## **مروری بر بیماری زایی و سقط جنین خودبخودی ناشی از** *لیستریا مونوسیتوژنز*

### مانوش زنده دل<sup>۱</sup>، سمانه کاظمی<sup>۲</sup>\*، امیررضا حاجتی ضیابری<sup>۳</sup>

۱. معاونت تحقیقات و فناوری، دانشگاه علوم پزشکی گیلان، رشت، ایران ۲. معاونت تحقیقات و فناوری، دانشگاه علوم پزشکی گیلان، رشت، ایران ۳. گروه علوم بالینی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

#### چگیدہ:

*لیستریا مونوسیتوژنز* به عنوان یکی از پاتوژن های غذایی، عامل ایجاد لیستریوز است. *لیستریا* در همه جای محیط زیست وجود دارد و می تواند بیوفیلم هایی را در محیط تولید مواد غذایی ایجاد کند، و بنابراین می تواند غذاهای آماده به طبخ (RTE) را که معمولاً به صورت خام یا بدون پردازش بیشتر مصرف می شوند، آلوده کند. *لیستریا مونوسیتوژنز* یک باکتری سازگار با محیط است. یکی از مهم ترین ویژگی های آن تحمل سرما و مقاومت آن در برابر غلظت نمک بالا و **PH** پایین است. از سوی دیگر، ویژگی های آن تحمل سرما و مقاومت آن در برابر غلظت نمک بالا و **PH** پایین است. از سوی دیگر، مبتلا به سرطان است. یکی از مهم ترین می شوند، آلوده کند. *لیستریا مونوسیتوژنز* یک باکتری سازگار با محیط است. یکی از مهم ترین ویژگی های آن تحمل سرما و مقاومت آن در برابر غلظت نمک بالا و **PH** پایین است. از سوی دیگر، مبتلا به سرطان است. سقط جنین خودبخودی به از دست دادن جنین قبل از هفته بیستم بارداری گفته مبتلا به سرطان است. سقط جنین خودبخودی به از دست دادن جنین قبل از هفته بیستم بارداری گفته می شود که به طور طبیعی و بدون هیچ گونه مداخله جراحی یا دارویی اتفاق می افتد. براساس مطالعات قبلی به نظر می رسد که بروز *لیستریا مونوسیتوژنز* در بین زنان باردار بالا باشد. می توان

كليد واژه ها: *ليستريا مونوسايتوژنز*، بيمارى زايى، سقط جنين

\*نویسنده مسئول: سمانه کاظمی

e-mail: samaneh.kazemi@ymail.com

# A review of Pathogenicity and Spontaneous Abortion caused by Listeria monocytogenes

#### Manoush Zendehdel<sup>1</sup>, Samaneh Kazemi<sup>2\*</sup>, Amirreza Hajati Ziabari<sup>3</sup>

- 1. Deputy of Research and Technology, Guilan University of Medical Sciences, Rasht, Iran
- 2. Deputy of Research and Technology, Guilan University of Medical Sciences, Rasht, Iran
- 3. Department of Clinical Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

#### Abstract

*Listeria monocytogenes*, as one of the foodborne pathogens, is a causative agent of listeriosis. *Listeria* is ubiquitous in the environment and can produce biofilms in the food production environment and thus contaminate ready-to-eat (RTE) products, which are typically consumed raw or without further processing. *L. monocytogenes* is an adaptable environmental bacterium. One of the most important characteristics of *L. monocytogenes* is its cold tolerance and resistance to high salt concentrations and low pH. On the other hand, this bacterium is an important pathogen in pregnant women, neonates, elderly individuals, immunocompromised individuals and patients with cancer. Spontaneous abortion is the loss of a fetus before the 20<sup>th</sup> week of pregnancy, when occurring naturally without any surgical or pharmaceutical intervention. Based on previous studies, it appears that *L. monocytogenes* incidence is high among pregnant women. It can be concluded that, pregnant women and their health care providers should be informed about listeriosis during pregnancy.

Key words: Listeria monocytogenes, Pathogenicity, Abortion

\*Corresponding Author: Samaneh Kazemi e-mail: samaneh.kazemi@ymail.com

#### Introduction

The organism now called *Listeria monocytogenes* was called Bacterium monocytogenes and was first described by Murray based on cases of sudden death in six young rabbits. Then, thanks to the efforts of the English surgeon and founder of disinfection, Joseph Lister, he changed the genus to *Listeria* (1-3). Due to the proliferation of monocytes in infected experimental animals, the name monocytogenes was chosen for this species (4). *L. monocytogenes* is a Gram-positive, non-spore forming, short rod, non-encapsulated, catalasepositive, oxidase-negative, facultative anaerobic, with a diameter of 0.4 to 0.5 micrometers and a length of 0.5 to 2 micrometers with a rounded end. It grows well at a temperature of  $37^{\circ}$ C and a pH equal to 6-9 (5,6). It is a facultative anaerobic intracellular pathogen that spreads throughout the environment and has been isolated from soil, feed, water, feces, animals, and humans. Although most bacteria do not grow or grow poorly below 4°C, *L. monocytogenes* survives at refrigeration temperatures. Therefore, L. monocytogenes is an important foodborne pathogen in refrigerated prepared foods (7,8). Since 1980, many cases of *L. monocytogenes* infection have been reported as epidemics or sporadic infections caused by consumption of contaminated food (9).

Center for Control Diseases (CDC) estimates that *Listeria* is the third leading cause of death from foodborne illness in the United States. *Listeria* can also cause an intestinal illness that is usually mild. When *Listeria* bacteria spread beyond the intestines, we call the infection invasive (10). Listeriosis, a serious infection usually caused by eating food contaminated with the bacterium *L. monocytogenes*. This agent is an important pathogen in pregnant women, neonates, elderly individuals, immunocompromised individuals and patients with cancer. Listeriosis in pregnant women cause mild illness in mothers but can be devastating to the fetus and leading to a clinical syndrome known as granulomatosis infantiseptica and death (11). The presence of older patients, primary bacteremia, central nervous system involvement, non-hematological malignancies, alcoholism, chronic kidney disease, cardiovascular disease, and pulmonary disease should alert physicians to the higher risk of mortality (12).

One of the most important characteristics of *L. monocytogenes* is its cold tolerance and resistance to high salt concentrations and low pH (13). This bacterium can grow well at the temperature of the refrigerator and is able to tolerate a concentration of 2.5% salt at a temperature of 4 °C for 4 months. Therefore, it can easily be transferred to humans through food stored in the refrigerator and cause food poisoning (14).

*L. monocytogenes* is an adaptable environmental bacterium (7). Our previous studies demonstrated that different doses of environmental stresses have lethal or sub-lethal roles in the survival of these bacteria and lead to different changes in its characteristics. The bacteria at pH $\leq$ 4 and pH $\geq$ 10 achieved by HCl and, NaOH, respectively, died. Also, concentrations ethanol at  $\geq$ 15% vol/vol, H2O2  $\geq$ 0.3% vol/vol, NaCl  $\geq$ 14% wt/vol, and heat  $\geq$ 50°C were lethal for the bacteria. Unlike other stresses, sucrose did not kill bacteria but decreased their growth. The phenotypical and biochemical characteristics of them changed when exposed to each stress (15). *L. monocytogenes* PTCC 1297 could be resistant to lead. By learning more about tolerant bacteria to heavy metals, researches may find better ways to use them for bioremediation of water and waste-water treatments and reduction of Chemical Oxygen Demand (COD). Microbial bioremediation is an efficient strategy due to its low cost and efficient alternative to target heavy metals (16). Adaptation to some stresses including hydrogen peroxide and heat increase resistance to antibiotics. Stresses such as ethanol,

hydrochloric acid and sodium chloride act in adverse. Exposing to some sub-lethal stresses increased cell survival when lethal doses of the same stress such as acid, ethanol and sodium chloride were used. But when we treating the cells to sublethal doses of H2O2 and heat the cell survival decreased (17).

#### **Pathogenicity mechanisms**

The disease caused by this bacterium was called "listeriosis". It is also a cause of meningitis, encephalitis, septicemia, endocarditis, abortion, abscesses and local wounds in humans (18, 19). In addition to humans, it can also cause diseases such as spontaneous abortion, meningitis and listeriosis in animals. Listeriosis in sheep is called "circling disease". The gastrointestinal tract is the main entry point for *Listeria* (20).

*L. monocytogenes* induces cell death in vitro and in vivo in various cell types including hepatocytes (21), lymphocytes (22), and dendritic cells (23).

*L. monocytogenes* is a facultative intracellular pathogen whose virulence depends on several factors, including host immunity, size of inoculum, strain-specific pathogenic factors, and coordinated expression of a number of specific proteins (20,24). Entry, reproduction, and pathogenesis of *L. monocytogenes* are described below (Figure 1):

- Adhesion in Listeria is done by proteins such as FbpA and LapB (25).
- This bacterium has internalin proteins (InIA and InIB) on the surface of its cell wall, which react with a receptor called E-cadherin on the surface of epithelial cells and lead the phagocytosis of the microorganism into the epithelial cells (26).
- After phagocytosis, the bacterium is enclosed inside the phagolysosome, where it is activated due to the low pH and produces listeriolysin O or LLO (encoded by the hly gene) (27). Listeriolysin O is an important virulence factor. This protein is a cytolysin/hemoglobin similar to streptolysin and performs its action by destroying cell membranes (28). In other words, it lyses the phagolysozyme membrane and allows Listeria to move through the phagolysosome towards the cytoplasm of the epithelial cell (27). In addition, two other main factors also play a role in the virulence of this bacterium, which allow it to escape from the vacuole/phagosome. These two factors are; Phospholipase A (encoded by plcA gene) Phospholipase B (encoded by plcB gene) (29, 30).
- After leaving the phagolysosome, the bacterium in the cytosol begins to express several genes, including hpt and IPL, in order to adapt to the environment (25).
- ➤ In addition, the bacterium in the cytosol begins to polymerize actin on its surface with the help of the ActA protein. It has been shown that ActA is not only necessary but also sufficient to cause movement in bacteria in the absence of other bacterial factors (31).
- Actin polymerization causes the microorganism to be pushed forward towards the membrane of eukaryotic cells (32).
- And finally, by creating pressure on the host cell membrane, it causes the formation of long protrusions called "cracks". The cracks are ingested in the adjacent cells, macrophages and hepatocytes, and the Listeria in them are released in the adjacent cells and the cycle begins again. In this way, L. monocytogenes can move from one cell to another cell without being exposed

to antibody, complement and polymorphonuclear cells (27). Spreading from one cell to another is done by a cellular mechanism called "parasitophagy" (33).

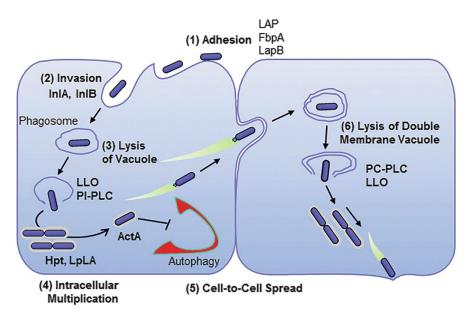


Figure 1. Entry, reproduction and pathogenesis of Listeria monocytogenes

#### **Spontaneous abortion**

A variety of pathogens, including bacteria, viruses, and protozoa, can cause abortions in humans and animals. However, little is known about the mechanisms of infectious abortion. (34).

Spontaneous abortion is the loss of a fetus before the 20th week of pregnancy when it occurs naturally without surgery or treatment. On the other hand, listeriosis is caused by L. *monocytogenes*, one of the foodborne pathogens. Transmission of L. *monocytogenes* to pregnant women presents as self-limiting flu-like symptoms that can cause miscarriage, stillbirth, or premature birth of infected infants (11).

During pregnancy, there is a natural suppression of the generalized adaptive immune system, characterized by significantly weakened cell-mediated immunity and a reduced T helper (Th) 1 response, to prevent maternal fetal rejection. Unfortunately, this immunosuppressive state can lead to increased susceptibility of the mother to certain infectious agents (35). Indeed, the placenta provides a protective niche for the growth of L. monocytogenes and can cross the intestinal, blood-brain and placental barriers, causing gastroenteritis, meningoencephalitis and maternal-fetal infections, respectively (36). On the other hand, *L. monocytogenes* has the ability to evade the killing mechanisms of phagocytic host cells and spread to placental tissues without exposure to antibiodies, neutrophils or antibiotics in the extracellular fluid. Bacterial colonization of placental trophoblast cells through production of IFN- $\gamma$  can cause abortion (37).

The CDC only tracks invasive *Listeria*. Invasive disease in pregnant women is usually mild. However, invasive disease during pregnancy usually results in miscarriage, stillbirth, premature birth, or life-threatening infection in the newborn. Infection during pregnancy causes fetal loss in about 20 percent of cases and neonatal death in about 3 percent of cases. Other people with invasive disease—mostly adults age 65 and older with weakened immune systems—usually have inflammation of the bloodstream (sepsis) or inflammation of the brain (meningitis or encephalitis). *Listeria* can sometimes infect other parts of the body. For invasive diseases not related to pregnancy, most people end up in hospital (about 87% of cases) and about one in six die (10). Every year, 4/100,000 pregnant people in the U.S. get sick with *Listeria*. But it can harm for baby. Sadly, 1/4 pregnant people who get this illness lose their pregnancy or their baby shortly after birth. This is because bacteria can spread and harm your baby during pregnancy, even if you don't feel very sick (38). Additionally, pregnant women are said to be responsible for 27 percent of all *Listeria* infections (39). Because *Listeria* infection can have serious consequences in causing spontaneous abortion and infant mortality, it is very important that obstetricians, midwives, and pregnancy care providers know about *L. monocytogenes* transmission, prevention, and associated risk factors.

Although listeriosis is rare, the fatality rate can be as high as 30% (7). Infection in pregnant women is important for two reasons. First, the placenta is a suitable niche for the growth of *L. monocytogenes*. The incidence of listeriosis in the general population is 0.7/100,000, but in pregnant women the incidence is 12/100,000 (i.e. a 17-fold increase) (40).

A variable prevalence of *L. monocytogenes* has been reported in several countries. The prevalence of *L. monocytogenes* (9%) in Iran (41) was higher than previous reports of isolation of *L. monocytogenes* in three of 100 (42), four of 305 (40), and one of 958 (43). The difference reported between studies may be due to differences in the study population, including race, culture, geographic region, diet, and laboratory diagnostic methods.

Ahmadi et al., (2022) reported that the frequency of *L. monocytogenes* infection was 4.109 (3.66%) observed among women with spontaneous abortion, 2.109 (1.83%) among women with normal delivery, 3.100 (3%) in fertile women, and 0.99 (0%) among infertile women (44).

Lotfollahi et al., (2019) isolated 3 (16.66%), 0 (0%), 1 (4%), 3 (12%) and 2 (8%) out of 9 isolates of *L. monocytogenes* from placental tissue. blood, urine, vaginal and rectal swabs. Resistance to penicillin G (77.8%) was the highest. However, the highest sensitivity was to trimethopyrim, norfloxacin and erythromycin (100%) (41). Because of high incidence of *L. monocytogenes*, diagnosis procedures for detection of *L. monocytogenes* and on time treatment is recommended. Additionally, because of high resistance rate of bacteria to antibiotic, antibacterial susceptibility before initiation of treatment is recommended especially in women with history of abortions or bad obstetric history. Pourkaveh et al., (2016) showed that identifying the risk factor of L. monocytogenes during pregnancy and considering it in pregnancy information and counseling can be crucial to reduce the prevalence of this bacterium and thus its side effects during pregnancy (11).

#### Conclusion

The prevalence of *L. monocytogenes* appears to be high among pregnant women; therefore, pregnant women and their health care providers should be informed about listeriosis during pregnancy. Healthcare providers for pregnant women, such as midwives and birth attendants, should be trusted sources of information for pregnant women and provide them with accurate advice about the risks associated with listeriosis. Most cases of listeriosis during pregnancy could be prevented not only by avoiding eating certain foods and avoiding contact with certain animals, but also by proper food preparation. In addition, it is recommended to raise awareness among people, including pregnant women, about not consuming unpasteurized

dairy products, in order to significantly reduce listeriosis and its spread by health and treatment centers and gynecologists.

#### **Conflict of interest**

No conflict of interest declared.

#### References

- 1. Fauci, A.S., et al (2008). Harrison's principles of internal medicine. 17<sup>th</sup>ed. United States: McGraw-Hill Professional.
- 2. Harvay, P.J.H. (1940). Listeria: change of name for genus of bacteria. Nature; 145(3668):264.
- 3. Murray, E.G.D., Webb, R.E., Swann, M.B.R. (1926). A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *bacterium monocytogenes* (n.sp.). J Pathol Bacteriol; 29:407\_439.
- 4. Nowrozi, J. (1996). Applied methods in the identification of bacteria, Publisher: Hayan, Tehran, pp. 49-50 [Text in Persian].
- 5. Cliver, D.O. (1990). Foodborne diseases. Academic press; 248-256.
- 6. Tabouret, M., Rycke. de.J., Dubray, G. (2003). Analysis of surface proteins of *listeria* in relation to species, serovar and pathogenicity. J Gen Microbiol; 138(4): 743-753.
- Ramaswamy, V., Cresence, V.M., Rejitha, J.S., Lekshmi, M.U., Dharsana, K.S., Parsad, SP., Vijila HM (2007). Listeria review of epidemiology and pathogenesis. J. Microbiol. Immunol. Infect., 40: 4-13
- 8. Salyers, A.A., Whitt, D.D. (2002). Bacterial pathogenesis. A molecular approach. 2nd ed. Washington D.C: ASM press, pp. 398-40
- 9. Iwamoto, M., Ayers, T., Mahon, B.E., Swerdlow, D.L. (2010). Epidemiology of seafoodassociated infections in the United States. Clin Microbiol Rev, 23(2):399-411
- 10. <u>https://www.cdc.gov/listeria/faq.html#control</u>
- 11.Pourkaveh, B., Ahmadi, M., Eslami, G., Gachkar, L. Factors contributes to spontaneous abortion caused by *Listeria monocytogenes*, in Tehran, Iran, 2015. Cell Mol Biol (Noisy-le-grand). 2016 Aug 29;62(9):3-10.
- 12. Huang, C., Lu, T.L., Yang, Y. Mortality risk factors related to listeriosis A meta-analysis. J Infect Public Health. 2023 May;16(5):771-783.
- Vazquez-Boland, J.A., Kuhn, M., Berchf, P., Chakraβorty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez-Zorn, B., Wehland, J., Kreft, J. (2001). *Listeria* pathogenesis and molecular virulence determinats. Clin Microbiol Rev; 4: 584-640.
- 14. Bollag, D.M., Edelstein, S.J. (1991). Protein methods. New York. Wiley -Liss; 150-197.
- 15. Kazemi S, Faezi-Ghasemi M. An In Vitro Study on Impact of Environmental Stresses on Growth, Morphological and Biochemical Features of *Listeria monocytogenes* PTCC 1297. JoMMID 2015; 3 (1 and 2):11-17
- Kazemi, S., Faezi-Ghasemi M. (2015). Effect of Heavy Metals Stresses on Growth, Surface Structure and Biochemical Features of *Listeria monocytogenes* PTCC 1297: An in Vitro Study, 2(4): e31023.

- Faezi-Ghasemi, M., Kazemi, S. (2015). Effect of Sub-Lethal Environmental Stresses on the Cell Survival and Antibacterial Susceptibility of *Listeria monocytogenes* PTCC1297. Zahedan J Res Med Sci. 17(1):e1915.
- Bortolussi, R., W. F. Schlech, Lly W. Albritton (1985). Listeria Manual of clinical microbiology. 4th Ed. American Society for Microbiology, Washington D.C. P.205-208.
- 19. Gilchrist, M. J. R. (1988). Listeriosis. Laboratory diagnosis of infectious diseases, principles and practice. Volume 1. P. 353-359. Springer-Verlag, New York.
- 20. Baron, E.J., Finegold, S.M. (1990). Aerobic, Non-Spore-Forming, Gram-Positive Bacilli. Bailey & Scott's Diagnostic Microbiology. 8<sup>th</sup> Ed. Baltimore, Maryland. CV Mosβy company; 458-461.
- 21. Rogers, H.W., Callery, M.P., Deck, B., Unanue, E.R. (1996). Listeria monocytogenes induces apoptosis of infected hepatocytes. J Immunol 156: 679–684
- 22. Merrick, J.C., Edelson, B.T., Bhardwaj, V., Swanson, P.E., Unanue, E.R. (1997). Lymphocyte apoptosis during early phase of *Listeria* infection in mice. Am J Pathol 151: 785–792
- Guzman, C.A., Domann, E., Rohde, M., Bruder, D., Darji, A., et al. (1996). Apoptosis of mouse dendritic cells is triggered by listeriolysin, the major virulence determinant of Listeria monocytogenes. Mol Microbiol. 20: 119–126
- 24. Esvan, H., Minet, J., Laclie, C., Cormier, M. (2000). Short communication Proteins variations in *listeria monocytogenes* exposed to high salinities. J Food Microbial. 55,151-155.
- 25. Stavru, F., Archambaud, C., Cossart, P. Cell biology and immunology of *Listeria monocytogenes* infections: novel insights. Immunol Rev 2011;240(1):160-84.
- 26. Werbrouck, H., Grijspeerdt, K., Botteldoom, N., Van Pamel, E., Rijpens, N., Van Damme, J., et al. (2006). Differential inl A and inl B expression and interaction with human intestinal and liver cells by *Listeria monocytogenes* strains of different origis. Appl Environ Microbial. 72: 3862-71.
- 27. Rahimi, M.K., et al. (2008). Javatz Medical Microbiology, Publisher: Aeezh, Tehran, Iran. [Text in Persian].
- 28. Ekhtiari, H., et al. (2011). Javatz-Zinser-Moray-Baron Principles of Medical Microbiology, Publisher: Ketab Mir, Tehran, Iran. [Text in Persian].
- 29. Schmid, M. W., Ng E. Y. W., Lampidis, R., Emmerth, M., Walcher, M., Kerft, J., Goebel, W. et al. (2005). Evolutionary history of the genus *Listeria* and it's virulence genes. Sys App Microbiol. 28(1):1-18.
- Zhang, C., Zhang, M., Ju J., Wise, J., Terry, P. M., Olson, M. et al. (2003). Genome Diversification in Phylogenrtic Lineages I and II of *Listeria monocytogenes*: Identification of Segments Unique to Lineage II Populations. J Bacteriol. 185(18):5573-5584.
- 31. Zigmond, S.H. (2004). Formin-Induced Nucleation of actin filaments. Current Opinion in Cell Biol. 16(1),99-105.
- 32. Grundling, A., Burrack, L. S., Bouwer, H. G. A., Higgins, D. E. (2004). *Listeria monocytogenes* regulates flagellar motility gene expression through Mog R, a transcriptional repressor required for virulence. Pro C. Natl. Acad. Sci. USA 101:12316-12323.
- Robbims, J.R., Barth, A.I., Marquis, H., de Hostos, E.L., Nelson, W.J., Theriot, J.A. (1999). *Listeria monocytogenes* Exploits normal Host cell Processes to Spread from Cell to cell. J Cell Biol 146(6):1333-50.
- Tachibana, M., Hashino, M., Nishida, T., Shimizu, T., Watarai, M. (2011). Protective Role of Heme Oxygenase-1 in *Listeria monocytogenes* Induced Abortion. PLoS ONE 6(9): e25046.
- 35. Mor, G., Cardenas, I. Review article: the immune system in pregnan- cy: a unique complexity. Am J Reprod Immunol. 2010; 63(6):425-33

- Barikbin, P., Sallmon, H., Huseman, D., Sarioglu, N., Weichert, A., von Weizsacker, K., et al. Clinical, Laboratory, and Placental Findings in Perinatal Listeriosis. Fetal Pediatr Pathol 2016; 21:1-8.
- 37. Abram, M., Schlüter, D., Vuckovic, D., Waber, B., Doric, M., Deckert, M. Effects of pregnancy-associated *Listeria monocytogenes* infection: necrotizing hepatitis due to impaired maternal immune response and significantly increased abortion rate. Virchows Archiv 2002;441(4):368-79
- 38. <u>https://www.cdc.gov/listeria/pregnant-people.html</u>
- 39. Goldstein, E.J., Overturf, G.D. Indications for the immunological evaluation of patients with meningitis. Clin Infect Dis 2003;36(2):189-94.
- 40. Kaur, S., Malik, S.V., Vaidya, V.M., Barbaddhe, S.B. (2007). *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. J. Appl. Microbiol., 103: 1889-1896.
- 41. Lotfollahi, L., et al. Prevalence and antimicrobial resistance profiles of Listeria monocytogenes in spontaneous abortions in humans Advanced Journal of Microbiology Research 2019, 13 (3), 001-004
- 42. Bhujwala, R.A., Hingorani, V., Chandra, R.K. (1973). Genital listeriosis in Delhi: a pilot study. Indian. J. Med. Res., 63: 1503–1508
- 43. Stepanovih, S., Vukovih, D., Djukic, S., Cirkovic, I., Svabic-Vlahovic. M. (2007). Long term analysis of *Listeria monocytogenes* vaginal carriage ferequency in Belgrade, Serbia (shortcommunication). Acta. Microbiol. Immunol. Hung, 54(2): 195-9.
- 44. Ahmadi, A., Ramazanzadeh, R., Derakhshan, S. et al. (2022). Prevalence of *Listeria monocytogenes* infection in women with spontaneous abortion, normal delivery, fertile and infertile. BMC Pregnancy Childbirth 22, 974.