

Improving Diagnostic Accuracy for *Listeria Monocytogenes* Infections in Pregnant Women and Children

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ABSTRACT

Background: *Listeria monocytogenes* is a gram-positive, facultative intracellular bacterium responsible for listeriosis, a severe infection affecting humans and animals. Listeriosis predominantly impacts immunosuppressed individuals, newborns, and the elderly, causing septicemia, meningitis, encephalitis, spontaneous abortions, and, in severe cases, death. **Objective:** This study aimed to detect virulence genes (*hly* and *prf*) in *Listeria monocytogenes* isolated from children and pregnant women in hospitals in Baghdad. **Methods:** The study focused on molecular detection of key virulence genes associated with *Listeria monocytogenes* pathogenicity, emphasizing their diagnostic significance in high-risk populations, including pregnant women and children.

Keywords: Virulence Genes, *Listeria Monocytogenes*, Children, Pregnant Women

INTRODUCTION

Listeriosis caused by *Listeria monocytogenes*, is one of the important bacterial zoonotic infections worldwide [1]. This results in severe, life-threatening infection that primarily affects high-risk groups, including pregnant women, newborns, elderly patients, and patients with a compromised immune system [2]. The mortality rate in high-risk groups is possible to be as high as 30% [3]. Research results indicate that the frequency of listeriosis is 20 times greater in pregnant women than it is in the general population [4]. Pregnant women have a heightened susceptibility to infection, and the presence of hidden or obvious bacteria in the bloodstream can lead to chorioamnionitis, which in turn can cause early-onset newborn listeriosis [5].

Ordinarily, pregnant women who acquire listeriosis may not present symptoms or show clinical signs that are not specific; but mimicking flu symptoms. Despite this inconspicuous beginning, it has the potential to trigger miscarriage or premature birth— leading to fetal death or newborn illness such as septicemia plus meningitis and encephalitis which can escape observation [6]. Around 20% of pregnancies that are affected by listeriosis have a spontaneous abortion or stillbirth. Additionally, two-thirds of newborns that survive have clinical neonatal listeriosis, this condition is caused by bacteria that are either present or have a history of infection, including pneumonia and sepsis. Additionally, listeriosis is responsible for 20% of all instances of neonatal meningitis [4]. The mortality rate associated with early-onset listeriosis is extremely high, despite treatment, this is stillbirth is also common in this context. Autopsies conducted on cases of early-onset listeriosis reveal notable inflammation of the placental remains known as chorioamnionitis, as well as the presence of granulomas in several organs, most notably the liver and spleen, in infected newborns [7].

Empirical evidence indicates that prompt intervention in the form of therapy for the mother with *Listeria* sepsis can effectively prevent the transmission of the infection to the fetus through the placenta, or alternatively, treat the fetus while still in the womb, resulting in the

subsequent birth of a healthy, uninfected baby [8]. Later-onset neonatal meningitis, which is produced by *L. monocytogenes*, exhibits the typical symptoms of the condition other organisms would produce in this situation, such as fever, irritability, fontanelle bulging, and meningismus.

The onset of these symptoms often occurs 1 to 2 weeks after giving birth. The woman often experiences a straightforward pregnancy, birth, and postpartum period without any indications of sepsis. A lumbar puncture is often performed based on the clinical symptoms, and in 50% of instances, the organism may be identified using Gram's stain analysis of the cerebrospinal fluid (CSF). CSF cultures often yield positive results, however in certain situations the organism may only be recovered from the blood or may be separated from both the blood and CSF concurrently. The cerebrospinal fluid (CSF) exhibits other features of bacterial meningitis, such as a high count of polymorphonuclear leukocytes, increased protein levels, and reduced glucose levels together with a drop in the ratio of CSF-serum glucose [7]. The aim of this study was Detection of virulence genes (*hly*, *prf*) in *Listeria monocytogenes* in children and pregnant women in Baghdad's hospitals.

MATERIALS AND METHODS

After visiting three hospitals in Baghdad, which were Al-Alawiya Maternity Hospital, the Medical City, and Al-Kadhimiya Hospital, 200 samples were collected, 100 of which were from infected women and children, 50 from women and 50 from children, and 100 of them from uninfected women and children, 50 from women and 50 from children.

Finding the bacteria was very difficult, as it was attempted many times. Samples were collected in large numbers until suspicious samples were obtained and sent to the laboratory for testing, the samples were taken with swab and incubated for 24 hours and then began cultured.

Table 1: Materials, Tools and Devices

Item	Company	Origin
1.5ml, 0.5ml and 0.2ml Tube JET BIOFIL, Singapore	Centrifuge Fisher Scientific,	USA
Autoclave	Daikyo	Japan
Biohazard Cabinet (Hood) LV2	BIOAIR	Italy
Bottle 500ml	Eppendorf	Germany
Burner	Satorins	Germany
Centrifuge	Hettich	Germany
Conical Flask	Had	China
Cool Box	Nunc	Denmark
Deep Freeze	GFL	Germany
Distillator	LabTech	Korea
Electrical Balance	Shlmadzu corporation	Japan
Electrophoresis (PCR)	Apelex	France
Elisa System	Elisis uno	Germany
Eppendorf Tubes	Eppendorf	Germany
Exospin Microcentrifuge	BioNEER	Latvia
Filter Paper	Gema medical	Spain
Filter Piece	Sun	Jordan
Gel Electrophoresis Apparatus	Hellabio	Greece
Gel Tank	Cleaver	Netherland
Gratuated Cilynder 250ml	Eppendorf	Germany
Hot Plate	Ekds	Japan
Incubator 40 L	Memmert-	Germany
Kindle Jar	BioNEER	Latvia
Loop	Hi-media	India
Medical Scalpel	WINLAB®	Egypt
Micro-Centrifuge	Eppendorf-	Germany
Micropipette 20-200 MI	WINLAB®	Egypt
Micropipette (0.5-10µl, 5-50 MI	Sartorius	Germany
Micropipette100-1000 MI	WINLAB®	Egypt
Micropipette100-1000 MI	WINLAB®	Egypt
Micropipette10-100 MI	WINLAB®	Egypt
Microwave Oven	LG	Korea
Multichannel Micropipette 50-300 MI	LabSysteme -	Hungary
Narrow Neck Flask	Eppendorf	Germany
Petri Dishes 9 Cm	Afco-Dispo	Jordan
Petry Dish	Cleaver	Netherland
Plastic Disposable Loop	Afco	Jordan
Plastic Tubes 10 MI	Sun	Jordan
Refrigerator	Sanyo	Japan
Single Channel Micropipette (Different Sizes)	Eppendorf	Germany
Sterile Swab	China	China
Sterilized Needles (5,3 MI)	Shanchuan	China
Swab Lab.	Servic	Spain
Syringe 10 MI	Hayat	Turkey
Thermo Cycler (Pcr)	Applied biosystem-	Egypt
Thermo Shaker (T-Shaker)	Biometra	Germany
Tips Sterellin	Ltd	UK
Tubes (Plain, Gel, And Screw-Capped)	Sail Brand	China
Universal Tube	WINLAB®	Egypt
UV Viewer Illuminator System	Cleaver scientific	UK
Volumetric Cylinder	HAD	China
Vortex	Eppendorf	Germany
Waterbath (10 And 20 L)	LabTech	Korea

Statistical Analysis

This study designed by that used in the analysis of variance for data of virulence genes values independent t-test, Dunnett's test at a 5% level of significance. Moreover, all frequency data was analyzed by Pearson's chi-squared test and Fisher's exact test. Data were processed and analyzed by using statistical program social science (SPSS-v 26)2019 and the results [9].

DNA Extraction

DNA extraction of *Listeria monocytogenes* was done using BioBasic DNA extraction kit. The following steps were followed for DNA extraction. 1) *Listeria monocytogenes* samples were cultured and incubated at 37°C for 18 hours. 2) The samples were centrifuged for 30 seconds at 12,000 rpm and the supernatant was discarded. 3) 180 ml of ACL buffer and 20 ml of proteinase K were added to the sediment

and the sample was vortexed. The sample was incubated for 1 hour at 56°C. 4) 200 ml of CL buffer was added to the sample and mixed well with it. 5) 200 ml of pure alcohol abide added to the sample and assimilated into it. 6) The composition of the mixture abides relayed to the Rotation Column.

7) The sample prevail then centrifuged at 12,000 rpm for 1 minute, the lowermost solution was then discarded. 8) 500 ml of CW1 solution was added to the column, the sample was then centrifuged for a minute at 12,000 rpm and the bottom solution was discarded. 9) 500 ml of the CW2 buffer was more to the column as a supplement,

the sample abide then centrifuged for a minute at 12,000 rpm and the bottom solution was discarded. 10) The column with the pure substance was centrifuged for two proceedings at 12,000 revolutions per minute and the bottom solution was discarded. 11) The column's spin was moved to a 1.5 ml microtube and placed at room temperature for 2-2.5 minutes to evaporate the alcohol. 12) 50-100 ml of Ce buffer was supplementary to the column and placed in room temperature for 2 minutes. The sample was then spun at 12,000 RPM for 2 minutes. 13) The undermost solution that had been extracted DNA was gathered [10].

Table 2: Kits for Molecular Detection.

Types of kits	Company	Origin
DNA Extraction Kit	Promega	U.S. A
DNA Ladder Kit		
PCR Master Mix		

Statistical Significance: $P \leq 0.05$

Results

Among the total of 250 samples collected from pregnant women, 12 samples (4.8%) and among the 250 samples collected from children, 60 samples (24%) detected as *Listeria monocytogenes*. In total, out of 500 clinical samples, 72 samples were identified as *Listeria monocytogenes* (14.4%). Based on the gel electrophoresis results of Multiplex PCR products obtained from the samples collected from pregnant women, the frequency of the *iap* gene is equal to 100% (12

samples), the frequency of the *hly* gene is equal to 100% (12 samples) and *prfA* is equal to It is 100% (12 samples).

The frequency of simultaneous presence of *iap*, *hly* and *prfA* genes is equal to 100% (12 samples) (fig. 1), the frequency of simultaneous presence of *iap* and *hly* genes is equal to 100% (12 samples) (fig. 2), the frequency of simultaneous presence of *prfA* gene is equal to 100% (12 samples) and the frequency of simultaneous presence of *hly* and *prfA* genes was equal to 100% (12 samples).

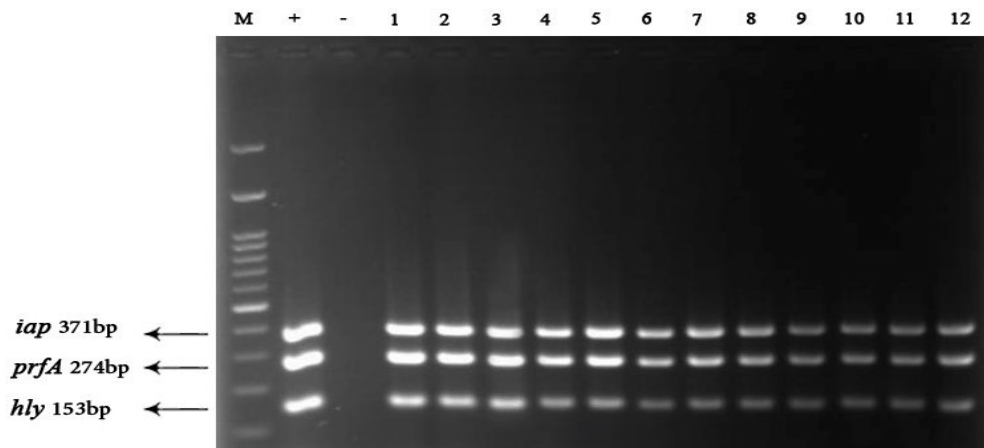


Figure 1: Gel Electrophoresis to Identify Virulence Genes in *Listeria Monocytogenes* Isolates (Samples 1 to 12). M well (100 to 1500 bp indicator), + Well: Positive Control Indicator, - Well: Negative Control Indicator, Wells 1 to 12: *Listeria Monocytogenes* Samples (Pregnant Women). All samples had all three genes *iap*, *prfA* and *hly*.

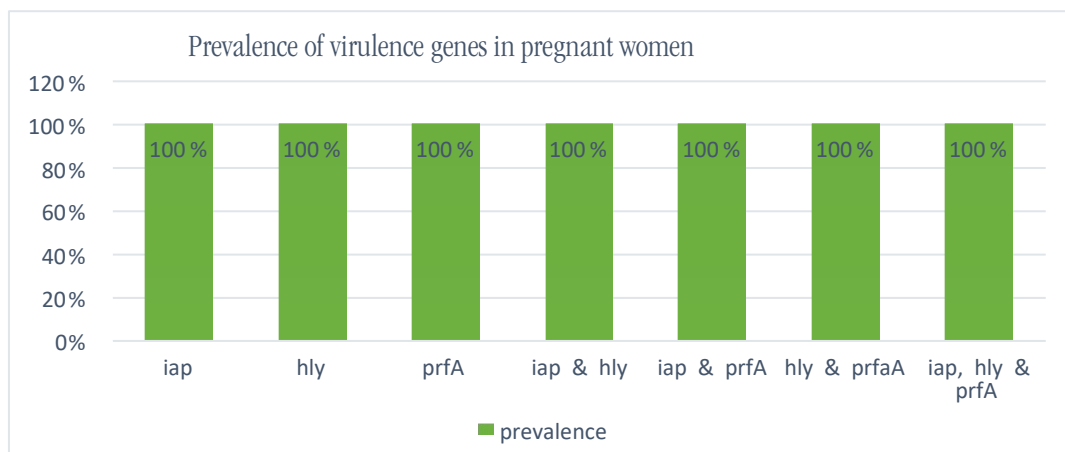


Figure 2: The Prevalence of *iap*, *hly* and *prfA* Genes in *Listeria monocytogenes* in Pregnant Women

Discussion

Listeria monocytogenes is known as a non-spore forming, non-branching, regular, short rod, gram-positive, and facultative anaerobic bacterium isolated from soil, animal food, water, feces, animals, and humans [11, 12]. *Listeria monocytogenes* is a food borne pathogenic bacteria causing the infection listeriosis, which possibly affects all people, particularly immunocompromised persons and pregnant women [13]. Since 1980, many cases of *L. monocytogenes* infection have been reported as a series of epidemic or sporadic infections due to the consumption of contaminated food [14-16]. In spite of low incidence of infection in the past, increased interest for lightly preserved and/or ready-to-eat (RTE) food products has recently led to increasing of *L. monocytogenes* prevalence which has become a public health concern [17]. *Listeria monocytogenes* with a vast range of natural reservoirs is more known for being a food-borne pathogen. Human infections have shown an impact on pregnancy outcomes [18, 19], so, this study surveyed the detection of virulence genes (hly, prf) in *Listeria monocytogenes* in children and pregnant women in Baghdad's hospitals [20]. For this purpose, 250 urine samples were collected from pregnant women and 250 urine samples from children who were hospitalized in Baghdad government hospitals. Then, the collected samples were analyzed using selective culture medium, gram staining and biochemical tests to identify *Listeria monocytogenes* [21]. Among the total of 250 samples collected from pregnant women, 12 samples (4.8%) and among the 250 samples collected from children, 60 samples (24%) detected as *Listeria monocytogenes*. In total, out of 500 clinical samples, 72 samples were identified as *Listeria monocytogenes* (14.4%). DNA of 72 samples which confirmed as *Listeria monocytogenes* were analyzed by using

Multiplex PCR method to check the presence or absence of virulence genes (hly and prfA). Based on the gel electrophoresis results of Multiplex PCR products obtained from the samples collected from pregnant women, the frequency of the hly gene is equal to 100% (12 samples) and prfA is equal to 100% (12 samples). The frequency of simultaneous presence of hly and prfA genes is equal to 100% (12 samples), the frequency of simultaneous presence of hly gene is equal to 100% (12 samples), the frequency of simultaneous presence of prfA gene is equal to 100% (12 samples) and The frequency of simultaneous presence of hly and prfA genes was equal to 100% (12 samples) [22].

Conclusions

Basically, low-tech diagnostics such are specific to the subatomic leveled provide a superior alternative to traditional diagnostics like the blocking of hemagglutination and the aestheticism of microorganisms. By strategically combining and integrating multiple molecular diagnostic methods based on the user's preferences, it is possible to achieve a rapid and accurate identification of the infectious inflammation pathogens. This method can also facilitate targeted treatment, which leads to successful management of inflammation. The Multiplex PCR 56 mechanism is highly advanced, budget-friendly, and appropriate for both qualitative and quantitative analysis of the typical prevalence of pathogens in typical laboratory settings. Multiplex PCR is capable of absolute gene quantification in samples. Additionally, the perturbation genes iap, hly and prfA were present in all unemotional samples, this indicates that these genes have an effect on the disease uninventive by *Listeria monocytosense* and are associated with the degree of pathogenicity.

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