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Molecular detection of *Listeria monocytogenes* from aborted women

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Abstract

Abortion, an involuntary and spontaneous termination of pregnancy, can be influenced by various factors, including potentially unknown ones. Bacterial infections play a significant role in some cases. *Listeria monocytogenes*, a facultatively pathogenic microorganism, can lead to the serious illness known as listeriosis, a global foodborne disease. This study aimed to assess the presence of *Listeria monocytogenes* in placental tissue and vaginal swabs of women experiencing abortion through biochemical and molecular analyses. The study was conducted in 2023 in Al-Diwaniyah, Iraq, and 125 samples were collected. The molecular testing involved PCR amplification of the 16S rRNA gene specific to L. monocytogenes. The study yielded 125 bacterial isolates (10.4% of samples), with all *L. monocytogenes* isolates (100%) containing virulence genes (inlA, plcA, iapA). The detection of *L. monocytogenes* is a matter of concern and should be considered by healthcare providers for appropriate management.

Keywords: *L. monocytogenes*, inlA, plcA, iap, PCR.

Introduction

Approximately 175 million pregnancies are recorded globally each year, with around 45 million pregnancies ending in abortion (1). Several common causes of hypertension and miscarriages in pregnant women include the microorganisms *Streptococcus agalactiae*, *Mycoplasma hominis*, and *Listeria monocytogenes* (2, 3). *Listeria monocytogenes* is a rod-shaped, strictly anaerobic, gram-positive foodborne pathogen that does not generate spores. This opportunistic intracellular bacterium is the source of listeriosis, one of the most common and deadly foodborne infections in the world (4). Most people with listeriosis also have another condition that affects T-cell-mediated immunity (5). This bacterium often infects immunocompromised individuals. As such, pregnant women are twenty times more vulnerable than the public. Unfortunately, pregnant women are associated with listeria in 27 percent of instances; this increases the risk of blood infection in the fetus, spontaneous miscarriage, stillbirth, and premature delivery. In

newborns, *L. monocytogenes* can potentially cause meningitis, pneumonia, and blood infections (6). Maternal blood cultures can be used to identify *L. monocytogenes* early on. Other methods include looking for the organism in respiratory tract secretions, amniotic fluid, placental or skin swabs, gastrointestinal aspirates, blood, or baby feces. The pathogenicity of *L. monocytogenes* is characterized by cell-to-cell transmission, cytosolic replication and motility, and host-cell adhesion and invasion (7). The transcriptional regulator PrfA controls the six primary virulence genes (prfA, plcA, hly, mpl, actA, and plcB) found on Listeria Pathogenicity Island (LIPI)-1 (8).

Well-characterized virulence elements that subvert host cell activities are responsible for each stage's dependence (9). Molecular techniques such as in vitro nucleic acid amplification and hybridization, serological testing that detect the presence of bacterial antigens or antibodies in blood and serum, and conventional procedures that require isolating and identifying the agent are the methods commonly used to diagnose listeriosis (10, 11).

METHODS

Collection of samples

A total of 125 samples were taken from women who had abortions between the start of 2023 and the end of the same year for the current investigation. the placental tissue and vaginal swab samples from women who had previously or currently had spontaneous abortions and were sent to gynecological departments. There was no known reason why these ladies were having abortions. All of the married women in the study ranged in age from 21 to 36.

The isolation and identification of *L. monocytogenes*

Vaginal swabs and 25g of placental tissue specimens were aseptically inoculated into 10 ml and 225 ml of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) broth, respectively. Following the inoculation in TSBYE, the samples underwent homogenization and were subsequently subjected to incubation for 24 hours at 37°C. After this incubation period, a loopful of the resulting culture was streaked onto the surfaces of a variety of agar plates, such as Listeria agar supplemented with 10 mg of ceftazidime, 20 mg of acriflavin, and 5 mg of polymyxin B, as well as PALCAM and Listeria agar. Afterward, the agar plates were incubated using the AOAC method for a duration of 24 to 48 hours at 35°C. To identify the bacteria, various techniques were used, including biochemical assays, oxidase, catalase, CAMP, motility tests at 25°C, and streaking on blood agar, all following Hitchins' methodology (12).

The extraction of bacterial genomic DNA

L. monocytogenes genomic DNA was extracted using the PrestoTM Mini gDNA Bacteria Kit Quick Protocol and performed following the instructions provided by the company.

Detection by polymerase chain reaction (PCR).

Molecular identification of the isolates was done for the detection of the 16S rRNA gene specific for *L. monocytogenes* and its virulence factors gene primers used in this study. PCR amplification was done using a conventional thermal cycler and was made according to the conditions in Table (1):

Table 1: PCR amplification conditions for detecting 16s rRNA, *plcA*, *inlA*, and *iapA* genes.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
16s rRNA						
plcA	$95C^0$ /3 min	$95C^0$ / 30 sec	58 C^0 /30sec	72 C^0 /1 min	72 C^0 /5 min	30
inlA	$95C^0$ /2 min	$95C^0$ / 30 min	57 C^0 /45sec	72 C^0 / 2min	72 C^0 /5min	30
iapA	$95C^0$ /3 min	$95C^0$ / 30 sec	58 C^0 /30sec	72 C^0 /1 min	72 C^0 /5min	30

Using specific primers for this study according to the mentioned conditions as illustrated in Table (2).

Table 2: Primer sets used to detect 16s rRNA, *plcA*, *inlA*, and *iapA* genes.

Gene	Sequence (5′-3′)	Product size (bp)
16s rRNA	F- GCTATGACG GGTATCC R- GAT TTTACC CCTACACCA	**1500bp**
plcA	F-TTGCTCGTGTCAGTTCTGGG R- TCTCAACATTTACCATGGGCCA	**210 bp**
inlA	F- TAGCACCACTGTCGGGTCTA R- AGCTGGTGCAATTAAAGCGC	**373 bp**
iapA	F- CGAATCTAACGGCTGGCACA R- ACAGGTGCAGCTTGTTGAGT	**160 bp**

Statistical analysis

The results were employed for assessing the variables' significance, utilizing Version 26 of SPSS software for statistical analyses. Results with $P < 0.05$ are deemed statistically significant.

Results

The microbiological examination of 125 samples yielded positive results for *L. monocytogenes* in 13 samples, representing a prevalence rate of 10.4%. Among the isolates obtained from clinical specimens, eight were identified, originating from a single placental tissue sample and five from vaginal swab samples, as detailed in Table 3.

To isolate *L. monocytogenes*, each isolate was cultivated on the selective medium PALCAM. Furthermore, as indicated in Table 4, the identification of *L. monocytogenes* was verified by evaluating their catalase activity, demonstrating positive CAMP reaction with *Staphylococcus aureus*, demonstrating motility at 25°C, obtaining negative results in the oxidase test, and exhibiting β-hemolysis on blood agar.

On performing PCR amplification, utilizing the 16S rRNA gene and PCR amplification, L. monocytogenes was evaluated in samples collected from aborted women (refer to Figure 1). Out of the 125 samples analyzed, 13 (10.4%) tested positive for *L. monocytogenes*, with every isolate containing the virulence genes plcA, iap, and inlA, as detailed below in figures (1-4).

Table 3: The Prevalence of L. monocytogenes in different samples

Sample	Total N. of samples	Positive samples (%)
Vaginal swabs	67	5 (4.7%)
Placenta tissue	58	8 (13.7%)
Total	125 (100%)	13 (10.4)
Statistical significance	$p < 0.05$*	

*: significance

Table 4: The biochemical tests on isolates

Samples	Hemolysis	Catalase test	Oxidase test	Motility at 25°C	CAMP test
Vaginal swabs	β-hemolysis	+	–	+	+S
Placental tissue	β-hemolysis	+	–	+	+S

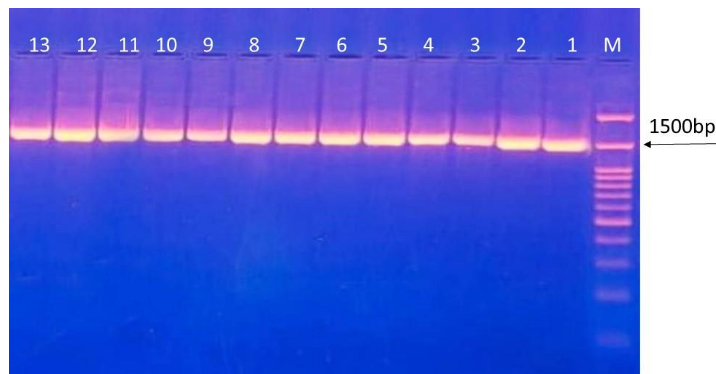

Figure (1): PCR amplification of 16S rRNA gene of the *L. monocytogenes* isolates. The DNA size marker used ranged from 100 to 2000 bp; lanes 1-13 positive isolates.

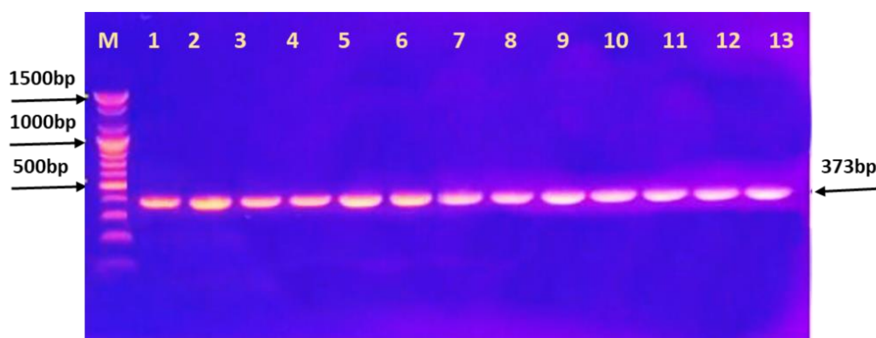

Figure (2): PCR amplification of the *inlA* gene (373 bp) was conducted in *L. monocytogenes* isolates. The DNA size marker used ranged from 100 to 1500 bp; lanes 1-5 positive isolates from vaginal swabs samples; Lanes 6-13, positive isolates from Placenta tissue sample.

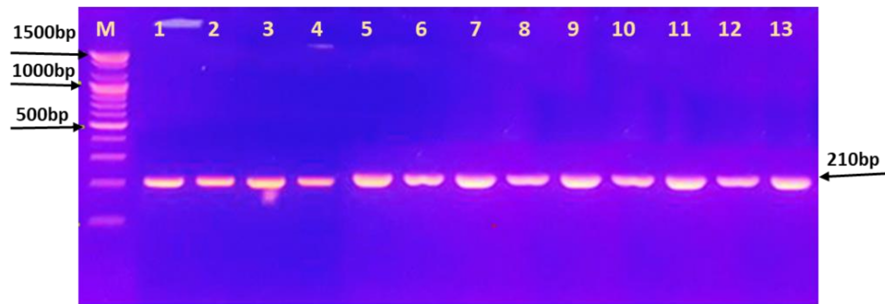

Figure (3): PCR amplification of the *plcA gene* (210 bp) in L. monocytogenes isolates. M, 100-1500 bp DNA size marker; lanes 1-5 positive isolates from vaginal swabs samples; Lane 6-13, positive isolates from Placenta tissue sample.

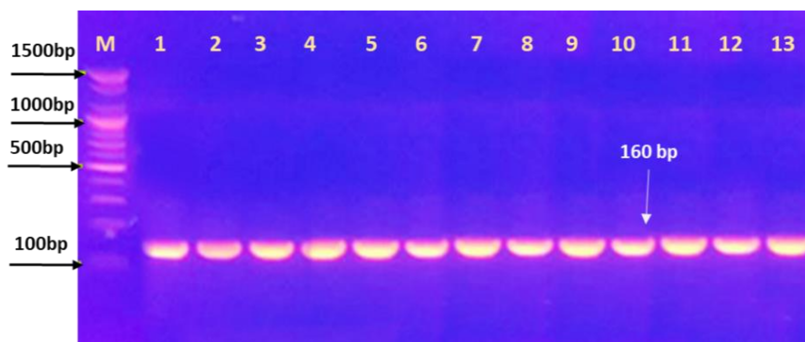

Figure (4): PCR amplification of the *iap* gene (160 bp) in *L. monocytogenes* isolates. M, 100-1500 bp DNA size marker; lanes 1-5 positive isolates from vaginal swabs samples; Lane 6-13, positive isolates from Placenta tissue sample**.**

Discussion

The pressing necessity to create tailored therapeutics has made it a top priority for studies on molecular and cellular etiology. A pregnant woman may be at risk for early pregnancy loss for several reasons, the most significant of which is infection. It is possible to stop more miscarriages in women by diagnosing bacterial infections (13).

In this study, PCR targeting the 16S rRNA gene was employed to detect *L. monocytogenes* in women who experienced abortion, revealing a detection frequency of 10.4%. This finding suggests the presence of *L. monocytogenes* in a subset of women who had experienced abortion, emphasizing the importance of understanding the role of this pathogen in pregnancy complications. According to Ahmadi et al. (14), the incidence of *L. monocytogenes* infection was 3.66% in women who had spontaneous abortions, 1.83% in women who had normal deliveries, 3% in fertile women, and 0% in infertile women. Eslami et al. (15) reported the detection of *L. monocytogenes* in 16.7% of aborted women, while Al-Mayahi et al. (16) identified L. monocytogenes in 4.8% of aborted women. The various outcomes could be attributed to factors such as the immunity of women, medical histories, dietary practices, geographical location, interaction with contaminated animals, and level of awareness regarding the spread of disease.

The type of virulence genes present in the bacterial strain, the infection dose, and the immunity of the infected subjects all affect the likelihood of contracting *L. monocytogenes* infection. On the other hand, individuals with immunodeficiency had a higher risk of infection (17). Our study utilized conventional PCR to identify virulence genes in *L. monocytogenes* isolates obtained from aborted women, revealing that all isolates harbored virulence genes including inlA, IapA, and plcA, with a prevalence of 100%. This finding highlights the significant presence of these virulence factors in *L. monocytogenes* strains associated with pregnancy complications. Further investigation is required to elucidate the role of these genes in *L. monocytogenes* pathogenicity during pregnancy and their potential impact on pregnancy outcomes. Our results are in agreement with Soni, et al., (18) demonstrated that all the isolates were positive for virulence genes tested i.e. inlA, inlC, inlJ, plcA, prfA, actA, hlyA, and iap. All *L. monocytogenes* isolates tested positive for the presence of the InlA gene in our results, these were in agreement with those obtained by Hamid, et al. (19) revealed the inlA gene was detected in all *L. monocytogenes* isolates (100%). Also, Hassanien and Shaker (20) detected hylA, and InlA genes from food and abortion women in Egypt. The Invasive associated protein (inlA) is vital for the pathogenicity of *L. monocytogenes* as it facilitates entry into cell types that express human E-cadherin, particularly in epithelial cells located at the luminal surface of intestinal villi (21).

Phosphatidylinositol-specific phospholipase C, encoded by the plcA gene, has a complementary role with listeriolysin O (LLO) in the dissolution of the primary and secondary vacuole that occurs after pathogen internalization [22]. Our findings on the plcA gene coincide with those of Throat et al. (23) who found that *L. monocytogenes* isolated from diverse sources, including humans, food, animals, and mosquitoes, have the prfA, actA, hlyA, inlC, flaA, plcA, inlJ, and luxS genes. Through the establishment of an intracellular niche that allows the bacteria to multiply and evade the immune response, invasive associated protein (Iap) facilitates Listeria's invasion into host cells. In *L. monocytogenes* isolates from women who experienced spontaneous abortions, the prfA, hylA, and Iap genes were detected, according to Kaur et al. (24). These genes increase *L. monocytogenes* attachment, invasion, development, and survival inside human cells, which increases its pathogenicity, particularly in immunocompromised patients. It is also a good indicator of the virulence level of *L. monocytogenes* (25). These genes are present in the isolated strains. Hence, the identification of multiple virulent genes through PCR assays is advantageous as it minimizes the time required and proves valuable for extensive investigations aimed at detecting pathogenic strains of Listeria (26).

Conclusion

The results of the study revealed a concerning prevalence of *Listeria monocytogenes* in the samples collected, with all isolates of *L. monocytogenes* found to contain virulence genes (inlA, plcA, iapA), which are associated with increased and enhanced pathogenicity. To effectively treat and control listeriosis infections, it is imperative to accurately identify *L. monocytogenes* and ascertain the risk factors associated with infection in women who have experienced abortions.

Conflict of interest: None

Funding: None

References

1- Wall, K. M., Lathrop, E., & Haddad, L. B. (2022). Post-abortion contraceptive prevalence rate as a sexual and reproductive health indicator. Women's Health, 18, 17455057221122498.

2- 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.

3- 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.

4- Yousif, M. G., & AL-Shamari, A. K. (2018). Phylogenetinc characterization of Listeria monocytogenes isolated from different sources in Iraq. Asian J Pharm Clin Res, 11(2), 1-4.

5- Allerberger, F., & Wagner, M. (2010). Listeriosis: a resurgent foodborne infection. Clinical Microbiology and Infection, 16(1), 16-23.

6- Bayat, A., Ahadi, A. M., Doudi, M., & Ghasemi Tehrani, H. (2024). Frequency of Listeria monocytogenes and Brucella abortus Infections in the Vaginal Secretions of Women with Spontaneous Abortion: A case study. Microbiology, Metabolites and Biotechnology, (Articles in Press).

7- Camejo, A., Carvalho, F., Reis, O., Leitão, E., Sousa, S., & Cabanes, D. (2011). The arsenal of virulence factors deployed by Listeria monocytogenes to promote its cell infection cycle. Virulence, 2(5), 379-394.

8- Chen, M., Cheng, J., Wu, Q., Zhang, J., Chen, Y., Zeng, H., ... & Ding, Y. (2018). Prevalence, potential virulence, and genetic diversity of Listeria monocytogenes isolates from edible mushrooms in Chinese markets. Frontiers in Microbiology, 9, 1711.

9- Pizarro-Cerdá, J., Kühbacher, A., & Cossart, P. (2012). Entry of Listeria monocytogenes in mammalian epithelial cells: an updated view. Cold Spring Harbor perspectives in medicine, 2(11), a010009.

10- Akca, D., & Şahİn, M. (2011). Investigation of Listeria species isolated from milk and vaginal swab samples of cows in the province of Kars, Turkey.

11- Matto, C., d'Alessandro, B., Mota, M. I., Braga, V., Buschiazzo, A., Gianneechini, E., ... & Rivero, R. (2022). Listeria innocua isolated from diseased ruminants harbour minor virulence genes of L. monocytogenes. Veterinary Medicine and Science, 8(2), 735-740.

12- Hitchins, A. D. (1996). Assessment of alimentary exposure to Listeria monocytogenes. International Journal of Food Microbiology, 30(1-2), 71-85.

13- Wu HL, Marwah S, Wang P, Wang QM, Chen XW. Misoprostol for medical treatment of missed abortion: A systematic review and networkmeta-analysis. Sci Rep. 2017;7(1):1664. [PubMed ID: 28490770]. [PubMed Central ID: PMC5431938]. https://doi.org/10.1038/s41598-017- 01892-0

14- Ahmadi, A., Ramazanzadeh, R., Derakhshan, S., Khodabandehloo, M., Farhadifar, F., Roshani, D., ... & Taheri, M. (2022). Prevalence of Listeria monocytogenes infection in women with spontaneous abortion, normal delivery, fertile and infertile. BMC pregnancy and childbirth, 22(1), 974.

15- Eslami, G., Goudarzi, H., Ohadi, E., Taherpour, A., Pourkaveh, B., & Taheri, S. (2014). Identification of Listeria monocytogenes virulence factors in women with abortion by polymerase chain reaction. Archives of Clinical Infectious Diseases, 9(3).

16- Abd Al-Mayahi, F. S., & Jaber, S. M. (2020). Multiple drug resistance of Listeria monocytogenes isolated from aborted women by using serological and molecular techniques in Diwaniyah city/Iraq. Iranian journal of microbiology, 12(4), 305.

17- Wang, Y., Ji, Q., Li, S., & Liu, M. (2021). Prevalence and genetic diversity of Listeria monocytogenes isolated from retail pork in Wuhan, China. Frontiers in Microbiology, 12, 620482.

18- Soni, D. K., Singh, D. V., & Dubey, S. K. (2015). Pregnancy-associated human listeriosis: Virulence and genotypic analysis of Listeria monocytogenes from clinical samples. Journal of Microbiology, 53, 653-660.

19- Hamid, H. H., Ghaima, K. K., Atheab, M. I. M., Mohammed, G. A., Khalaf, I. A., & Qasim, A. A. (2022). Analysis of hlyA gene sequencing of Listeria monocytogenes isolated from Iraqi women with recurrent miscarriage. HIV Nursing, 22(2), 3277-3282.

20- Hassanien, A. A., & Shaker, E. M. (2021). Virulence potential of listeria monocytogenes recovered from ice cream and aborted women samples in sohag city, egypt. Adv. Anim. Vet. Sci, 9(11), 1829-1837.

21- Gyanwali, G. C. (2021). The role of host exocytosis in Internalin A (InlA)-mediated entry of Listeria monocytogenes into human cells (Doctoral dissertation, University of Otago).

22- Pizarro-Cerdá, J., Kühbacher, A., & Cossart, P. (2012). Entry of Listeria monocytogenes in mammalian epithelial cells: an updated view. Cold Spring Harbor perspectives in medicine, 2(11), a010009.

23- Thorat SR, Rawool BD, Sonkusale PM, Warke SR, Choudhari SP, Kurkure NV (2019). Virulence profiling of Listeria monocytogenes isolated from different sources. Int. J. Curr. Microbiol. App. Sci. 8(5): 2010-2017. https://doi. org/10.20546/ijcmas.2019.805.233

24- Kaur S, Malik S, Vaidya V, Barbuddhe S. Listeria monocytogenes in spontaneous abortions in humans and its detection by multiplex PCR. J Appl Microbiol. 2007;103(5):1889–1896.

25- Liu, D., Lawrence, M. L., Austin, F. W., & Ainsworth, A. J. (2007). A multiplex PCR for species-and virulence-specific determination of Listeria monocytogenes. Journal of Microbiological Methods, 71(2), 133-140.

26- Abdeen, E. E., Mousa, W. S., Harb, O. H., Fath-Elbab, G. A., Nooruzzaman, M., Gaber, A., ... & Abdeen, A. (2021). Prevalence, antibiogram and genetic characterization of Listeria monocytogenes from food products in Egypt. Foods, 10(6), 1381.