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Prevalence and molecular characterization of *Listeria* spp. and *Listeria monocytogenes* isolated from fish, shrimp, and cooked ready-to-eat (RTE) aquatic products in Iran

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ABSTRACT

The prevalence of *Listeria* spp. and *Listeria monocytogenes* was investigated by biochemical and molecular methods in a total of 201 fish, shrimp, and ready-to-eat seafood samples collected from Iranian supermarkets. Thirty-six samples were also collected from a seafood processing plant. Twenty-one (8.86%) of the total retail and processing plant samples (237) were positive for *Listeria* spp., confirmed by a simplex PCR assay for the *prs* gene. Seven (2.95%) of the total samples were also positive for *L. monocytogenes*. The presence of four virulence-associated genes in the seafood isolates (*inlA*, *inlC*, *inlJ*, and *hlyA*) was examined using PCR and the results were compared with seven clinical *L. monocytogenes* strains. All virulence genes were detected in six fish isolates. One fish isolate did not show amplification of the *inlJ* and *inlC* genes. However, all seven clinical strains were positive for internalin genes. Furthermore, a multiplex PCR assay was employed to evaluate the major *L. monocytogenes* genoserogroups' distribution. The results revealed that the serotypes of lineage II are most frequently present in clinical and food isolates. In summary, PCR screening for both the major *L. monocytogenes* serovars and virulence genes revealed the potential public health risk posed by *L. monocytogenes* in aquatic products.

1. Introduction

Listeria monocytogenes is a pathogenic microorganism transmitted to man mostly through food. This pathogen is responsible for human listeriosis, a severe disease that may result in meningitis, encephalitis, septicemia, or abortion, with a considerable mortality rate (Laksanalamai et al., 2012; Vázquez-Boland et al., 2001). *L. monocytogenes* has the capacity to growth in a wide range of pH values (4.1–9.6), low water activity, and high salt concentrations (10%) (Paul et al., 2014). It can also be found on cooked ready-to-eat (RTE) food processing equipment, due to its ability to form biofilms (Ferreira, Almeida, Delgadillo, Saraiva, & Cunha, 2015).

Outbreaks of listeriosis have been associated with consumption of ready-to-eat food products (Nelson et al., 2004). During 1998–2008, 24 confirmed listeriosis outbreaks were reported in the United States (Cartwright et al., 2013). The number of listeriosis cases in the European Union increased 19.1% in 2009 (n = 1645) compared to 2008, and remained almost at the same level in 2012 (n = 1642). Consequently, in Europe, listeriosis has caused great concern about public health because of its high mortality rate (15–30%, with about 198 deaths in 2012 due to compared with 61 deaths due to salmonella), with clinical severity discernable in the hospitalization rate, >90% (EFSA & ECDC, 2014). Therefore, the presence of pathogenic serotypes of *L. monocytogenes* is one of the most important concerns of the seafood industry and public health agencies.

Listeria serotyping has been performed for many years and employed extensively in epidemiological investigations. However,

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classical serotyping by agglutination method has several disadvantages, such as the high cost of antisera and presence of non-typeable isolates. To overcome these problems, several molecular techniques have been suggested (Chenal-Francisque et al., 2015; Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004; Kerouanton et al., 2010) for the rapid identification of *L. monocytogenes* genoserogroups. These approaches are more sensitive, specific, and cost effective for the discrimination of *L. monocytogenes* genotypes, and could be used as first-level characterization of *L. monocytogenes*, especially in epidemiological investigations and food safety monitoring programs.

Many studies have reported the incidence of *L. monocytogenes* in fruits and vegetables (Hadjilouka, Andritsos, Paramithiotis, Mataragas, & Drosinos, 2014; Montero et al., 2015), dairy products (Dalzini et al., 2015; Karthikeyan, Gunasekaran, & Rajendhran, 2015; Soni, Singh, Singh, & Dubey, 2013), meat (Hadjilouka et al., 2014; Kovacevic, Mesak, & Allen, 2012; Montero et al., 2015), fish (Chou & Wang, 2006; Kovacevic et al., 2012; Wang et al., 2013), seafood products (Boerlin, Boerlin-Petzold, Bannerman, Bille, & Jemmi, 1997; Li et al., 2015; Miya, Takahashi, Ishikawa, Fujii, & Kimura, 2010; Momtaz & Yadollahi, 2013), and food processing environments (Chen, Pyla, Kim, Silva, & Jung, 2010; Li et al., 2015). However, the occurrence of *L. monocytogenes* in seafood has received less attention in Iran and very limited information is available on the prevalence and contamination levels of *Listeria* spp. and *L. monocytogenes*.

In the current study, a molecular method was used to serogroup *L. monocytogenes* strains isolated from naturally contaminated fish, shrimp, and cooked aquatic products. Additionally, a multiplex PCR was employed to detect simultaneously virulence-associated genes in food and clinical isolates.

2. Materials and methods

2.1. Bacterial strains

The standard strain of *L. monocytogenes* PTCC 1163 was obtained from Persian Type Culture Collection, Tehran, Iran, and used in PCR assays. The strain was reactivated from a glycerol stock culture. With two consecutive transfers, the bacterium was inoculated into 15 ml Tryptic Soy Broth (TSB; Liofilchem, Italy) and incubated under shaking (150 rpm) for 24 h at 37 °C.

Staphylococcus aureus and seven clinical *L. monocytogenes* strains, previously isolated by Lotfollahi et al. (2011), were obtained from the Microbiology Department of the Iran University of Medical Sciences, Tehran, Iran.

2.2. Sampling at retail level

From October 2014 to August 2015, a sampling program was carried out as part of this study in Karaj and Tehran, Iran, to identify the presence of *L. monocytogenes* in seafood samples. A total of 201 samples of seafood were collected from 14 retail food stores including shrimp nuggets, fish nuggets, kilka fish (Caspian tyulka, *Clupeonella caspia*), raw packed shrimp, and rainbow trout (*Oncorhynchus mykiss*). All of the samples were kept below 4 °C and transferred to the microbiology laboratory of Iran University of Medical Sciences.

2.3. Sampling at processing plant

Sixteen samples from edible materials of a seafood plant (raw fish and shrimp, n = 6; cooked RTE final products; n = 5, and additives, n = 5); and 20 surface swabs from floors, conveyors, and fryers (Table 2) were collected and transferred to the laboratory.

2.4. Enrichment, and biochemical and morphological identification

25 g of the seafood samples were homogenized and inoculated into 225 ml of TSBYE (Tryptic Soy Broth + 0.6% yeast extract). All of the samples were incubated at 4 °C (cold enrichment). After 7, 14, and 30 days of incubation, aliquots (100 μl) of the enriched cultures were streaked onto Listeria Oxford Agar plates (Liofilchem, Italy) and incubated at 37 °C for 48 h. Colonies appearing black were considered to be presumptive *Listeria* spp. Confirmation of the isolates was performed by routine laboratory test, including Gram staining, motility test, catalase reaction, oxidase test, and fermentation of sugars. Hemolysis of the positive isolates was tested on sheep blood agar (CAMP test). The isolates of *L. monocytogenes* were stored at −20 °C in TSB +25% glycerol.

2.5. DNA extraction

The isolated species were retrieved from glycerol stocks, kept at −20 °C, and cultured on Mueller-Hinton agar (Liofilchem, Italy). The colonies of each strain were inoculated into 7 ml of TSB. The inoculated tube was incubated at 37 °C for 24 h. Without using enzyme, genomic DNA was extracted from the cultured bacteria via a modified phenol-chloroform extraction protocol. Briefly, bacteria (500 μl) were pelleted and resuspended in 300 μl of lysis buffer (3.27 g sucrose, 0.036 g Tris base, 0.3 g SDS, and 0.0141 g $MgCl_2$ in 30 ml distilled water). Next, the microtubes were shaken by vortex mixer and incubated at 65 °C for 30 min. After incubation, the supernatants were collected by centrifugation at 8000 rpm for 5 min and suspended in an equal volume of equilibrated phenol. After quick agitation in the vortex mixer, the microtubes were centrifuged (8000 rpm, 5 min, room temperature). Then the supernatants were resuspended in an equal volume of chloroform and mixed. Following centrifugation (8000 rpm, 5 min, room temperature), the supernatants were transferred to new microtubes (2 ml). One-tenth volume of the collected supernatant, 3 M sodium acetate was added. The microtubes were then filled with 1.5 ml of cold ethanol (96%) and stored at −20 °C for 24 h. The microtubes were centrifuged (1200 rpm, 10 min, at 7 °C) and the supernatants were completely removed. Then, 100 μl of ethanol (70%) was added to the microtubes. The tubes were centrifuged (1200 rpm, 2 min, at 7 °C), and the supernatant was decanted and discarded. Finally, the tubes were dried at room temperature. The purified DNA was dissolved in 50 μl sterile distilled water. The DNA concentrations were determined at 260/280 nm using a spectrophotometer.

2.6. Molecular Listeria spp. identification

PCR assays were conducted in a 25 μl reaction mixture under the previously described PCR conditions (Doumith et al., 2004; Liu, Lawrence, Austin, & Ainsworth, 2007). *Prs* and *inlA* primers were employed to detect *Listeria* spp. and *L. monocytogenes*, respectively (Table 1).

2.7. Virulence genes

For each isolate, three virulence genes, *inlC*, *inlA*, and *inlJ*, of *L. monocytogenes* were tested by multiplex PCR using the primers designed by Liu et al. (2007) (Table 1). PCR was performed in a DNA thermal cycler (Bio-Rad, USA) in a volume of 25 μl. The cycling program consisted of 1 × 92 °C for 2 min; 29 × 94 °C for 27 s; 51 °C for 27 s, 72 °C for 50 s; and 1 × 72 °C for 2 min. The 4 μl PCR product was mixed with 1 μl safe stain and separated on 1% agarose gel for 60 min at 90 V. The presence of *hlyA* gene was also investigated by simplex PCR.

Table 1
List of primers used for determination of virulence marker genes and genoserogroups of *L. monocytogenes*.

Primer name	Primer sequence (5′-3′)	Size of amplicon (bp)	Target/encoded protein	References
lmo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	Serovars 1/2a, 1/2c, 3a, and 3c	Doumith et al. (2004)
lmo1118	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	Serovars 1/2c and 3c	Doumith et al. (2004)
ORF2819	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	Serovars 1/2b, 3b, 4b	Doumith et al. (2004)
ORF2110	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	Serovars 4b, 4d, and 4e	Doumith et al. (2004)
prs	F:GCTGAAGAGATTGCGAAAGAAG R:CAAAGAAACCTTGGATTTGCGG	370	All *Listeria* spp.	Doumith et al. (2004)
inlA	F: ACGAGTAACGGGACAAATGC R: CCCGACAGTGGTGCTAGATT	800	Internalin A	Liu et al. (2007)
inlC	F: AATTCCCACAGGACACAACC R: CGGGAATGCAATTTTTCACTA	517	Internalin C	Liu et al. (2007)
inlJ	F: TGTAACCCCGCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA	238	Internalin J	Liu et al. (2007)
hlyA	F: CGGAGGTTCCGCAAAAGATG R: CCTCCAGAGTGATCGATGTT	234	Listeriolysin O	Aznar and Alarcon (2003)

2.8. Genoserotyping of L. monocytogenes

The multiplex PCR assay method, adapted from Doumith et al. (2004), was used to identify the major *L. monocytogenes* serovars. For this purpose, the following primers were used: *prs*, ORF2110, ORF2819, *lmo1118*, and *lmo0737*. The primer sequences are presented in Table 1. The cycling program consisted of 1 × 94 °C for 3 min; 35 × 94 °C for 40 s; 53 °C for 1.15 min; 72 °C for 1.15 min; and one final cycle of 72 °C for 7 min. 4 μl of the PCR product was mixed with 1 μl of safe stain and electrophoresed (99 V, 1 h) in 1.5% agarose gel.

2.9. Statistical analysis

Fisher's exact test was employed to compare the rate of *Listeria* spp. contamination among various categories of retail products (SPSS 16.0, SPSS Inc., USA). The differences among various groups were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Listeria spp. and Listeria monocytogenes in raw fish, shrimp, and retail and processing environments

A total of 237 retail products and processing environment samples were collected. This formed the population for screening for the presence of *Listeria* spp. and *L. monocytogenes*. Twenty-one (8.86%) samples were positive for *Listeria* spp. Seven (2.95%) samples were positive for *L. monocytogenes*. Table 2 summarizes the occurrences of *Listeria* spp. in marketplace fish, shrimp, and cooked RTE seafood products. All samples in which *L. monocytogenes* had been isolated at room temperature displayed β-hemolytic activity on blood agar and umbrella type growth in motility agar. Among the retail raw fish samples (rainbow trout and Caspian tyulka), the prevalence of *Listeria* spp. varied significantly (Fisher's exact test $p = 0.004$) and the highest incidence of *Listeria* spp. (28%) and *L. monocytogenes* (16%) was observed in a single brand of retail fresh fish, commonly known as kilka (Caspian tyulka, *Clupeonella caspia*). Moreover, as captured in Table 2, the prevalence of *Listeria* spp. (including *L. monocytogenes*) varied significantly among three major categories (raw fish, raw shrimp, and cooked RTE samples) of retail products (Fisher's exact test, $p = 0.0366$; raw fish samples > cooked RTE samples > shrimp fish samples). Nine raw fish samples (14.28%) and one raw shrimp (1.69%) sample from the retail markets were positive for *Listeria* species. In addition, 5 fresh fish samples (7.9%) collected from 14 retail stores were positive for *L. monocytogenes*.

Several studies have been conducted about the incidence of *Listeria* spp. in raw fish and shrimp samples, recently reviewed in Jami, Ghanbari, Zunabovic, Domig, and Kneifel (2014). In these reports, the rate of *L. monocytogenes* contamination in raw fish and shrimp samples ranged from 43.3% (Chen et al., 2010) to 0% (Kuzmanović et al., 2011; Minami et al., 2010; Wang, Chern, Li, Yan, & Hsieh, 2012).

Overall, 36 samples were collected from one of the largest Iranian seafood plants and tested for the presence of *L. monocytogenes*. 33.3% incidence of *L. monocytogenes* was observed in raw food samples (tilapia fillet). Moreover, one *Listeria* contamination (20%) was detected in the final cooked RTE shrimp products. It seems cross-contamination during fish handling and removal of intestines are two possible routes for *Listeria* spp. contamination of raw fish and shrimp (Jami et al., 2014).

To date, few reports on the prevalence of *Listeria* spp. in raw fish and shrimp in Iran have been published. Unfortunately, listeriosis is not a reportable disease in the Iranian health program and the real situation of listeriosis in Iran is unknown (Jalali & Abedi, 2008). Moreover, there are no strict criteria for monitoring *L. monocytogenes* in the Iranian food processing plants. Momtaz and Yadollahi (2013) reported that *Listeria* spp. (all species), and *L. monocytogenes* were detected in 10.45% and 7.25% of fresh fish (in the cities of Isfahan and Shahrekord, Iran), respectively. They also reported one (2.5%) positive shrimp sample for *Listeria* contamination. Based on results of Modaresi, Mardani, Tukmechi, and Ownagh (2011) in the city of Urmia (northwestern Iran) the incidence of all species of *Listeria* spp. and *L. monocytogenes* were 12.37% and 2.57% in raw fish, including farmed samples of *Oncorhynchus mykiss*, farmed *Cyprinus carpio*, farmed *Hypophthalmichthys molitrix*, *Sander lucioperca*, *Abramis brama*, *Astacus leptodactylus*, and *Silurus glanis*, respectively. Compared with the previous studies, a higher prevalence of *Listeria* spp. (21.3%) has been reported from raw fish in the northern region of Iran (Jamali et al., 2015). Jamali et al. (2015) indicated that 6.7% of farmed carp (*Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*) are naturally contaminated with *L. monocytogenes*. These findings are in agreement with the results of the present study. However, Zarei, Maktabi, and Ghorbanpour (2012) reported lower prevalence of *L. monocytogenes* (1.4%) in raw saltwater fish. To the best of our knowledge, this is the first study to report the rate of

Table 2
Prevalence of *Listeria* spp. and *L. monocytogenes* collected from retail market and food processing plant.

Category	Seafood	Brand	N	All *Listeria* spp.	*L. monocytogenes*
1) Retail market: Raw fish samples	Rainbow trout	Stores	18	2 (11.11%)	1 (5.55%)
	Caspian tyulka	A	25	7 (28%)	4 (16%)
	Caspian tyulka	B	20	–	–
Fisher's exact test (for all *Listeria* spp., including *L. monocytogenes*)				*p = 0.004*	
		Total:	**63**	**9 (14.28%)**^{A}	**5 (7.93%)**
2) Retail market: Shrimp fish samples	Shrimp	C	19	–	–
	Shrimp	A	20	1 (5%)	–
	Shrimp	D	20	–	–
Fisher's exact test (for all *Listeria* spp.)				*p = 1.00*	
		Total:	**59**	**1 (1.69%)**^{B}	–
3) Retail market: Cooked RTE samples	Fish nugget	E	20	3 (15%)	–
	Shrimp nugget	C	20	–	–
	Shrimp nugget	E	20	2 (10%)	–
	Shrimp nugget	F	19	3 (15.78%)	–
Fisher's exact test (for all *Listeria* spp.)				*p = 0.30*	
		Total:	**79**	**8 (10.12%)**^{A}	–
Fisher's exact test (for 3 retail categories; n = 201)				*p = 0.036*	
Total retail products			**201**	**18 (8.95%)**	**5 (2.48%)**
Samples from the processing plant	Floor		10	–	–
	Equipment		10	–	–
	RTE final products		5	1 (20%)	–
	Additives		5	–	–
	Raw material (tilapia)		6	2 (33.3%)	2 (33.3%)
Total:			**36**	**3 (8.33%)**	**2 (5.55%)**
Total samples			**237**	**21 (8.86%)**	**7 (2.95%)**

Superscript letters represent statistically significant differences ($P < 0.05$) among results, based on Fisher's exact test.

L. monocytogenes contamination in aquatic products in Karaj and Tehran, Iran.

Iran has acquired the first rank of aquacultured freshwater trout in the world (Kalbassi, Abdollahzadeh, & Salari-Joo, 2013). In this study, *Listeria* spp. and *L. monocytogenes* were found in 11.11% and 5.55% of the rainbow trout samples. All rainbow trout samples collected from retail markets originating from fish farms. In Finland, Miettinen and Wirtanen (2005) reported that the incidence of *Listeria* spp. and *L. monocytogenes* in farmed *Oncorhynchus mykiss* is 35% and 14.6%, respectively. Although both studies focused on similar species collected from fish farms, the contamination rates are very different. The differences between these two studies may be due to the number of samples, sampling methods, and methods of experiment. Furthermore, Miettinen and Wirtanen (2006) showed that weather conditions have a strong effect on the prevalence of *Listeria* spp. in fish farms.

A previous study (Miya et al., 2010) suggested that the cell numbers of *L. monocytognes* in seafood product increase rapidly under inappropriate storage temperatures, from 10/g MPN to 10^4/g over the course of 2 days. Moreover, the results of the latter study revealed that at day 2, all seafood products were judged to be unspoiled by a panel of five judges. Therefore, due to the rapid growth of *L. monocytoges* in aquatic products at low temperature, the presence this pathogen in aquatic products can be considered an important public health hazard. These data raise the concern that raw aquatic products available at retail markets in Iran are at risk for foodborne listeriosis.

3.2. *Listeria spp. and Listeria monocytogenes in cooked RTE seafood products*

Although *L. monocytogenes* was not detected in 25 g of the cooked RTE seafood samples, other *Listeria* species were found in two different cooked RTE seafood brands (up to 15.78%; average = 10.12%). These observations are in agreement with the results of Zarei et al. (2012). In a survey conducted in central Iran (Fallah, Saei-Dehkordi, & Mahzounieh, 2013), 14.5% of cooked RTE seafood samples were contaminated with *L. monocytogenes*; however, cooked RTE shrimp samples were free of *L. monocytogenes*. This observation is partially consistent with the results of present study. The occurrence of *Listeria* spp. in cooked RTE seafood samples could be from the cross-contamination during fish handling, incomplete cleaning and disinfection procedures, and incomplete implementation of HACCP principles in processing plants.

3.3. *Genoserotyping of the L. monocytogenes isolates*

L. monocytogenes generally consists of three genetic lineages, and each of these lineages is comprised of distinct serotypes. Although there are 13 described serotypes of *L. monocytogenes*, the vast majority of listeriosis cases (98%) are caused by serotypes of lineages I and II (1/2c, 1/2a, 1/2b, and 4b). Other serotypes of lineage III are not associated with the listeriosis outbreaks (Orsi, den Bakker, & Wiedmann, 2011; Paul et al., 2014). In other words, at least 95% of the strains isolated from food products belong to 1/2a, 1/2b, 1/2c, and 4b serotypes.

In total, three genoserogroups were identified among the seafood and the clinical isolates (Fig. 1). Most of the isolates belonged to 1/2a, 3a and 1/2b, 3b, 7 serogroups. In contrast, all of the clinical isolates belonged to serogroups 1/2c, 3c (Table 3). These observations illustrate that differentiation of the major *L. monocytogenes* serovars could be a fast and useful technique for epidemiological purposes. Our results revealed that the serotypes of lineage II (1/2a, 1/2c, 3a) are most frequently present in clinical and seafood isolates. Similar findings have also been reported elsewhere (Ebner et al., 2015; Kanki, Naruse, Taguchi, & Kumeda, 2015; Leong, Alvarez-Ordóñez, & Jordan, 2014; Miya et al., 2010; Obaidat, Bani Salman, Lafi, & Al-Abboodi, 2015; Vitas, Garcia-Jalon, & Aguado, 2004).

Some studies show that serotype 4b is responsible for the major outbreaks (Cartwright et al., 2013). However, new studies indicate that the pathogenic serotype distribution among patients changes in time and in space. For instance, in Italy, Finland, Denmark, Great Britain, and Switzerland, there is an increase of listeriosis cases due to 1/2a during the last decade (Pontello et al., 2012). Listeriosis

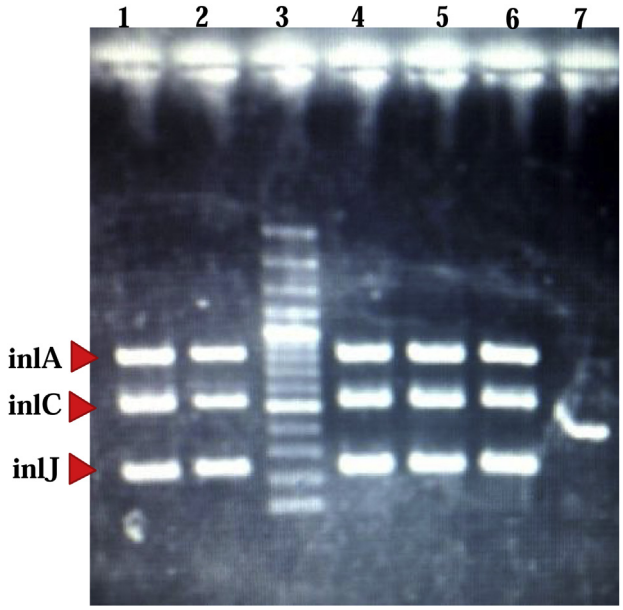

Fig. 1. Agarose gel electrophoresis of DNA products generated by multiplex PCR with seafood and clinical *Listeria monocytogenes* isolates using *lmo0737*, *lmo1118*, ORF2819, ORF2110, and *prs* primers. Lane 1, standard strain; lanes 2, 9, and 16, DNA molecular weight marker (100 bp); lane 3, *Listeria* spp.; lane 4, isolated *L. monocytogenes* from rainbow trout; lanes 5 and 8, *L. monocytogenes* isolated from raw material of the seafood processing plant; lanes 6 and 7, *L. monocytogenes* isolated from Caspian tyulka (*Clupeonella caspia*) fish; and lanes 10 to 17, the clinical *L. monocytogenes* strains.

Table 3
Prevalence of *L. monocytogenes* genoserogroups in raw fish and clinical isolates.

Sample	Frequency of genoserogroups[a]		
	1/2b, 3b, 7	1/2a, 3a	1/2c, 3c
Rainbow trout		1 (100%)	
Caspian tyulka	1 (50%)	1 (50%)	
Tilapia fillet	1 (25%)	1 (25%)	
Clinical strains			7 (100%)

[a] Frequency data represent the number of isolates (percent) in each genoserogroup.

outbreaks have also been caused predominantly by 1/2a isolates in Canada (Knabel et al., 2012). Furthermore, in Sweden, Parihar et al. (2008) illustrated that during 2000–07, only 13% of human listeriosis infections were attributable to serovar 4b, and most listeriosis cases (71%) have been caused by serovar 1/2a.

3.4. *L. monocytogenes* virulotyping

The presence of virulence genes, which differs among *L. monocytogenes* strains, is another important factor in listeriosis risk. Although the presence of internalin gene inlJ can be a valuable indicator for the pathogenicity of *L. monocytogenes* isolates, more recent studies indicate some strains contain no inlJ, yet have the potential to cause mouse mortality (Liu et al., 2006). Therefore, in order to evaluate the pathogenicity of *L. monocytogenes* strains, it is necessary to assay other internalin genes, *inlA* and *inlC* (Liu et al., 2007). The pathogen invades intestinal epithelial cells via an 800-amino-acid protein internalin A, which is encoded by *inlA* (Kanki et al., 2015).

In the current study, all of the food and clinical strains were tested for the presence of virulence genes *inlA*, *inlC*, *inlJ* and *hlyA*. The virulence genes were observed in all of the clinical strains and the seafood isolates, except in one seafood strain that did not show amplification of the inlJ and inlC genes (Fig. 2). These results are consistent with previous studies that showed that *inlA*, *inlC*, and *inlJ* genes are widely distributed among raw fish, shrimp, and cooked RTE seafood products in the northern region of Iran (Jamali et al., 2015), China (Chen et al., 2009), Malaysia (Jamali & Thong, 2014), and Canada (Kovacevic et al., 2012). Consistent with our results, Soni, Singh, and Dubey (2015) previously demonstrated that all clinical strains are positive for internalin genes.

In summary, our results regarding the occurrence of *L. monocytogenes* in aquatic products and the presence of virulence-associated genes indicate that contamination of seafood products may be a public health concern.

4. Conclusions

The findings of this study provide information about the

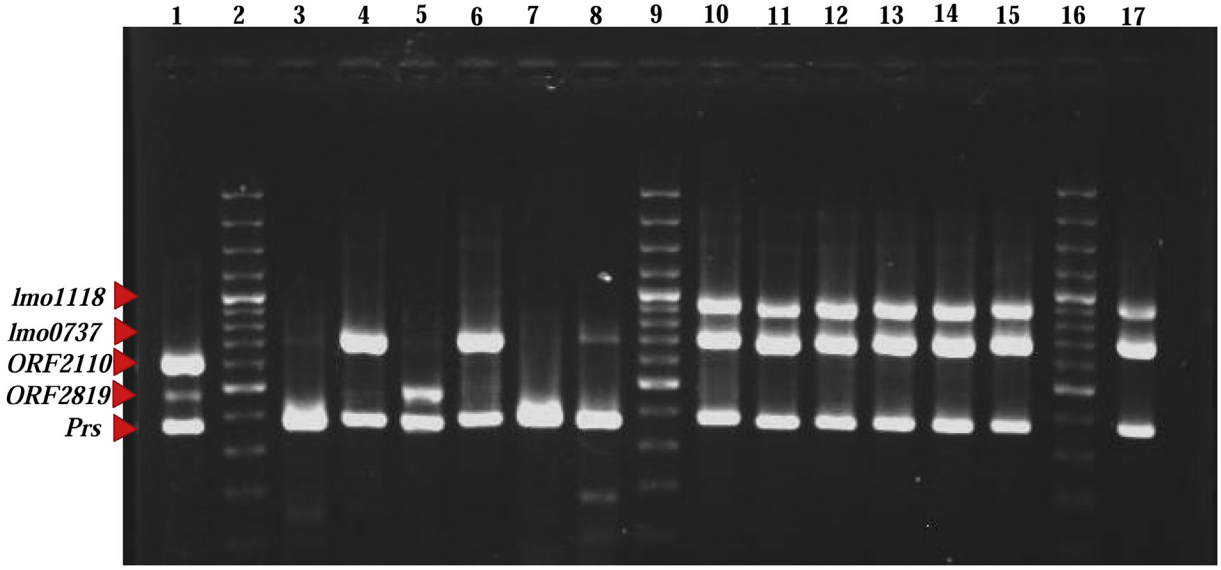

Fig. 2. Agarose gel electrophoresis of DNA fragments generated by multiplex PCR for internalin genes. The *inlA*, *inlC*, and *inlJ* gene products are 800, 517, and 238 bp, respectively. Lane 1, products amplified from DNA of seafood isolated *Listeria monocytogenes;* lane 2, *L. monocytogenes* reference strain; lane 3, DNA molecular weight marker (100 bp); and lanes 4–6, products amplified from DNA of clinical strains.

presence of *L. monocytogenes* in raw and cooked RTE seafood samples in Iran. Although the number of the samples was limited in the present study, the high prevalence of *L. monocytogenes* in raw materials and the presence of *Listeria* spp. in cooked RTE food products can be an alarm for monitoring this pathogen in the food industry.

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