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Prevalence of *Listeria monocytogenes* Infection in Iranian Pregnant Women with and without a History of Abortion: A Systematic Review and Meta-analysis

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Abstract

Background: *Listeria monocytogenes* (LM) is an important foodborne pathogen that can cause miscarriage, stillbirth, or premature birth of the fetus in pregnant women. This study aimed to investigate the prevalence of LM infection in Iranian pregnant women with and without a history of abortion.

Methods: A systematic search was performed in national (Iranian Scientific Information Database [SID], Magiran, IranMedex) and international (PubMed, Scopus, Web of Science) electronic databases for literature published between January 1, 1999, to the end of January 2022. Data analysis was done using R Studio software version 1.4.1717.

Results: In total, 17 studies, including 2553 women with a history of abortion and 1065 women without a history of abortion (3168 Iranian pregnant women), were included for the final analysis. The prevalence of LM infection in Iranian pregnant women with and without a history of abortion was estimated at 14% (95% CI: 13%–16%) and 5% (95% CI: 4%–7%), respectively. In addition, the incidence of the *hlyA* gene in Iranian pregnant women with and without a history of abortion was 11% (95% CI; 5%–22%).

Conclusion: The results of this study show that the prevalence of LM was higher in women who had a history of abortion compared to women without a history of abortion. Therefore, it seems that one of the possible etiological factors of abortion among Iranian pregnant women is the high prevalence of infection with LM.

Keywords: Iranian woman, Listeriosis, *Listeria monocytogenes*, Pregnant woman, Prevalence

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Introduction

Listeria monocytogenes (LM) is a gram-positive, non-sporulating, facultative anaerobic bacillus that is catalase-positive and oxidase-negative and is mainly transmitted to humans through the consumption of foods contaminated with this bacterium. This infection (listeriosis) is the leading cause of human foodborne infections worldwide (1-3). Recent assessments indicate that listeriosis ranks England's leading cause of food-related deaths (4). Furthermore, in 2010, LM was identified as the third most commonly transmitted foodborne pathogenic bacterium in the United States, following *Vibrio vulnificus* and *Clostridium botulinum*, resulting in many hospitalizations and deaths (5).

Epidemiological studies have shown that listeriosis is a rare and dangerous disease that leads to bacteremia, sepsis, meningoencephalitis, splenomegaly, and hepatomegaly, especially during pregnancy, as it may lead to abortion, fetal death, premature birth, and death of the baby after birth (6,7). Published reports show that 27 to 43% of all Listeria infections are associated with pregnancy listeriosis, with about 14% occurring in the third trimester of pregnancy (8,9). In pregnant women, the incidence of listeriosis is 12 per 100,000. Various studies have described

the risk of listeriosis in pregnant women as 10 to 20 times higher than in the general population (10,11).

Central nervous system (CNS) infections are one of the most common and well-known forms of listeriosis, accounting for 30.7% of all non-perinatal listeriosis cases (12,13). Bacteremia caused by LM presents as an acute febrile illness and is often accompanied by symptoms such as myalgia, arthralgia, backache, and headache. This condition can manifest at any stage of pregnancy; however, it is most commonly observed during the third trimester, probably due to a decrease in cellular immunity between the 26th and 30th weeks of pregnancy (12,14). In pregnancy-associated listeriosis, the most common complication is preterm delivery (64% of cases), and 22% of cases of listeriosis result in stillbirth or neonatal death (8). In children with LM, the bacterium infects the brain, especially in the brainstem, and causes mental disorders (15). Brain abscesses have been reported in 10% of patients in the thalamus and medulla oblongata (16).

Evidence suggests that the incidence of listeriosis has declined in industrialized countries. The prevalence of listeriosis has decreased by 40% in the United States and by 68% in France, reaching 2.7 and 1.4 cases per million people, respectively (17). However, in recent years, the incidence of listeriosis in most countries has either remained unchanged or has become more severe. For example, in South Africa in 2017–2018, following the consumption of Listeria-contaminated meat, severe listeriosis resulted in the death of 200 people, which was the largest Listeria outbreak to date (18). Researchers believe that changes in eating habits and the increasing interest in consuming ready-to-eat foods are among the causes of the increased incidence of listeriosis (2). In Iran, listeriosis is not classified as a reportable disease within the health system, and there are currently no established guidelines for managing listeriosis in the food industry (3).

Although various studies have documented the prevalence of LM in Iranian pregnant women, there is no comprehensive systemic review to summarize the results and provide an overview regarding the prevalence of Listeria in pregnant women. The current meta-analysis aimed to determine the prevalence of LM infection in Iranian pregnant women with and without a history of abortion.

Methods

Search strategy

We searched all published studies for both English and Persian language articles from January 1999 to January 1, 2022, in international electronic databases: PubMed, Scopus, and Web of Science, as well as national databases, including the Iranian Scientific Information Database (SID), IranMedex and Magiran, using the following keywords or terms: "listeriosis," "Listeria," "*Listeria monocytogenes*," "prevalence," "incidence," "frequency," "outbreaks," "occurrence," "epidemiology," "pregnant woman," and "Iranian woman."

Selection criteria and quality assessment

The present systematic review is based on the PRISMA 2020 guideline (19). Briefly, in the evaluation process, after the exclusion of duplicates, two researchers independently assessed the titles, abstracts, and full texts of the retrieved articles from databases to determine the articles that met the inclusion criteria; irrelevant articles were excluded, and in the event of disagreement, the two researchers consulted with a third researcher to reach consensus. All English and Persian language articles with available full texts that reported data on the infection, prevalence, and expression of virulence genes in LM in Iranian pregnant women with and without a history of abortion and used standard methods for Listeria detection in Iranian pregnant women were included. Studies that investigated infection and prevalence of other microorganisms in Iranian pregnant women, studies that investigated the infection and prevalence of LM in foreign nationals and non-Iranian pregnant women, review articles, congress abstracts, case reports, and articles without clear sample size were excluded. The quality of eligible studies was checked using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist (20). The references cited in the eligible articles were also investigated.

Data extraction

Data, including the first author's name, year of study, year of publication, sample source, sample size, number of cases positive for LM (prevalence), the type of virulence genes and antibodies, the diagnostic method, study design, mean age and age range, were extracted by two researchers independently and any disagreements were resolved by consensus.

Statistical analysis

Data analysis was done using R Studio software version 1.4.1717. The Cochrane Q and I^2 statistics were used to compute statistical heterogeneity in the studies. I^2 values above 75% were considered high heterogeneity, based on the recommendations provided by Higgins et al (21). In the absence of heterogeneity, the fixed-effects model and in the presence of heterogeneity, the random-effects model (22,23) were applied to obtain an overall effect size (prevalence) and 95% confidence intervals (CIs). The error was calculated for each study using binomial distribution. In all statistical analyses, P values of < 0.05 were considered significant for all tests.

Results

A summary of the article selection process based on a PRISMA flow diagram is depicted in Figure 1. A total of 353 articles were retrieved from the database search.

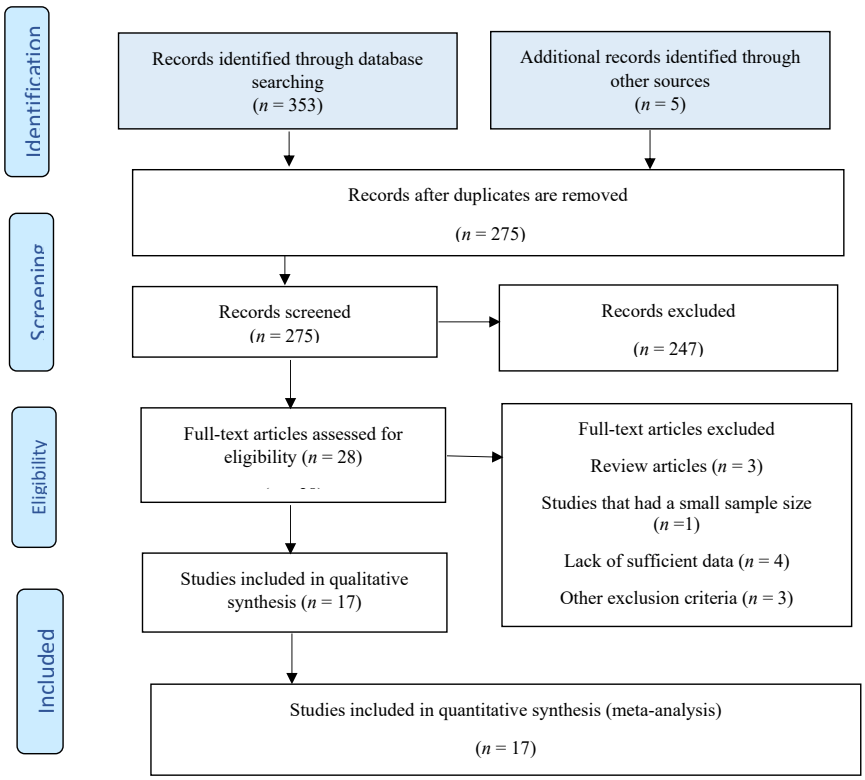

Figure 1. The PRISMA flow diagram for selection of published articles about the prevalence of LM infection in Iranian pregnant women with and without a history of abortion

A further five articles were identified by reviewing the literature referenced in the retained articles. After removing duplicates and irrelevant citations based on the title and abstract, 28 articles were selected for quality assessment. Finally, 11 articles were excluded on the basis of the criteria listed in Figure 1, and 17 eligible articles were retrieved for this systematic review and meta-analysis. The characteristics of the selected studies for the final analysis are reported in Table 1.

In the current meta-analysis, a total of 3168 Iranian pregnant women, including 2553 women with a history of abortion and 1065 women without a history of abortion in the age range of 14 to 50 years were examined.

Prevalence of LM infection in Iranian pregnant women with a history of abortion

Due to heterogeneity among the selected studies ($Q = 279.49$, $P < 0.001$, $I^2 = 95\%$), a random-effect model was used. The prevalence of LM infection in Iranian pregnant women with a history of abortion was 14% (95% CI; 13–16%) (Figure 2). The results of subgroup analysis for the prevalence of LM infection in Iranian pregnant women with a history of abortion by type of diagnostic method are presented in Table 2.

Prevalence of LM infection in Iranian pregnant women without a history of abortion

Due to heterogeneity among the selected studies ($Q = 93.43$, $P < 0.001$, $I^2 = 88\%$), a random-effect model was used. The prevalence of LM infection in Iranian pregnant women without a history of abortion was 5% (95% CI = 4–7%) (Figure 3). The results of subgroup analysis for the prevalence of LM infection in Iranian pregnant women without a history of abortion by type of diagnostic method are presented in Table 3.

Prevalence of LM virulence genes in Iranian pregnant women with and without a history of abortion

The fixed or random effects model was used to estimate the prevalence of LM virulence genes based on the presence or absence of heterogeneity among the selected studies using the Cochran Q-test and I^2 index. The subgroup analysis results for the prevalence of these genes are presented in Table 4.

Discussion

LM is a significant foodborne pathogen associated with the risk of miscarriage in pregnant women. The occurrence of listeriosis among pregnant women is 12 cases per 100 000, compared to 0.7 cases per 100 000 in the general population (41). One-third of human listeriosis is reported to be related to spontaneous abortion and often occurs as early as the third trimester of pregnancy (39).

In the current meta-analysis, it was shown that the prevalence of LM infection in Iranian pregnant women with a history of abortion (2553 women) and without a history of abortion (1065 women) is 14% (95% CI; 13%–16%) and 5% (95% CI = 4%–7%), respectively. Studies in

Table 1. Characteristics of studies included in the meta-analysis

First author (Reference)	Publication year	Years of study	Study design	Diagnostic method	Sample source	Type of gene / AB	Sample size		Number of positive cases for LM (Prevalence)		Range of age	Mean of age
							Case (with a history of abortion)	Control (without a history of abortion)	Case (with a history of abortion)	Control (without a history of abortion)		
Farajzadeh Sheikh (24)	2004	1999-2000	Case-control study	IFA	Blood	IgG IgM	120	60	12 (10)	2 (3.3)	14-45	-
Jamshidi (25)	2009	2002-2003	Case-control study	IFA	Blood	IgG	250	200	89 (35.6)	35 (17.5)	-	25.6±7.6
Saeedi (26)	2009	2005-2006	Case-control study	IFA Culture	Blood Placenta	IgGIgM -	118 120	99 60	9 (7.6) 3 (2.5)	3 (3.03) 0 (0)	-	-
Shayan (27)	2009	-	Cross-sectional	PCR Culture	Vaginal swab	*hlyA* -	100	-	36 (36) 7 (7)	-	20-49	-
Tahery (28)	2009	-	Case-control study	IFA	Blood	Listeria-specific antibody	102	102	12 (11.8)	3 (2.9)	16-45	30.7±8.2
Jahangiri Sisakht (29)	2012	2008-2009	Cross-sectional	PCR Culture	Blood Placenta Urine Cervix	*hlyA* -	107	-	11 (10.28) 0 (0)	-	15-38	26.7
Eslami (30)	2014	2012-2013	Cross-sectional	PCR Culture	Vaginal swab	*hlyA* & *plcA* -	96	-	16 (16.7) 4 (4.1)	-	-	33.5±7.2 30.9±4.7
Haghroosta (31)	2014	-	Case-control study	IFA	Blood	IgG IgM	120	60	12 (10)	2 (3.3)	14–45	27.6
Sobhani Lari (32)	2014	–	Cross-sectional	PCR	Urine	*hlyA*	100	-	30 (30)	-	19–49	29±7.03
Eslami (33)	2015	2011–2012	Cross-sectional	PCR	Vaginal swab	*actA*, *prfA*, and *inlB*	96	-	23 (24)	-	-	-
Seify (34)	2016	2014–2015	Case-control study	Culture	Vaginal swab Urine	-	270	270	8 (2.96)	6 (2.2)	15-44	-
Pourkaveh (35)	2016	2015–2016	Cross-sectional	PCR	Vaginal swab	*hlyA* & *plcA*	317	-	54 (17)	-	18-35	26.5±3.9
Tajedini (36)	2017	2016–2017	Cross-sectional	IFA	Blood	IgG IgM	58	-	21 (36)	-	20-50	-
Heidarzadeh (37)	2018	2015–2017	Cross-sectional	PCR	Vaginal swab	*hlyA, inlC inlJ, prfA inlA, actA*	400	-	22 (5.5)	-	-	-
Heidari (38)	2018	2016–2017	Case-control study	PCR	Vaginal swab	*hlyA*	52	48	3 (5.7)	1 (2)	-	-
Zahirnia (39)	2019	2015–2016	Case-control study	PCR Culture	Vaginal swab	*hlyA, inlB prfA, actA* -	124	76	31 (25) 1 (0.8)	28 (36.8) 8 (10.5)	18–42	-
Rezaei (40)	2019	2016–2018	Case-control study	PCR	Vaginal swab Placenta Blood	*hlyA, iap*	123	150	52 (42.2)	6 (4)	-	-

Abbreviations: AB, antibody; IFA, indirect immunofluorescence assay; PCR, polymerase chain reaction; IgG, immunoglobulin G; IgM, immunoglobulin M

Table 2. The prevalence of LM infection in Iranian pregnant women with a history of abortion by type of diagnostic method

Diagnostic method	Prevalence of LM (95% CI)	Test for heterogeneity		
		Q-statistic	*P* value	I^2
Culture	0.03 (0.02–0.04)	6.27	0.28	52%
PCR	0.18 (0.13–0.25)	84.40	<0.001	88%
IFA	0.18 (0.08–0.35)	100.09	<0.001	95%

CI, confidence interval.

Table 3. The prevalence of LM infection in Iranian pregnant women without a history of abortion by type of diagnostic method

Diagnostic method	Prevalence of LM (95% CI)	Test for heterogeneity		
		Q-statistic	*P* value	I^2
Culture	0.05 (0.02–0.13)	8.71	<0.001	77%
PCR	0.08 (0.01–0.33)	36.92	<0.001	91%
IFA	0.05 (0.02–0.11)	25.80	<0.001	73%

CI, confidence interval.

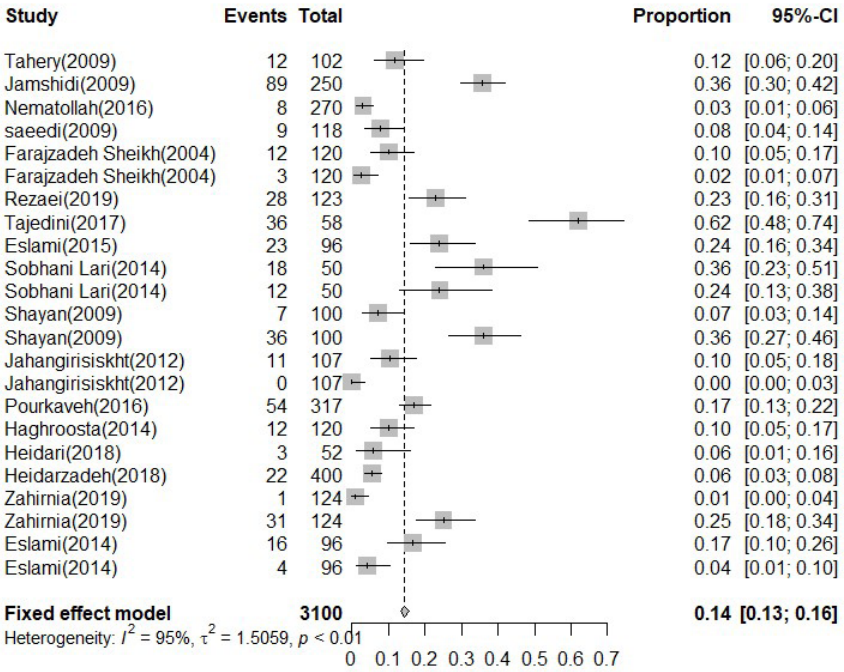

Figure 2. The prevalence of LM infection in Iranian pregnant women with a history of abortion

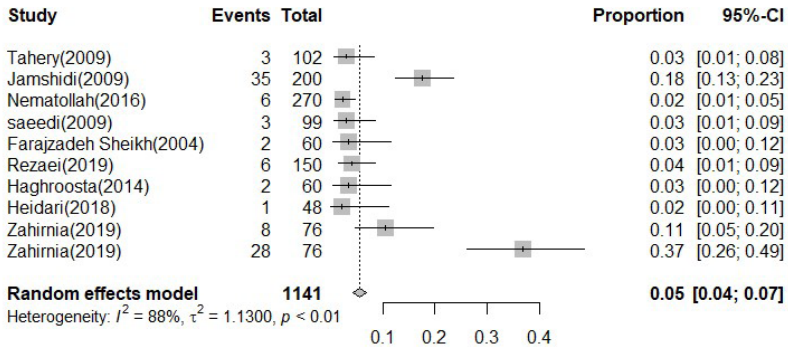

Figure 3. The prevalence of LM infection in Iranian pregnant women without a history of abortion

other parts of the world show very different statistics for infection with this bacterium and prevalence of infection in pregnant women, with the percentage of pregnant patients with confirmed Listeria compared to the total population of patients with Listeria reported as 17.7% in France (42), 16.9% in the United States (43), 16% in Spain (44), 15% in Germany (45), 12% in England and Wales (4), 11% in Italy (46), and 9% in Austria (47). In addition, in China, 41.1% to 52% of listeriosis cases were associated with pregnancy, indicating the widespread impact of the disease (48). Variations in prevalence in different communities can be due to differences in the characteristics of the study population, including culture, race, nutrition, geographical area, and laboratory diagnostic methods (49).

Rapid and accurate diagnosis of the disease is one factor that reduces mortality and hospital costs. Currently, traditional culture-based methods are still the gold standard in detecting infectious agents such as Listeria. However, culture sensitivity is significantly reduced due

Table 4. Prevalence of LM virulence genes in Iranian pregnant women with and without a history of abortion

Type gene	Prevalence of LM (95% CI[1])	Test for heterogeneity		
		Q-statistic	*P* value	I^2
actA	0.10 (0.06, 0.16)	12.82	0.001	75%
prfA	0.08 (0.04, 0.15)	16.43	<0.001	79%
hlyA	0.11 (0.05, 0.22)	104.06	<0.001	94%
inlB	0.06 (0.02, 0.22)	7.49	0.006	76%
hlyA/plcA	0.17 (0.14, 0.21)	0.007	0.93	0%

CI, confidence interval.

to the use of antibiotics in animal feed and the potential for intracellular growth of this bacterium. Furthermore, diagnosis with this method takes more than a few days (50). Infection with LM is usually asymptomatic, and the first sign is often, stillbirth, or severe infection in sensitive adults. Therefore, the importance of rapid diagnosis of this bacterium is emphasized in order to start antimicrobial treatment in time to prevent abortion and reduce pregnancy complications (32). Evidence suggests that PCR can confirm the presence of LM with more than 90% accuracy. Following new advances in molecular methods, selecting specific genes for LM and differentiating this bacterium from other species is possible. Many studies based on the PCR on LM have been based on tracing the *hlyA* gene. This gene encodes listeriolysin O (LLO), a critical factor disrupting the host's vacuolar membrane. It is found in all strains of LM, playing a vital role in its complete virulence (51-55). Other important genes in the LM infection cycle include the *plcA* gene, which encodes phosphatidylinositol-specific phospholipase C (PI-PLC), the *plcB* gene, which encodes phosphatidylcholine-specific phospholipase C (PC-PLC), the *actA* gene, which encodes actin polymerization protein, and the *inlA* and *inlB* genes, which encode internalin A and internalin B, respectively (56). The existing literature indicates that the *hlyA* gene has frequently been chosen as a target for PCR detection, and the primer/probe sets corresponding to regions of this gene are highly specific for LM. Nevertheless, a limitation of the PCR assay based on the *hlyA* gene is that certain strains of serovar 4c do not possess this gene, resulting in negative results (10, 54). According to our analysis, the incidence of the *hlyA* gene in Iranian pregnant women with and without a history of abortion is 11% (95% CI = 5%–22%).

In a study conducted in Iran, culture and molecular methods reported the frequency of infection at 7% and 36%, respectively (27). This is consistent with the results of the current meta-analysis, which found that in pregnant women with a history of abortion, the prevalence of LM infection using culture and PCR methods was 3% (95% CI = 2%–4%) and 18% (95% CI = 13%–25%), respectively. Also, the prevalence of LM infection in pregnant women without a history of abortion using culture and PCR methods was 5% (95% CI = 2%–13%) and 8% (95% CI = 1%–33%), respectively. PCR is commonly used for rapid, sensitive, and specific screening as well as confirmation of LM. Furthermore, culture methods are still applicable and are used in many studies. Cultural and molecular techniques are consistently being developed to enhance the sensitivity and specificity of LM detection. Progress in molecular methodologies has facilitated the rapid detection of LM in food and clinical samples with high sensitivity and specificity, replacing traditional and time-consuming detection approaches. While molecular methods offer numerous benefits, they also present certain limitations, including the requirement for sophisticated and expensive technology in comparison to conventional techniques (57).

Listeriosis is now recognized as a foodborne illness with a high mortality rate (58). The presence of LM in food is a major threat and concern for public health, especially for pregnant women. Therefore, stricter regulations on food production, more preventive measures, and better healthcare for pregnant women are recommended to reduce the incidence of Listeria.

The limitations of the present study include 1) significant heterogeneity, 2) diversity of diagnostic methods with different sensitivity and specificity, and 3) exclusive use of full texts in English and Persian articles.

Conclusion

This systematic review and meta-analysis revealed that Iranian pregnant women with a history of abortion have a high prevalence of LM infection compared to Iranian pregnant women without a history of abortion, and it seems to be one of the possible etiological factors of abortion among Iranian pregnant women. Since rapid diagnosis and timely initiation of antimicrobial therapy can prevent miscarriage and reduce pregnancy complications, it is suggested that PCR techniques using specific gene primers be used to identify LM in clinical samples. Also, implementation of measures to prevent food contamination by LM through public education by midwives and obstetricians is suggested to pregnant women to consume healthy food.

Authors' Contribution

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Visualization: Siavash Hamzeh Pour, Reyhane Sefidkar, Fateme Akrami-Mohajeri.
Writing–original draft: Siavash Hamzeh Pour, Reyhane Sefidkar.

Competing Interests
The authors declare they have no conflict of interest.

Ethical Approval
Not applicable.

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