



POLYMERASE CHAIN REACTIONS (PCR) TECHNIQUE FOR MOLECULAR IDENTIFICATION OF HIGH-RISK LISTERIA MONOCYTOGENES INFECTIONS IN PREGNANT WOMEN

Nawar Al-Janabi

Department of Biotechnology, College of Biotechnology,
Al-Qasim Green University, Babylon, Iraq.

*Correspondence: dr.nawar@biotech.uoqasim.edu.iq

Abstract

Listeria monocytogenes is a facultative, non-spore forming, rod-shaped intracellular pathogen. *L. monocytogenes* is varying from 0.5 μm to 2.0 μm in length and 0.4 μm to 0.5 μm in diameter. Stillbirths, diarrhea, fever, and headache are the most common symptoms of infections with *L. monocytogenes*. This pathogen is threatening pregnant women, new-borns, elderly and individuals with weak immune system more than others. Infections with this microorganism cause a high mortality rate and significant economic loss. Early identification of this pathogen could minimize or even prevent the infections. Polymerase chain reactions technique could identify and diagnose the high-risk *L. monocytogenes* infection. Therefore, in the current research, A Polymerase Chain Reactions technique for molecular identification of *L. monocytogenes* infections from fifteen swab samples collected from pregnant woman were performed. The results of our study identified *L. monocytogenes* in 10 swab samples. Each sample gave clear band with amplicon size of 320 bp. We determined that Polymerase chain reactions technique should be used more frequently in any suspected cases of *L. monocytogenes* infections especially in pregnant woman.

Keywords: Listeria monocytogenes, Pregnant women, Stillbirths, PCR Technique, Molecular identification.

Introduction

Listeria Monocytogenes is Gram positive bacterium pathogen and the causative agent of Listeriosis. *L. monocytogenes* is a rod-shaped bacterium and it has been reported to cause a high death rate and significant economic loss for many countries [1]. *L. monocytogenes* infections has been reported in many developing and developed countries due to its highly rates of transmission ways either from one person to another or from domestic animals to people which makes it a real life-threatening infections around the world [2]. *L. monocytogenes* has high ability to survive harsh environmental conditions, such as very low or relatively high temperatures as well as in drying atmosphere [3]. This bacterium can cause listeriosis and they are usually occurs in two forms. The first form named as non-invasive gastrointestinal listeriosis and the second form is named as invasive listeriosis [4]. Invasive listeriosis causes a serious and dangerous infections in human and it's very difficult to be treated [5]. *L. monocytogenes* has many ways to establish infections in their host and it also can be transmitted from infected women to their fetus through the placenta. On other hand, non-invasive listeriosis establishes



as typical febrile gastroenteritis and the symptoms that caused by this from include septicemia, neurolisteriosis [6]. *L. monocytogenes* can grow so fast and spread easily from one to another individuals by any means of contact, which enable it to cause a high mortality rate due to its pathogenicity and their incidence of human listeriosis [7]. The percentage of death rate caused by this disease is considered the highest among food-borne diseases. People of old aged, pregnant women, and weak immune system are the most susceptible to infections with listeriosis, which make this disease, is at high concern [8].

Prevention of this disease is necessary to reduce the high rate of mortality and minimize the economic loose. Listeriosis in poor countries are significantly high due to a weak hygienic procedures and due to lack of food safety monitoring [9]. In the United States, nearly 2500 people get listeriosis that resulting in nearly 500 death cases because of this disease [10, 11]. The most way of transmission of *L. monocytogenes* to humans is from domestic animals in which this pathogen resides more frequently in their intestine. For, example, people who eats raw or not well cooked meat are more frequently infected by *L. monocytogenes* [12]. Also, pregnant women are more probably infected than normal adults in about 20 times to this sickness. During pregnancy, newborns suffer the serious effects of infection than the pregnant women themselves [11]. However, in the current research, we aimed to identify *L. monocytogenes* in fifteen pregnant women who had serious threat of getting stillbirths. We synthesized specific 16s ribosomal RNA primers that only match to *L. monocytogenes* microorganism and we used PCR technique to accurately identifying this pathogen.

Materials and Methods

Samples collections

This study includes fifteen swab samples collected from pregnant women by competent sampling doctor and according to the guideline and research ethics.

Bacterial isolation and growth media

Swab samples were directly cultured in brain heart infusion (BHI) agar or broth (Difco, Sparks, MD) and incubated at 37 °C for 24 h. Then pure and single colony was taken from each sample for performing our experiment.

Extraction of *L. monocytogenes* DNA

L. monocytogenes genomic DNA from each swab sample was isolated using the FastDNA™ SPIN Kit for Microbes and the FastPrep-24™ Instrument (MP Biomedicals, Santa Ana, CA) following manufacturer's instructions.

Extracted *L. monocytogenes* DNA quality check

The purity of extracted DNA from *L. monocytogenes* was checked by using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA) for measuring the DNA concentration and purity.

Primer design and polymerase chain reaction (PCR) conditions

Molecular identification of *L. monocytogenes* was done based on 16s ribosomal RNA gene. The specific primers for *L. monocytogenes* 16s RNA gene were designed using NCBI-Genbank database and primer3 software (http://www-ename.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and synthesized



commercially (MWG Eurofins Genomics) Table 1. PCR procedure for all fifteen isolates was performed by using (GoTaq® Green PCR master kit) according to the manufacture procedures. PCR was done by thermo-cycler (BioRad-USA) with following conditions; 95°C, for 2 min. 95°C, for 30 sec. 57.9°C, for 30 sec. 72°C, for 40.0 sec. 72°C, for 5 min. and stored at 4°C. Finally, PCR products were analyzed by agarose gel electrophoresis and visualized by ultra-violet transilluminator.

Table 1: The PCR primers of 16s ribosomal RNA gene of *Listeria monocytogenes*

Accession No.	Primers		Product Size
X56153.1	F	GGTGGAGCATGTGGTTAATTC	320bp
	R	TTCGCGACCCTTGTACTATC	

Results and Discussion

In the 1930s, *L. monocytogenes* was discovered as a pathogen that causes a serious illness for humans and animals. *L. monocytogenes* was reported to cause of stillbirth and abortion in early pregnancy [13]. Therefore, the goal of this study was to use a rapid, specific, and sensitive PCR technique for molecular identification of *L. monocytogenes* in pregnant woman who are suspected of having *L. monocytogenes* infections. Fifteen swab samples were collected from pregnant women by competent sampling doctor and PCR primers were specifically designed to band only with the 16s ribosomal RNA of *L. monocytogenes* with product size of 320 bp. Our results indicated that, 10 swab samples gave a positive result with clear bands Figure 1.



Figure 1. Agarose gel with PCR amplification product of 16s ribosomal RNA gene (320 bp).

10 positive results from fifteen swab samples are considered a high incidence of *L. monocytogenes* infections in pregnant woman. Our results suggest that many stillbreaths cases may cause by *L. monocytogenes* rather than other possible cause. In the diagnostic laboratories, the test for the presence of this pathogen in pregnant woman is not given high attention. Also, diagnostic of *L. monocytogenes* identification is very difficult and that could be the reason of hiding the high incidence of the *L. monocytogenes* infections. Here, we show the importance of accurate PCR technique to report the real infections rate of such pathogen among pregnant woman. *L. monocytogenes* characterized by their high



pathogenicity and have a specific ability to sneak, multiply, and replicate in many different types of eukaryotic cells including humans and animals. It also has the ability penetrate the intestinal barrier, brain barrier, and the placenta barrier [14]. This pathogen also can resist many different eukaryotic immune cells. It has been reported that bacterial attachment to the host cell surfaces is important for the persistence and survival of this pathogen [15, 16].

Commonly, *L. monocytogenes* flagella promote the attachment of this bacterium to the host cell surfaces [17]. In addition, it has been shown that flagella are critical for biofilm formation and adhesion of *L. monocytogenes* on host cell surfaces [18]. It has been reported that *L. monocytogenes* has four to six peritrichous flagella, which are modified by β -O-linked glycosylation [19]. However, *L. monocytogenes* attachment process is mediated by cell wall surface anchor proteins. The bacterial cell surface anchor proteins are contributing to the bacterial adhesion to many host cell surfaces [20-22]. Many numbers of predicted bacterial cell surface proteins are encoded by *L. monocytogenes* compared to other bacteria. The large numbers of this protein enable this pathogen to infect and colonize many hosts including the placenta [23]. This may be a good explanation of the high incidence of *L. monocytogenes* infections in pregnant woman. However, the use of PCR technique for early identification *L. monocytogenes* is of high importance for early detection and prevention of stillbirths in pregnant woman. Generally, this technique is not well considered in the diagnostic laboratories although it gives a specific and accurate result for the presence of this pathogen. Consequently, we propose that PCR technique must be used more frequently in pregnant woman for early detection and identification of *L. monocytogenes* infections. This strategy may contribute to minimize the high incidence of *L. monocytogenes* rate of infections in pregnant woman as well as in other individuals.

References

1. Barton Behravesh, C., et al., Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996–2005. *Journal of Infectious Diseases*, 2011. **204**(2): p. 263-267.
2. Zhao, X., et al., Antimicrobial kinetics of nisin and grape seed extract against inoculated *Listeria monocytogenes* on cooked shrimps: Survival and residual effects. *Food Control*, 2020. **115**: p. 107278.
3. Reddy, S., et al., The role of *Listeria monocytogenes* cell wall surface anchor protein LapB in virulence, adherence, and intracellular replication. *Microbial pathogenesis*, 2016. **92**: p. 19-25.
4. Maury, M.M., et al., Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. *Nature genetics*, 2016. **48**(3): p. 308-313.
5. Lüth, S., et al., Backtracking and forward checking of human listeriosis clusters identified a multiclonal outbreak linked to *Listeria monocytogenes* in meat products of a single producer. *Emerging Microbes & Infections*, 2020. **9**(1): p. 1600-1608.
6. Lecuit, M., *Listeria monocytogenes*, a model in infection biology. *Cellular microbiology*, 2020. **22**(4): p. e13186.



7. Truchado, P., et al., Post-process treatments are effective strategies to reduce *Listeria monocytogenes* on the surface of leafy greens: A pilot study. *International journal of food microbiology*, 2020. **313**: p. 108390.
8. Vázquez-Boland, J.A., et al., *Listeria* pathogenesis and molecular virulence determinants. *Clinical microbiology reviews*, 2001. **14**(3): p. 584-640.
9. Han, Y., Z. Sun, and W. Chen, Antimicrobial susceptibility and antibacterial mechanism of limonene against *Listeria monocytogenes*. *Molecules*, 2020. **25**(1): p. 33.
10. Montero, D., et al., Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. *Frontiers in microbiology*, 2015. **6**: p. 384.
11. Muriana, P. and K. Kushwaha, Food pathogens of concern: *Listeria monocytogenes*. 2010, Oklahoma Cooperative Extension Service.
12. Goh, S., et al., *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. *Poultry science*, 2012. **91**(10): p. 2686-2690.
13. Akgul, A., et al., Small molecules targeting LapB protein prevent *Listeria* attachment to catfish muscle. *Plos one*, 2017. **12**(12): p. e0189809.
14. Reis, O., et al., LapB, a novel *Listeria monocytogenes* LPXTG surface adhesin, required for entry into eukaryotic cells and virulence. *The Journal of infectious diseases*, 2010. **202**(4): p. 551-562.
15. Lundén, J.M., et al., Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *Journal of food protection*, 2000. **63**(9): p. 1204-1207.
16. Mørerø, T. and S. Langsrud, *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. *Biofilms*, 2004. **1**(2): p. 107-121.
17. Vatanyoopaisarn, S., et al., Effect of flagella on initial attachment of *Listeria monocytogenes* to stainless steel. *Applied and Environmental Microbiology*, 2000. **66**(2): p. 860-863.
18. Lemon, K.P., D.E. Higgins, and R. Kolter, Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of bacteriology*, 2007. **189**(12): p. 4418-4424.
19. Schirm, M., et al., Flagellin from *Listeria monocytogenes* is glycosylated with β -O-linked N-acetylglucosamine. *Journal of bacteriology*, 2004. **186**(20): p. 6721-6727.
20. Parkar, S., et al., Factors influencing attachment of thermophilic bacilli to stainless steel. *Journal of applied microbiology*, 2001. **90**(6): p. 901-908.
21. Flint, S., J. Brooks, and P. Bremer, The influence of cell surface properties of thermophilic streptococci on attachment to stainlesssteel. *Journal of applied microbiology*, 1997. **83**(4): p. 508-517.
22. Navarre, W.W. and O. Schneewind, Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiology and molecular biology reviews*, 1999. **63**(1): p. 174-229.
23. Popowska, M. and Z. Markiewicz, Classes and functions of *Listeria monocytogenes* surface proteins. *Polish journal of microbiology*, 2004. **53**(2): p. 75.