

# DETECTION OF *INLB* GENES OF *LISTERIA MONOCYTOGENES* ISOLATED FROM WOMEN WITH SPONTANEOUS ABORTIONS

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## Abstract

**Background:** *Listeria monocytogenes* is a food-borne intracellular bacterium which possesses many virulence factors that enable it to overcome the host immune system and of particular importance are surface proteins InIA and InIB which have a crucial role in initiating infection. The aim of this study is to detect the incidence of *L. monocytogenes* infection in placental tissue from women with spontaneous abortions by targeting *InIB* genes-based polymerase chain reaction.

**Methods:** In one hundred and eleven pregnant women suffering from spontaneous abortions, about 25 grams of the placental tissue from each person was homogenized and centrifuged for about 15 min at 5000 rpm at 2-8°C. A specific set of primers was used for detection of *L. monocytogenes InIB* gene using conventional PCR technique.

**Results:** Out of 111 placental tissue harvested from women with abortions, only 11 (9.9%) were proven to be positive for *InIB* gene. The highest rate of positivity (18.9%) was observed in the age group 20-29 years old. Of the 11 cases reported to be positive for listeriosis, 8 (72.7%) cases had their abortions in the first trimester.

**Conclusion:** *Listeria monocytogenes* may have a noteworthy role in pregnancy loss and should be considered when there are spontaneous abortions.

**Keywords:** Listeriosis, *Listeria monocytogenes*, *InIB* Gene, Spontaneous Abortions, Placental Tissue

## Introduction

*Listeria monocytogenes* is a saprophytic Gram-positive rod-shaped bacterium. Listeriosis, a disease caused by this bacterium, is a food-borne disease characterized by severe septicemia and meningoenzephalitis in immunocompromised individuals; and a placental infection which can lead to meningoenzephalitis of the newborn and abortions in pregnant women (1). The ability of *L. monocytogenes* to cause disease correlates with its capacity to internalize, survive and replicate within target cells. Furthermore, this bacterium can cross the intestinal, the blood-brain, and the fetoplacental barriers (2, 3).

Two surface proteins are mainly responsible for this potential, namely InIA and InIB which are encoded by *InIA* and *InIB* genes respectively in the major virulence

locus. InIA initiates entry of *L. monocytogenes* to the limited types of epithelial cells, while InIB promotes introducing of these bacteria into many cell types, such as endothelial cells, hepatocytes, and epithelial cells (4). Specific host cell receptors are involved in the bacterium tropism and internalization. InIA is a ligand for E-cadherin, a cell adhesion molecule presenting in epithelial tissues and involved in the formation of intercellular junctions, whereas InIB recognizes the receptor tyrosine kinase Met (c-Met) on the surfaces of various epithelial cells (5). Accordingly, the presence of encoding gene determines the target cells with a subsequent actin polymerization, membrane extension and bacterial uptake (6).

Listeriosis presents in two forms according to the immune status. In immunocompetent subjects, the disease usually is non-invasive, and mainly associated with gastroenteritis.

On the other hand, an invasive form of the disease occurs in immunocompromised individual which can develop to serious sequels such as meningoencephalitis (7).

When this bacterium infects pregnant women, it may be associated with dread complications such as abortion or stillbirth. Moreover, the bacterium can cross the placenta causing fetal listeriosis (8). Listeriosis as a food-borne disease was extensively investigated; however, the relationship of this disease with abortion has drawn less attention (8). This study, proposed to investigate the presence of *L. monocytogenes* infection in placental tissue of women with spontaneous abortions by targeting *InlB* genes using PCR.

**Materials and Methods**

**Subjects**

This study included a total of 111 pregnant women with spontaneous abortions who attended the Gynaecology wards and Emergency Unit in Al-Imamain Al-Khadhmain Medical City, Baghdad during the period from December 2017 to May 2018.

Placental tissue samples were collected from each woman who participated in the study. Informed consent after explaining the aims of the study was obtained from each woman. Women with chromosomal and/or uterine abnormalities and those with incompetent cervixes were excluded from this study. Data including age, number of previous abortions, gestational age, and history of congenital abnormalities were extracted from these women either through direct interview or from their medical records. This study was approved by the Institutional Review Board (IRB), College of Medicine, AL-Nahrain University under the ethics number MMM 213.

**Preparation of tissue homogenate**

One hundred and eleven placental tissue samples (about 25 grams each) were brought to the laboratories of Microbiology department, College of Medicine, Al-Nahrain University, where they were homogenized with 10 ml of phosphates buffer using tissue homogenizer according to Bhattacharya et al. (8).

The homogenate was centrifuged for about 15 min at 5000 rpm and 2-8°C. The supernatant was collected carefully and used for genomic DNA extraction using SYNC™ DNA Extraction Kit, Genaid, Taiwan. Two primers were used to amplify the gene *InlB* gene. These were: forward primer 5'-TGATGCTTTTGCAGAAACAATC -3'and reverse primer 5'-ATCACTTATACCATTATGCTCC -3' (9).

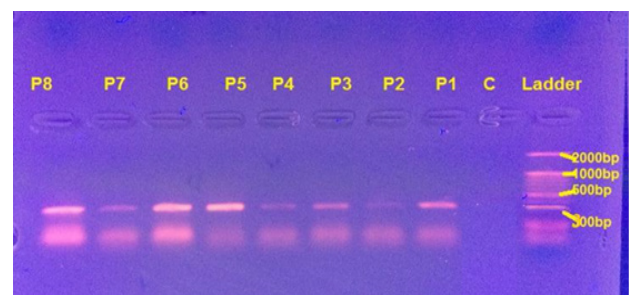
The reaction was performed in a 25 µL of reaction mixture containing 2 µL of DNA template, 200 µM of each deoxynucleotide triphosphate (dNTP), 1.5–2.5 mM MgCl<sub>2</sub>, 1 µL of each primer, and 0.6 unit Taq DNA polymerase (Bioneer, Korea). The volume was adjusted to 25 µL

with deionized water. The reaction was amplified by a thermocycler PCR system (Hybaid, UK). The PCR conditions were as that reported previously by Eslami et al. (9). The PCR products were stained with ethidium bromide and undergone 2% gel electrophoresis. The results were detected through UV transluminator with camera.

**Results**

**Principle finding**

Out of 111 placental tissue harvested from aborted women, only 11 (9.9 %) were proven to be positive for *InlB* gene through the PCR amplification and respective amplicon product of 319 bp (Figure 1).



**Figure 1:** Detection of *InlB* gene in *Listeria monocytogenes*. Ladder: 100 bp DNA; Lane P1-P8: positive samples (319 bp); Lane C: negative control.

The distribution of *L. monocytogenes* among the age groups is presented in Table 1. Of the 11 positive women, 7 were observed within age group 20-29 years old (18.9 %). The association between age and *L. monocytogenes* positive pregnant women was significant (p=0.019).

**Table 1:** The distributions of *Listeria monocytogenes* among the age groups

Age group	Positive		Negative		χ <sup>2</sup>	P-value
	No.	%	No.	%		
<20 years old (n= 19)	1	5.3	18	94.7	9.847	0.019*
20-29 years old (n= 37)	7	18.9	30	81.1		
30-39 years old (n= 48)	1	2.1	47	97.9		
≥40 years old (n=7)	2	28.6	5	71.4		
N= 111	11		100			

\* p<0.05

### Association of listeriosis with gestational age and abortion

Distribution of samples according to the gestational age showed that the highest percentage of *L. monocytogenes* was from women who aborted in the first trimester (8 cases, 72.7%), while 2 cases (18.1%) were in the second trimester with significant association ( $p=0.01$ ) as shown in Table 2.

**Table 2:** Association of listeriosis with gestational age

Variable	Positive		$\chi^2$	P-value
	No.	%		
Abortion at 1 <sup>st</sup> trimester	8	72.7	6.6	0.010*
Abortion at 2 <sup>nd</sup> trimester	2	18.2		

\*  $p<0.05$

### Association of listeriosis with adverse pregnancy outcome

Analysis of the association of *L. monocytogenes* with adverse pregnancy outcome showed a significant association as shown in Table 3 with the chi-square statistic of 8.6826. The  $p$ -value was 0.013.

**Table 3:** Distribution of positive cases in relation to adverse pregnancy outcome

Type of adverse pregnancy outcome	Positive cases		$\chi^2$	P-value
	No.	%		
Abortion	7	63.6	8.6826	0.013*
Still birth	3	27.3		
Congenital abnormalities	1	9.1		
Total	11	100.0		

\*  $p<0.05$

### Discussion

*Listeria monocytogenes* is a Gram-positive bacterium that causes a wide range of disease. During pregnancy, females become more prone to infection with a variety of infectious agents such as bacterial infection. *Listeria monocytogenes* infection can be asymptomatic during pregnancy or may present with subclinical symptoms such as a nonspecific fever. However, infection with these bacteria may lead to placentitis which causes stillbirth, abortion and granulomatosis infantiseptica (10).

In the present study, 11 (9.9 %) out of 111 women with spontaneous abortions were found to be positive with *L. monocytogenes* infection using conventional PCR targeting *InlB* gene. This result was lower than the results reported by Al-Taii, who obtained seventeen isolates (12%) of *L. monocytogenes* from 132 samples (11). The result of this study is higher than the percentage reported by Qassim et al. who reported that 6 % out of 250 placental tissues were positive for *L. monocytogenes* (12). Also, the present rate was higher than that reported by AL-Dosh who isolated 8 isolates of *L. monocytogenes* from 140 samples of women with recurrent abortions (13). Globally, Jackson et al. mentioned that the percentage of *L. monocytogenes* isolated from pregnant women was 1.6% (14).

This discrepancy of the outcomes can be attributed to the differences in the number and sample types in each study and methodology used for diagnosis of *L. monocytogenes*. Even when PCR technique is used, various factors can lead to false-negative PCR results such as low DNA yield which resulted from incomplete cell lysis during DNA extraction particularly from tissue samples.

Our data showed that age group of 20-29 years old has highest number of *L. monocytogenes* infection and there is significant association between infections with age. Younger age groups are more likely to develop vaginosis due to sexual activity and these results are in harmony with *Listeria* Annual Summary which states that the median age of pregnancy-associated cases was 28 years old (15). In contrast, the results of the present study do not concur with a study by Yaghoob et al. who reported that age group of 41-46 years old was more prone to infection with *L. monocytogenes* (16). Factors such as methods of diagnosis, variation in life style and socioeconomic status may explain the variation in the results.

The present study found that the highest fetal losses took place in the first trimester and that 8 positive cases (72.7%) had an abortion in the first trimester while the remainder had abortion in the second trimester. This result is in harmony with a study done by Qassim et al. (12) who mentioned that most cases of the abortions in pregnant women with listeriosis occurred in the first trimester of pregnancy. Results obtained in this study showed a statistically significant ( $p=0.013$ ) association between infection with *L. monocytogenes* with adverse pregnancy outcome. A study conducted by Jamshidi et al. (17) showed that placentitis as well as chorioamnionitis with multiple placental abscesses are associated with listeriosis. It appears that almost all cases of pregnant women infected with *L. monocytogenes* have adverse pregnancy outcome.

### Conclusion

*Listeria monocytogenes* may have a noteworthy role in pregnancy loss and it should be considered as a risk factor for abortion. Diagnosis at the right time can prevent and decrease abortion and would enable the possibility of a successful future pregnancy.

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## Competing Interests

The authors declare that they have no competing interests.

## References

- Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect.* 2010;16(1):16-23.
- Lecuit M. Understanding how *Listeria monocytogenes* targets and crosses host barriers. *Clin Microbiol Infect.* 2005;11(6):430-6.
- Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes*. *Proc Natl Acad Sci.* 2011;108(49):19484-91.
- Kargar M, Ghasemi A. Role of *Listeria monocytogenes* hly- A gene isolated from fresh cheese in human habitual abortion in Marvdasht. *J Clinic Infect Dis.* 2009;4(4):214-8.
- Zhang Y, Xia M, Jin K, Wang S, Wei H, Fan C, *et al.* Function of the c-Met receptor tyrosine kinase in carcinogenesis and associated therapeutic opportunities. *Mol Cancer.* 2018;17(1):45.
- Hamon M, Bierne H, Cossart P. *Listeria monocytogenes*: a multifaceted model. *Nat Rev Microbiol.* 2006;4(6):423-34.
- Chenal-Francois V, Lopez J, Cantinelli T, Caro V, Tran C, Leclercq A, *et al.* Worldwide distribution of major clones of *Listeria monocytogenes*. *Emerg Infect Dis.* 2011;17(6):1110.
- Bhattacharya D, Pandit S, Mukherjee R, Das N, Sur TK. Hepatoprotective effect of Himolive, a polyhedral formulation in rats. *Indian J Physiol Pharmacol.* 2003;47(4):435-40.
- Eslami G, Samadi R, Taherpanah R, Taherpor A, Baseri N. Detection of actA and InlB genes in *Listeria monocytogenes* isolated from women with spontaneous abortions. *NBM.* 2014;2(1):18-21.
- Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, Wing EJ. Listeriosis during pregnancy: a case series and review of 222 cases. *Medicine.* 2002;81(4):260-9.
- Al-Taii MA. Physiological and pathological aspects of *Listeria monocytogenes* isolates from different clinical cases in Mosul City. Thesis. Iraq: University of Mosul. 2004.
- Qassim KW, Al-Attraq AA, Edrees Y. Detection of *Listeria monocytogenes* in placenta of aborted women. *Iraqi JMS.* 2017;15(4):404-10.
- Al-Dosh ND. Pathogenic and antigenic study on *Listeria monocytogenes* lipopolysaccharide in compare to some Gram-negative bacteria. Thesis. Iraq: University of Baghdad. 2017.
- Jackson KA, Iwamoto M, Swerdlow D. Pregnancy-associated listeriosis. *Epidemiol Infect.* 2010;138(10):1503-9.
- Centers for Disease Control and Prevention (CDC). Outbreak of invasive listeriosis associated with the consumption of hog head cheese - Louisiana, 2010. *MMWR.* 2011;60(13): 401-5.
- Yaghoob T, Farshid K, Yadollah AM. *Listeria monocytogenes* and abortion: A case study of pregnant women in Iran. *Afr J Microbiol Res.* 2009;3(11):826-32.
- Jamshidi M, Jahromi AS, Davoodian P, Amirian M, Zangeneh M, Jadcafeh F. Seropositivity for *Listeria monocytogenes* in women with spontaneous abortion: a case-control study in Iran. *Taiw J Obstet Gynecol.* 2009;48(1):46-8.