Microbiology, Metabolites and Biotechnology
MMB
The former ARMMT

Frequency of *Listeria monocytogenes* and *Brucella abortus* Infections in the Vaginal Secretions of Women with Spontaneous Abortion: A case study

Atefeh Bayat[1], Ali Mohammad Ahadi*[2], Monir Doudi[1], Hatav Ghasemi-Tehrani[3]

[1] *Depatment of Microbiology, Falavarjan Branch, Islamic Azad University, Falavarjan, Isfahan, Iran*
[2] *Department of Genetics, Shahrekord University, Shahrekord, Iran*
[3] *Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

Article Info

Document Type:
Research Paper

Received 29/10/2023
Primary Acceptation
21/11/2023
Final Acceptation
21/12/2023
Published
12/05/2024

Keywords:
Listeria monocytogenes,
Brucella abortus,
Vaginal secretions,
Abortion

Abstract

Abortion is an involuntary and spontaneous termination of pregnancy. Various factors can be involved in abortion, and many of them are currently unknown. Among these, bacterial infections are of particular importance. This study aimed to investigate the presence of *Listeria monocytogenes* and *Brucella abortus* in the vaginal secretions of women suffering from abortion by biochemical and molecular methods. In the present study, a total of 110 samples of vaginal secretions from women suffering from abortion were collected in 2019 in Isfahan, Iran. The samples were collected from patients referred to gynecologists, obstetricians, and infertility specialists. Then, an antibiotic sensitivity test for bacterial isolates was done using the standard antibiogram method. The molecular detection of bacteria was performed by PCR reaction using specific primers designed for 16S rRNA gene amplification of *L.monocytogenes* and *B. abortus*. Based on the results, a total of 110 isolates of bacteria were obtained in the present study from women with and without abortion (control). Among the isolates, three belonged to *L. monocytogenes* (10.3%), and one belonged to *B. abortus* (3.4%). *B. abortus* was sensitive to all antibiotics used, and only one of the three *L. monocytogenes* isolates was resistant to tetracycline, ampicillin, and gentamicin antibiotics. The results of molecular identification showed that designing a species-specific primer to amplify the 16S rRNA gene by PCR assay may be a fast, low-cost, and reliable tool for screening for bacterial agents involved in abortion, which needs more study in the population. The high prevalence of *L. monocytogenes* in the vaginal secretions of women suffering from abortion is cause for concern and should be taken into consideration by treatment staff.

1. Introduction

According to published global statistics, 175 million pregnancies are registered worldwide every year, of which 45 million cases result in abortion (Wall et al., 2020). According to the latest research reported in Iran, 35% of women have experienced at least one abortion before

*Corresponding author. Ali Mohammad Ahadi. Department of Genetics, Shahrekord University, Shahrekord, Iran, E-mail address: ahadi_al@sku.ac.ir
DOI: 10.22104/MMB.2023.6391.1115
Please cite this article as: Ali Mohammad Ahadi, Microbiology, Metabolites and Biotechnology (MMB),
https:// DOI: 10.22104/MMB.2023.6391.1115

reaching the age of 44 (Erfani, 2009). *Listeria monocytogenes*, *Mycoplasma hominis*, and *Streptococcus agalactiae* bacteria are one of the most common causes of hypertension and miscarriage in women during pregnancy (Aljičevič et al., 2005; Amir et al., 2020; Ncib et al., 2022). Characterization of these bacteria in women with miscarriages and at gestational age, as well as administration of antibiotics and treatment of these infections, can prevent costs incurred by the patient and the healthcare system (Filippi et al., 2021). *L. monocytogenes* causes local and general infections in humans and other vertebrates and causes a wide range of infections in humans, including cold-like infections, primary meningitis, encephalitis, and septicemia (Vázquez-Boland et al., 2001; Inoue et al., 2017; Ahmadi et al., 2022). This bacterium often causes infection in people with immune system defects. Therefore, pregnant women are 20 times more at risk than the general population. Twenty-seven percent of all listeriosis is related to pregnant women, which unfortunately increases the possibility of premature birth, spontaneous abortion, stillbirth, and blood infection in the baby. In addition, *L. monocytogenes* can cause pneumonia, blood infection, and infant meningitis (Janakriman, 2008). Early detection of *L. monocytogenes* can be done through maternal blood culture, detection of the organism in cerebrospinal fluid, blood, amniotic fluid, respiratory tract secretions, placental or skin swabs, gastrointestinal aspirates, or infant feces (Wang et al., 2021). The diagnostic methods of this bacterium include culture, immunoassay test, nucleic acid hybridization, PCR, IUX phage, and combined culture and molecular tests (Le Monnier et al., 2011). Gram staining can be useful in up to 33% of cases, but due to the intracellular presence of the organism, it may cause a diagnostic error. There is also the possibility of being confused with Pneumococcus spp., diplococci, diphtheroids, and Haemophilus spp. (Shayan et al., 2009; Charlier et al., 2020). Brucella causes abortion and fetal abnormalities in livestock due to its tendency to grow more in the embryonic tissues (which contain erythritol) of cows, goats, sheep, and pigs (Mesner et al. 2007), resulting in heavy economic losses due to reduced production (Franc et al., 2018; Ntivuguruzwa et al., 2022). Since this substance has not been found in humans, it is believed that Brucella is not capable of causing complications such as abortion in humans. Conversely, the presence of anti-Brucella antibodies in the amniotic fluid has weakened Brucella's role in causing pregnancy complications (Al-Tawfiq & Memish, 2013; Bosilkovski et al., 2020). However, in endemic areas, the fate of human pregnancies with brucellosis is similar to that of infected animals (Hull & Schumaker, 2018), with results reported ranging from normal delivery to abortion, intrauterine fetal death, premature delivery, and placental retention. Therefore, it is thought that abortion and early delivery in pregnant women with brucellosis may be caused by inflammation caused by this febrile disease (Bosilkovski et al., 2020; Majzoobi et al., 2023). In some cases, Brucella has been found to result from pregnancy, indicating the transfer of Brucella through the placental barrier and intrauterine contamination of the human fetus (Byndloss et al., 2019). There have even been reported cases of human infections that were clearly transmitted through the placenta that caused congenital Brucella infection at birth, subsequently causing contamination of the delivery team due to exposure to amniotic fluid. It should be noted that the results of different studies from different parts of the world show that there is no definitive agreement about the effect of this infection on human abortion (Karcaaltincaba et al., 2010). Hence, the purpose of this study was to investigate the frequency of *L. monocytogenes* and *B. abortus* in the vaginal secretions of women with abortion by biochemical and molecular methods in several medical centers in Isfahan.

2. Materials and Methods

2.1 Collection of samples from vaginal secretions and bacteria isolation

In the present study, a total of 220 swab samples of vaginal secretions were collected from women suffering abortions and healthy from the beginning of 2019 to the end of the same year.

samples in this study included 110 samples of vaginal secretions from women who were referred to gynecological departments because of a present sudden abortion. A proven cause of abortions in these women had not been detected. Also, 110 samples were taken from pregnant women without reproductive problems or genital infections as a control group. The studied women were all married ages between 21 to 35 years old with no assisted reproductive methods for pregnancy. These women had been referred to different medical centers with a gynecology department in Isfahan, Iran. The samples were taken after abortion, from the middle third area of the vagina with sterile swabs by gynecologists. Then, the samples were transferred in tubes containing transfer medium to the microbiology laboratory of Islamic Azad University, Falavarjan Branch. The study was approved by the Islamic Azad University Ethics Committee, Falavarjan Branch, Isfahan, with the code IR.IAU.FALA.REC.1398.025. The bacteria were inoculated on Listeria LEB enrichment medium, Oxford agar, Blood agar, and Brucella agar and incubated at 37 ºC for 24 hours. The bacterial colonies were purified using the same media (Riedel et al., 2019; Bayat et al., 2023).

2.2 Standard bacterial strains

To confirm the identification of bacterial isolates, standard bacterial strains (*L. monocytogenes* PTCC: 1163 and *B. abortus* PTCC: S19) were obtained from the Persian Type Culture Collection (PTCC): ptcc@irost.org and Pasteur Institute of Iran, Tehran.

2.3. Investigation of microscopic and macroscopic characteristics of the isolates

The macroscopic characteristics of the purified bacterial colonies, including color, shape, and size, were evaluated after growth on the culture media. Also, the microscopic characteristics of the isolates were investigated following Gram staining (Riedel et al., 2019; Bayat et al., 2023).

2.4 Molecular identification of the isolates

Molecular identification of the isolates was done using specific Listeria monocytogenes and Brucella abortus primers (Bayat et al., 2023). (Table 1) shows the characteristics of the primers used in this study.

Table 1: Sequence Characteristics of the Primers Used in the Present Study.

Strains	Sequence	Target sequence	Fragment length	Primers
L. monocytogenes	5'-TGACATCCTTTGACCACTCTG-3' 5'-TGTGACGGGCGGTGTGTAC-3'	16S	417	FLSAB RMHCTAB
B. abortus	5'-TGTGTATAATTCGAAGCAACG-3' 5'-TGTGACGGGCGGTGTGTAC-3'	16S	520	FBAAB RMHCTAB

2.5 Phylogenetic analyses

To draw the phylogenetic trees of DNA sequences, *L. monocytogenes* and *B. abortus* and related strains were put through advanced analysis using comparison search BLAST (http://www.ncbi.nlm.nih.gov/blast) and phylogenetic estimating by ClustalW (http://www.ebi.ac.uk/clustalw) for each concerned DNA sequence. MEGA7 determined the best-fit model of evolution.

2.6 Antibiotic sensitivity testing

The antibiotic discs used in this research included amoxicillin 30 µg, penicillin 2 U, cephalexin 30 µg, ampicillin 10 µg, ceftazidime 30 µg, clindamycin 2 µg, vancomycin 30 µg, tobramycin 10 µg, erythromycin 15 µg, ciprofloxacin 5 µg, rifampin 5 µg, doxycycline 30 µg, tetracycline 30 µg, norfloxacin 32 µg, and streptomycin 10 µg. All were obtained from the Padtan Teb Medicine Company, Iran. The Kirby-

Bauer disk diffusion method was used for antibiotic susceptibility determination in the isolates (Bayat et al., 2023).

2.7 Statistical analysis

Statistical analysis (including number, percentage, mean, and standard deviation) and inferential (analytical) statistics (including chi-square tests, independent t-tests, and Fisher's exact test) were conducted. SPSS 24 software program was used to measure the data statistically. Tables and graphs were drawn using the Excel 2016 software program.

3. Results and discussion

3.1 The frequency of bacterial isolates and their relationship with abortion in pregnant women

The results of the frequency distribution of abortion-causing bacteria in two groups (with abortion and control) are presented in (Table 2). Based on Fisher's exact statistical test results, *B. abortus* (P=0.941) and *L. monocytogenes* (P=0.622) showed a significant difference between the two groups.

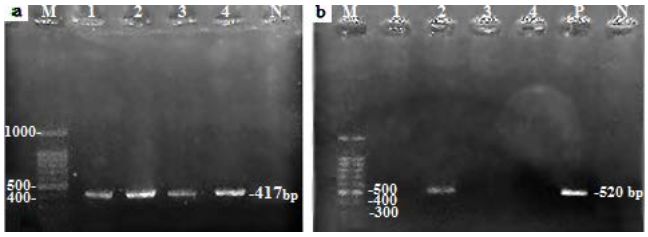
Table 2: The results of the distribution of the frequency of abortion-causing bacteria in two groups (control and abortion).

Strains	Group	Abortion		P value
		No.	Percentage	
L. monocytogenes	Negative	107	97.3	0.622
	Positive	3	2.7	
B. abortus	Negative	109	99.1	0.941
	Positive	1	0.9	
Total		110	100	

3.2 The results of molecular detection

According to Fig. 1 a and b, the agarose gel electrophoresis results of all PCR products related to the *L. monocytogenes* and *B. abortus* DNAs show the amplified fragment size of 417 bp in 3 samples for *L. monocytogenes* (Fig. 1a) and 520 bp in 1 sample for *B. abortus* (Fig. 1b) in comparison to a 100 bp DNA size marker.

Figure 1: Specific PCR Products of L. monocytogenes**(a)**, Electrophoresis Results of B. arbutus **(b)**.



Note: **(a)** M: marker (100 bp), 1 to 3: PCR product of 1 to 3 isolates. P: Positive control (standard sample), N: Negative control. **(b)** M: marker (100 bp), 2: PCR product of isolate 1 (no. 2). P: Positive control (standard sample), N: Negative control.

3.3 The results of 16S rRNA gene sequencing in the bacterial isolates

In this stage, after the identification of bacterial isolates by molecular analysis, the obtained sequences were confirmed and recorded in the World Gene Bank and BLAST, with 99-100% similarity to *B. abrutus* and *L. monocytogenes*. These bacteria are available on the NCBI site under accession numbers OQ102142.1 and OQ104737.1, respectively. The molecular identification results showed that designing the species-specific primer to amplify the 16S rRNA gene using PCR assay may be a fast, low-cost, and reliable tool to screen for bacterial agents involved in abortion.

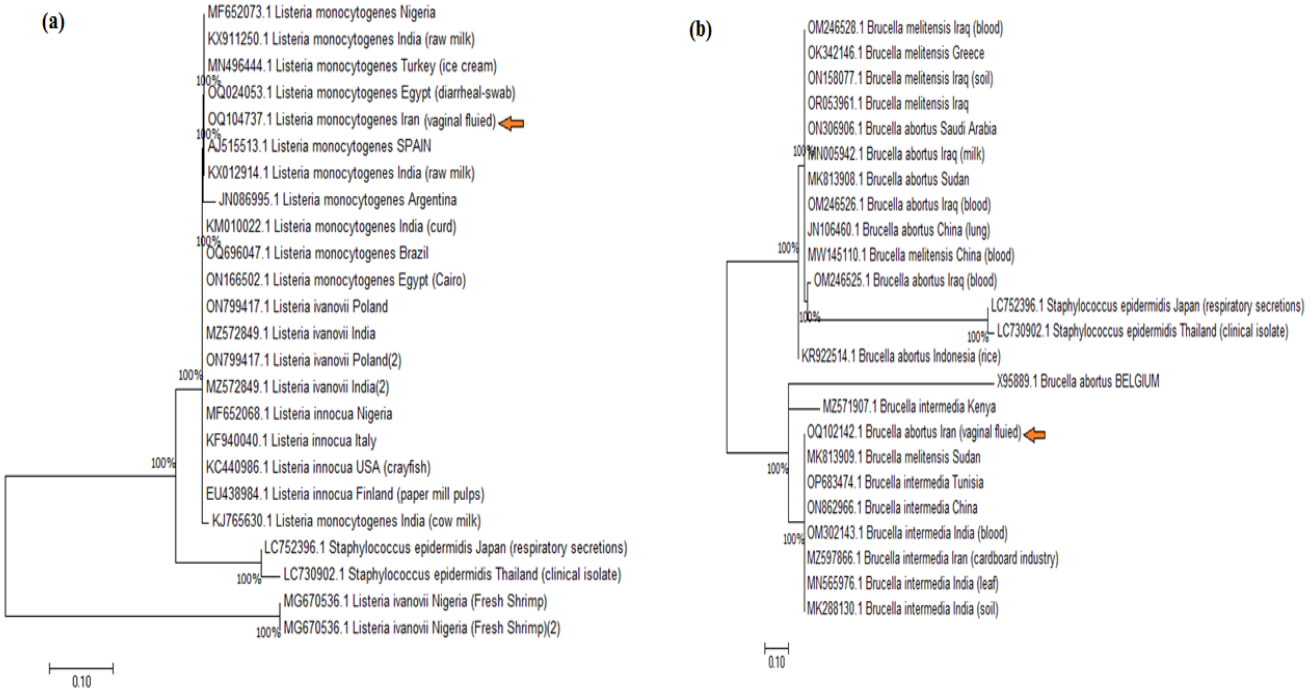
3.4 The results of phylogenetic analyses

Figure 2 shows the phylogenetic tree obtained from the ClustalW alignment using MEGA7 software for two of the novel sequences (as indicated by orange arrows) and representative lines from most of the previously described species (as indicated by GenBank accession numbers). A consensus of the two trees based on 16S rRNA means that both novel bacteria associated with abortion do not form a monophyletic group. The phylogram presented three major groups for *L. monocytogenes* and *B. abortus* species. *L. monocytogenes* was grouped with *L. ivanovii* and *L. inocua* (Fig. 2a), and *B. abortus*, with *B. intermedia* and *B. melitensis*

(Fig. 2b). The sequences were deposited in GenBank with the accession numbers OQ104737.1 for *L. monocytogenes* and OQ102142.1 for *B. abortus*.

In the phylogram, the sequence of *L. monocytogenes* (OQ104737.1) identified in this study clustered with the *L. monocytogenes* species previously identified from Spain and Turkey as the nearest neighbors. Sequence phylogenetic analysis for the *B. abortus* (OQ102142.1) isolate showed that this isolate had the most sequence similarity with *B. melitensis* isolated from Sudan. This phylogeny relationship is very important because although both strains cause abortion, *B. abortus* has been isolated and identified from human clinical samples and *B. melitensis* from livestock samples.

Figure 2. Dendrograms of L. monocytogenes (a) and B. abortus (b) Strains Isolated from the Vaginal Secretions of Women with Spontaneous Abortion.

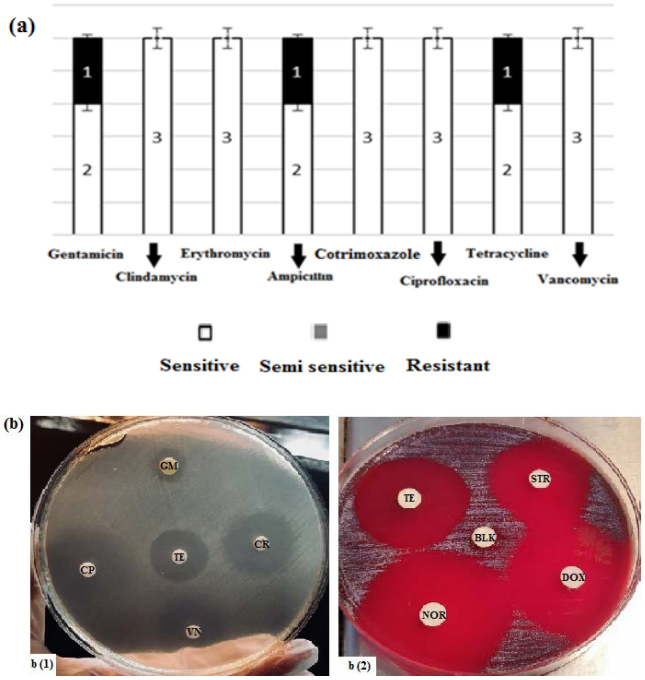


Note: DNA sequences of the 16S rRNA region were aligned using MEGA7 software. Orange arrows indicate themes identified in this study (GenBank accession numbers OQ104737.1 and OQ102142.1).

3.5 Antibiogram test results on Listeria and Brucella isolates

This study used five antibiotics, including rifampin, tetracycline, doxycycline, norfloxacin and streptomycin, to perform the antibiogram test for a *B. abortus* isolate. The test results showed that only the isolate of *B. abortus* was sensitive to all these antibiotics. Eight antibiotics, including vancomycin, tetracycline, ciprofloxacin, cotrimoxazole, ampicillin, erythromycin, clindamycin, and gentamicin, were used to perform the antibiogram test for three isolates of *L. monocytogenes*. The results of antibiotic resistance and susceptibility of the three strains of *L. monocytogenes* are presented in Fig. 3 (a). As can be seen, only one isolate was resistant to tetracycline, ampicillin, and gentamicin antibiotics. The results of the antimicrobial resistance test using the antibiogram test for *L. monocytogenes* and *B. abortus* isolates are presented in Fig. 3 (b).

Figure 3: Antibiotic Resistance and Sensitivity of 3 Isolates of *L. monocytogenes* **(a),** Antibiogram Test Results in the Isolates L. monocytogenes (Fig. b (1)) and B. abortus (Fig. b (2)) **(b)**



Note: The numbers on the graphs show the number of resistant and sensitive strains.

In this study, three samples were isolated and identified as *L. monocytogenes*. However, in a previous study investigating the prevalence of *L. monocytogenes* in spontaneous abortions, vaginal samples were collected from women with a history of abortion admitted to Shariati Hospital in Tehran, Iran, during the years 2009 to 2010. Among 100 isolates, nine isolates belonged to *L. monocytogenes*, of which six isolates were obtained from placenta tissue and vaginal swabs, two isolates were obtained from anal swabs, and one isolate had been obtained from urine. The frequency of *L. monocytogenes*, known as the cause of abortion, was reported as 9%, which is higher than the frequency of this bacterium in the present study. One of the reasons for the difference in the abundance of this bacteria may be due to the different sampling locations, different methods of identifying bacteria, and target populations in different provinces (Lotfollahi et al., 2011). In another study by Goudarzi et al. (2013), among 87 samples of vaginal swabs of women suffering abortion, five isolates of the species *L. monocytogenes* were identified using the culture method; however, seven isolates of *L. monocytogenes* were detected using the PCR method. Therefore, it was suggested that the molecular method is better to avoid incorrectly identifying the abortion-causing bacteria (Goudarzi et al., 2013).

The frequency of *L. monocytogenes* in the present study was 10.3%. Jami et al. (2010) collected 305 samples (including blood, urine, vaginal swab, rectal swab, and fetal tissue) in a study on 61 women with abortions. After performing phenotypic tests, ten samples were detected to be infected with Listeria, and by performing differential tests, four samples were found to belong to the *L. monocytogenes* species. The frequency of Listeria in their study is higher than the frequency of this bacterium in the present study. The results of many Iranian studies show that the frequency of *L. monocytogenes* is higher than that in the present study. This may be due to the difference in the number and the manner of sampling, different cultural and economic levels in different societies, lack of hygiene, lack of antibiotic use, especially after tests, and using different diagnostic tests performed by different personnel with different diagnostic devices.

Based on the results of macroscopic and microscopic identification of isolates in this study, one isolate (3.4%) was detected as Brucella, which has the lowest frequency among the isolates. It has been shown that Brucella is the cause of abortion and fetal abnormalities in livestock, although they have also played a very small role in human abortion (Ghaznavi et al., 2012; Bosilkovski et al., 2019).

In general, Brucella causes heavy economic losses and loss of life to livestock and poultry. This is due to its tendancy to grow more in the fetal tissues of cows, goats, sheep, pigs, and even dogs because these tissues contain erythritol, which is highly favored by this bacterium. Since erythritol is fortunately not been found in humans, It is widely believed that Brucella is less able to cause serious complications such as

abortion in humans; however, it can occasionally cause complications. In addition, the presence of anti-Brucella antibodies in human amniotic fluid has weakened Brucella's role, making it less likely to cause complications during pregnancy or abortion in humans.

Nevertheless, in endemic areas, pregnancy in humans infected with Brucella can be similar to that of infected animals, ranging from normal delivery to abortion, with the death of the fetus in the womb, premature births, and placental retention associated with brucellosis in pregnant women being reported. It is thought that inflammation caused by this febrile disease may cause abortion and early delivery in pregnant women with brucellosis(Majzoobi et al., 2023).

In some cases, researchers have found Brucella in pregnant women, which indicates the transfer of Brucella from the placental barrier and intrauterine infection of the human fetus. Even cases of human infection explicitly through the placenta have been reported, which caused congenital Brucella infection at birth and also contamination of the delivery team due to exposure to amniotic fluid (Ghaznavi-Rad & Zarinfar, 2012; Byndloss et al., 2019). Roushan et al. (2011) followed 19 pregnant women with confirmed Brucella infection, among whom 10 (53%) suffered from spontaneous abortion in the first trimester of pregnancy; they were able to treat the infection with a combination of antibiotics (rifampin plus cotrimoxazole) in 13 patients. As a result, nine healthy babies were born, and only four mothers (31%) had spontaneous abortion complications despite the treatment. They emphasized the role of screening in preventing severe complications of brucellosis during pregnancy. Kurdoglu et al.'s study (2010) investigated the consequences of brucellosis on pregnancy in 29 pregnant women with brucellosis. In their study, they first ruled out the role of other microorganisms such as the herpes virus, cytomegalovirus, rubella virus, and toxoplasma parasite using the ELISA method and then followed up with the patients. Among the investigated patients, seven patients (24.11%) had spontaneous abortions, intrauterine death occurred in one patient (3.5%), and premature delivery was observed in two patients (6.9%). The researchers were only able to isolate Brucella from the blood cultures of two patients. A noteworthy point in this research was the delivery of healthy babies in 19 patients (65.5%) (Kurdoglu et al., 2010).

4. Conclusion

In this study, we used a combination of morphological, molecular, and phylogenetic analysis techniques to investigate the frequency of *L. monocytogenes* and *B. abortus* infection in the vaginal secretions of women with spontaneous abortion in Isfahan. The proposed design of the species-specific primer for amplifying the 16S rRNA gene by PCR assay would be a fast, low-cost, and reliable tool to screen for bacterial agents involved in abortion, which needs more study in the population. The high prevalence of *L. monocytogenes* in the vaginal secretions of women suffering from abortion is cause for concern and should be taken into consideration by the treatment staff.

Author contribution:

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Fereshteh Hozoorbakhsh. The first draft of the manuscript was written by Fereshteh Hozoorbakhsh, and all authors commented on and edited subsequent versions. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

Not Applicable.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

Not Applicable.

References

[1] Ahmadi, A., Ramazanzadeh R, Derakhshan S, Khodabandehloo M, Farhadifar F, Roshani D, Mousavi A, Hedayati MA, & Taheri M (2022). Prevalence of *Listeria monocytogenes* infection in women with spontaneous abortion, normal delivery, fertile and infertile. Bmc Pregnancy Childbirth, *22*(1):974. https://doi.org/10.1186/s12884-022-05330-6.

[2] Aljičevič, M., Bešlagić, E., Zvizdić, Š., Hamzič, S., & Mahmutovič, S. (2005). *Listeria monocytogenes* as the possible cause of the spontanous abortion in female of the fertile age. *Bosnian Journal of Basic Medical Sciences*, *5*(4), 89. https://doi.org/10.17305/bjbms.2005.3241.

[3] Amir, M., Brown, J. A., Rager, S. L., Sanidad, K. Z., Ananthanarayanan, A., & Zeng, M. Y. (2020). Maternal microbiome and infections in pregnancy. *Microorganisms*, *8*(12), 1996. https://doi.org/10.3390/microorganisms8121996.

[4] Bayat, A., Doudi, M., Ahadi, A. M., & Ghasemi-Tehrani, H. (2023). Isolation and characterization of *Streptococcus agalactiae* and its capsular antigen, along with *Mycoplasma hominis* and *Listeria monocytogenes*, as the abundant infections in the women with abortion in Iran. *Jundishapur Journal of Microbiology, 16*(10): e141748. https://doi.org/10.5812/jjm-141748.

[5] Bosilkovski, M., Arapović, J., & Keramat, F. (2020). Human brucellosis in pregnancy–An overview. *Bosnian journal of Basic Medical Sciences*, *20*(4), 415. https://doi.org/10.17305/bjbms.2019.4499.

[6] Byndloss, M. X., Tsai, A. Y., Walker, G. T., Miller, C. N., Young, B. M., English, B. C., ... & Tsolis, R. M. (2019). *Brucella abortus* infection of placental trophoblasts triggers endoplasmic reticulum stress-mediated cell death and fetal loss via type IV secretion system-dependent activation of CHOP. *MBio*, *10*(4), 10-1128. https://doi.org/10.1128/mBio.01538-19.

[7] Charlier, C., Disson, O., & Lecuit, M. (2020). Maternal-neonatal listeriosis. *Virulence*, *11*(1), 391-397. https://doi.org/10.1080/21505594.2020.1759287

[8] Erfani, A. (2016). Levels, trends and correlates of abortion in Tehran, Iran: 2009–2014. *International Perspectives on Sexual and Reproductive Health*, *42*(2), 93-101. https://doi.org/10.1363/42e1316

[9] Filippi, V., Dennis, M., Calvert, C., Tunçalp, Ö., Ganatra, B., Kim, C. R., & Ronsmans, C. (2021). Abortion metrics: a scoping review of abortion measures and indicators. *BMJ Global Health*, *6*(1), e003813. https://doi.org/10.1136/bmjgh-2020-003813

[10] Franc, K. A., Krecek, R. C., Häsler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health*, *18*(1), 1-9. https://doi.org/10.1186/s12889-017-5016-y.

[11Ghaznavi-Rad, E., & Zarinfar, N. (2012). Brucellosis in pregnancy. *Arak Medical University Journal*, *14*(7), 100-108. http://jams.arakmu.ac.ir/article-1-1416-.pdf

[12] Goudarzi, E., Harzandi, N., & Yousefi, J. (2013). Survey of PCR efficiency in the detection of *Listeria*, *Brucella* and *Mycoplasma* in culture negative samples obtained from women with abortion. *Journal of Mazandaran University of Medical Sciences*, *23*(105), 61-9. http://jmums.mazums.ac.ir/article-1-2770-en.html

[13] Hull, N. C., & Schumaker, B. A. (2018). Comparisons of brucellosis between human and veterinary medicine. *Infection Ecology & Epidemiology*, *8*(1), 1500846. https://doi.org /10.1080/20008686.2018.1500846.

[14] Inoue, T., Itani T, Inomata N, Hara K, Takimoto I, Iseki S, Hamada K, Adachi K, Okuyama S, Shimada Y, Inoue, T., Itani, T., Inomata, N., Hara, K., Takimoto, I., Iseki, S., ... & Mimura, J. (2017). *Listeria Monocytogenes septicemia* and *meningitis* caused by *Listeria enteritis* complicating ulcerative colitis. *Internal Medicine*, *56*(19), 2655-2659. https://doi.org/10.2169/internalmedicine.8654-16.

[15] Jami, S., Jamshidi, A., & Khanzadi, S. (2010). The presence of *Listeria monocytogenesin* raw milk samples in Mashhad, Iran. *Iranian Journal of Veterinary Research*, *11*(4), 363-367. https://journals.shirazu.ac.ir/article_108_83d8eca24a78493312723308969e82c5.pdf

[16] Janakiraman, V. (2008). Listeriosis in pregnancy: diagnosis, treatment, and prevention. *Reviews in Obstetrics and Gynecology*, *1*(4), 179. PMC2621056

[17] Karcaaltincaba, D., Sencan, I., Kandemir, O., Guvendag -Guven, E. brucellosis in human pregnancy increase abortion risk? Presentation of two cases and review of literature. *Journal of Obstetrics and Gynaecology Research*, *36*(2), 418-423. https://doi.org/10.1111/j.1447-0756.2009.01156.x

[18] Kurdoglu, M., Adali, E., Kurdoglu, Z., Karahocagil, M. K., Kolusari, A., Yildizhan, R., ... & Akdeniz, H. (2010). Brucellosis in pregnancy: a 6-year clinical analysis. *Archives of Gynecology and Obstetrics*, *281*, 201-206. https://doi.org/10.1007/s00404-009-1106-0. Epub 2009 May 12

[19] Le Monnier, A., Abachin, E., Beretti, J. L., Berche, P., & Kayal, S. (2011). Diagnosis of *Listeria monocytogenes* meningoencephalitis by real-time PCR for the hly gene. *Journal of Clinical Microbiology*, *49*(11), 3917-3923. https://doi.org/10.1128/JCM.01072-11.

[20] Lotfollahi, L., Nowrouzi, J., Irajian, G., Masjedian, F., Kazemi, B., Eslamian, L., ... & Ramez, M. (2011). Prevalence and antimicrobial resistance profiles of *Listeria*

monocytogenes in spontaneous abortions in humans. *African Journal of Microbiology Research*, *5*(14), 1990-3. https://doi.org/ 10.5897/AJMR11.498

[21] Majzoobi, M. M., Teimori, R., Nouri, S., Karami, M., Bosilkovski, M., & Saadatmand, A. (2023). Maternal, fetal, and neonatal outcomes of gestation in women with and without *Brucella* Infection. *Journal of Research in Health Sciences*, *23*(1). https://doi.org/10.34172/jrhs.2023.110.

[22] Mesner, O., Riesenberg, K., Biliar, N., Borstein, E., Bouhnik, L., Peled, N., & Yagupsky, P. (2007). The many faces of human-to-human transmission of brucellosis: congenital infection and outbreak of nosocomial disease related to an unrecognized clinical case. *Clinical Infectious Diseases*, *45*(12), e135-e140. https://doi.org/10.1086/523726

[23] Ncib, K., Bahia, W., Leban, N., Mahdhi, A., Trifa, F., Mzoughi, R., ... & Donders, G. (2022). Microbial diversity and pathogenic properties of microbiota associated with aerobic vaginitis in women with recurrent pregnancy loss. *Diagnostics*, *12*(10), 2444. https://doi.org/10.3390/diagnostics12102444

[24] Ntivuguruzwa, J. B., Kolo, F. B., Mwikarago, E. I., & Van Heerden, H. (2022). Characterization of *Brucella* spp. and other abortigenic pathogens from aborted tissues of cattle and goats in Rwanda. *Veterinary Medicine and Science*, *8*(4), 1655-1663. https://doi.org/10.1002/vms3.805.

[25] Riedel, S., Morse SA, Mietzner TA, Miller S (2019). *Jawetz Melnick & Adelbergs medical microbiology, 28e*. McGraw Hill Professional, USA.

[26] Roushan, M. R. H., Baiani, M., Asnafi, N., & Saedi, F. (2011). Outcomes of 19 pregnant women with brucellosis in Babol, northern Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *105*(9), 540-542. https://doi.org/10.1016/j.trstmh.2011.06.003

[27] Shayan, R., Satari, M., & Forouzandeh, M (2009). Isolation and identification of *Listeria monocytogenes* in vaginal samples by PCR. *Pathobiology Research (Modares Journal of Medical Sciences) 12*(1), 51-58. https://pesquisa.bvsalud.org/portal/resource/pt/emr-93845

[28] Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., ... & Kreft, J. (2001). *Listeria* pathogenesis and molecular virulence determinants. *Clinical microbiology reviews*, *14*(3), 584-640. https://doi.org/10.1128/CMR.14.3.584-640.2001.

[29] Wall, D. J., Reinhold, C., Akin, E. A., Ascher, S. M., Brook, O. R., Dassel, M., ... & Glanc, P. (2020). ACR appropriateness criteria® female infertility. *Journal of the American College of Radiology*, *17*(5), S113-S124. https://doi.org/10.1016/j.jacr.2020.01.018

[30] Wang, Z., Tao, X., Liu, S., Zhao, Y., & Yang, X. (2021). An update review on *Listeria* infection in pregnancy. *Infection and Drug Resistance*, *14*, 1967-1978. https://doi.org/10.2147/IDR.S313675.

[31] A Al-Tawfiq, J., & A Memish, Z. (2013). Pregnancy associated brucellosis. *Recent Patents on Anti-Infective Drug Discovery*, *8*(1), 47-50. https://doi.org/10.2174/1574891x11308010009.