

## Original Article

# Detection of actA and InlB genes in *Listeria monocytogenes* Isolated from women with Spontaneous abortions

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## Abstract

**Background & Objective:** Assessment of the extent of the role of *L. monocytogenes* in human spontaneous abortions, using isolation methods and PCR analysis for the presence of actA and InlB genes.

**Methods and Materials:** In this descriptive study, vaginal swabs were collected from 96 women with spontaneous abortions who referred to Tehran's hospitals. The samples were cultured in to *Listeria* specific media (PALCAM agar). Then, the *Listeria* genus was verified by differentiation biochemical tests, such as, hemolysis on Blood agar, Catalase and Oxidase reactions, and motility at room temperature. PCR technique was performed on all samples and detected the actA and InlB genes of *L. monocytogenes*.

**Results:** In culture, 7 of 96 samples were positive for *L. monocytogenes*. With the PCR technique, actA and InlB genes were detected from 12 and 2 vaginal samples, respectively.

**Conclusions:** The occurrence of pathogenic *L. monocytogenes* in cases of spontaneous abortions was 12.5%. It seems that the actA and InlB genes have important roles as essential virulence factors in pathogenic *L. monocytogenes*. The results show the PCR method is more sensitive, easier and faster than culture to detect *L. monocytogenes*.

**Key words:** *Listeria monocytogenes* , spontaneous abortions , actA gene , InlB gene

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## Introduction

*L. monocytogenes* is a gram positive, non- spore forming, facultative intracellular and adaptable environmental bacterium. Although most of bacteria do not grow or grow weakly at temperatures below 4 °C, *L. monocytogenes* survives in low temperatures. Therefore, *L. monocytogenes* is an important food born pathogen in ready-to-eat foods that have been refrigerated.<sup>1,2</sup>

*L. monocytogenes* has been found in 10% or more of healthy people, usually in the gut<sup>3</sup>. All the 13 serovars of *L. monocytogenes* are reported to cause human listeriosis, but serovars 1/2a, 1/2b and 4b are

implicated with most of the cases<sup>4</sup>. Pregnant women are particularly prone to infection. The placenta provides a protective niche for the growth of *L. monocytogenes*, thereby resulting in spontaneous abortions, stillbirth neonatal infection, severe necrotizing hepatitis, placental necrosis and increased risk of postimplantation loss<sup>5,6</sup>. Latent listeriosis in pregnant women leads to habitual abortions, intrauterine deaths and fetal malformations<sup>7,8</sup>. The incidence of listeriosis in general population is 0.7 in 100000 but this prevalence is 12 in 100000 in pregnant women (which is a 17-fold increase)<sup>9</sup>. The fetus suffers more damage than the pregnant women, leading to a clinical syndrome known as granulomatosis

infantiseptica<sup>10</sup>. *L. monocytogenes* causes meningitis and hydrocephalus in children born of infected mothers<sup>11</sup>. These reports highlight the importance of the pathogen as a cause of spontaneous abortions and infant mortality<sup>12</sup>. The reliable diagnosis of listeriosis is made by isolation of the pathogen<sup>13</sup>. The microbiological assays as well as the serological profile of the infected subjects by conventional tests remain a time-consuming and tedious task and may be insufficient to discriminate between pathogenic and nonpathogenic strains. Polymerase chain reaction (PCR) is the best technique for detection of virulence markers. ActA and InB are necessary virulence factors of *L. monocytogenes*. ActA, 90-KDa surface protein is required for actin polymerization and thus allows intracytoplasmic movement of *Listeria monocytogenes*<sup>14,15</sup>. InlB, which is a 67-KDa surface and secreted protein is sufficient for entry of the bacterium into cell. InlB can promote the entry of noninvasive bacterial cell into hepatocyte of mammalian cell and cause internalization of inert particles<sup>16</sup>.

Unlike developed countries, systematic studies done on the association of pathogenic *L. monocytogenes* with spontaneous abortions are lacking, especially in the Iran context. In Iran, *L. monocytogenes* has been isolated from foods of animal origin and clinical cases in animals. The present study describes the detection of actA and InlB genes in pathogenic *L. monocytogenes* isolated from women with spontaneous abortions<sup>17</sup>.

## Methods and Materials

In this descriptive study, vaginal swabs were collected from 96 women with spontaneous abortions who referred to Tehran's hospitals, during 2011. Vaginal swabs were inoculated into 10 ml of TSBYE (Tryptical

**Table 1:** *L. monocytogenes*' actA and InlB primers

Target gene	Primer sequence	Product size(bp)	Reference
<b>actA</b>			
Forward:	5'- AACACAGATGAATGGGAAGAAG -3'	278	in this study
Reverse:	5'-TCCACTTGTATAGCTGGTCG -3'		
<b>InlB</b>			
Forward:	5'-TGATGCTTTTGCAGAAACAATC -3'	319	in this study
Reverse:	5'-ATCACTTATACCATTATGCTCC -3'		

soy broth plus 0/6% yeast extract, Merck, Germany). All of samples were incubated at 4 °C. After two months, aliquots from enrichment broth (TSBYE) were streaked on to PALCAM agar (Merck, Germany) and plates were incubated at 35 °C for 5 days. After 5 days the green shiny colonies with black shadow were seen on PALCAM. Confirmation of isolates was done by gram staining, haemolysis on Blood agar, catalase and oxidase reaction, motility at room temperature. The confirmed *L. monocytogenes* was stored in TSB (10%) and glycerol (5%).

In PCR reaction, the primers for detection of actin gene (actA) and Internalin B- gene (InlB) are shown in table 1.

ATCC7644 was standard strain of pathogenic *L. monocytogenes*. DNA extraction was done by the Genomic DNA Purification Kit (Fermentas Kit), the

obtained lysate (3  $\mu$ l) was used as a DNA template in PCR reaction mixture. For the standardized PCR protocol for 25  $\mu$ l reaction mixture included 12.5  $\mu$ l

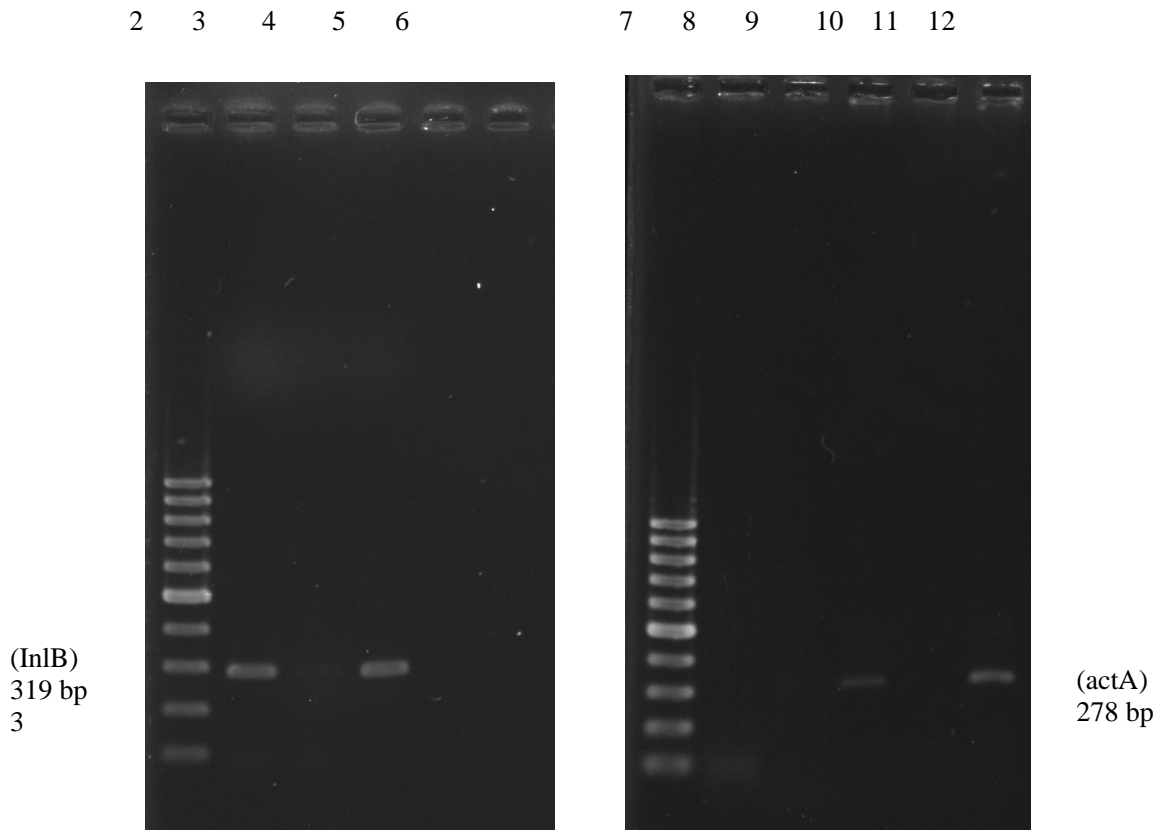
Master mix (Sinaclon), 7.5  $\mu$ l DDW, 1  $\mu$ l forward primers, 1  $\mu$ l reverse primers and 3  $\mu$ l DNA to make up the reaction volume. The cycling conditions for PCR included an initial denaturation of DNA at 94 °C for 5 min followed by 36 cycles each of 1 min denaturation at 94 °C, 1 min annealing at 53 °C (for actA and InlB genes) and 1 min extension at 72 °C, followed by final extension of 5 min at 72 °C and held at 4 °C. The reaction was performed in 36 cycles. The PCR products run on 1.5% agarose gel electrophoresis. The

gel was stained with ethidium bromide (0.5  $\mu$ g/ml) and visualized by UV transilluminator.

## Results

The microbiological and biochemical analysis of 96 vaginal swabs from the patients with spontaneous abortion revealed that 7(7.2%) isolates were *L. monocytogenes*. The standardized PCR allowed amplification of virulence-associated genes of *L.*

*monocytogenes* namely *actA* and *InlB* to their respective base pairs, 276, 319bp PCR products. With PCR method, 12 (12.5%) of the 96 samples were positive for *L. monocytogenes*. The *ActA* gene was detected in all of 12 (100%) positive samples, while, only 2 (16.6%) samples were positive for *InlB* gene (Figure 1).



**Figure 1:** Detection of *InlB* and *ActA* genes in *L. monocytogenes*

Lane 1,7, DNA Marker (100-1000 bp)

Lane 2,3,11, negative samples

Lane 4,9,10, positive samples

Lane 5,12, negative control

Lane 6,8, positive control

## Discussion

About one-third of reported human listeriosis cases happen during pregnancy, which may result in spontaneous abortion<sup>18</sup>. In the present study, using PCR, 12.5% isolates of *L. monocytogenes* were recovered from 96 human abortion cases; but in culture method 7.2% isolates of *L. monocytogenes* were detected. These findings are in agreement with the

earlier reports on the isolation of *L. monocytogenes* from 9 of 100<sup>18</sup> and 22 of 428 patients with bad obstetric history<sup>19</sup>, therefore highlighting the role of *L. monocytogenes* as a causative agent of human abortions. Reports of listeriosis from humans in Iran are scanty, either because of failure to identify the isolate, its rarity, low incidence rate or lack of awareness<sup>20</sup>. The listeria infection in pregnant women is asymptomatic or occurs as influenza-like syndrome.

Following delivery, mothers of infected newborns may shed *L.monocytogenes* for 7-10 days in vaginal secretions or urine. We isolated a pathogenic *L.monocytogenes* in the present study from the vaginal swabs of women with spontaneous abortion in the second trimester of pregnancy<sup>9</sup>. Antibiotic treatment of pregnant women or immunocompromised people who have eaten food contaminated by *L.monocytogenes* can prevent the most serious consequences of listeriosis, but only if the infection is diagnosed in time. Another complication is that listeria is able to grow well at low temperatures. Thus, refrigeration is not as effective in preventing growth of listeria in food as it is for most other bacteria that cause food-borne disease<sup>19</sup>. The results show that PCR is a more sensitive, easier and faster method in comparison to culture for detecting *L. monocytogenes*. It is obvious that quick diagnostic and starting antimicrobial therapy at the right time can prevent and decrease abortion's complications, so it is suggested that using PCR in detecting *L.monocytogenes* can be more effective<sup>20</sup>.

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