Avicenna J Clin Microb Infec. 2015 August; 2(3): e26905. DOI: 10.17795/ajcmi-26905

Published online 2015 August 1. **Research Article**

Phenotypic and Genotypic Characteristics of *Listeria monocytogenes* Isolated From Dairy and Meat Products

Abbas Bahador [1]; Behrooz Sadeghi Kalani [2]; Firuzeh Valian [3]; Gholamreza Irajian [2,*] ; Lida Lotfollahi [4,*]

[1]Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, IR Iran
[2]Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, IR Iran
[3]Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, IR Iran
[4]Department of Microbiology, Faculty of Medicine, UrmiaUniversity of Medical Sciences, Urmia, IR Iran

[*]*Corresponding authors*: Gholamreza Irajian, Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188058649, Fax: +98-2188058649, E-mail: Dr.irajian@gmail.com; Lida Lotfollahi, Department of Microbiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, IR Iran. Tel: +98-9144470366, Fax: +98-2188058649, E-mail: liphd83@yahoo.com

Received: January 11, 2015**; Revised:** March 11, 2015**; Accepted:** April 15, 2015

Background: *Listeria monocytogenes* is a foodborne pathogen and a serious threat to the public health in the world. Consumption of traditional foods such as dairy and meat products can be a major reason for relative abundance and isolation of these bacteria.
Objectives: The purpose of this study was to determine the phenotypic and genotypic characteristics of *L. monocytogenes* strains isolated from dairy and meat products.
Materials and Methods: A total of 317 dairy products and meat-processed samples were collected. Antibiotic susceptibility test was performed on each sample by the disk diffusion method (Kirby Bauer). Five reference loci were used for typing of *L. monocytogenes* strains by MLVA (Multiple Locus VNTR Analysis) Technique.
Results: A total of 24 *L. monocytogenes* isolates were collected from the dairy and meat products. Resistance of isolated *L. monocytogenes* strains to penicillin G were 54.54% (from dairy products) and 46.15% (from processed meat). Genetic relatedness of isolates were assessed by MLVA. Out of 13 different types, type 2 with 6 strains and type 3 with 4 strains, were the most common types.
Conclusions: MLVA analysis showed that samples obtained from different sources could have similar genetic profile. As a result, administration of penicillin in patients with listeriosis (especially pregnant women) and antibiotic susceptibility test are recommended. The fast and accurate methods such as MLVA for tracking of pollution sources of *L. monocytogenes* are recommended during outbreaks.

Keywords: Genotypic; Dairy Products; Meat; *Listeria monocytogenes*

1. Background

Listeria monocytogenes is an omnipresent bacterium that can cause severe disease in humans and animals (1). For instance, although there were only 2500 ailments each year in the US, *L. monocytogenes* infections account for 4% of all hospitalizations and 28% of all deaths from foodborne illnesses (2). According to food net US, listeriosis is responsible for 30% of foodborne deaths from 1996 to 2005 and had a high case fatality rate of 16.9% (3). The main mode of transmission of *Listeria* is through environmental impurity of ready-to-eat food products throughout their production and processing (4-6). In recent years, several outbreaks of listeriosis have been related to contaminated trading nutrients, for instance vegetables, milk, and meat foodstuffs (7, 8).

Listeria infection typically needs antimicrobial treatment to heal. Recommendations are penicillin G or ampicillin combined (or not) with an aminoglycoside. However, little information is available on the resistance of *L. monocytogenes*, predominantly for non-human-origin isolates (9). Isolates of *L. monocytogenes* should be characterized in order to precisely diagnose their infection, comprehend the epidemiology of infection, consider outbreaks, and efficiently prevent and reduce the spread of *Listeria* through the food chain and other routes. Since many strains of *L. monocytogenes* exist, it is indispensable to have a sound system of subtyping so that the most effective strategies can be implemented for the control of outbreaks (10). The phenotypic subtyping methods are usually less sensitive, have low distinctive ability, and are not easy to reproduce, whereas the genotypic methods are more sensitive and reliable (10). In recent years, multiple Locus VNTR analysis (MLVA) is used for subtyping *L. monocytogenes* strains (11). "VNTR" is a "variable-number tandem repeat". MLVA is a method employed for the genetic analysis of particular microorganisms such as pathogenic bacteria. It takes advantage of the polymorphism of tandemly repeated DNA sequences and utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found

in many different loci in the genome of a variety of organisms. This method is widely used to assess the molecular fingerprint of microorganisms such as bacteria and perform molecular typing of particular bacteria. There are several methods for *L. monocytogenes* typing, including repetitive element palindromic PCR (rep-PCR), multilocus sequence typing (MLST), esterase electrophoresis (zymotyping), random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE), and restriction fragment length polymorphism (RFLP) of ribosomal DNA (ribotyping), however, MLVA method has a high discriminatory power with high-throughput screening that is fairly inexpensive, easy to perform, rapid, and reliable. Therefore, MLVA technique is well suited to interlaboratory comparisons during epidemiological investigations of infection and preferentially it is used in molecular typing of bacteria. According to previous studies, MLVA has a sensitivity equal to that of PFGE and a specificity superior to that of PFGE (12-15).

2. Objectives

The purpose of this study was to determine the phenotypic and genotypic characteristics of *L. monocytogenes* strains isolated from dairy and meat products.

3. Materials and Methods

3.1. Bacterial Strains

During November 2010 to December 2013, a total of 317 samples (including dairy products and meat processed) were collected from Tehran, Iran (Table 1). All samples were transferred to TSBYE (Tryptic soy broth positive 0.6% yeast extract, Merck, Germany) and incubated at 4°C. After 7 - 16 days till 6 months incubation, samples were cultured on PALKAM Agar (Merck, Germany) and *Listeria* selective agar (Himedia, India), then, plates were incubated at 35°C for 24-48 h. Grown colonies were recognized using microbiological and biochemical tests, including Gram staining, catalase reaction, oxidase test, hemolysis on Sheep Blood Agar, Christie Atkins Munch Petersen (CAMP) test, Voges-Proskauer (MR-VP), Methyl Red tests and fermentation of sugars (xylose, rhamnose, mannitol, and methyl α-D-mannopyranoside).

3.2. Antimicrobial Susceptibility Test

Antibiotic susceptibility test was done by the disk diffusion method (Kirby Bauer) (16). The turbidity of broth after incubation was adjusted with sterile saline to achieve turbidity comparable to 0.5 McFarland standards.

Results were interpreted according to the clinical and laboratory standards institute (guidelines M45-A2). Eight antibiotic disks, including penicillin G (10U), chloramphenicol (10 μg), tetracycline (25 μg), trimethoprim (5 μg), streptomycin (10 μg), ampicillin (10 μg), ciprofloxacin (5 μg), and erythromycin (15 μg) (Himedia, India) were used. *L. monocytogenes* ATCC 7644 was used as the reference strain.

3.3. Multiple-Locus Variable Number of Tandem Repeat Analysis Typing

DNA extraction was prepared from full-grown colonies at 37°C overnight in brain heart infusion broth (BHI) by using a DNA extraction kit protocol (Roche Co, New York, USA). Five reference loci (Lm10, Lm11, Lm23, Lm32, and LM-TR6) and 5 corresponding primer pairs previously described (Table 2), were used to perform typing of *L. monocytogenes* strains by MLVA technique (17). Specificity of all primers was verified using the basic local alignment search tool (BLAST). PCR technique was used for amplification of each locus. The reaction mixture consisted of 2 μL of extracting DNA, 2.5 μL of 10 × PCR buffer, 1.5 μL MgCl2 (50 mM), 0.5 μL dNTP (10 mM), 1.25 μL of each primer, 0.4 μL of Taq DNA polymerase (5 U/μL) and deionized water to a final volume of 25 μL. The reaction mixture was amplified in a thermocycler (Eppendorf, Germany) in the following PCR conditions: denaturation at 94°C for 5 minutes, 31 cycles with denaturation at 94°C for 30 s, annealing at 52°C for 20 s, extension at 72°C for 45 seconds, and final extension at 72°C for 5 minutes. The results of PCR products were further analyzed by electrophoresis in 3% agarose gel for 120 minutes in tris-acetate buffer, visualized by ethidium bromide staining, illuminated by UV-Transilluminator and accepted by a gel documentation apparatus (UVP Gel Seq Software, England). A 50 bp DNA ladder (fermentas) was used as a size reference.

Table 1. Resources, the Number and Percentage of Samples Examined in This Study [a]

Sources	Number of Sample	Number of Isolated Bacteria
Dairy		
Cheese	70 (4.65)	5 (1.7)
Cream	20 (7.18)	3 (15)
Curd	17 (9.15)	3 (6.17)
Total	107	11 (2.10)
Processing Meat		
Sausage	90 (9.42)	6 (6.6)
Meat extracts	55 (2.26)	4 (2.7)
Chicken extract	65 (9.30)	3 (6.4)
Total	210	13 (1.6)

[a] Values are presented as No. (%).

Table 2. Characteristics of Selected TR Loci for MLVA Sub-Typing of *L. monocytogenes*

VNTR Locus	Primers	TR Sequence	Protein Description or Function
Lm10	F-CAGATATCGATACGATTGAC	-GAAGAACCAAAA-	ATP-dependent metalloprotease FtsH
	R-CAGTTAGTATTTCCAACGTC		
Lm11	F-GAATAAAATGCTAGATGTGG	-TTGCTTGTTTTTG-	Cell wall surface anchor family protein
	R-CCGATTCAAAAATAGTAAAC		
Lm23	F-TATTTACGGAAAAGACGTAG	-CATCGG-	Putative peptidoglycan bound protein (LPXTG motif)
	R-CGTAACTGTCCTACCATTAG		
Lm32	F-AAAGCTTTGCCAGTGCAAGT	-AACACC-	Hypothetical protein
	R-TTGTGACTTGGCACTTCTGG		
LM-TR6	F-AAA AGC AGC GCC ACT AAC G-	-CCAGACCCAACA-	Hypothetical protein
	R-TAA AAA TCC CAA TAA CAC TCC TGA-		

3.4. Calculation of the Number of Tandem

The following formula was used to calculate the number of tandem repeats (Equation 1):

$$(1)\ \text{Tandem repeat} = \frac{\text{PCR product size} - (\text{The size of the right flank region} + \text{The size of the left flank region})}{\text{Tandem repeat size}}$$

The flanking regions with known sizes were previously described (11, 15). In addition, to calculate the PCR product molecular weight from the images obtained on the gel, GeneTools from SynGene Version 3. 08 Software were used (Figure 1).

3.5. Charting of Phylogenic Tree

After converting the number of tandem repeat of each locus to the corresponding sequences of isolates, evolutionary strain diagram was drawn by using of MEGA 6.06 software.

4. Results

4.1. Bacterial Isolation

Out of 317 samples, 24 *L. monocytogenes* isolates were collected from the dairy and meat products (Table 1).

4.2. Antimicrobial Susceptibility Testing of L. monocytogenes

The response of *L. monocytogenes* to the various antimicrobial agents is listed in Table 3. High resistance to penicillin was found amongst isolates. Resistance to penicillin G among strains isolated from dairy samples and processing meat were 54.54% and 46.15%, respectively. Antibiotic resistance was not found for tetracycline, trimethoprim, ciprofloxacin, ampicillin, and erythromycin.

4.3. Multiple Locus VNTR Analysis Typing

After analysis of gel photo PCR product by Genetools software, the number of tandem repeats was calculated by using the described formula (Table 4) and the number of repeats per locus isolates was typed. To this effect, strains that have 80% or more than 80% similarity were placed at one type and other strains were placed on the different types. Finally, 13 different types were obtained as well as type 2 with 6 strains and type 3 with 4 strains, respectively, which were the most abundant types (Table 4).

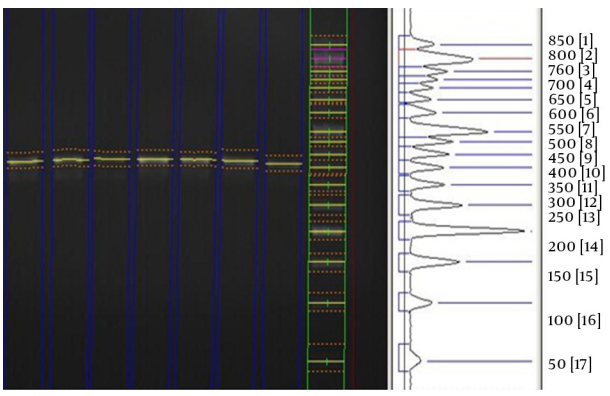

Figure 1. Photo of GeneTools Software (Version 3. 08) Environment to Calculate the Size of the PCR Product

Table 3. Susceptibility of *L. monocytogenes* Strains to 8 Antimicrobial Agents [a,b]

Antibiotic	Dairy Samples (Total = 11)			Processing Meat Samples (Total = 13)			Total Samples (24)		
	R	I	S	R	I	S	R	I	S
Chloramphenicol	1 (9.09)	4 (36.36)	6 (54.54)	0 (0)	6 (46.15)	7 (53.84)	1 (4.16)	10 (41.66)	13 (54.16)
Penicillin G	6 (54.54)	4 (36.36)	1 (9.09)	6 (46.15)	4 (30.76)	3 (23.07)	12 (50)	8 (33.33)	4 (16.66)
Streptomycin	1 (9.09)	2 (18.18)	8 (72.72)	0 (0)	6 (46.15)	7 (53.84)	1 (4.16)	8 (33.33)	15 (62.5)
Tetracycline	0 (0)	1 (9.09)	10 (90.90)	0 (0)	3 (23.07)	10 (76.9)	0 (0)	4 (16.66)	20 (83.33)
Trimethoprim	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	24 (100)
Ciprofloxacin	0 (0)	1 (9.09)	10 (90.90)	0 (0)	3 (23.07)	10 (76.9)	0 (0)	4 (16.66)	20 (83.33)
Ampicillin	0 (0)	1 (9.09)	10 (90.90)	0 (0)	1 (7.69)	12 (92.3)	0 (0)	2 (8.33)	22 (91.66)
Erythromycin	0 (0)	2 (16.6)	9 (81. 81)	0 (0)	1 (7.69)	12 (92.3)	0 (0)	3 (12.5)	3 (87.5)

[a] Abbreviations: I, intermediate resistance; R, resistant; and S, susceptible.
[b] Values are presented as No. (%).

Table 4. Types, Number, Sources, and the Number of Tandem Repeats per Lux of *L. Monocytogenes* Isolates

Strain	Source	LM10	LM11	LM23	LM32	LM-TR6	Type Number
1	Cheese	3	4	28	19	0	1
2	Cheese	3	4	28	24	0	1
3	Cheese	4	0	28	21	0	5
4	Cheese	4	3	28	24	3	6
5	Cheese	5	3	30	23	3	2
6	Cream	5	3	30	15	3	2
7	Cream	5	3	30	15	3	2
8	Cream	5	3	31	15	3	2
9	Curd	5	3	0	15	3	7
10	Curd	4	3	31	15	3	3
11	Curd	4	3	31	21	3	3
12	Sausage	0	4	38	16	0	8
13	Sausage	4	3	31	16	3	3
14	Sausage	4	3	31	16	1	3
15	Sausage	5	3	30	16	1	2
16	Sausage	4	4	31	19	0	4
17	Sausage	4	4	31	19	0	4
18	Meat extracts	5	3	30	15	2	2
19	Meat extracts	4	4	31	19	0	4
20	Meat extracts	6	3	30	13	2	9
21	Meat extracts	5	1	30	22	2	10
22	Chicken extract	6	1	30	21	2	11
23	Chicken extract	5	2	30	22	3	12
24	Chicken extract	5	2	0	22	3	13

4.4. Phylogenic Tree

Evolutionary relationships tree of strains are shown in Figure 2.

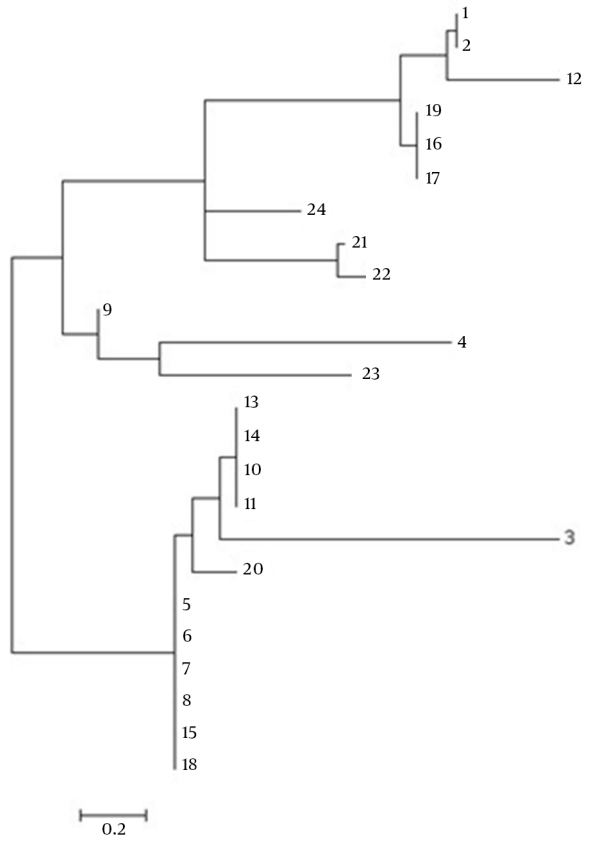

Figure 2. Dendrogram of the MLVA Results for the *L. monocytogenes* Isolates

5. Discussion

The actual prevalence of *Listeria monocytogenes* in Iran is unknown and little information about *L. monocytogenes* is available. In addition, this disease has not been reported in the Iranian health system. In this study, the prevalence of *L. monocytogenes* was 7.5%. In a study in 2009 by Morobe (18), the prevalence of *L. monocytogenes* in food samples was reported at 3.4%, which was not consistent with our study. In addition, in a study during 2002 - 2004 conducted in Brazil, the bacteria were isolated from 3.4% of food samples (19). Arslan and colleagues studied the prevalence of *L. monocytogenes* in Turkish white cheese house-cooked. The overall prevalence of various strains of *Listeria* in cheese was 1.33% and *L. monocytogenes* was isolated from 2.9% of the samples (20). In the study by Jalali et al. on the frozen meat, fresh meat, beef and mutton samples, totally 4 (2.1%) *L. monocytogenes* were isolated (21).

In Northern Ireland in 2003, 205 samples of poultry meat packed two large sales were made in 14 cases (18%) of *L. monocytogenes* was isolated (125) that corresponded with the present study. These various findings could be due to differences in sample size, geographic area, or processing of food production. Until now, it seemed that *L. monocytogenes* remains sensitive to the antibiotics used to treat listeriosis, however, in recent years, resistant strains of bacteria to antibiotics have been reported (22).

In the present study, *L. monocytogenes* strains isolated from food samples were susceptible to trimethoprim, erythromycin, ciprofloxacin, ampicillin, and tetracycline. The highest sensitivity (100%) was to trimethoprim. In addition, strains resistant to penicillin (50%) were observed. Prazak and Murano in their study reported the isolation of bacteria susceptible to all antibiotics except penicillin, which corresponds to the results of our study (23). Arslan and Ozdemir (20) in their study reported resistance to penicillin and chloramphenicol, which are in agreement with the results of the present study. Morvan et al. (24) did not report any case of resistance to penicillin in France (24). Penicillin resistance in our study (but susceptibility to these antibiotics in Morvan et al. (24) study) is possibly due to differences in drug use practices between two countries and the indiscriminate use of antibiotics, and sometimes without a doctor's prescription is in our country.

In the present study, we used the MLVA technique. In this study, 5 loci (Lm10, Lm11, Lm 23, Lm 32, and LM-TR6) were used for MLVA-typing. By using this technique, strains were successfully differentiated from each other and 13 different types were obtained. Type 2 with 6 strains and type 3 with 4 strains were the most abundant types, respectively. In this study, some strains isolated from cheese, cream, and sausage were at one type (type 1), also some strains isolated from meat and meat extracts were at one type (type 4). These findings show that the source of infection could be the same. In addition, strains isolated from chicken extracts were in different types, which show the different sources of pollution.

In 2006, Murphy and colleagues, for the first time were able to differentiate 45 foodborne *L. monocytogenes* strains using MLVA (11). They used 6 loci and their results were consistent with the results of current study. In another study, Chen et al. (14) and colleague isolated a total of 46 strains of *L. monocytogenes* from food samples and typed them by MLVA. Their results were in agreement with the results of the present study too. The results of our study show the high frequency of *L. monocytogenes* in dairy and meat products and resistance to penicillin is significant too. Processing of these foods to eliminate this bacterium is not suitable, and consumption of these foods for people at risk, especially pregnant women is very dangerous. Moreover, because penicillin with gentamicin is the treatment of choice for listeriosis in Iran and other countries, antibiotic susceptibility testing for on time and precise treatment is crucial. Given the importance of identifying potential sources of pollution, particularly the incidence of epidemics, utilizing a quick

and inexpensive technique with high contrast resolution is necessary. In this regard, MLVA technique is perfect for these purposes, and is recommended due to its simplicity. According to study conducted by Noller et al. (25), MLVA method has a sensitivity equal to that of PFGE and a specificity superior to that of PFGE in outbreak detection.

MLVA analysis showed that samples obtained from different sources could have similar genetic profile. As a result, administration of penicillin in patients with listeriosis (especially pregnant women) is suggested and antibiotic susceptibility test recommended. The fast and accurate methods such as MLVA for tracking of pollution sources of *L. monocytogenes* are recommended during outbreaks because of its ideal properties, high sensitivity, and specificity comparing with other methods.

Acknowledgements

All authors appreciated Department of Microbiology, Tehran University of Medical Sciences.

Authors' Contributions

This study designed, drafted, analyzed, and supervised by Gholamreza Irajian and Abbas Bahador. The results were analyzed by Behrooz Sadeghi Kalani. Abazar Pournajaf and Mansour Sedighi reviewed the draft. Tahmineh Narimani and Behzad Emadi performed the laboratory procedures

Funding/Support

This study supported by Tehran University of Medical Sciences, Tehran, IR Iran.

References

1. Farber JM, Peterkin PI. Listeria monocytogenes, a food-borne pathogen. *Microbiol Rev.* 1991;**55**(3):476–511.
2. Mead PS, Slutsker L, Griffin PM, Tauxe RV. Food-related illness and death in the united states reply to dr. hedberg. *Emerg Infect Dis.* 1999;**5**(6):841–2.
3. Barton Behravesh C, Jones TF, Vugia DJ, Long C, Marcus R, Smith K, et al. Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996-2005. *J Infect Dis.* 2011;**204**(2):263–7.
4. Bernbom N, Vogel BF, Gram L. Listeria monocytogenes survival of UV-C radiation is enhanced by presence of sodium chloride, organic food material and by bacterial biofilm formation. *Int J Food Microbiol.* 2011;**147**(1):69–73.
5. de Oliveira MM, Brugnera DF, Alves E, Piccoli RH. Biofilm formation by Listeria monocytogenes on stainless steel surface and biotransfer potential. *Braz J Microbiol.* 2010;**41**(1):97–106.
6. Gueriri I, Cyncynatus C, Dubrac S, Arana AT, Dussurget O, Msadek T. The DegU orphan response regulator of Listeria monocytogenes autorepresses its own synthesis and is required for bacterial motility, virulence and biofilm formation. *Microbiology.* 2008;**154**(Pt 8):2251–64.
7. Meldrum RJ, Ellis PW, Mannion PT, Halstead D, Garside J, Welsh Food Microbiological F. Prevalence of Listeria monocytogenes in ready-to-eat foods sampled from the point of sale in Wales, United Kingdom. *J Food Prot.* 2010;**73**(8):1515–8.
8. Uyttendaele M, Busschaert P, Valero A, Geeraerd AH, Vermeulen A, Jacxsens L, et al. Prevalence and challenge tests of Listeria monocytogenes in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. *Int J Food Microbiol.* 2009;**133**(1-2):94–104.
9. Granier SA, Moubareck C, Colaneri C, Lemire A, Roussel S, Dao TT, et al. Antimicrobial resistance of Listeria monocytogenes isolates from food and the environment in France over a 10-year period. *Appl Environ Microbiol.* 2011;**77**(8):2788–90.
10. Jadhav S, Bhave M, Palombo EA. Methods used for the detection and subtyping of Listeria monocytogenes. *J Microbiol Methods.* 2012;**88**(3):327–41.
11. Murphy M, Corcoran D, Buckley JF, O'Mahony M, Whyte P, Fanning S. Development and application of Multiple-Locus Variable number of tandem repeat Analysis (MLVA) to subtype a collection of Listeria monocytogenes. *Int J Food Microbiol.* 2007;**115**(2):187–94.
12. Miya S, Kimura B, Sato M, Takahashi H, Ishikawa T, Suda T, et al. Development of a multilocus variable-number of tandem repeat typing method for Listeria monocytogenes serotype 4b strains. *Int J Food Microbiol.* 2008;**124**(3):239–49.
13. Kerouanton A, Brisabois A, Denoyer E, Dilasser F, Grout J, Salvat G, et al. Comparison of five typing methods for the epidemiological study of Listeria monocytogenes. *Int J Food Microbiol.* 1998;**43**(1-2):61–71.
14. Chen S, Li J, Saleh-Lakha S, Allen V, Odumeru J. Multiple-locus variable number of tandem repeat analysis (MLVA) of Listeria monocytogenes directly in food samples. *Int J Food Microbiol.* 2011;**148**(1):8–14.
15. Sperry KE, Kathariou S, Edwards JS, Wolf LA. Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping Listeria monocytogenes strains. *J Clin Microbiol.* 2008;**46**(4):1435–50.
16. Clinical Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing, twenty-first informational supplement. M02-A10 and M07-A8," CLSI Document M100-S21.*Wayne: CLSI; 2011.
17. Li X, Huang B, Eglezos S, Graham T, Blair B, Bates J. Identification of an optimized panel of variable number tandem-repeat (VNTR) loci for Listeria monocytogenes typing. *Diagn Microbiol Infect Dis.* 2013;**75**(2):203–6.
18. Morobe IC. *Prevalence, antimicrobial profiles, molecular serotyping and toxigenicity of" listeria monocytogenes" isolated from food in Gabarone, Botswana*. Botswana: University of South Africa; 2009.
19. de Souza VM, Franceschini SA, Martinez RCR, Ratti RP, De Martinis ECP. Survey of Listeria spp. in matched clinical, food and refrigerator samples at home level in Brazil. *Food Control.* 2008;**19**(10):1011–3.
20. Arslan S, Ozdemir F. Prevalence and antimicrobial resistance of Listeria spp. in homemade white cheese. *Food Control.* 2008;**19**(4):360–3.
21. Jalali M, Abedi D. Prevalence of Listeria species in food products in Isfahan, Iran. *Int J Food Microbiol.* 2008;**122**(3):336–40.
22. Charpentier E, Gerbaud G, Jacquet C, Rocourt J, Courvalin P. Incidence of antibiotic resistance in Listeria species. *J Infect Dis.* 1995;**172**(1):277–81.
23. Prazak MA, Murano EA, Mercado I, Acuff GR. Antimicrobial resistance of Listeria monocytogenes isolated from various cabbage farms and packing sheds in Texas. *J Food Prot.* 2002;**65**(11):1796–9.
24. Morvan A, Moubareck C, Leclercq A, Herve-Bazin M, Bremont S, Lecuit M, et al. Antimicrobial resistance of Listeria monocytogenes strains isolated from humans in France. *Antimicrob Agents Chemother.* 2010;**54**(6):2728–31.
25. Noller AC, McEllistrem MC, Pacheco AG, Boxrud DJ, Harrison LH. Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic Escherichia coli O157:H7 isolates. *J Clin Microbiol.* 2003;**41**(12):5389–97.