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**Original Article**

# Occurrence of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in goat and sheep milk from four rural and tribal regions, Fars province, Iran

**Sayyadi, M.[1]; Rahmdel, S.[2]; Pashangeh, S.[3]; Hosseinzadeh, S.[4**]; Fani, F.[5]; Zare, M.[6] and Shekarforoush, S. S.[4*]**

[1]DVM Student, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [2]Ph.D. Student in Food Hygiene, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [3]Department of Food Science and Technology, Faculty of Agriculture, Jahrom University, Jahrom, Iran; [4]Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [5]Department of Microbiology, Infectiology and Immunology, Laval University, Quebec, Canada; [6]MSc in Microbiology, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]***Correspondence:*** *S. S. Shekarforoush, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: shekar@shirazu.ac.ir*

[**]***Co-correspondence:*** *S. Hosseinzadeh, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: hosseinzadeh@shirazu.ac.ir*

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## Abstract

**Background:** Raw sheep and goat milk may harbor major foodborne pathogens of public health concern, including *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7. The rising issue of antimicrobial resistance further underscores the need for monitoring these contaminants. **Aims:** This research paper describes a survey of the frequency and antimicrobial susceptibility of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in milk samples from goat and sheep herds, located in Fars province, Iran. **Methods:** A total number of 110 milk samples from 50 goat and sheep herds located in tribal and rural regions were investigated for pathogenic organisms. The antimicrobial susceptibility of the isolates was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** None of the samples showed the presence of *Salmonella*. Four samples (3.6%) from three rural herds contained *L. monocytogenes*, all positive for the species-specific virulence gene *hlyA*. *E. coli* O157:H7 was detected in five milk samples (4.5%) belonging to four rural herds. The four virulence genes (*stx*$_1$, *stx*$_2$, *eae*, and e*hlyA*), and two serotyping genes (*rfbE*, and *fliC*) were found in all *E. coli* O157:H7 isolates. The somatic cell count of contaminated samples was lower than the US legal limit of $1.0 \times 10^6$ cells/ml; milk contamination was not, therefore, related to the mastitis. All *L. monocytogenes* isolates were resistant to penicillin, ampicillin, and trimethoprim-sulfamethoxazole. The *E. coli* O157:H7 isolates exhibited full or intermediate resistance to ampicillin, cefixime, and chloramphenicol, but they were susceptible to gentamicin, kanamycin, streptomycin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, and trimethoprim. **Conclusion:** Despite the low occurrence, the presence of foodborne pathogens in raw milk reinforces the need to control the sale and consumption of raw milk dairy products in rural and tribal regions where goat and sheep farming is of great economic importance.

**Key words:** Antibiotic resistance, *Escherichia coli* O157:H7, Goat milk, *Listeria monocytogenes*, *Salmonella*

## Introduction

The microbiological quality of raw materials used in food preparation can affect the safety of the end product. In the case of dairy foods prepared from raw milk, consumer safety concerns arise from possible contamination with human pathogens. *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are among the most important raw milk-related pathogens, as they have been implicated in worldwide outbreaks associated with raw milk and its derivatives (Jamali *et al*., 2013; Gonzales-Barron *et al*., 2017). In the United States, the annual burden of diseases caused by these foodborne pathogens has been estimated to be 76 million acute illnesses, 325,000 hospitalizations, and 5000 deaths (Pires *et al*., 2010). Salmonellosis and

listeriosis are also responsible for the high rate of mortality due to foodborne illnesses in Europe (Fagundes *et al*., 2011). A matter of greater concern is the antimicrobial resistance of the pathogens, making treatment more difficult. In Iran, the wide use of antibiotics in human and veterinary medicine has led to a rise of antibiotic resistance in pathogens (Rahimi *et al*., 2010; Jamali *et al*., 2013; Lotfollahi *et al*., 2017).

Although bovine milk is the most produced and used milk in Iran, goat, and sheep farming is still of great economic importance in the rural and tribal regions. A majority of the milk from these farms is converted to dairy products, either consumed by the producers themselves or sold. The production and consumption of raw milk and dairy foods in these regions, which is in turn accompanied by human health risks. The presence of foodborne pathogens in goat and sheep milk from Iranian herds has been previously addressed in a few studies (Rahimi *et al*., 2010; Farzan *et al*., 2012; Momtaz *et al*., 2012; Rahimi *et al*., 2012; Jamali *et al*., 2013; Shaigan Nia *et al*., 2014; Lotfollahi *et al*., 2017; Ranjbar *et al*., 2018). No similar work has been, however, performed on the herds from Fars province with a considerable population of nomads and peasants.

Therefore, the present preliminary study, aimed to investigate the frequency and antimicrobial susceptibility of *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 in milk samples from goat and sheep herds located in Fars Province, Iran.

## Materials and Methods

### Sample collection

According to the Nomadic Affairs Organization of the Ministry of Agricultural Jihad, the main areas where nomadic livestock are settled in Fars Province include 12 main areas (clusters). Four clusters were randomly selected. Three nomadic areas included Firuzabad (A), Marvdasht (B) and Jahrom (C), and one rural area (Bidzard - D). In each area, the settlement of nomadic herds was determined and about 10% of them were randomly selected. Details of the regions of sampling and the number of herds (including sheep and goats) are given in Table 1. One hundred and ten samples of raw milk from 70 individual goats and 40 individual sheep were collected from 50 randomly selected herds with 40 to 323 animals. Out of 50 herds, 47 herds contained sheep and goats, one herd included sheep and two herds just included goats. From each herd, depending on the number and type of animals, one to three milking animals were arbitrarily selected. Milk samples were directly taken under sterilized conditions. In the study areas, the feeding system was a combination of indoor feeding and part-time grazing. The only milking procedure was traditional hand milking. While tribal grazing lands were far from human activities, the rural pastures were contaminated with sewage and human waste.

Following teat disinfection and discarding the first milk streams, a milk sample of about 50 ml from each udder half was collected in a sterile plastic vial. The samples were then transported on ice to the microbiology laboratory and analyzed immediately.

### Microbiological analyses

*Somatic cell count (SCC) of milk samples*

A portion of the milk sample intended for SCC testing was preserved in 0.5% potassium dichromate and tested within 24 h of sampling. Before testing, the samples were heated to 40°C in a water bath and held at this temperature for 15 min. SCC was measured using an automated cell counter (Fossomatic; Foss Electric, Hillerod, Denmark).

*Isolation of pathogenic bacteria*

The milk samples were first homogenized by vortexing and then divided into 10-ml portions and screened for *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7. The analyses were conducted on the sediments from centrifugation of 10-ml milk samples (10,000 × g for 15 min at 4°C).

The modified Bacteriological Analytical Manual method was used for *Salmonella* isolation (Andrews *et al*., 2007). In brief, the sample sediment was suspended in 10 ml of lactose broth (Merck, Darmstadt, Germany). Following 24 h of incubation at 35°C, 0.1 and 1 ml of the culture were transferred to Rappaport-Vassiliadis (Merck) and tetrathionate (Merck) broths and incubated for 24 h at 42 and 35°C, respectively. Both broths were subsequently streaked onto the xylose-lysine-desoxycholate agar (Merck), Salmonella-Shigella agar (Merck), and bismuth sulfite agar (Merck). After incubation at 35°C for 24-48 h, the suspected colonies were purified and subjected to biochemical identification tests, including Triple Sugar Iron (TSI) agar, Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC), lysine decarboxylase, and urease tests.

**Table 1:** Details of the regions of sampling and somatic cell count (SCC) of milk samples

| Region | Number of herds | Sample | | SCC (cells/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Median | | | Mean ± SD | | |
| | | Sheep | Goat | Sheep | Goat | Total | Sheep | Goat | Total |
| A | 20 | 22 | 24 | 611 | 1237 | 1115 | $2.1\times10^3\pm4.3\times10^3$ | $3.0\times10^3\pm3.6\times10^3$ | $2.6\times10^3\pm3.9\times10^3$ |
| B | 10 | 9 | 11 | 178 | 1644 | 420 | $9.6\times10^2\pm1.8\times10^3$ | $3.6\times10^3\pm5.6\times10^3$ | $2.4\times10^3\pm4.4\times10^3$ |
| C | 11 | 5 | 16 | 122 | 383 | 163 | $1.9\times10^3\pm3.9\times10^3$ | $5.3\times10^2\pm8.0\times10^2$ | $8.6\times10^2\pm2.0\times10^3$ |
| D | 9 | 4 | 19 | 151 | 491 | 446 | $2.5\times10^3\pm4.1\times10^3$ | $2.1\times10^3\pm3.4\times10^3$ | $2.2\times10^3\pm3.4\times10^3$ |
| Total | 50 | 40 | 70 | 271 | 700 | 511.5 | $1.8\times10^3\pm3.6\times10^3$ | $2.3\times10^3\pm3.6\times10^3$ | $2.1\times10^3\pm3.6\times10^3$ |

Excel 2016 software was used for descriptive statistical analysis

To detect *L. monocytogenes*, the sediment was enriched in 10 ml of buffered *Listeria* enrichment broth (Merck). At the 4th h of incubation at 30°C, the selective agents including acriflavin (10 mg/L), sodium nalidixate (40 mg/L), and natamycin (25 mg/L) were added to the enrichment broth, and the incubation was continued for an additional 44 h. The enrichment broth was sub-cultured at 24 and 48 h onto the modified PALCAM agar (Merck) consisting of basal medium supplemented with 5 mg/L acriflavin and 40 mg/L sodium nalidixate. Presumptive isolates were then identified by Gram-staining, motility, methyl red, and Voges-Proskauer tests (Hitchins and Jinneman, 2013).

*E. coli* O157:H7 was identified as described by Chye *et al*. (2004). The enrichment procedure was performed by transferring the sediment to 10 ml of tryptic soy broth (Merck) containing 1.5 g/L bile salts No. 3 (Oxoid Ltd., Basingstoke, UK) and 20 mg/L novobiocin (Sigma-Aldrich, St Louis, MO, USA). After overnight incubation at 35°C, the culture was plated onto sorbitol MacConkey agar (Merck) supplemented with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L). The colorless colonies were purified on eosin-methylene blue agar (Merck); colonies with a metallic green sheen were further characterized by the following biochemical tests: IMViC and TSI tests. Serotyping of O- and H-antigens was carried out using standard methods at Prof. Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences.

All stock cultures were maintained in brain-heart infusion (Merck) broth containing 30% (v/v) glycerol at -80°C. When required, the isolates were inoculated into brain-heart infusion (BHI) broth and grown at 37°C for 24-48 h.

*Polymerase chain reaction (PCR) confirmation of the isolates*

All the primer pairs used to amplify the virulence genes are listed in Table 2. The *L. monocytogenes* isolates were tested for the presence of the *hlyA* gene encoding α-haemolysin (Furrer *et al*., 1991; Hosseinzadeh *et al*., 2012). The PCR mixture was prepared in a final volume of 25 µL with 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 100 µM dNTP, 5 U of Taq deoxyribonucleic acid (DNA) polymerase, and 2 µL DNA template. The thermal profile consisted of initial denaturing at 95°C for 5 min, followed by 40 cycles of 30 s at 95°C, 60 s at 56°C, and 60 s at 72°C and a final extension step of 72°C for 5 min.

The *E. coli* O157:H7 isolates were subjected to PCR detection of four virulence genes (*stx*$_1$, *stx*$_2$, *eae*, and e*hlyA*), and two serotype genes (*rfbE*, and *fliC*) (Bai *et al*., 2010). A duplex PCR was used to simultaneously amplify the genes *stx*$_1$ and *stx*$_2$; amplification was conducted in a reaction volume of 25 µL containing 1 × PCR buffer, 0.75 mM $MgCl_2$, 0.2 µM of each primer, 62.5 µM dNTP, 2.5 U of Taq DNA polymerase, and 2 µL DNA template. The PCR thermal program was 95°C for 3 min, 35 cycles of 94°C for 45 s, 58°C for 90 s, and 72°C for 90 s, and 72°C for 7 min. For e*hlyA* detection, 2 µL of DNA template was added to a mixture of 10 µL of Taq DNA Polymerase 2.0 × Master Mix RED (1.5 mM $MgCl_2$; Ampliqon, Copenhagen, Denmark) and 0.4 µM of each primer. The gene was amplified under the following thermal conditions: 95°C for 3 min; 35 cycles of 95°C for 20 s, 58°C for 40 s, and 72°C for 90 s; 72°C for 5 min. The genes *eae*, *rfbE*, and *fliC* were co-amplified according to Bai *et al*. (2010).

*L. monocytogenes* American Type Culture Collection (ATCC) 1297 and *E. coli* O157:H7 ATCC 43895 were used as positive control strains.

## Antimicrobial susceptibility tests

The minimal inhibitory concentrations (MIC) of penicillin (0.5-16 µg/ml), ampicillin (0.5-16 µg/ml), and trimethoprim/sulfamethoxazole (0.5/9.5-16/304 µg/ml) against *L. monocytogenes* isolates were measured using the broth microdilution method and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines M45-P breakpoints (CLSI, 2005).

**Table 2:** The primers used for PCR confirmation of *L. monocytogenes* and *E. coli* O157:H7

| Target gene | Primer sequence (5′→3′) | PCR product (bp) | Reference |
|---|---|---|---|
| *hlyA* | CGGAGGTTCCGCAAAAGATG<br>CCTCCAGAGTGATCGATGTT | 234 | Furrer *et al*. (1991) |
| *stx*$_1$ | ATAAATCGCCATTCGTTGACTAC<br>AGAACGCCCACTGAGATCATC | 180 | Lefebvre *et al*. (2009) |
| *stx*$_2$ | GGCACTGTCTGAAACTGCTCC<br>TCGCCAGTTATCTGACATTCTG | 255 | Lefebvre *et al*. (2009) |
| *eae* | CATTATGGAACGGCAGAGGT<br>ACGGATATCGAAGCCATTTG | 375 | Bai *et al*. (2010) |
| *ehlyA* | ACGATGTGGTTTATTCTGGA<br>CTTCACGTGACCATACATAT | 165 | Dipineto *et al*. (2006) |
| *rfbE* | CAGGTGAAGGTGGAATGGTTGTC<br>TTAGAATTGAGACCATCCAATAAG | 296 | Bai *et al*. (2010) |
| *fliC* | AGCTGCAACGGTAAGTGATTT<br>GGCAGCAAGCGGGTTGGTC | 949 | Bai *et al*. (2010) |

**Table 3:** Frequency of *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 in 50 herds and 110 goat and sheep milk samples

| Pathogens | No. (%) of positive herd | | | | | No. (%) of positive samples | | |
|---|---|---|---|---|---|---|---|---|
| | Region A (N=20) | Region B (N=10) | Region C (N=11) | Region D (N=9) | Total (N=50) | Goat milk (N=70) | Sheep milk (N=40) | Total (N=110) |
| *Salmonella* | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| *L. monocytogenes* | 0 (0%) | 0 (0%) | 0 (0%) | 3 (33.3%) | 3 (6.0%) | 4 (5.7%) | 0 (0%) | 4 (3.6%) |
| *E. coli* O157:H7 | 0 (0%) | 0 (0%) | 0 (0%) | 4 (44.4%) | 4 (8.0%) | 4 (5.7%) | 1 (2.5%) | 5 (4.5%) |

**Table 4:** The origin of foodborne pathogens isolated from goat and sheep milk

| Pathogens | Isolate code | Region | Herd | Origin | Somatic cells of milk (cells/ml) |
|---|---|---|---|---|---|
| *L. monocytogenes* | R4S88 | D | 42 | Goat | $7.2\times10^4$ |
| | R4S90 | D | 43 | Goat | $3.6\times10^5$ |
| | R4S91 | D | 43 | Goat | $2.0\times10^5$ |
| | R4S110 | D | 50 | Goat | $1.4\times10^5$ |
| *E. coli* O157:H7 | R4S93 | D | 44 | Goat | $9.5\times10^4$ |
| | R4S94 | D | 45 | Goat | $5.4\times10^5$ |
| | R4S96 | D | 46 | Sheep | $1.5\times10^5$ |
| | R4S109 | D | 50 | Goat | $1.5\times10^5$ |
| | R4S110 | D | 50 | Goat | $1.4\times10^5$ |

The susceptibility of *E. coli* O157:H7 isolates to ampicillin (10 µg), cefixime (5 µg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), trimethoprim (5 µg), and chloramphenicol (30 µg) was determined by the disk diffusion method proposed by Clinical and Laboratory Standards Institute (CLSI, 2015).

## Results

### Isolation of pathogenic bacteria and detection of virulence genes

The frequency of foodborne pathogens in 50 herds from 4 regions of Fars province, Iran, and 110 milk samples is shown in Table 3. *Salmonella* was absent from all the samples analyzed. The frequency of *L. monocytogenes* and *E. coli* O157:H7 in the herd was 6.0% and 8.0%, respectively. As shown in Tables 3 and 4, all contaminated samples belonged to the rural region (D), while no pathogen was isolated from milk samples from tribal areas (regions A, B, and C).

All *L. monocytogenes* isolates retrieved by culturing methods were positive for the species-specific virulence gene *hlyA* (Fig. 1). All five isolates of *E. coli* O157:H7 harbored the virulence genes ($stx_1$, $stx_2$, *eae*, e*hlyA*) (Fig. 2), and the serotyp genes (*rfbE*, and *fliC*).

### Antimicrobial susceptibility

The antimicrobial resistance of *L. monocytogenes* and *E. coli* O157:H7 isolates are summarized in Tables 5 and 6, respectively. All *L. monocytogenes* isolates were resistant to the beta-lactam antibiotics (penicillin and ampicillin) tested and exhibited resistance to trimethoprim-sulfamethoxazole.

All five *E. coli* O157:H7 isolates were resistant to ampicillin. They also showed intermediate resistance to cefixime and chloramphenicol. The isolates were, however, susceptible to the other antimicrobials tested.

### SCC of milk samples

The SCC of the samples was in the range of $1.3 \times 10^2$ and $1.8 \times 10^7$ cells/ml, with the median value of $5.1 \times 10^5$ cells/ml (Table 1). The SCC levels of 37% of the samples were above the US legal limit of $1.0 \times 10^6$ cells/ml (Paape *et al*., 2007). The SCC of contaminated samples was lower than the US legal limit of $1.0 \times 10^6$ cells/ml (Table 4).

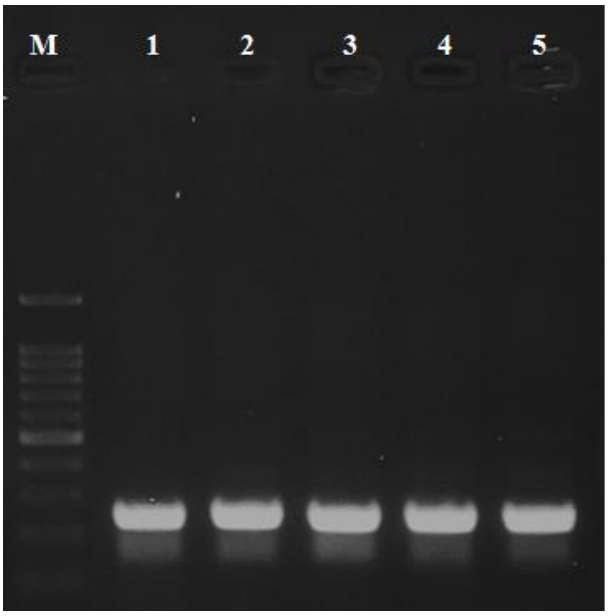


**Fig. 1:** PCR amplification of a 234 Base pair (bp) fragment of the gene *hlyA* in *Listeria monocytogenes* isolates. Lane M: 100-bp DNA ladder, Lane 1: Positive control (*L. monocytogenes* ATCC 1297), and Lanes 2-5: *L. monocytogenes* R4S88, R4S90, R4S91, and R4S110, respectively

**Table 5:** Minimal inhibitory concentration (µg/ml) of penicillin G, ampicillin and trimethoprim/sulfamethoxazole for *L. monocytogenes* isolates from goat and sheep milk

| Isolate code | Antimicrobial agents | | |
|---|---|---|---|
| | Penicillin (range: 0.5-16 µg/ml) | Ampicillin (range: 0.5-16 µg/ml) | Trimethoprim/Sulfamethoxazole (range: 0.5/9.5-16/304 µg/ml) |
| R4S88 | 8 | 4 | 8/152 |
| R4S90 | 8 | 8 | 8/152 |
| R4S91 | 8 | 8 | 8/152 |
| R4S110 | 8 | 4 | 4/76 |

**Table 6:** Antimicrobial susceptibility profiles of *Escherichia coli* O157:H7 isolates from goat and sheep milk

| Isolate code | Antimicrobial agents[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Amp | Cef | Gen | Kan | Strep | Tet | Cip | Tmp/Smx | Tmp | Clo |
| R4S93 | R | I | S | S | S | S | S | S | S | I |
| R4S94 | R | I | S | S | S | S | S | S | S | I |
| R4S96 | R | I | S | S | S | S | S | S | S | I |
| R4S109 | R | I | S | S | S | S | S | S | S | I |
| R4S110 | R | I | S | S | S | S | S | S | S | I |

[a]Antimicrobials used: Amp: Ampicillin, Cef: Cefixime, Gen: Gentamicin, Kan: Kanamycin, Strep: Streptomycin, Tet: Tetracycline, Cip: Ciprofloxacin, Tmp/Smx: Trimethoprim/sulfamethoxazole, Tmp: Trimethoprim, and Clo: Chloramphenicol. S: Sensitive, I: Intermediate, and R: Resistant (CLSI, 2015)

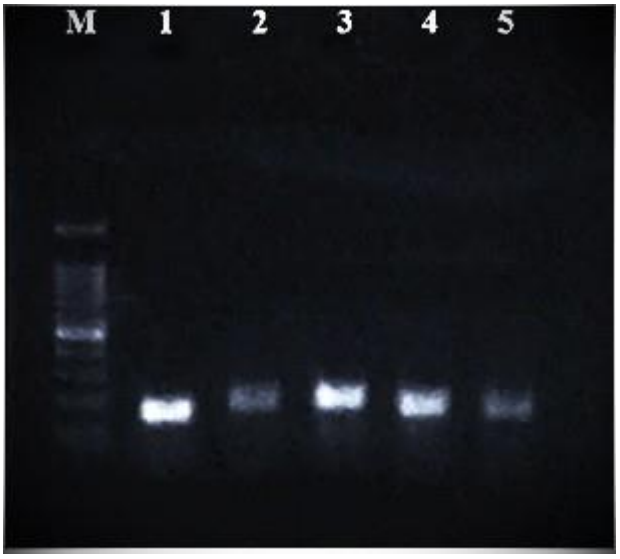


**Fig. 2:** PCR amplification of a 165 bp fragment of the gene *ehlyA* in *Escherichia coli* O157:H7 isolates. Lane: M: 100-bp DNA ladder, Lane 1: Positive control (*E. coli* O157:H7 ATCC 43895), and Lanes 2-5: *E. coli* O157:H7 R4S93, R4S94, R4S96, and R4S110, respectively

## Discussion

In this study, the frequency of *L. monocytogenes* and *E. coli* O157:H7 contamination in milk from some rural and nomadic sheep and goat herds in Fars province, Iran, was 6.0 and 8.0%, respectively. *Salmonella* contamination was not observed in any of the studied herds. The rate of raw milk contamination with *Salmonella* varied depending on the geographic region, herd size, farming practices, farm environmental contamination, and fecal excretion (Hill *et al*., 2012). Scarce information is available on goat and sheep milk contamination with *Salmonella* in Iran (Shaigan Nia *et al*., 2014). The present study was a comprehensive survey of contamination with *Salmonella* in the sheep and goat herds of rural and tribal areas in Fars province, Iran. In thisstudy, *Salmonella* was not detected in any sample, which is consistent with reports from other countries (Foschino *et al*., 2002; Morgan *et al*., 2003; Mühlherr *et al*., 2003; Ekici *et al*., 2004; Cortés *et al*., 2006; Bogdanovičová *et al*., 2015; Cavicchioli *et al*., 2015; Bogdanovièová *et al*., 2016). Although unable to compete with the indigenous microbiota of raw milk, *Salmonella* can persist in the hostile environment of milk. Failure to isolate this pathogen may be due to the interference effect of autochthonous microbiota (Nero *et al*., 2008). The possibility of transmission to consumers through raw milk dairy foods should, therefore, not be ignored, as such products have been implicated in several outbreaks worldwide (Desenclos *et al*., 1996; Mazurek *et al*., 2004; Dominguez *et al*., 2009; Mungai *et al*., 2015). Four goat milk samples collected from three rural herds contained *L. monocytogenes* (Table 3). The frequency was comparable to that of other parts of the country (Rahimi *et al*., 2010; Jamali *et al*., 2013; Lotfollahi *et al*., 2017) or the world (Barbuddhe *et al*., 2000; Osman *et al*., 2014; Bogdanovičová *et al*., 2015; Bogdanovièová *et al*., 2016). However, a much higher contamination rate (11.5%) has been reported in sheep milk samples from Karak district, Jordan (AL-Tahiri *et al*., 2008). On the other hand, in some studies, the organism was found to be absent from goat and sheep milk (Foschino *et al*., 2002; Morgan *et al*., 2003; Atil *et al*., 2011; Cavicchioli *et al*., 2015). Such discrepancies could be attributed to differences in husbandry and feeding strategies, on-farm hygienic practices, herd size, and isolation procedures (Dalzini *et al*., 2016). In line with this explanation, the *L. monocytogenes* isolates were all from the rural region, where indoor housing was more common than in tribal regions. Furthermore, the rural pastures were

contaminated with untreated sewage and human waste, which may favor fodder crop contamination with *L. monocytogenes*. Among the foodborne pathogens, *L. monocytogenes* seems to be a major concern to the food industry because of its ubiquity in the environment and resistance to the damaging effects of low pH, low temperature, and high salt concentration (Lotfollahi *et al*., 2017). For instance, *L. monocytogenes* has been found to survive a mandatory ripening period of two months in raw milk cheese (Nair *et al*., 2004). Precautions should, therefore, be taken to minimize the risk of contamination.

The efficiency of the mandatory 60-day ripening period is also questionable concerning the elimination of *E. coli* O157:H7 from raw milk cheese (Nair *et al*., 2004). Among Shiga toxin-producing *E. coli* (STEC), the O157:H7 serotype is the most common cause of human infection and death. Although cattle is considered the main reservoir of STEC, these organisms have been frequently isolated from the feces of goats, sheep, pigs, dogs, and pigeons (Picozzi *et al*., 2005). Previous studies on the prevalence of *E. coli* O157:H7 in raw goat and sheep milk have reported rates ranging from as low as 0% to more than 7% (Foschino *et al*., 2002; Cortés *et al*., 2005; Picozzi *et al*., 2005; Solomakos *et al*., 2009; Lye *et al*., 2013; Bogdanovičová *et al*., 2015). In Iran, the contamination of goat and sheep milk with STEC serotype O157 has been previously reported; however, a few isolates subjected to flagellar typing were not of the H7 subtype and were classified as O157, non-H7 strains (Farzan *et al*., 2012; Momtaz *et al*., 2012; Rahimi *et al*., 2012; Ranjbar *et al*., 2018). In this study, *E. coli* O157:H7 was isolated from five samples belonging to four rural herds. The presence of STEC in milk may be due to fecal contamination or excretion from the infected udder (Stephan *et al*., 2008). The SCC values of contaminated milk samples did not exceed $5.4 \times 10^5$ cells/ml. Thus, milk contamination might be associated with fecal contamination but not mastitis. The contamination occurred in Region D because it was close to the rural areas with high contamination to sewage. The remaining areas (A, B, and C) were tribal regions far from the contaminated sources.

Some foodborne pathogens, such as *L. monocytogenes* may lead to severe infections needing antibiotic therapy. Their antimicrobial resistance can, therefore, give rise to treatment failure. Apart from penicillins (penicillin and ampicillin) and folate pathway inhibitors (trimethoprim-sulfamethoxazole), CLSI does not define any clinical breakpoint for *Listeria* susceptibility testing (Conter *et al*., 2009). Limited information on the test antibiotics and susceptibility test method has led the researchers to use CLSI criteria for staphylococci. While some of these studies have indicated a high prevalence of antibiotic resistance among *L. monocytogenes* isolates from raw milk and dairy products (Jamali *et al*., 2013; Sharma *et al*., 2017; Akrami-Mohajeri *et al*., 2018), low prevalence has been found in others (Rahimi *et al*., 2010; Lotfollahi *et al*., 2017). Penicillin and ampicillin are still the treatment of choice for listeriosis; the second-choice therapy is a combination of trimethoprim and sulfamethoxazole (Conter *et al*., 2009). Resistance of the isolates to last-resort antibiotics serves as a warning for public health and reinforces the need to manage the persistent and widespread use of antibiotics in Iran. Using the same assessment method, much lower resistance rates have been reported in a few surveys (Conter *et al*., 2009; Prieto *et al*., 2016; Lotfollahi *et al*., 2017).

The resistance rates of *E. coli* O157:H7 isolates in the present study are similar to those reported by Solomakos *et al*. (2009). A higher resistance frequency has been obtained in several studies (Momtaz *et al*., 2012; Msolo *et al*., 2016; Ranjbar *et al*., 2018). Although some uncertainties exist over the antibiotic treatment of STEC-related infections, monitoring their resistance to the current antibiotics is crucial. Such data would help us to take steps toward controlling the widespread distribution of antibiotic resistance among the organisms of different species (Solomakos *et al*., 2009).

In the present study, *L. monocytogenes* and *E. coli* O157:H7 were isolated from four and five milk samples belonging to the rural region, respectively. More intensive housing and pasture contamination in the rural region would provide favorable conditions for milk contamination. The isolation of foodborne pathogens, albeit at low levels, raises concerns over infections arising from the consumption of raw milk and its derivatives. Antimicrobial non-susceptibility of the isolates has made the situation much worse. Efforts should, therefore, be made to increase public awareness of the necessity of pasteurization to eliminate microbial safety hazards from milk.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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