Prevalence of *Listeria* species and *Listeria monocytogenes* serotypes in ready mayonnaise salads and salad vegetables in Iran

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Three hundred samples of ready mayonnaise salads and salad vegetables were used for isolation of *Listeria* spp. by ISO 11290-1. The isolates were identified using biochemical tests, further confirmed by duplex PCR and differentiated via conventional agglutination serotyping. A total of 8.7% of the samples harboured *Listeria* spp., including 7% *Listeria monocytogenes*, 1% *Listeria innocua* and 0.7% *Listeria welshimeri*. The *L. monocytogenes* isolates were divided into four serotypes of 1/2a (61.9%), 1/2b (19%), 3b (4.8%) and 4b (14.3%). The prevalence of *L. monocytogenes*, especially serotype 4b indicates that ready mayonnaise salads and salad vegetables could be potent sources of listeriosis.

**Key words:** *Listeria* spp., *Listeria monocytogenes*, serotyping, ready mayonnaise salads, salad vegetables.

**INTRODUCTION**

The genus *Listeria* contains 10 species: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri*, *Listeria grayi*, *Listeria marthii*, *Listeria rocourtiae*, *Listeria fleischmannii* and *Listeria weihenstephanensis* (Zhang et al., 2007; Halter et al., 2012). Among these species only *L. monocytogenes* and *L. ivanovii* are pathogenic, and the rest are non-pathogenic (Volokhov et al., 2002; Liu, 2006). *L. monocytogenes* is an intracellular foodborne pathogen that causes listeriosis and severe infections in humans with high mortality rate, mainly in high risk groups including pregnant women, elderly people, babies, HIV and cancer patients. It has been isolated from surface water, soil, vegetation, environments, and different food categories (Cocolin et al., 2005; Kuhn et al., 2008; Liu, 2008).

Approximately, 2500 cases of listeriosis occur in the United States every year, from which about 20% lead to death (Wilks et al., 2008). The incidence of listeriosis was reported as about 0.3 cases per 100 000 population in the European Union, in 2007 (Lindbäck, 2011). Although, several sporadic cases of human listeriosis have been reported in Iran (Nazari, 1963; Shayanfar and Jalilvand, 2004), there is no data on outbreak of listeriosis and the sources of contamination were unknown. Various food surveys conducted in Iran had reported on the detection of *L. monocytogenes* in different food products, including vegetables, ready to eat foods (Jalali and Abedi, 2008),...
raw meat (Rahimi et al., 2012b), quail (Dorcheh et al., 2013), and dairy (Mahmoodi, 2010; Rahimi et al., 2012a; Jamali et al., 2013a) products. The reports on its prevalence in different parts of Iran depicts a slight upward trend during the last decade (Moshtaghi et al., 2007; Jalali and Abedi, 2008; Rahimi et al., 2012a; Dorcheh et al., 2013). Despite all these studies, the data for prevalence of L. monocytogenes in ready salads and salad vegetables in Iran is limited and needs to be further investigated, as these products are highly consumed.

There are 13 different serotypes of L. monocytogenes, from which only three serotypes, 1/2a, 1/2b and 4b, have been detected from listeriosis cases in human (Zhang et al., 2007), which has been detected from listeriosis cases in human (Moshtaghi et al., 2007; Jalali and Abedi, 2008; Rahimi et al., 2012a,b; Dorcheh et al., 2013). Despite all these studies, the objectives of this study were to determine the prevalence of Listeria spp. and L. monocytogenes in ready mayonnaise salads and salad vegetables in Tehran city, Iran and to serotype the isolates using antisera against O and H antigens.

### MATERIALS AND METHODS

#### Isolation and identification

Two hundred and fifty (250) samples of individual salad vegetables including tomatoes, cabbages, lettuces, cucumbers and carrots (without any other ingredients) and ready mayonnaise salads were purchased from wet markets in Northern and Eastern parts of Tehran city, Iran. For detection of L. monocytogenes, ISO 11290-1 method was applied as described by Ennaji et al. (2008). Twenty-five grams of each sample was homogenized in 225 ml of primary enrichment culture of Listeria (Half Fraser broth, Merck, Germany) in sterile stomacher bag and was incubated at 30°C for 24 h. One ml of the primary enrichment culture was then added to 9 ml of enrichment broth (Fraser broth, Merck, Germany), and it was incubated at 37°C for 24 h. A loopfull of the enrichment broth was streaked onto Palcam agar (Merck, Germany) as selective agar and RAPID L. mono (BIO-RAD, France) as chromogenic media and the plates were incubated for 24 to 48 h at 37°C.

The presumptive colonies from each culture medium were spread on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) (Merck, Germany) and were identified using cultural, morphological and biochemical tests. Gram staining, oxidase, catalase, motility, Methyl Red and Voges-Proskauer (MR-VP), hemolysis test and CAMP test were used for identification of isolates and API Listeria kit (BioMerieux) was applied for characterization of them.

#### PCR protocol

DNA extraction and PCR amplifications were used as described by Rossmanith et al. (2006). Two pairs of primers including U1/L1 and LM1/LM2 were applied for simultaneous identification and confirmation of Listeria at genus level and L. monocytogenes, respectively. The optimized PCR conditions consisted of an initial denaturation of 95°C for 4 min and 30 cycles of 95°C, 1 min, 52°C, 45 s, 72°C, 2 min and a final elongation 72°C, 8 min. The U1/L1 and LM1/LM2 primers allowed the amplification of 16S rRNA (938 bp) and LLO gene (701 bp), respectively.

#### Serotyping

All isolates of L. monocytogenes were serotyped using antisera against O and H antigens according to the manufacturer (DenkaSeiken, Tokyo, Japan), with slight modifications. Briefly, the isolates were inoculated onto TSAYE (Merck, Germany) for the determination of O antigens. A portion of the bacterial colony on the agar plate was picked up and suspended in 0.2% normal saline solution (1ml). The bacterial suspension was then heated at 100°C for 1 h. For typing of H antigen, the bacterial colony on motility medium was inoculated into 1 mL TSB, followed by overnight incubation at 30°C. Formalin was then added to each culture (1%) and mixed gently. 20 µl of H antisera was added into each well of a 96-well microtitre plate. The cell suspension which was fixed by formalin was added appropriately to the wells containing antisera. After 2 min of agitation the plate was incubated at 50°C for an hour before visual observation (Indrawattana et al., 2011).

### RESULTS AND DISCUSSION

The prevalence of Listeria spp. and L. monocytogenes obtained from ready mayonnaise salads and salad vegetables samples has been shown in Table 1, based

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**Table 1. Prevalence of Listeria species and different serotypes of the L. monocytogenes isolates in salad vegetables and ready salads.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Listeria spp.</th>
<th>L. monocytogenes</th>
<th>L. innocua</th>
<th>L. welshimeri</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2a</td>
<td>1/2b</td>
<td>3b</td>
<td>4b</td>
</tr>
<tr>
<td>Tomato</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cabbage</td>
<td>50</td>
<td>3(6%)</td>
<td>3(6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lettuce</td>
<td>50</td>
<td>6(12%)</td>
<td>3(6%)</td>
<td>2(4%)</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>50</td>
<td>9(18%)</td>
<td>3(6%)</td>
<td>1(2%)</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Carrot</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ready mayonnaise salad</td>
<td>50</td>
<td>8(16%)</td>
<td>4(8%)</td>
<td>1(2%)</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>26(8.7%)</td>
<td>13(4.3%)</td>
<td>4(1.3%)</td>
<td>3(1%)</td>
</tr>
</tbody>
</table>

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Figure 1. A representative gel of PCR-amplified products of Listeria spp. Listeria spp. is indicated by a single band at 938 bp (16s rRNA) while L. monocytogenes is indicated by two bands, 938 bp and 701 bp (LLO gene). Lane 1, 100 bp molecular size marker; lane 2, positive control (L. monocytogenes, ATCC 35152); lanes 3, 5, 11 and 14: non-L. monocytogenes; lanes 4, 6 to 10, 12, 13, 15, L. monocytogenes; lane 16: negative control.

on the PCR result, as final confirmation of the presumptive isolates identity (Figure 1). Listeria species were isolated from 8.7% (26/300) of the samples, and the isolates included three species, L. Monocytogenes (21 isolates), L. innocua (three isolates), and L. welshimeri (two isolates). Seven percent of the samples were L. monocytogenes positive, including cabbage, lettuce, cucumber and ready mayonnaise salad samples, while tomato and carrot samples were free of the pathogen. L. monocytogenes had been previously detected in vegetables and salad vegetables such as lettuce, tomato, carrot, cucumber, cabbage, potato and parsley (Odumeru et al., 1997; Ponniah et al., 2010; Jamali et al., 2013b).

In 2008, Jalali and Abedi reported a low prevalence of L. monocytogenes (1.2%) in vegetables in Isfahan province, Iran (Jalali and Abedi, 2008). The results obtained from the current study indicated a comparatively higher incidence (6%) in Tehran city, which reveals a significant possibility of contamination for the consumers. Comparison of the occurrence rates of Listeria spp. in Iran and in different food sources, with the findings of the present study indicates that its population has undergone an upward trend during the recent years (Moshhtaghi et al., 2007; Jalali and Abedi, 2008; Rahimi et al., 2012a,b; Dorcheh et al., 2013). However, it is still less frequent than compared to Malaysia (Ponniah et al., 2010). Among the studied samples, cucumbers showed the highest prevalence (18%) of Listeria species. The isolates were dispersed between L. monocytogenes (14%), L. innocua (2%), and L. welshimeri (2%). High prevalence of L. monocytogenes in cucumber samples was previously reported by Ponniah et al. (2010) in Malaysia. In the current study, Listeria species were detected in 16% of ready mayonnaise salads where 75% of the contaminated samples were L. monocytogenes positive. Earlier findings by Pinto et al. (2010) and Uyttendaele et al. (2009) also indicated significant existence of L. monocytogenes in mayonnaise based deli salads. Among 50 samples of lettuce, 12% were contaminated with Listeria spp. and 83.3% of the contaminated samples were L. monocytogenes positive.

The prevalence of Listeria species in ready mayonnaise salads and salad vegetables, obtained from this study might be linked to the presence of the Listeria genus in natural environment, soil and surface water (Nightingale et al., 2004). It indicates the susceptibility of agricultural products to L. monocytogenes contaminations (Welshimer and Donker-Voet, 1971), which may lead to listeriosis in human and animals when the contaminated raw vegetables are consumed.

Four different serotypes (1/2a, 1/2b, 3b and 4b) were identified for the L. monocytogenes isolates in this study (Table 1). The results indicate higher prevalence of serotypes 1/2a, 1/2b and 4b among the L. monocytogenes isolates, which concurred with the previous investigations on food samples (Kathariou, 2002; Zhang et al., 2007). The most prevalent serotype among the isolates was 1/2a, which was also reported in other studies (Aarnisalo et al., 2003; Wallace et al., 2003; Gorski, 2006; Chemaly et al., 2008; Pan et al., 2009).

In summary, the persence of L. monocytogenes in ready mayonnaise salads and salad vegetables indicated that they could be potential sources of listeriosis in humans and animals because these types of foods are commonly eaten raw. There is a need for a more strict control measures in food hygiene and processing of agricultural produce. From another point of view, the prevalence of Listeria spp. might alter based on the sam-
pling time, due to the seasonal changes in its population, previously reported (Guerini et al., 2007). Hence, systematic studies of the seasonal incidence of this pathogen especially in ready to eat food can give a clear image of the contamination risks for the consumers.

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REFERENCES


