future studies should be aimed to investigate the distribution of this isolate in other food products in the region.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Ahmadi, E.
Acquisition of data: Ahmadi, E.; Amiri, A.
Analysis and interpretation of data: Ahmadi, E.
Drafting of the manuscript: Ahmadi, E.
Critical revision of the manuscript for important intellectual content: Mardani, K.
Statistical analysis: Mardani, K.
Administrative, technical, and material support: Ahmadi, E.

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References

Batchelor, M., Knutton, S., Caprioli, A., Huter, V., Zanial, M., Dougan, G., *et al.*, 1999. Development of a universal intimin antiserum and PCR primers. J Clin Microbiol 37, 3822-3827.

Brenjchi, M., Jamshidi, A., Farzaneh, N., Bassami, M.R., 2011. Identification of shiga toxin producing Escherichia coli O157:H7 in raw cow milk samples from dairy farms in Mashhad using multiplex PCR assay. Iran J Vet Res 12, 145-149.

Cebula, T.A., Payne, W.L., Feng, P., 1995. Simultaneous identification of strains of Escherichia coli serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J Clin Microbiol 33, 248-250.

CLSI, 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animal; approved standard, Clinical and Laboratory Standards Institute, Wayne, PA.

Dormanesh, B., Safarpoor Dehkordi, F., Hosseini, S., Momtaz, H., Mirnejad, R., Hoseini, M.J., *et al.*, 2014. Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian pediatric patients. Iran Red Crescent Med J 16, e14627.

Güler, L., Gündüz, K., 2007. Virulence properties of Escherichia coli isolated from clinical bovine mastitis. Turk J Vet Anim Sci 31, 361-365.

Guler, L ,.Gunduz, K., Ok, U., 2008. Virulence factors and antimicrobial susceptibility of Escherichia coli isolated from calves in Turkey. Zoonoses Public Health 55, 249-257.

Ivbade, A., Ojo, O.E., Dipeolu, M.A., 2014. Shiga toxin-producing Escherichia coli O157:H ᵛin milk and milk products in Ogun State, Nigeria. Vet Ital 50, 185-191.

Iweriebor, B.C., Iwu, C.J., Obi, L.C., Nwodo, U.U., Okoh, A.I., 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, 213.

Kobori, D., Rigobelo, E., Macedo, C., Marin, J.M., Avila, F., 2004. Virulence properties of Shiga toxin-producing Escherichia coli isolated from cases of bovine Mastitis in Brazil. Rev Elevage MedVet Pays Trop 57, 15-20.

Lamey, A., Ammar, A.M., Zaki, E., Khairy, N., Moshref, B.S., Refai, M.K., 2013. Virulence factors of Escherichia coli isolated from recurrent cases of clinical and subclinical mastitis in buffaloes. Int J 4, 86-94.

Lanz, R., Kuhnert, P., Boerlin, P., 2003. Antimicrobial resistance and resistance gene determinants in clinical

Escherichia coli from different animal species in Switzerland. Vet Microbiol 91, 73-84.

Lira, W.M., Macedo, C., Marin, J.M., 2004. The incidence of Shiga toxin-producing Escherichia coli in cattle with mastitis in Brazil. J Appl Microbiol 97, 861-866.

Mahanti, A., Samanta, I., Bandopaddhay, S., Joardar, S.N., Dutta, T.K., Batabyal, S*., et al.*, 2013. Isolation, molecular characterization and antibiotic resistance of Shiga Toxin-Producing Escherichia coli (STEC) from buffalo in India. Lett Appl Microbiol 56, 291-298.

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, e62288.

Mohammadi, P., Abiri, R., Rezaei, M., Salmanzadeh-Ahrabi, S., 2013. Isolation of Shiga toxin-producing Escherichia coli from raw milk in Kermanshah, Iran. Iran J Microbiol 5, 233-238.

Momtaz, H ,.Farzan, R., Rahimi, E., Safarpoor Dehkordi, F., Souod, N., 2012a. Molecular characterization of Shiga toxin-producing Escherichia coli isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J 2012, 231342.

Momtaz, H., Safarpoor Dehkordi, F., Taktaz, T., Rezvani, A., Yarali, S., 2012b. Shiga toxin-producing *Escherichia coli* isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. Sci World J 2012, 618709.

Mora ,A., Blanco, J.E., Blanco, M., Alonso, M.P., Dhabi, G.,

Echeita, A.*, et al.*, 2005. Antimicrobial resistance of Shiga toxin (verotoxin)-producing Escherichia coli O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res Microbiol 156, 793-806.

Osek, J., 2003. Development of a multiplex PCR approach for the identification of Shiga toxin-producing Escherichia coli strains and their major virulence factor genes. J Appl Microbiol 95, 1217-1225.

Pan, T.M., Chen, L.M., Su, Y.C., 2002 .Identification of Escherichia coli O157:H7 by multiplex PCR with primers specific to the hlyA, eaeA, stx1, stx2, fliC and rfb genes. J Formos Med Assoc 101, 661-664.

Rangel, P., Marin, J.M., 2009. Analysis of Escherichia coli isolated from bovine mastitic milk. Pesqui Vet Bras 29, 363-368.

Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M., Lagace, J., 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J Clin Microbiol 39, 2584-2589.

Tavakoli, M., Pourtaghi, H., 2017. Molecular detection of virulence genes and multi-drug resistance patterns in Escherichia coli (STEC) in clinical bovine mastitis: Alborz province, Iran. Iran J Vet Res 18, 208-211.

Zafarane, S., Houri, H., Kazemian ,H., Heidari, H., Amiri, P., Tabarraei, B., 2017. Characterization of virulence genes, serogroups and antimicrobial susceptibility of Shiga toxin producing Escherichia coli isolated from bovine mastitic milk in Tehran, Iran. Tropl Biomed 34, 295-304.