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Comparison of Shedding, and Antibiotic Resistance Properties of *Listeria monocytogenes* Isolated From Milk, Feces, Urine, and Vaginal Secretion of Bovine, Ovine, Caprine, Buffalo, and Camel Species in Iran

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ABSTRACT

Background: *Listeria monocytogenes* causes listeriosis characterized by encephalitis, septicaemia, and abortion or stillbirth. Its traditional diagnosis is based on serological responses, whileseveral molecular methods have been developed for safer and more rapid, sensitive, and accurate detection.The epidemiology, prevalence, shedding routes, and antibiotic resistance properties of *L. monocytogenes* are essentially unknown in various animals species.

Objectives: The present study was performed to study the shedding routes, and antibiotic resistance properties of *L. monocytogenes* isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel in Iran.

Materials and Methods: A total of 596 milk, 619 feces, 443 vaginal swab, and 522 urine samples were collected from various animal species. Samples were examined by culture, conventional and real-time PCR for evaluating the presence of *L. monocytogenes*. Finally antimicrobial resistance properties were studied using the simple disc diffusion method.

Results: The culture method showed that 186 of 2180 samples (8.53%) had positive results for *L. monocytogenes*. In total,61 (10.23%) milk, 40 (6.46%) feces, 43 (9.7%) vaginal swab, and 48 (9.19%) urine samples had positive results for *L. monocytogenes* using conventional PCR. After the Light Cycler real-time PCR it was recognized that 69 (11.57%) milk, 48 (7.75%) feces, 53 (11.96%) vaginal swab and 57 (10.91%) urine samples hadpositive results for the presence of *L. monocytogenes*. The sensitivity and specificity of conventional and real-time PCR were 94% and 99.1%, and 100% and 97.9%, respectively. *L. monocytogenes* had the highest shedding in bovine milk (10.83%), ovine urine (16.98%), caprine feces (14.38%), buffalo milk (11.11%), and camel vaginal secretion (15.18%). Antibiotic resistance to tetracycline (71.3%) was the highest, while the resistance to nitrofurantoin (5.72%) had the lowest frequency.

Conclusions: Shedding of *L. monocytogenes* in different animal species, and different samples are different. Due to antibiotic resistance, especially in *L. monocytogenes*, veterinarians should pay more attention to prescribe antibiotics. We recommend using the real-time PCR for safe, sensitive, and rapid detection of *L. monocytogenes* in clinical samples, and using the disk diffusion methods to prescribe suitable antibiotics.

Keywords: Listeria monocytogenes; Shedding Routes; Antibiotic Resistance; Clinical Samples

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Implication for health policy/practice/research/medical education:
Listeria monocytogenes may have different shedding patterns in various animal species. Besides, the detection of antibiotic resistance properties of *L. monocytogenes* is important from clinical and epidemiological points of view.

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1. Background

Listeria monocytogenes is a rod-shaped, opportunistic intracellular, Gram-positive, facultative anaerobic, non-spore-forming bacterium of the genus *Listeria,* which is widely spread in the environment, and can causesevere invasive diseases such as septicemia, meningoencephalitis, mastitis, and abortion in humans and animals, primarily affecting pregnant, new-born, and immunocompromised individuals(1, 2). In addition to increased incidence of Listeriosis caused by *L. monocytogenes* in the recent years (1), and zoonosis, public health and food-born aspects of the disease, damages and economic losses of thebacterium due to abortion, mastitis, and meningoencephalitis in livestock, play an effective and vital role in increasing the importance of the disease in the world.

Listeria species are tolerant to extreme conditions such as low pH, low temperature, and high salt conditions (3, 4). Therefore, the *Listeria* species can be found in a variety of environments, including sewage, water, silage, soil, effluents and foods, which doubles the problem. In total *L. monocytogenes* can cause mastitis (5), metritis, keratoconjunctivitis, encephalitis, reproductive diseases, and iritis (6-8). Several reports showed that the *L. monocytogenes* can be detected in various animal samples, such as meat (9), milk (9), and feces (10).

Previous study showed that the *L. monocytogenes* is a causative agent for 3.58% of the cases of bovine abortion (11). A previous study from Turkey showed that the prevalence of *L. monocytogenes* was 4.36% in chickens, 0.58% in sheep, and 1.53% in cattle fecal samples (12). Several studies in Australia (13), Spain (14), the USA (15), Canada (16),and Iran (17)showed 1.5% to 4.1% prevalence of *L. monocytogenes* in raw milk.

There are various methods for the diagnosis of Listeriosis such as culture, serology and molecular methods. The standard analytical methods for the detection of *L. monocytogenes* are culture and biochemical assays, but they may have some dangers for laboratory workers, and usually require at least 10 days for the complete *L. monocytogenes* identification (18). Moreover, the detection of this pathogen in clinical samples using these standard methods is difficult due tothe sporadic or low levels of contamination (<100 CFU/ g), the presence of a high level of background microflora, and competitor organisms whichcould mask the presence of *L. monocytogenes* (19).

The presence of cross contamination with other bacteria, and even fungi may exist in these traditional diagnostic methods. Despite the popularity of serological methods, they have low sensitivity and specificity for the detection of *L. monocytogenes* in clinical samples, and always take days to be completed (20). In these years DNA-based methods such as conventional PCR and real-time PCR have been developed as safe, useful, sensitive, and accurate methods for the detection of *L. monocytogenes* in clinical specimens (21, 22).

Despite these challenges, classical cultivation techniques have been still remained as the official methods used. To our knowledge, in most cases infected with *L. monocytogenes*, if the treatment be not sufficient, in addition to weakening the body immunity and susceptibility to other diseases, it can lead to death. Treatment of diseases caused by this bacterium often requires antimicrobial therapy; however antibiotic-resistant strains of bacteria cause more severe diseases for longer periods of time than their antibiotic-susceptible counterparts. Several studies have shownthat antibiotic resistantof *L. monocytogenes* is increasing day by day (23-25). Therefore, identification of antibiotic resistance properties of bacterium is very essential in the reduction of treatment costs.

2. Objectives

The epidemiology of Listeriosis is essentially unknown in Iran, and it seems that the prevalence rate, comparing-shedding routes, and antimicrobial resistance properties of *L. monocytogenes* in bovine, ovine, caprine, buffalo, and camel species in Iran havenever been reported. Therefore the two-fold purposeof the current study wasto study and compare shedding and antibiotic resistance properties of *L. monocytogenes* isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species.

3. Materials and Methods

3.1. Sample Collection

From April to November of 2011, a total of596 milk, 619 feces, 443 vaginal swab, and 522 urine samples were collected from 101 bovine, 100 ovine, 98 caprine, 91 buffalo, and 65 camel herds in various parts of Iran (*Table 1*). The animals which their milk, feces, urine, and vaginal secretions samples collected for this study were clinically healthy, and the samples hadnormal findings in physical characteristics examinations. Samples were collected under sterile conditions, and were immediately transported at 4°C to laboratory in a cooler with ice packs. All samples were kept at -20 °C until the processing.

In this study the fecal samples were placed in separate sterile plastic bags to prevent spilling, and cross contamination, and immediately transported to laboratory in an ice box. Fecal suspensions were prepared at10% to 20% (w/v) in0.01 Mphosphate-buffered saline (PBS) pH 7.2, and centrifuged at 3,000 x g for 15 min at 4 °C. The supernatants were used for DNA extraction. The vaginal secretions of each species were collected using sterile wet vaginal swabs, and immediately transported to laboratory in an ice box too.

3.2. Culture

For *L. monocytogenes* detection in samples, microbiological standard methods were used (18). Briefly, 1 g or 1 mL of each sample was aseptically taken, cultured in 9 mL of *Listeria* enrichment broth (UVM I) (Merck, Germany), and incubated at 37 °C for 24 h. One mL of primary enrichments were transferred to 9 mL of UVM II (Frazer broth) (Merck, Germany), and incubated at 37 °C for 24 h. Secondly enrichments were streaked onto Oxford agar (Merck, Germany) and Palcam agar (Merck, Germany), and incubated at 35 °C for 48 h.

The plates were examined for *Listeria* colonies (black colonies with black sunken), and at least 3 suspected colonies were subcultured on Trypton Soy agar supplemented with 0.6% of yeast extract (TSAYE) (Merck, Germany), and incubated at 37 °C for 24 h. All the isolates were subjected to standard biochemical tests including gram staining, catalase test, motility test at 25 °C, and 37 °C, acid production from glucose, mannitol, rhamnose, xylose, α-methyl-D-mamoside, and nitrate reduction, hydrolysis of esculin, MR/VP test, ß-hemolytic activity, and CAMP test.

3.3. DNA Extraction

DNA extraction was performed based on the previous method (26),which is briefly as follows; after centrifuging the enrichment culture for 10 min at 12,000g, 200 µl of the sample were transferred to the microcentrifuge tube reaction containing the ready to use lysis reagent (Listeria Short- Prep Kit, Molecular Roche), each tube was mixed by inversion for 2s, and centrifuged for 5 min at 8000g, the supernatant was discarded, and 200 µl of resuspension reagent (Listeria ShortPrep Kit, Molecular Roche) was added. The reaction tubes were placed in the cell disruption unit (Roche, Molecular) for 8 min, and the lysates were incubated for 5 min at 95 °C, and centrifuged for 13,000g at 1 min.

3.4. Conventional PCR Technique

PCR assays were performed in 50 mL reaction volumes. The primer pair consisting of primer A [5'-CAT TAG TGG AAA GAT GGA ATG -3'], and primer B [5'-GTA TCC TCC AGA GTG ATC GA -3'] was used for the amplification of a 730 bp region of the *hly* gene (27). PCR was performed in the Perkin Elmer Gen Amp PCR system 2400 thermal cycler. Amplification conditions were optimized to the thermal cycler, and were as follows: 80°C for 10 min, an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing at55 °C for 30 sec, and extension at 72 °C for 30 sec, then a final extension at 72 °C for 2 min.

The amplified DNA was analyzed by gel electrophoresis on a 1.2 % agarose gel stained with ethidium bromide (3 mL/100 mL). A 100 bp ladder (Promega) was used as a reference marker. Tris-borate EDTA (0.5´) was used as the running buffer, and the gel was viewed using UV transillumination at a wavelength of 254 nm.

3.5. Real-Time PCR Technique

Used from the assay which was developed by O'Grady et al. (28). Real-time PCR amplification was performed on the Light Cycler using the "Light Cycler Fast Start DNA master hybridization probes" kit (Roche Diagnostics, Mannheim, Germany). PCR was performed in a final volume of 20 ml including 2 ml of template DNA in 10× Light Cycler hybridization buffer with MgCl2 adjusted to 5 mM concentration. PCR primers (0.5 mM concentration), and FRET hybridization probes for *L. monocytogenes* and IAC targets (0.2 mM concentration) were added to the reaction mixture, and the volume was increased to 20 ml by addingnuclease free dH2O.

The cycling parameters consisted of: 95 °C incubation for 10 min for enzyme activation and DNA denaturation, followed by 45 PCR amplification cycles consisting of 95 °C for 10 sec, 55 °C for 20 sec, and72 °C for 10 sec. The temperature transition rate for all cycling steps was 20 °C /sec. Fluorescence acquisition was at the end of the annealing stage of each cycle. The thermocycling program was followed by a melting program of 95 °C for 1 min (denaturation), 45 °C for 30 sec (annealing), and then 45-80 °C at a transition rate of 0.1 °C /ecs with continual monitoring of fluorescence.

All subsequent analysis was performed in the F2/BackF1 (ssrA gene target), and F3/ BackF1 (IAC) channels with color compensation using the second derivative maximum option of the Light Cycler software (version3.5). A no-template negative control was included in each run. For clinical samples with negative results for *L. monocytogenes* by the standard method which yielded a positive result with the rapid method, the ssrA gene PCR product generated in the rapid method for these samples was sequenced (Sequiserve, Vaterstetten, Germany).

The sensitivity and specificity of each test weredetermined using the formulaeas follows:

Sensitivity: True positive/True positive + false negative x 100

Specificity: True negative/True negative + positive x 100

3.7. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method using Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute guidelines (29). This medium contains MH agar, 5% defibrinated horse blood,20 mg/L β-NAD (MH-F). The antimicrobial agents tested and their corresponding concentrations were as follows: sulfamethoxazol (25 µg/disk), trimethoprim (5 µg/disk), chloramphenicol (30 µg/disk), enrofloxacin (5 µg/disk), tetracycline (30 µg/disk),

gentamycin (10 μg/disk), cephalothin (30 μg/disk), ampicillin (10 u/disk), and streptomycin (10 μg/disk). Isolates were cultured in trypticase-soy broth(TSB) supplemented with 0.6% yeast extract, and transferred to Mueller–Hinton agar (Oxoid CM 337). The plates were incubated at 35°C in 5% CO2 in air for 18 h. In this study *Staphylococcus aureus* ATCC 29213 was used as the quality control.

3.8. Statistical Analysis

Data wastransferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test analysis was performed, and differences were considered significant at values of $P < 0.05$.

4. Results

In this study a total of 596 milk, 619 feces, 443 vaginal swab, and 522 urine samples from 101 bovine, 100 ovine, 102 caprine, 91 buffaloes, and 65 camel herds of Iran were tested for the presence of *L. monocytogenes* by evaluation of culture, conventional and real-time PCR assays. The culture method showed that totally 186 of 2180 samples (8.53%) including 58 (2.66%) milk, 40 (1.83%) feces, 42 (1.92%) vaginal secretions, and 46 (2.11%) urine had positive results for *L. monocytogenes* (*Table 1*).

Quality of DNA extracted after agarose gel electrophoresis from milk, feces, vaginal swab, and urine of bovine, ovine, caprine, buffalo, and camel species were considered acceptable, and diagnosed suitable for PCR assay. Results indicated that from a total of 596 milk, 619 feces, 443 vaginal swab, and 522 urine samples, 61 (10.23%), 40 (6.46%), 43 (9.7%), and 48 (9.19%) samples showed the band with the size of 730 bp in conventional PCR, and were recognized as positive for the presence of *L. monocytogenes* (*Figure 1*).

After Light Cycler Real-time PCR, 69 (11.57%) milk, 48 (7.75%) feces, 53 (11.96%) vaginal swab, and 57 (10.91%) urine hadpositive results for the presence of *L. monocytogenes* (*Table 2*). Our study showed that the sensitivity and specificity of conventional PCR, and real-time PCR assays were 94% and 99.1%, and 100% and 97.9%, respectively (*Table 3*).

Table 1. Application of Culture Method to Detect *L. monocytogenes* in Milk, Urine, Feces, and Vaginal Secretion of Bovine, Ovine, Caprine, Buffalo, and Camel Species.

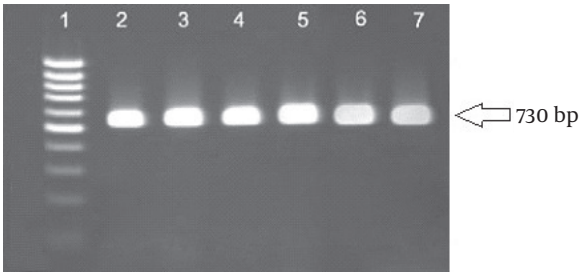
Species	Samples, No.				Culture Method (%)			
	Milk	Feces	Vaginal Swab	Urine	Milk	Feces	Vaginal Swab	Urine
Bovine	120	146	97	123	11 (9.16)	5 (3.42)	8 (8.24)	5(4.06)
Ovine	115	128	88	106	15 (13.04)	10 (8.59)	10 (11.36)	14(13.2)
Caprine	143	139	89	100	17 (12.58)	18 (13.66)	9 (11.23)	12(12)
Buffalo	108	106	81	98	9 (10.18)	4 (4.71)	5 (6.17)	7(7.14)
Camel	101	100	79	95	6 (5.94)	3 (4)	10 (13.92)	8(9.47)
Total	596	619	434	522	58 (9.73)	40 (6.46)	42(9.48)	46(8.81)

Table 2. Distribution of *L. monocytogenes* in Milk, Feces, Vaginal Secretions and Urine of Bovine, Ovine, Caprine, Buffalo, and Camel Species Using Conventional and Real-Time PCR Assays in Iran.

	Samples, No.				Conventional PCR (%)				Real-Time PCR (%)			
	Species	Samples, No.	Conventional PCR (%)	Real-Time PCR (%)	Milk	Feces	Vaginal Swab	Urine	Milk	Feces	Vaginal Swab	Urine
Milk	Feces	Vaginal Swab	Urine	Milk	Feces	Vaginal Swab	Urine	Milk	Feces	Vaginal Swab	Urine	7 (5.69)
Bovine	146	97	123	12 (10)	5 (3.42)	8 (8.24)	6 (4.87)	13 (10.83)	7 (4.79)	9 (9.27)	7 (5.69)	18 (16.98)
Ovine	115	128	88	106	16 (13.91)	10 (8.59)	10 (11.36)	15 (15.09)	17 (14.78)	11 (8.59)	13 (14.77)	18 (16.98)
Caprine	143	139	89	100	17 (12.58)	18 (13.66)	9 (11.23)	12 (12)	18 (12.58)	20 (14.38)	11 (12.35)	13 (13)
Buffalo	108	106	81	98	9 (10.18)	4 (4.71)	6 (7.4)	7(7.14)	12 (11.11)	6 (5.66)	8 (9.87)	9(9.18)
Camel	101	100	79	95	7 (6.93)	3 (4)	10 (13.92)	8(9.47)	9 (8.91)	4 (4)	12 (15.18)	10 (10.52)
Total	596	619	434	522	61 (10.23)	40 (6.46)	43 (9.7)	48(9.19)	69 (11.57)	48 (7.75)	53 (11.96)	57 (10.91)

Statistical analysis showed significant differences ($P < 0.05$) between the levels of bacterium in bovine milk andfeces and urine, ovine milk andfeces, buffalo milk andfeces, and finally camel vaginal secretions andfeces. In addition, the statistical analyses were significant ($P < 0.05$) between the levels of bacterium shedding in ovine milk and camel milk, caprine feces and camel and bovine feces, camel vaginal secretions and bovine and buffalo vaginal secretions, and finally ovine urine and bovine urine samples.

Figure 1. Conventional PCR for the Detection of *L. monocytogenes* in Various Animal Species.



1;100 bp ladder, 2-6 are positive samples for bovine, ovine, caprine, buffalo, and camel species, 7; positive control.

Our results showed that there were no significant differences between the ability of conventional PCR and real-time PCR for the detection of *L. monocytogenes* in milk, feces, vaginal swab, and urine of all species. This study showed the higher accuracy and sensitivity of real-time PCR than conventional PCR for the detection of *L. monocytogenes* in all clinical samples. Therefore the prevalence rates of *L. monocytogenes* in milk, feces, vaginal secretions, and urine of animals in Iran were 11.57%, 77.75%, 11.96%, and 10.91%, respectively (*Table 2*).

Results showed that the *L. monocytogenes* had the highest shedding in bovine milk (10.83%), ovine urine (16.98%), caprine feces (14.38%), buffalo milk (11.11%), and camel vaginal secretion (15.18%). In addition, the results of our study showed that ovine were the most sensitive, and buffalo was the most resistance species to *L. monocytogenes*, respectively.

Our results indicated that the total prevalence of re sistance to tetracycline, penicillin, streptomycin, sulfamethoxazol, gentamycin, and erythromycin were 71.3%, 43.6%, 34.8%, 33.9%, and 33.03%, respectively (*Table 4*). Also, antibiotic resistance to chloramphenicol, ampicillin, enrofloxacin, lincomycin, and cephalotin were 29.9%, 29.5%, 20.7%, 16.7%, and 15.85%, respectively (*Table 3*). Our results showed that the bacterium had a lowest antibiotic resistance to nitrofurantoin (5.72%), trimethoprim (11.01%) and ciprofloxacin (11.8%) while tetracycline had the highest antibiotic resistance (71.3%). Results showed that all *L. monocytogenes* isolated from milk, feces, urine, and vaginal secretions of bovine, ovine, caprine, buffalo, and camel species had resistance to one or more antibiotics. Statistical analysis showed significant differences ($P < 0.05$) between resistances to nitrofurantoin and tetracycline in all animal species.

Table 3. Evaluation of Sensitivity and Specificity of Conventional PCR and Real-Time PCR for the Detection of *L. monocytogenes* in All Clinical Samples. The Sensitivity andSpecificity of the Conventional Method Were 94% and 99.1%, and the Sensitivity and Specificity of the Real-Time PCR Were 100% and 97.9%.

	Culture Positive	Culture Negative	Total
Conventional PCR positive	175	17	192
Conventional PCR negative	11	1977	1988
Total	186	1994	2180
Real-time PCR positive	186	41	227
Real-time PCR negative	0	1953	1953
Total	186	1994	2180

5. Discussion

The present study was designed for the detection of *L. monocytogenes* in milk, urine, feces, and vaginal secretions of bovine, ovine, caprine, camel, and buffalo species using conventional and real-time PCR assays, and also evaluating their performance as sensitive, specific, and accurate diagnostic methods. Results showed that the real-time PCR assays due to higher sensitivity and accuracy was more suitable for the detection of *L. monocytogenes* in clinical samples. In addition, the real-time PCR assay had some advantages compared to the conventional PCR; it was demonstrated as an important diagnostic tool yielding reliable and reproducible results, and didnot require post-PCR analysis (gel electrophoresis, hybridization). Besides, the real-time PCR has a limited risk of cross contamination than conventional PCR. But the real-time PCR is more expensive than conventional PCR.

This study showed that the conventional PCR method is more technically time-consuming and labour-intensive

Table 4. AntibioticResistance Properties of *L. monocytogenes* Isolated From Milk, Feces, Urine, and Vaginal Secretion of Bovine, Ovine, Caprine, Buffalo, and Camel (Disk Diffusion Method).

L.monocytogenes(+)[a]	P10[b] (%)	TE30 (%)	S10 (%)	C30 (%)	SXT (%)	GM10 (%)	E15 (%)	NFX5 (%)	L2 (%)	CF30 (%)	CIP5 (%)	TMP5 (%)	F/M300 (%)	AM10 (%)
Bovine milk (13)	7	9	2	2	5	6	7	5	3	3	3	2	1	5
Bovine urine (7)	4	6	1	1	2	3	3	2	1	1	1	1	-	3
Bovine feces (9)	5	7	2	2	3	3	3	3	2	2	-	-	-	2
Bovine vaginal secretion (7)	4	5	2	2	1	2	2	1	1	-	-	1	-	3
Total Bovine (36)	20 (55.5)	27 (75)	7 (19.4)	7 (19.4)	11 (30.5)	14 (38.8)	15 (41.6)	11 (30.5)	7 (19.4)	6 (16.6)	4 (11.1)	4 (11.1)	1(2.7)	13()
Ovine milk (17)	10	15	9	9	10	10	10	8	3	3	2	2	2	9
Ovine urine (11)	6	8	4	4	6	5	5	3	2	1	1	1	-	5
Ovine feces(13)	6	10	3	3	3	4	4	1	2	1	2	1	1	3
Ovine vaginal secretion (18)	10	15	9	8	11	11	10	8	5	5	4	3	3	9
Total Ovine (59)	32 (54.2)	48 (81.3)	25 (42.3)	24 (40.6)	30 (50.8)	30 (50.8)	30 (50.8)	20 (33.8)	12 (20.3)	10 (16.9)	9 (15.2)	7 (11.8)	5(8.4)	26(44.06)
Caprinemilk (18)	9	15	6	5	8	8	7	4	3	3	3	3	3	5
Caprineurine (20)	12	17	12	8	10	10	8	3	5	4	3	3	2	8
Caprinefeces (11)	3	6	4	2	2	1	2	1	1	-	-	2	-	1
Caprinevaginal secretion (13)	2	7	2	1	1	2	3	-	-	1	1	1	-	2
TotalCaprine(62)	27 (43.5)	45 (72.5)	24 (38.7)	16 (25.8)	21 (33.8)	21 (33.8)	20 (32.2)	8 (12.9)	9 (14.5)	8 (12.9)	7 (11.2)	9 (14.5)	5(8.06)	16(25.8)
Buffalo milk (12)	7	11	7	8	2	2	2	1	2	3	1	-	-	2
Buffalo urine (6)	1	3	1	1	1	1	1	-	-	1	-	-	-	-
Buffalo feces (8)	1	3	2	2	1	1	-	-	-	-	1	1	-	-
Buffalo vaginal secretion (9)	2	4	2	1	2	2	1	1	2	1	1	1	1	2
Total Buffalo (35)	11 (31.4)	21 (60)	12 (34.2)	12 (34.2)	6 (17.1)	6 (17.1)	4 (11.4)	2 (5.7)	4 (11.4)	5 (14.2)	3 (8.5)	2 (5.7)	1(2.8)	4(11.4)
Camel milk (9)	2	5	2	1	1	2	1	2	1	3	-	-	-	3
Camel urine (4)	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Camelfeces (12)	3	8	4	3	4	2	2	3	2	2	3	1	-	3
Camel vaginal secretion (10)	3	7	4	5	6	2	3	1	3	2	1	2	1	2
Total camel (35)	9 (25.7)	21 (60)	11 (31.4)	9 (25.7)	11 (31.4)	6 (17.1)	6 (17.1)	6 (17.1)	6 (17.1)	7 (20)	4 (11.4)	3 (8.5)	1(2.8)	8(22.8)
Total (227)	99 (43.6)	162 (71.3)	79 (34.8)	68 (29.9)	79 (34.8)	77 (33.9)	75 (33.03)	47 (20.7)	38 (16.7)	36 (15.85)	27 (11.8)	25 (11.01)	13(5.72)	67(29.5)

[a] positive results were from the real-time PCR assay
[b] this table P10= penicillin (10 u/disk); TE30= tetracycline (30 μg/disk); S10= streptomycin (10 μg/disk); C30= chloramphenicol (30 μg/disk); SXT= sulfamethoxazol (25 μg/disk); GM10= gentamycin (10 μg/disk); E15= erythromycin (15 μg/disk); NFX5= enrofloxacin (5 μg/disk); L2= lincomycin (2 μg/disk); CF30= cephalothin (30 μg/disk); CIP5= ciprofloxacin (5 μg/disk); TMP5= trimethoprim (5 μg/disk); F/M300= nitrofurantoin (300 μg/disk); AM10= ampicillin (10 u/disk).

for detecting *L. monocytogenes* than the real-time PCR assay. The real-time PCR assay which was used in this study could simplify the procedure by testing presumptive *L. monocytogenes* genome taken directly from clinical samples. In addition, compared with culture, the PCR has the primary advantages of being more sensitive and more rapid. However, the conventional PCR had a higher specificity than the real-time method (99.1% versus 97.9%) but due to higher sensitivity, safety, lower risks to laboratories and time wasted we recommended using real-time PCR for the detection of *L. monocytogenes* in clinical samples.

Since, PCR has been developed for the detection of *L. monocytogenes* in a wide variety of clinical samples such as salads (30), meta (31), water (32), salmon products (33), cheese (34), food(35), milk (36), urine (37), fecal (37), vaginal swab (38),and in all of these studies PCR has been introduced as an accurate and sensitive assay for the detection of *L. monocytogenes*. Many studies showed that in most cases animal products such as unpasteurized milk, soft cheeses, raw milk, butter, chocolate milk, and even poultry products are the main sources for human listeriosis (39-42). Our results showed that the livestock resources such as milk, feces, urine, and vaginal fluids could contaminate the environment, animals and human with *L. monocytogenes*. Our results showed that bovine milk (10.83%), ovine urine (16.98%), caprine feces (14.38%), buffalo milk (11.11%), and camel vaginal secretions (15.18%) had the highest levels of *L. monocytogenes*. So it can be concluded that *L. monocytogenes* can be a potential factor for causing abortion in camel, and mastitis in bovine and buffalo. Besides, *L. monocytogenes* is able to substitution in ovine urine and caprine feces.

To our knowledge, *L. monocytogenes* can causes encephalitis, uterine infections (abortion and septicemia in neonates), eye infections, and keratitis in ruminants. *L. monocytogenes* can be shed in the fecal material of clinically affected animals; however, healthy animals also can be latent *L. monocytogenes* carriers (43). Previous study showed that up to 50% of fecal samples collected from animals with no clinical symptoms of listeriosis (including sheep, cattle, pigs, goats, and poultry) may contain *L. monocytogenes* (44). Although most animal listeriosis appears to be caused by ingestion of silage contaminated with high levels of *L. monocytogenes*, but not all cases are feedborne (45). It seems that, the *L. monocytogenes* are tolerant to extreme conditions such as low temperature, low pH, and even high salt conditions. Therefore, it can be found in a variety of environments, including sewage, soil, silage, effluents, water, and foods. Therefore, it is enough for thehuman and animalsto use these contaminated resources like foods and silages to become infected. It seems that the silages of infected animals of our study are contaminated with soil, sewage or had the low pH.

Unfortunately, there is no available data or study about the detection of *L. monocytogenes* in clinical samples such as milk, feces, urine, and vaginal secretions of various animal species, and our study is the first report of direct detection of bacterium in these clinical samples. Another study (46) showed that from the total 24 case farms enrolled, 16 had case animals with encephalitis, 4 had case animals with abortions, 3 had cows with clinical mastitis, and 1 had an animal with keratitis.

This previous survey indicated that a total of 414 samples (107 fecal, 120 soil, 87 feedstuff, and 100 water) hadpositive results for *L. monocytogenes*, yielding an overall prevalence of 20.1% (overall, 22.2% of samples collected on bovine farms, and 16.8% of samples collected on small-ruminant farms tested positive for *L. monocytogenes*) (46). Studies showed that the gastrointestinal tract of animals, environment, and skin of the teats are the most common sources for *L. monocytogenes* in raw milk (47, 48). Previous study showed that shedding of *Listeria* into bovine milk (49) is less frequent, which isin contrast with our results.

In the past, there wasno antibiotic resistance for the*Listeria* species, and all of them were uniformly susceptible to common antibiotics including ampicillin or penicillin, trimethoprim, tetracyclines, erythromycin, and gentamicin (50, 51). The first antibiotic-resistant in *L. monocytogenes* was described in 1988 (23), and then many more resistant strains have been detected in food and sporadic cases of listeriosis (24, 52). To our knowledge, these antibiotic resistances occurred duringseveralyears due to irregular andsteadyuse ofantibiotics. It seems that antibiotic resistances of animal species have beentransferred to human through foods with animal origin.

Study in Botswana showed that from the total 57 samples with positive results of *L. monocytogenes,* 31 (54.39%) were resistant to one or more antibiotics. This study on Botswana showed that resistance to penicillin G, sulphamethoxazole/trimethoprim, chloramphenicol, and tetracycline were 42.11, 29.82%, 28.30%, and 22.81%, respectively (53). Our study showed that *L. monocytogenes* had the highest antibiotic resistance to tetracycline (71.3%), which was higher than the USA (8.4%) (25). Previous study showed that tetracycline resistance is thought to originate from the use of antibiotic in animal production (54). In another study disc diffusion method showed that all strains of *L. monocytogenes* which were isolated from food samples were susceptible to penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin, and trimethoprim (55).

This study showed that the sensitivity of the only one *L. monocytogenes* against streptomycin was 12.2%, but our results indicated 34.8% resistance of the bacterium to streptomycin. Morobe *et al.* showed that resistance to streptomycin was 19.30% in various food samples (53). Our study showed that *L. monocytogenes* had variable resistance to different antibiotics in various species. For example resistance to streptomycin in bovine,

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cies. For example resistance to streptomycin in bovine, ovine, caprine, buffalo, and camel species were 19.4%, 42.3%, 38.7%, 34.2%, and 31.4%, respectively. In addition to above, resistance against one antibiotic was very different between various sources of bacterium in a species. For example resistance to penicillin in *L. monocytogenes* isolated from milk, feces, urine, and vaginal secretions of a species hadmanydisputes.

Our results indicated that resistance against chloramphenicol was 29.9%. Chloramphenicol is a forbidden antibiotic, and the high antibiotic resistance to chloramphenicol in our study indicated the irregular and unauthorized duse of this drugin veterinary treatment in Iran. Unfortunately, veterinarians in many fields of veterinary such as large animal internal medicine, poultry and even aquaculture, use this antibiotic as a basic one. Therefore, ina very short period of time, antibiotic resistancehas been appeared. In many cases of listeriosis in animals in Iran, penicillin is one the useful choices, but our results showed high resistance against this drug (43.6%), and it was similar to other previous studies which have reported 66.7% (56), 5% (57), and 83% (58) resistance to penicillin. Facinelli *et al.* (52) showed that four strains of *L. monocytogenes* were resistant to one or more antibiotics (erythromycin, kanamycin, gentamicin, rifampicin, SXT, tetracycline). According to the results of Yucel *et al.* among the *Listeria* isolates, only *L. monocytogenes* was resistant to SXT (66%) (59).

The multiple antibiotic resistances of *L. monocytogenes* which were seen in our study, and many other previous researches (60-62) showed that the veterinarians should pay more attention to prescribethe antibiotics

Our data suggests that (i) animals secretion such as milk, urine, vaginal fluid, and even feces can play important roles in the epidemiology and distribution of *L. monocytogenes* in environment; (ii) the shedding routes of *L. monocytogenes* differ between bovine, ovine, caprine, buffalo, and camel species; (iii) all animals of this study contributeto amplification and dispersal of *L. monocytogenes* into the farm environment; (iv) *L. monocytogenes* had the highest shedding in bovine milk (10.83%), ovine urine (16.98%), caprine feces (14.38%), buffalo milk (11.11%), and camel vaginal secretion (15.18%).; (v) *R*eal-time PCR was more rapid, safe, sensitive, and accurate than conventional PCR for the detection of *L. monocytogenes* in clinical samples; (vi) Preventionfromcutting down the pH of silage, and contamination of animal's silage with sewage and soil can be effective in the control of listeriosis in animals; (vii) thepresent study is the first report of *L. monocytogenes* shedding routes in bovine, ovine, caprine, buffalo, and camel species in the world; (viii) *L. monocytogenes* isolated from bovine, ovine, caprine, buffalo, and camel milk, feces, vaginal secretions, and urine samples wasresistantto all penicillin, tetracycline, streptomycin, chloramphenicol, sulfamethoxazol, gentamycin, erythromycin, enrofloxacin, lincomycin, cephalothin, ciprofloxacin, trimethoprim, nitrofurantoin, and ampicillin antibiotics, but due to highest resistance to tetracycline and lowest resistance to nitrofurantoin we recommend the use of nitrofurantoin instead of tetracycline in antibiotic prescription; (ix) we recommendto reduceantibioticprescribing, particularly in theveterinary medicine, and in prescribed cases use the disc diffusion method in Mueller–Hinton agar for evaluation theantibiotic resistance pattern.

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Authors' Contribution

None declared.

References

1. Choi WS, Hong CH. Rapid enumeration of Listeria monocytogenes in milk using competitive PCR. *Int J Food Microbiol.* 2003;**84**(1):79-85
2. Rossmanith P, Krassnig M, Wagner M, Hein I. Detection of Listeria monocytogenes in food using a combined enrichment/real-time PCR method targeting the prfA gene. *Res Microbiol.* 2006;**157**(8):763-71
3. Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Comparative assessment of acid, alkali and salt tolerance in Listeria monocytogenes virulent and avirulent strains. *FEMS Microbiol Lett.* 2005;**243**(2):373-8
4. Sleator RD, Gahan CG, Hill C. A postgenomic appraisal of osmotolerance in Listeria monocytogenes. *Appl Environ Microbiol.* 2003;**69**(1):1-9
5. Fedio WM, Schoonderwoerd M, Shute RH, Jackson H. A case of bovine mastitis caused by Listeria monocytogenes. *Can Vet J.* 1990;**31**(11):773-5
6. Erdogan HM, Cetinkaya B, Green LE, Cripps PJ, Morgan KL. Prevalence, incidence, signs and treatment of clinical listeriosis in dairy cattle in England. *Vet Rec.* 2001;**149**(10):289-93
7. Low JC, Donachie W. A review of Listeria monocytogenes and listeriosis. *Vet J.* 1997;**153**(1):9-29
8. Sanchez S, Studer M, Currin P, Barlett P, Bounous D. Listeria keratitis in a horse. *Vet Ophthalmol.* 2001;**4**(3):217-9
9. Ibrahim A, Mac Rae IC. Incidence of Aeromonas and Listeria

spp. in red meat and milk samples in Brisbane, Australia. *Int J Food Microbiol.* 1991;**12**(2-3):263-9
10. Fenlon DR. Wild birds and silage as reservoirs of Listeria in the agricultural environment. *J Appl Bacteriol.* 1985;**59**(6):537-43
11. Kirkbride CA. Bacterial agents detected in a 10-year study of bovine abortions and stillbirths. *J Vet Diagn Invest.* 1993;**5**(1):64-8
12. Kalender Hakan. Detection of Listeria monocytogenes in Faeces from Chickens, Sheep and Cattle in Elaz Province. *Turk J Vet Anim Sci.* 2003;**27**:449-451
13. Deutz A, Pless P, Koefer J. Examination of raw cows and ewes milk for human pathogens. *Ernaehrung.* 1999;**23**:359-362
14. Gaya P, Sanchez J, Medina M, Nuñez M. Incidence of Listeria monocytogenes and other Listeria species in raw milk produced in Spain. *Food Microbiol.* 1998;**15**(5):551-555
15. Barton W, Draughon FA, Davidson PM, Oliver SP. Prevalence of Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica, and Salmonella in bulk tank milk: risk factors and risk of human exposure. *J Food Prot.* 1992;**55**(2):93-97
16. Fedio WM, Jackson H. Incidence of Listeria monocytogenes in raw bulk milk in Alberta. *Canadian Institute Food Sci Technol.* 1990;**23**(4/5):236-238
17. Jami S, Jamshidi A, Khanzadi S. The presence of Listeria monocytogenes in raw milk samples in Mashhad, Iran. *Iran J Vet Res.* 2010;**11**(4):363-367
18. Ashraf Mohamed AbdEl-Malek , Sohaila Fathi Hassan Ali , Raafat Hassanein Moemen , Abdelazeem Mohamed , Khalid Ibrahim Elsayh . Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of Listeria monocytogenes. *Vet World.* 2010;**3**(8):353-359
19. Norton DM, McCamey MA, Gall KL, Scarlett JM, Boor KJ, Wiedmann M. Molecular studies on the ecology of Listeria monocytogenes in the smoked fish processing industry. *Appl Environ Microbiol.* 2001;**67**(1):198-205
20. Amagliani G, Giammarini C, Omiccioli E, Merati EG, Pezzotti G, Filippini G, et al. A combination of diagnostic tools for rapid screening of ovine listeriosis. *Res Vet Sci.* 2006;**81**(2):185-9
21. Bickley J, Short JK, McDowell DG, Parkes HC. Polymerase chain reaction (PCR) detection of Listeria monocytogenes in diluted milk and reversal of PCR inhibition caused by calcium ions. *Lett Appl Microbiol.* 1996;**22**(2):153-8
22. O' Grady J , Sedano-Balbas S, Maher M, Smith T, Barry T. Rapid real-time PCR detection of Listeria monocytogenes in enriched food samples based on the ssrA gene, a novel diagnostic target. *Food Microbiol.* 2008;**25**(1):75-84
23. Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P. Transferable plasmid-mediated antibiotic resistance in Listeria monocytogenes. *Lancet.* 1990;**335**(8703):1422-6
24. Roberts MC, Facinelli B, Giovanetti E, Varaldo PE. Transferable erythromycin resistance in Listeria spp. isolated from food. *Appl Environ Microbiol.* 1996;**62**(1):269-70
25. Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA, Meng J. Characterization of Listeria monocytogenes isolated from retail foods. *Int J Food Microbiol.* 2007;**113**(1):47-53
26. Ashraf Mohamed AbdEl-Malek , Sohaila Fathi Hassan Ali , Raafat Hassanein Moemen , Abdelazeem Mohamed , Khalid Ibrahim Elsayh . Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of Listeria monocytogenes. *Vet World.* 2010;**3**(8):353-359
27. Vanegas Maria Consuelo, Vásquez Elizabeth, Martinez Aida Juliana, Rueda Adriana M. Detection of Listeria monocytogenes in raw whole milk for human consumption in Colombia by real-time PCR. *Food Control.* 2009;**20**(4):430-432
28. Gilot P, Content J. Specific identification of Listeria welshimeri and Listeria monocytogenes by PCR assays targeting a gene encoding a fibronectin-binding protein. *J Clin Microbiol.* 2002;**40**(2):698-703
29. O'Grady J, Ruttledge M, Sedano-Balbas S, Smith TJ, Barry T, Maher M. Rapid detection of Listeria monocytogenes in food using culture enrichment combined with real-time PCR. *Food Microbiol.* 2009;**26**(1):4-7
30. National Committee for Clinical Laboratory Standards , National Committee for Clinical Laboratory Standards Villanova, PA. *Performance Standards for Antimicrobial Disc Susceptibility Test.* 1988.
31. Berrada H, Soriano JM, Pico Y, Manes J. Quantification of Listeria monocytogenes in salads by real time quantitative PCR. *Int J Food Microbiol.* 2006;**107**(2):202-6
32. Kawasaki S, Fratamico PM, Horikoshi N, Okada Y, Takeshita K, Sameshima T, et al. Evaluation of a multiplex PCR system for simultaneous detection of Salmonella spp., Listeria monocytogenes, and Escherichia coli O157:H7 in foods and in food subjected to freezing. *Foodborne Pathog Dis.* 2009;**6**(1):81-9
33. Nogva HK, Rudi K, Naterstad K, Holck A, Lillehaug D. Application of 5'-nuclease PCR for quantitative detection of Listeria monocytogenes in pure cultures, water, skim milk, and unpasteurized whole milk. *Appl Environ Microbiol.* 2000;**66**(10):4266-71
34. Rodriguez-Lazaro D, Jofre A, Aymerich T, Garriga M, Pla M. Rapid quantitative detection of, Listeria monocytogenes in salmon products: evaluation of pre-real-time PCR strategies. *J Food Prot.* 2005;**68**(7):1467-71
35. Rudi K, Naterstad K, Dromtorp SM, Holo H. Detection of viable and dead Listeria monocytogenes on gouda-like cheeses by real-time PCR. *Lett Appl Microbiol.* 2005;**40**(4):301-6
36. Koo K, Jaykus LA. Detection of Listeria monocytogenes from a model food by fluorescence resonance energy transfer-based PCR with an asymmetric fluorogenic probe set. *Appl Environ Microbiol.* 2003;**69**(2):1082-8
37. Amagliani G, Omiccioli E, Campo A, Bruce IJ, Brandi G, Magnani M. Development of a magnetic capture hybridization-PCR assay for Listeria monocytogenes direct detection in milk samples. *J Appl Microbiol.* 2006;**100**(2):375-83
38. Lotfollahi Lida, Nowrouzi Jamileh, Irajian Gholamreza, Masjedian Faramarz, Kazemi Bahram, Eslamian Laleh, et al. Prevalence and antimicrobial resistance profiles of Listeria monocytogenes in spontaneous abortions in humans. *African J Microbiol Res.* 2011;**5**(14):1990-1993
39. Kaur S, Malik SV, Vaidya VM, Barbuddhe SB. Listeria monocytogenes in spontaneous abortions in humans and its detection by multiplex PCR. *J Appl Microbiol.* 2007;**103**(5):1889-96
40. Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, et al. An outbreak of gastroenteritis and fever due to Listeria monocytogenes in milk. *N Engl J Med.* 1997;**336**(2):100-5
41. Headrick ML, Tollefson L. Food borne disease summary by food commodity. *Vet Clin North Am Food Anim Pract.* 1998;**14**(1):91-100
42. Maijala R, Lyytikainen O, Autio T, Aalto T, Haavisto L, Honkanen-Buzalski T. Exposure of Listeria monocytogenes within an epidemic caused by butter in Finland. *Int J Food Microbiol.* 2001;**70**(1-2):97-109
43. Ryser Elliot T, Marth Elmer H. *Listeria, listeriosis, and food safety.* 2007.
44. Pell AN. Manure and microbes: public and animal health problem? *J Dairy Sci.* 1997;**80**(10):2673-81
45. Meng J, Doyle MP. Emerging issues in microbiological food safety. *Annu Rev Nutr.* 1997;**17**:255-75
46. Wiedmann M, Mobini S, Cole JR, Jr, Watson CK, Jeffers GT, Boor KJ. Molecular investigation of a listeriosis outbreak in goats caused by an unusual strain of Listeria monocytogenes. *J Am Vet Med Assoc.* 1999;**215**(3):369-71
47. Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, et al. Ecology and transmission of Listeria monocytogenes infecting ruminants and in the farm environment. *Appl Environ Microbiol.* 2004;**70**(8):4458-67
48. Harvey J, Gilmour A. Occurrence of Listeria species in raw milk and dairy products produced in Northern Ireland. *J Appl Bacteriol.* 1992;**72**(2):119-25
49. Origin of bovine raw milk contamination by Listeria monocytogenes. Austria: Wolfpassing Co. 1996.
50. O'Donnell ET. The incidence of Salmonella and Listeria in raw milk from farm bulk tanks in England and Wales. *Int J Dairy Technol.* 1995;**48**(1):25-29
51. Rota Carmina, Yanguela Javier, Blanco Domingo, Carramilana

Juan J, Arino Agustin, Herrera Antonio. High Prevalence of Multiple Resistance to Antibiotics in 144 Listeria Isolates from Spanish Dairy and Meat Products. *J Food Prot.* 1996;**59**(9):938-943
52. Teuber M. Spread of antibiotic resistance with food-borne pathogens. *Cell Mol Life Sci.* 1999;**56**(9-10):755-63
53. Facinelli B, Giovanetti E, Varaldo PE, Casolari P, Fabio U. Antibiotic resistance in foodborne Listeria. *Lancet.* 1991;**338**(8777):1272
54. Morobe IC, Obi CL, Nyila MA, Gashe BA, Matsheka MI. Prevalence, antimicrobial resistance profiles of Listeria monocytognes from various foods in Gaborone, Botswana. *Afr J Biotechnol.* 2011;**8**(22)
55. Schroeder CM, Zhao C, DebRoy C, Torcolini J, Zhao S, White DG, et al. Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food. *Appl Environ Microbiol.* 2002;**68**(2):576-81
56. Altuntas Evrim Gunes, Kocan Deniz, Cosansu Serap, Ayhan Kamuran, Juneja Vijay K, Materon Luis. Antibiotic and Bacteriocin Sensitivity of Listeria monocytogenes Strains Isolated from Different Foods. *Food and Nutrition.* 2012;**3**:363-368
57. Santos Mantilla SP, Franco RM, Trindade de Oliveira LA, Santosi EB, Gouvea R. [Antimicrobial resistance in bacteria of the genus Listeria spp. isolated from ground beef cattle]. *Braz J Vet Res Anim Sci.* 2008;**45**(2):2
58. Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. Prevalence and antibiotic susceptibility of Listeria spp. isolated from raw meat and retail foods. *Food Control.* 2010;**21**(5):708-713
59. Chen BY, Pyla R, Kim TJ, Silva JL, Jung YS. Antibiotic resistance in Listeria species isolated from catfish fillets and processing environment. *Lett Appl Microbiol.* 2010;**50**(6):626-32
60. Yücel N, Çıtak S, Önder M. Prevalence and antibiotic resistance of Listeria species in meat products in Ankara, Turkey. *Food Microbiol.* 2005;**22**(2–3):241-245
61. Cosansu Serap, Kuleasan Hakan, Ayhan Kamuran, Materon Luis. antimicrobial activity and protein profiles of pediococcus spp. isolated from turkish "sucuk". *J Food Processing and Preservation.* 2007;**31**(2):190-200
62. Rodas-Suarez OR, Flores-Pedroche JF, Betancourt-Rule JM, Quinones-Ramirez EI, Vazquez-Salinas C. Occurrence and antibiotic sensitivity of Listeria monocytogenes strains isolated from oysters, fish, and estuarine water. *Appl Environ Microbiol.* 2006;**72**(11):7410-2
63. Wikler Matthew A. *Performance standards for antimicrobial susceptibility testing: sixteenth informational supplement.* 2006.