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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*, *L. 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),

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Table1. Prevalence of *Listeria* species and different serotypes of the *L. monocytogenes* isolates in salad vegetables and ready salads.

Sample	No. of samples	No (%) of samples positive for							
		Listeria spp.	*L. monocytogenes*					*L. innocua*	*L. welshimeri*
			1/2a	1/2b	3b	4b	Total		
Tomato	50	0	0	0	0	0	0	0	0
Cabbage	50	3(6%)	3(6%)	0	0	0	3(6%)	0	0
Lettuce	50	6(12%)	3(6%)	2(4%)	0	0	5(10%)	1(2%)	0
Cucumber	50	9(18%)	3(6%)	1(2%)	1(2%)	2(4%)	7(14%)	1(2%)	1(2%)
Carrot	50	0	0	0	0	0	0	0	0
Ready mayonnaise salad	50	8(16%)	4(8%)	1(2%)	0	1(2%)	6(12%)	1(2%)	1(2%)
Total	300	26(8.7%)	13(4.3%)	4(1.3%)	1(0.3%)	3(1%)	21(7%)	3(1%)	2(0.7%)

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,b; 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 (Borucki et al., 2003; Zhang et al., 2007). The most frequently isolated strain from human listeriosis cases is 4b, while 1/2a is the most prevalent serotype isolated from various food samples (Zhang et al., 2007). 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, Germay), 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/LI1 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/LI1 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 of 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 antiserum. 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

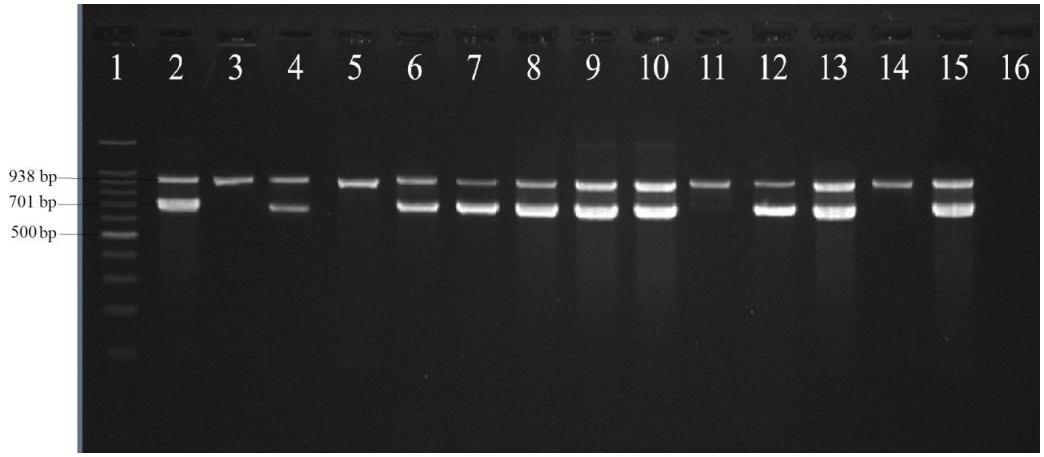

Figure 1. A representative gel of PCR-amplified products of *Listeria* spp. *Listeria* spp. is indicated by a single band at 938 bp (16s rRNA) while *L. monocytogenes* is indicated by two bands, 938 bp and 701 bp (LLO gene). Lane 1, 100 bp molecular size marker; lane 2, positive control (*L. monocytogenes*, 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 (Moshtaghi 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 existance 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 commenly 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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