Detection of *Listeria monocytogenes* in frozen food using a specific *inlB* virulence gene

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Abstract. This study was undertaken to detect the presence of *Listeria monocytogenes* in frozen food. A total of 200 different food samples were collected from Basrah markets, southern Iraq, during September 2015 to March 2016. These samples included frozen fish, frozen burger, frozen chicken and worker's hands swabs, 50 each. The polymerase chain reaction technique was used to evaluate the presence of *Listeria monocytogenes* by using of *inlB* specific gene. The PCR results revealed that only four samples (7.27%) were contaminated by *Listeria monocytogenes*. In conclusion frozen food may carry a dangerous type of bacteria and lead to human illness.

Keywords. *Listeria monocytogenes*, meat, burger, chicken, *inlB*, Basrah.

1. Introduction

Contamination of raw meat is one of the main sources of food-borne diseases [1, 2]. The presence of microbes in meat products cannot be detected visually [3]. This increases the risks of food borne microbes and the incidence of human illnesses [4]. Food associated bacteria are on two types; pathogenic bacteria which cause a disease and spoilage bacteria which may cause loss of quality of food and developing a bad smell or texture. Pathogenic bacteria that cause food-borne disease and cannot be detected during food consumption such as *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus*, etc.[5]. *Listeria* causes a food-borne disease called Listeriosis that has become a major problem for the public health in developed countries in addition to food industry [6, 7]. The disease also affects humans, and rised during the 1980s. There was rising in the numbers of human cases in several countries together with evidence for food-borne transmission of this disease [8]. The uterus of pregnant and the central nervous system or the blood stream are mostly affected by listeriosis. In non-pregnant women or in the immunocompromised or elderly people, listeriosis usually presents as meningitis or septicemia [9]. At the studied area less attention has been made for studying listeriosis or isolation of *Listeria* from imported meat and meat products. Milk was extensively studied previously including isolation of *Listeria* [10] and other types of bacteria [11, 12, 13, 14, 15, 16]. The aim of this study was to evaluate the microbial quality of imported frozen meat regarding Listerial contamination, which cause severe health problems.

2. Materials and Methods

2.1. Samples collection

Imported frozen food samples were collected from different local markets and workers in Basrah city for bacterial investigation of *Listeria monocytogenes*. A total of 200 samples were collected during the period from September 2015 to March 2016,including 50 samples of each frozen burger, frozen chicken, frozen fish and swabs from worker's hands.Twenty-five grams of the samples were collected in a sterile containers and immediately transported inside ice box to the laboratory for bacteriological analysis.

2.2. Culturing the samples

Cold enrichment procedure was applied by adding 10gm of each sample to 90 ml of TSB broth (Himedia, India) which was kept at 4ºC for 4 weeks [17]. The samples were streaked on TSB agar containing nalidixic acid (40µg/ml) and lithium chloride 0.5gm /L and incubated for 48 h at 37°C [18].

2.3. Detection of Listeria monocytogenes

Fifty-five samples showing bacterial growth including 15 frozen fish,15 frozen burgers,15 frozen chicken and 10 swaps of worker's hand were selected. These samples were subjected to detection of *Listeria monocytogenes* by detecting the presence of the *inlB* specific gene by PCR technique.

2.4. Extraction of bacterial DNA

This procedure was done by using commercially available DNA extraction and purification kits (Geneaid, Korea). Five hundred microlitres of the culture, containing approximately 1 x 109 cells, were transferred to a 1.5 ml microcentrifuge tube. The tubes were centrifuged for 1 minute at 14,000-16,000 xg to pellet the cells and the supernatant was then carefully discarded by pipetting or pouring . One hundred and eighty microlitres of GT buffer were added to the tubes, and mixed by pipetting, and then followed by adding 20 µl of proteinase K. The samples were then incubated at 60°C for at least 10 minutes. During incubation, tubes were inverted every 3 minutes.

2.5. Primers

Oligonucleotide primers of inlB gene with a length of 343bp were used in this study [19]. Sequences of primers were;

F: CTGGAAAGTTTGTATTTGGGAAA

R:TTTCATAATCGCCATCATCACT

The electrophoresis of amplified product was carried out in 1.5% agarose gel (Promega, USA) using7µl DNA ladder as molecular weight marker and 7µl of PCR reactions at 70V for 30 min,and then at 80V for 20 min. The gel was visualized by UV transilluminator (Verlber Louemal, EEC), and then photographed

3. Results

3.1. Enrichment procedure

Out of 200 samples only 55 samples showed bacterial growth after cold enrichment. These samples were 15 from frozen fish,15 from frozen burgers, 15from frozen chicken and 10 from worker's hand (Table 1).

Table 1. Number and percentage of positive bacterial culture isolated from frozen food and worker's hand.

Sample source	No. of samples	No. of culture positive samples	% culture Positive
Frozen fish	50	15	30
Frozen burger	50	15	30
Frozen chicken	50	15	30
Worker's hand	50	10	20
Total	200	55	27.5

3.2. PCR results for inlB gene in Listeria monocytogens

PCR amplification of 343bp of inlB gene was applied on the extracted DNA from all the culture positive samples (Figure 1). According to PCR results out of 55 examined samples, the positive PCR results were observed in 4 samples only at the rate of 7.27% (Table 2).

Table 2. Molecular identification of *Listeria monocytogenesin* studied samples according to PCR assay using *inlB* gene.

Sample	No. of culture positive samples	No. of *inlB* positive samples	% PCR Positive
Frozen fish	15	2	13.3
Frozen burger	15	1	6.6
Frozen chicken	15	1	6.6
Worker's hand	10	0	0
Total	55	4	7.27

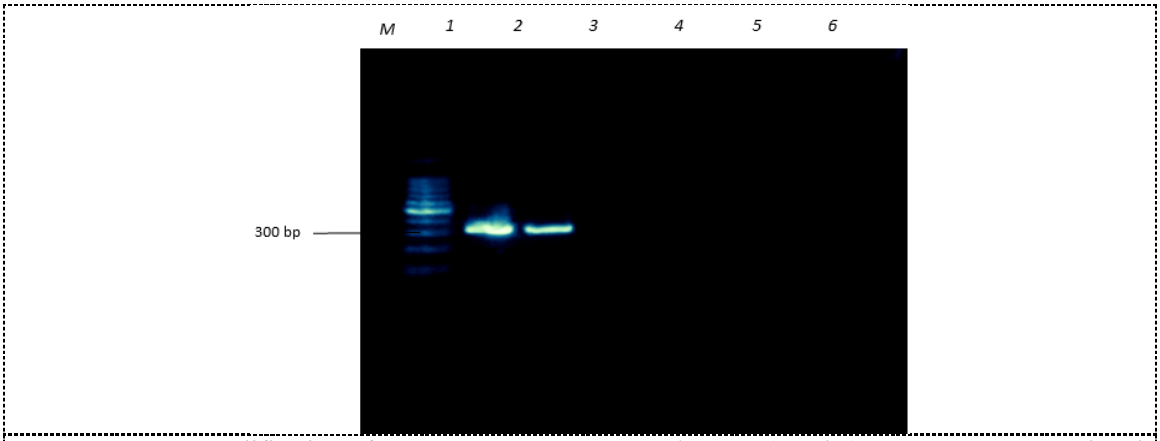

Figure 1. PCR amplification of *Listeria monocytogenes inlB* gene (343 bp) on 1.5% agarose gel and stained with ethidium bromide. Lane: M, Marker; Lane 1 and 2 *Listeria monocytogenes inlB* gene positive; Lane 3-6 negative results.

4. Discussion

Listeria monocytogenes considered as a cause of listeriosis disease in humans and animals [20,21]. Many deal with *Listeria* spp show that these bacteria can be found in a different food sources such as dairy products [22], meat and poultry [23], and in vegetables [24]. Different listeriosis outbreaks were found to be associated with milk consumption. This case is causing high concern in the industry of dairy due to the number of cases and increasing in the rate of mortality in these outbreaks to 30% [25]. Special groups of risk also observe in listeriosis outbreaks which include pregnant women, new born, immunecompromized patients and the old persons [26]. Polymerase chain reaction technique is a highly sensitive and more accurate than culture-based methods for detecting the pathogenmicroorganismsin the contaminated food. Many samples gave a positive result in their tests for *L. monocytogenes* by using the method of PCR while it gave a negative result by cultural methods. The attribute authors the sensitivity of PCR detection method increase over culturing methods to the truth that the former does not have selection initial step. Polymerase chain reaction detectionmethod solved the problem of some cells that cannot grow in the selective media when there is a small number present only. The false–negatives of some reported for the cultural detection methods are sometime these methods depended on using color changes for differentiate between species. These methods are influenced by the observer ability [27,28]. The target gene of *L. monocytogenes* is the primer pair,inlB-F and inlB -R of the inlBgene which detected by PCR [19;29; 30; 31] will amplify and produce a double-stranded fragment of 343bp. At this study, the rate of isolation *Listeria monocytogenes* was 4/55 isolates (7.27%) by inlB gene. this result is in agreement with [32] who found (6.2%) of *Listeria monocytogenes* by inlB gene.

5. Conclusion

From the above results, it can be concluded that the imported frozen food are not safe from a pathogenic bacteria such as *Listeria monocytogenes*. Culture method without more investigation is not sufficient for bacteria identification and PCR techniques is a rapid sensitive method for this purpose.

6. References

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