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Serotyping of *Listeria monocytogenes* Isolates from Women with Spontaneous Abortion Using Polymerase Chain Reaction Method

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ABSTRACT

Background: *Listeria monocytogenes* infection during pregnancy may cause spontaneous abortion or stillbirth, or the birth of babies with neonatal meningitis. The aim of this study was to serotype *L. monocytogenes* isolated from women with spontaneous abortion using polymerase chain reaction.

Methods: Vaginal swab samples were obtained from 96 women with spontaneous abortion. The microbial culture was performed on blood agar and PALCAM agar followed by differential biochemical tests for characterization of *L. monocytogenes* isolates. Besides, total DNA was extracted from each vaginal specimen, and PCR assay was then carried out using specific primers for target genes of this bacterial species.

***Results*:** Microbial culture and PCR revealed 4 and 16 *L. monocytogenes* isolates (out of 96 vaginal specimens), respectively. There was a significant association between consuming unsterilized dairy products and the risk of *Llisteria* infection ($P < 0.001$). Various serotypes of *L. monocytogenes* were detected as follows; the serotypes 1/2b, 3b (31.25%), the serotypes 1/2c, 3c (31.25%), the serotypes 1/2a, 3a (25%), and the serotypes 4 (12.5%).

***Conclusion*:** However, the difference between frequencies of observed serotypes was not statistically significant ($P < 0.05$). The 1/2b, 3b, and 1/2c, 3c serotypes of *L. monocytogenes* are more commonly seen in vaginal specimens of women with spontaneous abortion, and PCR technique as a convenient tool may be used for identifying of causative strains. However, further studies need to improve and optimize this approach.

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Introduction

Listeria monocytogenes is a Gram-positive, facultatively anaerobic, motile, rod-shaped intracellular bacterium belonging to the family of *Listeriaceae* (1, 2). *L. monocytogenes* has been found in more than 60 animal species, predominantly in mammalians and birds (3, 4). It can also be isolated from soil, silage, and some other environmental sources (4, 5). For example, various subtypes of *L. monocytogenes* have been detected in food samples and food-processing plants by means of molecular techniques (6-9).

As an emerging food-borne pathogen, *L. monocytogenes* can cause an invasive infectious disease termed as listeriosis or may lead to another non-invasive form of infection-related illnesses known as febrile gastroenteritis (10-12). Infection with *L. monocytogenes* during pregnancy may cause spontaneous abortion or stillbirth (12, 13), and sometimes leads to the birth of babies with neonatal meningitis (14, 15).

Several virulence determinants have been reported to be responsible for the pathogenicity of *L. monocytogenes* (16, 17). For instances, it is suggested that expression of the *plcA* gene as an essential virulence factor plays a crucial role in the pathogenicity of *L. monocytogenes* (18). The *hlyA* gene coding for listeriolysin O contributes to digestion of host-cell phagosomal membrane and thereby to the release and extensive multiplication of the bacterium into the cytosol (19). Like many other bacteria, genes coding for virulence-associated factors of *L. monocytogenes* have been considered as efficient targets and are widely used for serotyping of this bacterium (20).

L. monocytogenes strains are mainly serotyped based on the variation in flagellar (H) and somatic (O) antigens (21). Common serotypes of *L. monocytogenes* have been categorized into two main phylogenetic divisions using various molecular serotyping methods; division I encompasses serotypes 1/2b, 3b, 4b, 4d, and 4e, and Division II contains serotypes 1/2a, 3a, 1/2c, and 3c (22, 23). Less common serotypes of *L. monocytogenes* including 4a and 4c have been placed under division III (24). The serotypes 1/2a, 1/2b, and 4b are the most common serotypes of *L. monocytogenes* isolated from the vast majority of clinical samples (25, 26).

Regarding the clinical importance of *L. monocytogenes*, herein, we aimed to isolate the serotypes of this bacterium from vaginal specimens of women with spontaneous abortion in Iran and identify them using molecular methods.

Materials and Methods

Patients and Specimens

Study subjects consisted of 96 women with symptoms of bleeding and pain referred to medical centers of Shahid Beheshti University of Medical Sciences during June 2012 to May 2013, pre-diagnosed with spontaneous abortion, and underwent curettage before the 20th week of pregnancy. Demographic and clinical data of the patients including; age, history of infections, and consumption of disinfected versus non-disinfected vegetable and pasteurized versus local unpasteurized dairy products were recorded using a questionnaire.

A gynecologist in the operating room by means of vaginal swabs and conventional sampling method performed sampling. All the specimens were inoculated into sterile tubes containing 10 ml of Tryptic Soy Broth Yeast Extract (TSBYE) transport medium (Merck, Germany), and then were immediately transferred to microbiology research laboratory located at the university, under ice-cold sterile condition.

Cultivation Conditions and Identification Tests

After the lapse of three-month incubation at 4 °C, cultivation of all specimens was performed. Briefly, 0.1 ml of each sample aliquot was plated onto blood agar (Merck, Germany) and PALCAM agar (Merck, Germany), separately and incubated at 37 °C for 24 to 48 h. The greenish-black shiny colonies caused by Esculin hydrolysis on

PALCAM agar and yellow small colonies with ß-hemolysis on blood agar were isolated as listeria, and then were subjected to various confirmatory tests followed as; Gram-staining, oxidase, catalase, sugar use and fermentation (mannitol, rhamnose and xylose), methyl red–Voges-Proskauer (MR-VP) test, and motility on semisolid medium at 20-25 ºC (13).

DNA extraction

DNA of each aliquot from the TSBYE enrichment medium was extracted using Accuprep Genomic DNA Extraction Kit (Bioneer Co., Korea) according to the manufacturer's guideline. In details, 200 µl of each sample was individually added into 1.5 ml micro-tubes containing 200 µl of binding buffer and 20 µl of proteinase K, then mixed and incubated at 60°C for 20 min. Afterwards, 100 µl of isopropanol was admixed to each micro-tube, and the resultant lysate was centrifuged at 8000 rpm for one min. Next, the binding column was transferred to a new 1.5 ml micro-tube, and 500 µl of each washing buffer I and II were added, respectively, each followed by a centrifugation at 12000 rpm for one min. Then, 200 µl of the elution buffer was added to the binding column in new a 1.5 ml micro-tube and the mixture was centrifuged at 8000 rpm for one min. Finally, the extracted DNA was placed into a 0.5 ml micro-tube and stored at -20 °C until the polymerase chain reaction (PCR) assay was performed (13).

Molecular serotyping of listeria by PCR assay

In the first step, molecular detection of *L. monocytogenes* was carried out by recruiting two pairs of primers designed for two virulence-associated genes of the bacterium, *plcA*, and *hlyA*, as we have previously described (27). In the second step, *L. monocytogenesis*-positive samples were subjected to PCR using special primers for *D1* and *D2* for categorization into divisions I/III and II, respectively. Finally, the third PCR assay was performed using the special primers for *GLT* gene on D1-positive samples, and for *flaA* gene on *D2*-positive samples. All the primers designed and used in this study are presented in Table 1. The standard strain ATCC7644 of *L. monocytogenes* was used as positive control.

The PCR assay for each gene of interest was carried out in a final volume of 25 µl consisting of; 12 µl of PCR master mix (Ampliqon 2740, Denmark), 1 µl of forward primer, 1 µl of reverse primer, 3 µl of template DNA and 8 µl of double distilled water (ddH2O)(28).

Finally, PCR product together with 6X loading buffer was added onto ethidium bromide-containing 1.5 % agarose gel in Tris-Borate-Ethylen ediamine tetra acetic acid (Tris-Borate-EDTA or TBE) and was run at 95 V for 45 min, and was then visualized under Ultraviolet trans illuminator (DOC-008.XD, UV Tech, European Economic Community). Standard 50 and 100 bp ladders (GeneRuler, Fermentas, Thermo Fisher Scientific, and USA) were used for determination and verification of PCR product bands of interest.

Ethics

All women involved in the study read and signed an informed consent form. Sampling was performed by an expert gynecologist doctor. An identification code was assigned to each specimen to provide confidentiality and to avoid any disclosure of identity and personal information of the subjects in any stages of the study. Also, the Ethics Committee based at the university verified all the experimental methods and the information use. No financial cost was imposed to the participants.

Statistical Analysis

All data were analyzed by chi-square (χ^2) and proportional test using Stata/SE 11.0 software. Results are expressed as frequency and percentage. The differences with P value less than 0.05 were considered as statistically significant.

Result

A total of 96 women with mean age, 30.9 ± 4.7 years were included in the study. Demographic and clinical features of the patients are given in Table 2. Following culture and differential tests, *L. monocytogenes* was detected in only 4 of 96 vaginal specimens (4.2%), while the results of PCR assay for genes *hlyA* and *plcA* revealed that 16 of 96 vaginal specimens (16.7%) were positive for the bacterium. Following chi-square analysis, a significant difference (P<0.01) was found between the sensitivity of PCR assay compared to the culture (100% over 36.4%), although both methods demonstrated similar specificity calculated as 100%.

As shown in Table 2, with regard to the frequencies of listeria positivity, there was a significant difference between patients consuming sterilized and unsterilized dairy products (3 of 58 versus 13 of 38; P<0.001). But, frequencies of the listeria positive patients were not significantly different regarding the history of infections (3 of 58 versus 13 of 38; P>0.05), or type (disinfected versus non-disinfected) of vegetable consumption (5 of 44 versus 11 of 52; P> 0.05).

The results of PCR for serotyping of *L. monocytogenes* using *D1*- and *D2*-specific primers showed that among 16 listeria-positive specimens, 7 (43.8%) were positive for *D1* primers and 9 (56.2%) positive for *D2* primers, categorized into divisions I/III and II, respectively (Fig. 1a and b). However, no significant difference was observed between the frequencies of *L. monocytogenes* in divisions I/III and II (P= 0.479).

When *D1*-positive specimens were subjected to PCR using the primers specific for *GLT* gene, the results revealed that among 5 of 7 specimens (71.43%) were positive for *GLT*, hence recognized as serotypes 1/2b, 3b, and 2 others (28.57%) were negative for this gene which was recognized as serotypes 4 of *L. monocytogenes* (Fig. 2a). In the current study, further identification of sub-types of serotypes 4 was not pursued. Following the proportional test analysis, it was demonstrated that the frequency of 1/2b, 3b serotypes of *L. monocytogenes* was insignificantly higher than that of serotypes 4 (P= 0.109).

In addition, PCR assay using the primers specific for *flaA* gene detected 4 positive specimens for this gene out of 9 *D2*-positive specimens (44.44%) which were considered as 1/2a, 3a serotypes of *L. monocytogenes*. Also, 5 of 9 D2-positive specimens (55.56%) were negative for *flaA* amplification and thereby were regarded as serotypes 1/2c, 3c (Fig 2b). According to the results of the proportional test, no significant difference was observed between the frequencies of 1/2a, 3a serotypes, and 1/2c, 3c serotypes of *L. monocytogenes* (P= 0.637).

Moreover, among 16 positive specimens (16.7% of all studied specimens), frequency percentage of the serotypes 1/2b, 3b were calculated as 31.25%, the serotypes 4 as 12.5%, the serotypes 1/2a, 3a as 25% and the serotypes 1/2c, 3c as 31.25%. However, following proportional test no significant difference was observed between frequencies of serotypes 1/2b, 3b and 1/2c, 3c (P= 1.00), serotypes 1/2b, 3b and 1/2a, 3a (P= 0.200), serotypes 1/2b, 3b and 4 (P=0.694), and serotypes 1/2a, 3a and 4 (P=0.365).

Furthermore, regarding all 96 studied specimens, the serotypes 1/2b, 3b and 1/2c, 3c appeared with the highest overall frequency, each showed a prevalence of 5.2%. The overall frequency of serotypes 1/2a, 3a was estimated as 4.2%. The serotypes 4 exhibited the lowest overall frequency calculated as and 2.1%, using PCR screening technique. However, following proportional test there was no significant difference between overall frequencies of the serotypes 1/2b, 3b and 1/2c, 3c (P= 1.00), serotypes 1/2b, 3b and 1/2a, 3a (P= 0.733), serotypes 1/2b, 3b and 4 (P= 0.248), and serotypes 1/2a, 3a and 4 (P= 0.407).

Discussion

Listeria monocytogenes is a predominant food-borne pathogen causing listeriosis and leads to spontaneous abortion of a fetus in the second or

third trimester of pregnancy (29). Hence, serotyping of *L. monocytogenes* to define predominant strains might be of particular importance in the elucidation of the etiology and also in the clinical management of Listeria-related spontaneous abortions. It is clear that conventional serotyping methods of *L. monocytogenes* are noneconomical and reliable in terms of money, time, accuracy, and delicacy (30, 31). Besides, some molecular approaches particularly PCR have drawn much attention in today's clinical microbiology, and in the diagnosis of infectious diseases and related disorders (31). Thus, PCR seems to be a good candidate as a tool for characterization of *L. monocytogenes* serotypes in clinical samples (28).

To the best of our knowledge, the present study is the first to characterize and investigate the prevalence of various serotypes of L. monocytogenes isolates from women with spontaneous fetus abortion; we recruited both conventional methods and PCR assay for detection of *L. monocytogenes* followed only by PCR for identification of its serotypes.

Our results indicated that PCR technique is a more sensitive tool than microbial culture and biochemical tests for detection of *L. monocytogenes*. In addition, we found a significant association between consuming unsterilized dairy products and listeria infection, while no significant association was seen between neither history of infection nor consuming non-disinfected vegetables and risk of listeria infection in our study. In addition, we showed that detection of different serovars of *L. monocytogenes* is efficiently possible by PCR technique.

Our findings support many preceding studies suggesting application of molecular approaches to diagnosis and management of *L. monocytogenes*-related and non-related infections and disorders (28, 31). By way of example, Jeffers and colleagues detected 39 distinct strains of *L. monocytogenes* from animal and human isolates, using combination of automated ribotyping and restriction fragment length polymorphism (RFLP) typing methods (32). Shayan and colleagues detected *L. monocytogenes* in 36 out of 100 vaginal specimens from women using PCR technique, while only 7% of the case was detected by conventional culture method (32). However, Shalaby and colleagues demonstrated that there was no difference between sensitivity of the conventional methods and PCR technique in detection of *L. monocytogenes* isolates from different clinical samples, yet considering the PCR technique as a more suitable approach in terms of quick detection and identification of the bacterium (31); in agreement with our findings and also with those of Jeffers and colleagues (33), and Shayan and colleagues (32).

Table 1. Primers used for detection and serotyping of *L. monocytogenesis*.

Primers	Primer sequences	Annealing
HlyA	ATCAGTGAAGGGAAAATGCAAGAAG TTGTATAAGCAATGGGAACTCCTGG	**59**
PlcA	ATGTTAAGTTGAGTACGAATTGCTC TACGATGAGCTATAACGGAGACATG	**51**
D1	CGATATTTTATCTACTTTGTCA TTGCTCCAAAGCAGGGCAT	**59**
D2	GCGGAGAAAGCTATCGCA TTGTTCAAACATAGGGCTA	**59**
GLT	AAAGTGAGTTCTTACGAGATTT AATTAGGAAATCGACCTTCT	**59**
flaA	TTACTAGATCAAACTGCTCC AAGAAAAGCCCCTCGTCC	**59**

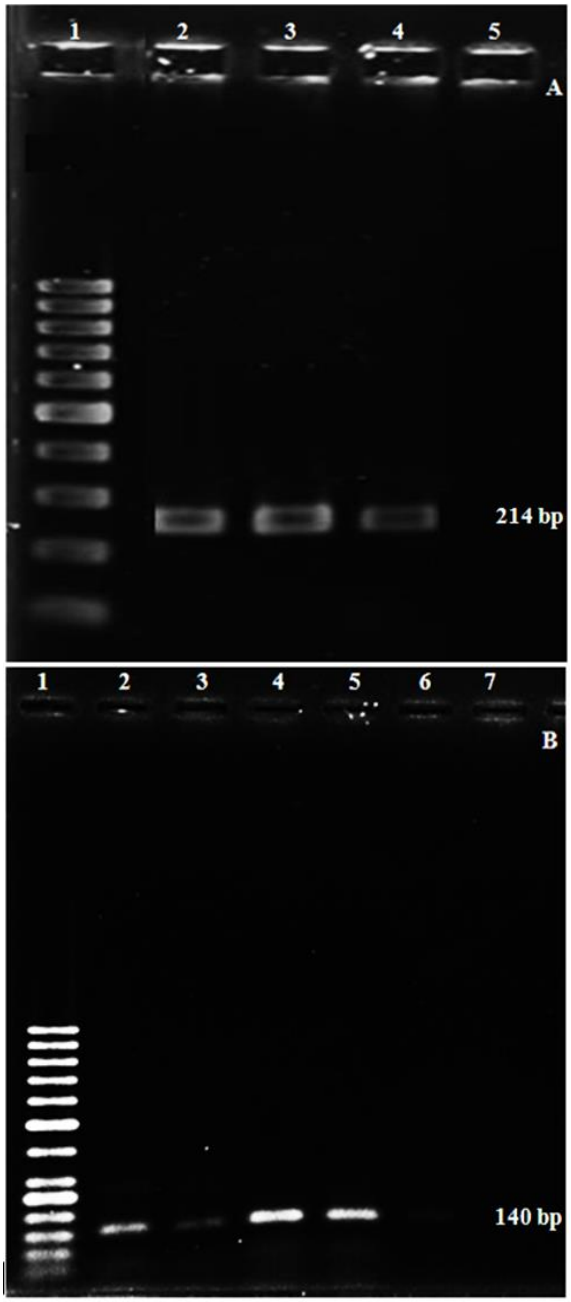

Figure 1. Gel electrophoresis analysis on PCR product of *D1* and *D2* primers of *L. monocytogenesis*: A) lane 1; DNA ladder 100 bp, lanes 2, 3, and 4; 214 bp bands of *D1*, lane 5; negative for *D1*. B) lane 1; DNA ladder 50 bp, lanes 2, 3, 4, 5 and 6; 140 bp bands of D2, lane 7; negative for *D2*.

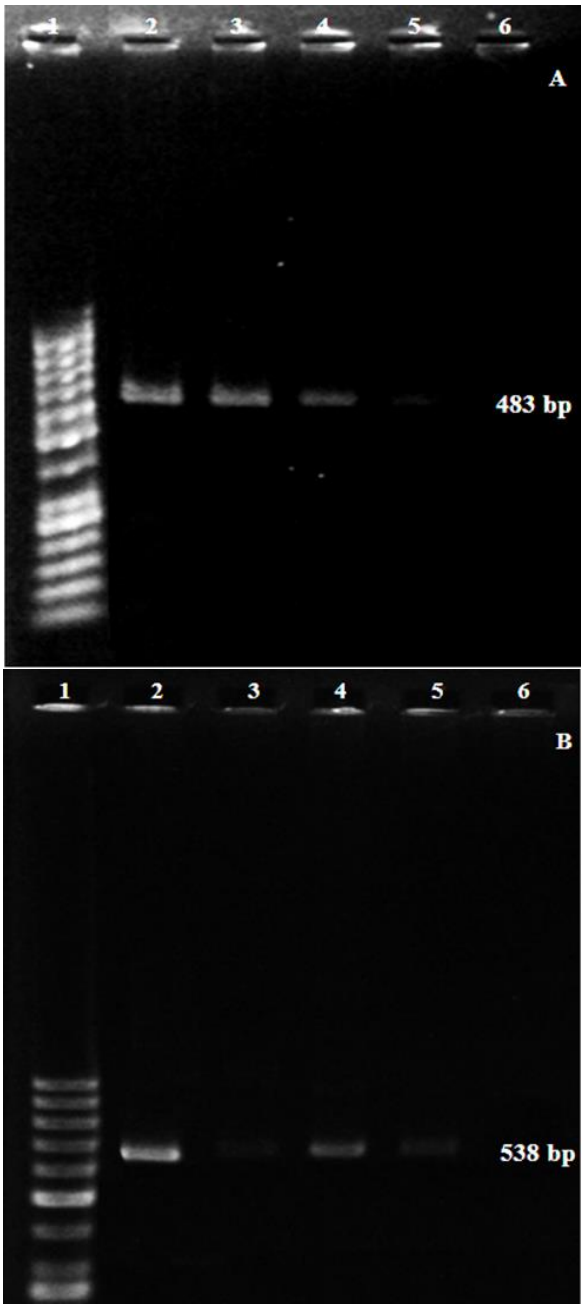

Figure 2. Gel electrophoresis analysis on PCR product of *GLT* and *flaA* primers of *L. monocytogenesis*: A) lane 1; DNA ladder 50 bp, lanes 2, 3, 4, and 5; 483 bp bands of GLT, lane 6; negative for *GLT*. B) lane 1; DNA ladder 100 bp, lanes 2, 3, 4, and 5; 538 bp bands of *flaA*, lane 6; negative for *flaA*.

Table 2. Results of the PCR assay for detection of *L. monocytogenesis* in vaginal swab samples from women with spontaneous abortion regarding demographic and clinical features of the patients (statistical analysis of each row is calculated together).

Variable		Total	Listeria Positive	Listeria Positive	P value
History of infections	Yes	58 (60.4%)	3 (18.8%)	55 (68.7%)	0.06
	No	38 (39.6%)	13 (81.2%)	25 (31.3%)	
Vegetable	Disinfected	44 (45.8%)	5 (31.3%)	39 (48.7%)	0.20
	Non-disinfected	52 (54.2%)	11 (68.7%)	41 (51.3%)	
Dairy products	Sterilized	58 (60.4%)	3 (18.8%)	55 (68.7%)	0.0002
	Unsterilized	38 (39.6%)	13 (81.2%)	25 (31.3%)	

Conventionally, serotyping and phage typing are used for typing of various strains of *L. monocytogenes* (30). However, serotyping as the first line of typing of *L. monocytogenes* is a difficult and expensive approach (33) and works based on the phenotypic characteristics of the bacterium (34). What's more, many strains of *L. monocytogenes* could not be typed by means of phage typing (34). Therefore, according to the results of present study, PCR is suggested as an efficient, cost-effective and rapid tool to be taken into consideration for serotyping of *L. monocytogenes* in the clinical management of related diseases.

Using PCR technique, we found that 16% of the studied women with spontaneous abortion harbored *L. monocytogenes* infection. Consistent with our study, Lotfollahi and colleagues have isolated *L. monocytogenes* from 9% of women with spontaneous abortion referring to an Iranian hospital with the highest rate of resistance of the bacteria to penicillin G using disk-agar antibiogram test (35). Our findings in accordance with those of Lotfollahi and colleagues may support the opinion regarding the role of *L. monocytogenes* in the occurrence of spontaneous abortion of fetuses. On the other hand, Jamshidi and colleagues have observed no relationship between the history of abortions and *L. monocytogenes* seropositivity among Iranian women which may cast doubt on the belief that this bacterium is one of the causative agents of spontaneous fetus abortions (36).

Moreover, in our study, the 1/2b, 3b, and 1/2c, 3c serotypes of *L. monocytogenes* appeared with the highest, and the serotypes 1/2a, 3a and 4 with the lowest frequency among Iranian women with spontaneous abortion. Our findings are not consistent with those of previous investigations among different populations (25, 26, 37). For instances, Althaus and colleagues isolated 93 various strains of *L. monocytogenes* from human infections in Switzerland, the majority of which belonged to the serotypes 1/2a (62.37%) and 4b (30.11%), but the 1/2b and 1/2c serotypes revealed the lowest prevalence of 5.38% and 2.15%, respectively (37). Besides, Aurora and colleagues using multiplex-PCR serotyping assay showed that 72.2% of *L. monocytogenes* isolates were belonging to serotypes 4b, 4d, 4e, while other serotypes including 1/2b, 3b, 1/2a, 3a appeared with low frequencies (30). Indrawattana and colleagues using enterobacterial repetitive intergenic consensus (ERIC)-PCR detected the serotypes belonging to division I of *L. monocytogenes* in the majority of raw meat samples in Thailand (38). Therefore, our results

are totally in contrast to those of Althaus and colleagues (37), Aurora and colleagues (30), and Indrawattana and colleagues (38).

One possible explanation for this controversy might be the difference between origin as well as source of the isolates used in our study and in that of Althaus et al., Aurora and et al., and Indrawattana et al. In other words, despite their low frequencies among populations, 1/2b, 3b and 1/2c, 3c serotypes of *L. monocytogenes* compared to 1/2a, 3a and 4b seem more likely to happen in pregnant women, causing spontaneous abortions. Another subtle explanation might be provided regarding the different molecular approaches adopted in each of the above-mentioned studies.

Conclusion

The results of present study showed that PCR possesses high specificity and sensitivity in detection of *L. monocytogenes* isolates from spontaneous abortion-experienced women. It seems that serotyping of *L. monocytogenes* by PCR technique could facilitate identification of a possible causative strain of the bacterium. In addition, there might be an association between consuming unsterilized dairy products and the occurrence of listeria infection. Moreover, the 1/2b, 3b, and 1/2c, 3c serotypes of *L. monocytogenes* compared to other serotypes are more likely involved in the occurrence of spontaneous fetus abortions. Therefore, identification of *L. monocytogenes* serotypes by PCR technique might provide valuable information and serve as an efficient tool for assessing the risk of listeria-related spontaneous fetus abortions. However, further confirmatory investigations are required to develop this idea, thoroughly.

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Conflict of interest

None declared conflict of interest

References

1. Low J, Donachie W. A review of *Listeria monocytogenes* and listeriosis. *Vet J* 1997; **153**(1):9-29.
2. Kalani BS, Irajian G, Lotfollahi L, et al. Putative type II toxin-antitoxin systems in *Listeria monocytogenes* isolated from clinical, food, and animal samples in Iran. *Microb Pathog* 2018; **122**:19-24.
3. Nightingale K, Schukken Y, Nightingale C, et al. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl Environ Microbiol* 2004; **70**(8):4458-67.
4. Pizarro-Cerdá J, Kühbacher A, Cossart P. Entry of *Listeria monocytogenes* in mammalian epithelial cells: an updated view. *Cold Spring Harb Perspect Med* 2012; **2**(11):a010009.
5. Tompkin R. Control of *Listeria monocytogenes* in the food-processing environment. *J Food Prot* 2002; **65**(4):709-25.
6. Momtaz H, Yadollahi S. Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. *Diagn Pathol* 2013; **8**(1):149.
7. Meloni D, Consolati SG, Mazza R, et al. Presence and molecular characterization of the major serovars of *Listeria monocytogenes* in ten Sardinian fermented sausage processing plants. *Meat Sci* 2014; **97**(4):443-50.
8. Kalani BS, Pournajaf A, Sedighi M, et al. Genotypic characterization, invasion index and antimicrobial resistance pattern in *Listeria monocytogenes* strains isolated from clinical samples. *J Acute Dis* 2015; **4**(2):141-

6.
9. Bahador A, Kalani BS, Valian F, et al. Phenotypic and genotypic characteristics of *Listeria monocytogenes* isolated from dairy and meat products. *Avicenna J Clin Microbiol Infect* 2015; **2**(3).e26905
10. Farber J, Peterkin P. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev* 1991; **55**(3):476-511.
11. Buchanan RL, Gorris LG, Hayman MM, et al. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* 2017; **75**:1-13.
12. Vázquez-Boland JA, Krypotou E, Scortti M. *Listeria* Placental Infection. *mBio* 2017; **8**(3):e00949-17.
13. Lotfollahi L, Chaharbalesh A, Rezaee MA, et al. Prevalence, antimicrobial susceptibility and multiplex PCR-serotyping of *Listeria monocytogenes* isolated from humans, foods and livestock in Iran. *Microb Pathog* 2017; **107**:425-9.
14. Sapuan S, Kortsalioudaki C, Anthony M, et al. Neonatal listeriosis in the UK 2004–2014. *J Infect* 2017; **74**(3):236-42.
15. Fayol L, Beizig S, Le Monnier A, et al. Neonatal meningitis due to *Listeria monocytogenes* after 3 weeks of maternal treatment during pregnancy. *Arch Pediatr* 2009; **16**(4):353-6.
16. Vázquez-Boland JA, Kuhn M, Berche P, et al. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001; **14**(3):584-640.
17. Schwarzkopf A. *Listeria monocytogenes*--aspects of pathogenicity. *Pathol Biol* 1996; **44**(9):769-74.
18. Portnoy DA, Chakraborty T, Goebel W, et al. Molecular determinants of *Listeria monocytogenes* pathogenesis. *Infect Immun* 1992; **60**(4):1263.
19. Soni DK, Dubey SK. Phylogenetic analysis of the *Listeria monocytogenes* based on sequencing of 16S rRNA and *hlyA* genes. *Mol Biol Rep* 2014; **41**(12):8219-29.
20. Soni DK, Singh M, Singh DV, et al. Virulence and genotypic characterization of *Listeria monocytogenes* isolated from vegetable and soil samples. *BMC Microbiol* 2014; **14**(1):241.
21. Seeliger H, Hönne K. Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol* 1979; **13**:31-49.
22. Aarts H, Hakemulder L, Van Hoef A. Genomic typing of *Listeria monocytogenes* strains by automated laser fluorescence analysis of amplified fragment length polymorphism fingerprint patterns. *Int J Food Microbiol* 1999; **49**(1):95-102.
23. Graves LM, Swaminathan B, Reeves MW, et al. Comparison of ribotyping and multilocus enzyme electrophoresis for subtyping of *Listeria monocytogenes* isolates. *J Clin Microbiol* 1994; **32**(12):2936-43.
24. Rasmussen OF, Skouboe P, Dons L, et al. *Listeria monocytogenes* exists in at least three evolutionary lines: evidence from flagellin, invasive associated protein and listeriolysin O genes. *Microbiol* 1995; **141**(9):2053-61.
25. Borucki MK, Call DR. *Listeria monocytogenes* serotype identification by PCR. *J Clin Microbiol* 2003; **41**(12):5537-40.
26. Tappero JW, Schuchat A, Deaver KA, et al. Reduction in the incidence of human listeriosis in the United States: effectiveness of prevention efforts? *JAMA* 1995; **273**(14):1118-22.
27. Eslami G, Goudarzi H, Ohadi E, et al. Identification of *Listeria monocytogenes* virulence factors in women with abortion by polymerase chain reaction. *Arch Clin Infect Dis* 2014; **9**(3).e19931.
28. Doumith M, Buchrieser C, Glaser P, et al. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J Clin Microbiol* 2004; **42**(8):3819-22.
29. Mohammadzadeh R, Shivaee A, Ohadi E, et al. In silico insight into the dominant type II toxin–antitoxin systems and Clp proteases in

Listeria monocytogenes and designation of derived peptides as a novel approach to interfere with this system. *Int J Pept Res Ther* 2019:1-11.
30. Aurora R, Prakash A, Prakash S. Genotypic characterization of *Listeria monocytogenes* isolated from milk and ready-to-eat indigenous milk products. *Food Control* 2009; **20**(9):835-9.
31. Shalaby MA, Mohamed MS, Mansour MA, et al. Comparison of polymerase chain reaction and conventional methods for diagnosis of *Listeria monocytogenes* isolated from different clinical specimens and food stuffs. *Clin Lab* 2010; **57**(11-12):919-24.
32. Shayan R, Satari M, Forouzandeh M. Isolation and identification of *Listeria monocytogenes* in vaginal samples by PCR. *Modares J Med* 2009; **12**(1):51-58.
33. Jeffers GT, Bruce JL, McDonough PL, et al. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. *Microbiol* 2001; **147**(5):1095-104.
34. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* 2006; **55**(6):645-59.
35. Lotfollahi L, Nowrouzi J, Irajian G, et al. Prevalence and antimicrobial resistance profiles of *Listeria monocytogenes* in spontaneous abortions in humans. *Afr J Microbiol Res* 2011; **5**(14):1990-3.
36. Jamshidi M, Jahromi AS, Davoodian P, et al. Seropositivity for *Listeria monocytogenes* in women with spontaneous abortion: a case-control study in Iran. *Taiwan J Obstet Gynecol* 2009; **48**(1):46-8.
37. Althaus D, Lehner A, Brisse S, et al. Characterization of *Listeria monocytogenes* strains isolated during 2011–2013 from human infections in Switzerland. *Foodborne Pathog Dis* 2014; **11**(10):753-8.
38. Indrawattana N, Nibaddhasobon T, Sookrung N, et al. Prevalence of *Listeria monocytogenes* in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. *J Health Popul Nutr* 2011; **29**(1):26.