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

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Abstract
Listeria monocytogenes is a facultative, non-spore forming, rod-shaped intracellular pathogen. L. monocytogenes is varying from 0.5 µm to2.0 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 loos. 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 Grame positive bacterium pathogen and the causativae gent 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

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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, ppregnant 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-enome.wi.mit.edu/cgibin/primer/primer3_www.cgi) and synthesized

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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	GGTGGAGCATGTGGTTTAATTC	320bp
	R	TTCGCGACCCTTTGTACTATC	

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.

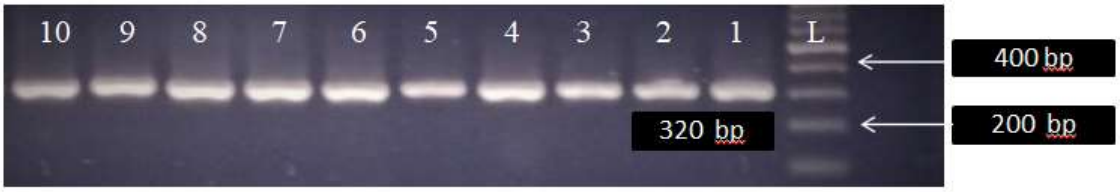

Figure **1.** Agarose gel with PCR amplification product of 16s ribosomal RNA gene (320 bp).

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

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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 well 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 is 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.

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