Isolation of *Listeria monocytogenes* of Karun River (Environmental Sources Rural and Urban) by Culture and PCR Assay

Nerssy Nassirabady 1,*; Hossein Meghdadi 2; Ameneh Alami 2

1Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, IR Iran
2Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

*Corresponding author: Nerssy Nassirabady, Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, IR Iran Tel: +98-6113331045, Fax: +98-64333045, E-mail: researcherirany@yahoo.com

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### Background

*Listeria monocytogenes* is a facultative intracellular pathogen thought to be widely distributed in the environment. Listeriolysin O (LLO) and internalin A are two major pathogenesis factors in this bacterium.

### Objectives

The purpose of this research was to isolate of *Listeria* from different parts of the Karun river in Iran. The bacteria were identified based on cultural characteristics, biochemical tests and by PCR assay.

### Materials and Methods

A total of 150 water samples from Karun river (rural and urban environment) were collected. The bacteria were identified based on cultural characteristics, biochemical tests and by PCR assay.

### Results

From total 150 samples, twenty *L. monocytogenes* were isolated and identified and amplification of two pathogenicity genes: *hlyA* and *inlA* in twenty *L. monocytogenes* were positive.

### Conclusions

Detection of *Listeria monocytogenes* with *hlyA* and *inlA* genes suggest that these strains may have the potential to invade host cells, and consumption of water contaminated with *L. monocytogenes* can cause human disease.

**Keywords:** *Listeria monocytogenes*; Cultural; Method; River

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1. **Background**

   *Listeria spp.* are facultative anaerobic, Gram-positive, non-spore-forming, small rods bacteria. This bacterium infects a wide variety of animal hosts including human, sheep, cattle, goats, pigs, rabbits, and mice. *Listeria monocytogenes* can cause listeriosis, encephalitis and abortions (1-3). Controlling this parasite is still a challenge for food business operators. *L. monocytogenes* is a facultative intracellular pathogen that uptake into phagocytic and non-phagocytic cells. Internalins such as *inlA* (encoded by *inlA* gene) and B (*inlB*), important pathogenesis genes mediating adhesion and invasion to eukaryotic cells (4). The pathogenicity of the *L. monocytogenes* is associated with factors like listeriolysin O (*llo*) (5-7). There are many target genes such as *inlA*, 16S rRNA, iap gene, *inlB* and *hlyA* that can be use for detection and pathogenesis of this bacterium (8-10). The hly product, was also the first virulence factor for which a precise role in the pathogenesis of Listeria infection was demonstrated (5-7).

2. **Objectives**

   Based on this, the aim of this study was isolation and identification of *Listeria monocytogenes* from different parts of the Karun river, Iran by culture, biochemical identification and proving to be invasive by PCR assay.

3. **Materials and Methods**

   3.1. **Sample Sites**

      As seen in Table 1, during three months period (March to May 2014), 150 water samples from different parts of Karun river were collected to analyze for the presence of *Listeria monocytogenes* according to MFHPB-30 method (11).

   3.2. **Isolation of Listeria monocytogenes**

      250 mL of water sample were filtered through 0.45 μm pore size, of which 1 mL was transferred into 9 mL of *Listeria Enrichment Broth* (LEB), and incubated at 37°C for 24 hours. Following inoculation 1 mL of culture into 10 mL of *Fraser Listeria Selective Enrichment Broth*, incubation at 37°C for 24 hours, and plated on CHROM agar (Merck, Germany) incubated at 37°C for 24 hours. The blue colonies with white halos were selected. Isolation and identification of the *Listeria monocytogenes* was done according to standard procedure such as catalase, oxidase, β haemolysis, the CAMP assay, mobility test at 25°C and Acid production from xylose and rhamnose (12). The presence of *hlyA* and *inlA* genes were detected by PCR.

   3.3. **PCR Amplification**

      The colonies on CHROM agar medium was transferred into microtubes containing distilled water then boiled
for 10 minutes at 95°C and centrifuged for 10 minutes at 3,000 × g. Supernatant included DNA was used as template in PCR. A set of primers (F:5'-GAATGTAAACTTCGGCAATCAG-3' and R:5’-GCCGTCGATGATTTGAACTTCATC-3’) was used to amplify hlyA (388 bp) DNA fragment (13). The reaction volume (25 μL) composed of 50 mM KCl, 10 mmol. Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.2 mM of each dNTP (deoxynucleoside triphosphate), 0.5 mM of each primer, 1 units of Taq polymerase (Cinnagen Co., Tehran, Iran), 14 μL of sterile distilled water and 5 μL of processed sample. Amplification was performed with an initial denaturation of 94°C for 4 minutes followed by 30 cycles each of denaturation at 94°C for 45 seconds, annealing at 56°C for 45s, and extension at 72°C for 1 minutes, followed by 7 minutes of final extension at 72°C was performed. A set of Primers (seq01: 5’-AATCTAGCACCACTGTCGGG 3’) and (seq02: 5’-TGTGACCTTCTTTTACGGGC 3’) was used to amplify inlA (733 bp) DNA fragment (14). The PCR was carried out in a 25 μL reaction using the 50 mM KCl, 10 mmoL Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.5 mM of each primer, 0.2 mM of each dNTP, 1 units of Taq polymerase (Cinnagen Co., Tehran, Iran), 14 μL of sterile distilled water and 5 μL of processed sample. The conditions of PCR were as follows 4 minutes at 94°C followed by 30 cycles (95°C for 30 seconds, 52°C for 1 minutes, 72°C for 2.5 minutes), and then 7 min at 72°C was performed. For analysis of the PCR products of each PCR assay performed, 12 mL of the reaction solutions from of amplification were resolved on 2% agarose gels containing safe stain (Cinnagen Co., Tehran, Iran), and the PCR products were visualized by UV transillumination after staining.

4. Results

A total of 150 (45 rural environment and 105 urban environment) water samples were collected from March to May 2014 from the Karun river region, Khozestan province, Iran. Of which 4 samples (rural environment) and 16 (urban environment) has blue colonies with a white halo. All the 20 samples were positive as Listeria monocytogenes (Tests of β hemolysis, CAMP, catalase, mobility at 25°C and acid production from rhamnosus were positive and oxidase and acid production from xylose tests were negative). The presence of hlyA gene and inlA genes was positive for 13.3% of the isolates (Figures 1 and 2). The results of this study by PCR method are presented in (Table 2).

<table>
<thead>
<tr>
<th>Site</th>
<th>Geographical Coordinates</th>
<th>Depth Line, m</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shushtar (Jam Kanar)-urban</td>
<td>32°97’ N; 48°77’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Shushtar (Islan park)-urban</td>
<td>31°31’ N; 48°46’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Shushtar (SikaPark)-urban</td>
<td>32°98’ N; 48°75’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Molasani (Ahvaz junction)-rural</td>
<td>31°97’ N; 48°71’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Zergan (Ahvaz functions)-rural</td>
<td>31°00’ N; 48°28’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Karoun river in Ahvaz (Naderi)-urban</td>
<td>31°78’ N; 48°74’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Water channels Shahid Chamran University-urban</td>
<td>31°20’ N; 48°40’ E</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>Khorramshahr (North Park saheli)-urban</td>
<td>30°25’ N; 48°10’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Khorramshahr (South Park saheli)-urban</td>
<td>30°29’ N; 48°15’ E</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Abadan-rural environment</td>
<td>30°22’ N; 48°20’ E</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>
5. Discussion

Water can play an important role in the transmission of the bacteria such as Listeria monocytogenes, which causes dangerous disease like listeriosis (15, 16). Although, L. monocytogenes has been isolated from a variety of sources, but its isolation from waters has been rarely reported (17, 18). In our study, 20 (13.3%) L. monocytogenes was isolated from 150 water samples from different parts of the Karun river, which is more than other report (15). In another study which is more than our study, 17.4% L. monocytogenes isolated from samples of milk, bulk tank swabs, cheese, feed, water, faeces and the environment (19). In the present study, hlyA and inlA in twent L. monocytogenes were positive, which is more than other report (91.7%) (20). The positive CAMP and haemolysis assay and cytogenes were positive, which is more than other report hlyA in twenty L. monocytogenes (19). In the present study, and inlA swabs, cheese, feed, water, faeces and the environment in the presence of L. monocytogenes has been isolated from a variety of sourc-

<table>
<thead>
<tr>
<th>Origin of Samples</th>
<th>Sites</th>
<th>Number of Samples</th>
<th>Number of positive isolates (hlyA gene)</th>
<th>Number of positive isolates (inlA gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment (rural)</td>
<td>Zergan (Ahvaz functions, Abadan, Molasani (Ahvaz junction)</td>
<td>45</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Environment (urban)</td>
<td>Shushtar (SikaPark), Water channels Shahid Chamran University, Karoun river in Ahvaz (Naderi), Khorramshahr (South Park saheli)</td>
<td>105</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>150</td>
<td>20</td>
<td>20</td>
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</tbody>
</table>

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Authors' Contributions

All authors participated equally in this article, especially in design, work, statistical analysis and manuscript writing.

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