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The Occurrence of Serotypes and Virulence Genes of *Listeria monocytogenes* in Various Food Products

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HIGHLIGHTS:

- Among 900 food samples, 136 (15.1%) were positive for *Listeria* spp.
- The most contaminated food was beef (35%) followed by chicken (29%) and green vegetables (23%).
- The most isolated *Listeria* spp included *L. monocytogenes* and *L. ivanovii*.
- Among *L. monocytogenes* isolates, dominant serogroups were 1/2a and 4b.

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Acronyms and abbreviations
PCR=Polymerase Chain Reaction
RTE=Ready-to-Eat

ABSTRACT

Background: Given that controlling *Listeria* contamination is very important in food chain system, the knowledge of their prevalence in food is very important. Therefore, this study aims to examine the prevalence of important *Listeria* species in various food types and evaluate serotype distribution, as well as the study of the virulence factors of *L. monocytogenes*.

Methods: During July 2018 and January 2020, 900 food samples were collected in the North of Iran, including beef, chicken, fish, shrimp, milk and yogurt, green vegetables, mixed vegetable salad, Olivier salad, and cottage cheese. After isolation and identification steps, each bacterial DNA was extracted. Then, using specific primers, species, serotypes, and virulence genes of *Listeria* isolates were evaluated in the samples by Polymerase Chain Reaction (PCR) method.

Results: The test results of 136 samples (15.1%) were positive for *Listeria* spp. and the most contaminated food was beef (35%) followed by chicken (29%) and green vegetables (23%). The most isolated *Listeria* spp. was *L. monocytogenes* and *L. ivanovii*. Among *L. monocytogenes* isolates, the dominant serogroups were 1/2a and 4b; furthermore, all of the isolates of this species harbored four virulence genes, including *hlyA*, *plc*, *iap*, and *actA*.

Conclusion: These reports highlighted the importance of food safety in various food products, particularly raw meats and vegetables. Moreover, contamination of healthy foods such as fish and vegetables with *Listeria* is an indicator of public health.

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Introduction

Some food-borne pathogens such as *Campylobacter*, *Listeria*, and other related bacteria have increased the incidence of food-borne diseases in recent years (Mohajer et al., 2021; Raeisi et al., 2017). Due to *Listeria* capacity to cause severe infections with high fatality rates in cases of human listeriosis, the Centers for Disease Control and Prevention (CDC) classified this bacterium as a significant food-borne pathogen (CDC, 2020). This report states that about 1,600 people get listeriosis each year, which has a mortality rate of 16%. *Listeria* is a Gram-positive facultative anaerobes bacterium and has several species. Its three main hemolytic species such as *L. monocytogenes*, *L. seeligeri*, and *L. ivanovii* are the most important species, and they can invade the host cells and cause listeriosis in both animals and humans (Snapir et al., 2006; Townsend et al., 2021). The immunocompromised individuals, pregnant women, infants, and elderly people, are the most susceptible targets of *Listeria* (Al et al., 2021). *L. monocytogenes* is the most frequent species related to human diseases and has the ability to grow slowly at refrigerator temperature (Todd and Notermans, 2011). Furthermore, it can survive in different extreme environmental conditions, namely wide temperature and pH ranges, and high levels of salt. Therefore, it has global expansion and is often isolated in various food types such as meat and meat products, dairy products, vegetables, and seafood. Hence, there is always the possibility that these foods can cause human listeriosis (Du et al., 2017; Lotfollahi et al., 2017; Nüesch-Inderbinen et al., 2021). *L. monocytogenes* has several serovars, such as 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7, among which, serovars, 1/2a, 1/2b, 1/2c, and 4b are more important compared to the other serotypes since they are commonly isolated from 95% of cases in the mentioned food types as well as clinical specimens (Liu, 2006). The serovar of 4b is responsible for all major outbreaks of listeriosis and the other three are frequently isolated from the contaminated food (Doumith et al., 2004).

Listeria species are different in pathogenicity due to their different mechanisms and virulence factors. Some of these virulence factors include the ability to produce hemolysin (encoded by *hlyA*), protein actin polymerization (*actA*), and proteins involved in the invasion (encoded by *iap*) of the bacterium, as well as inositol phosphatidylinositol phospholipase C encoded by *plcA* (Rawool et al., 2007). In this regard, *hlyA* indicates the main virulence factor (Listeriolysin O), which is directly associated with pathogenicity (Osman et al., 2014). Because of the ubiquitous nature of *Listeria* spp. and the importance of listeriosis in developing countries, continuous comprehensive investigations are required to update the information about the amount of food products that contaminated with *Listeria*. These data help reduceing and controling the incidence of listeriosis. Therefore, the current study was carried out to investigate the prevalence of *Listeria* species in a wide variety of food and to examine the molecular serotyping of *L. monocytogenes* and their potential virulence genes.

Materials and methods

Sampling

During July 2018 and January 2020, 900 samples of 10 types of food (90 samples of each type) were collected to determine the incidence of *Listeria*. The samples were selected considering their importance, amount of consumption, and availability for the community. Chicken and beef samples were obtained from the end of the line of some slaughterhouses located in Mazandaran and Golestan provinces, northern Iran. Fresh fish (*Salmo trutta*) and shrimp samples were collected from a local fish market in Mazandaran province. Furthermore, mixed packed salad (vegetable salads consisting of tomato, cucumbers, cabbage, and lettuce), Olivier salad (a type of Ready-to-Eat (RTE) food), green vegetables (including spinach, parsley, broccoli, coriander, and dill), were purchased from several supermarkets, along with samples of traditional yogurt and cottage cheese. Moreover, bulk milk tanks of different farms were used to collect milk samples. All raw milk samples were obtained from Mazandaran and Golestan provinces (about 30 ml) using a sterile syringe. In the case of meat samples, 2 g were collected by using a forceps and sterile scalpel from brisket or thigh sections of the carcasses and was placed in a sterile bag. Moreover, 50 g of cheese, yogurt, and RTE food samples were put in a sterile sample container with 100 cc capacity. The vegetable salads and green vegetables were also placed in a sterile bag. Samples were delivered to the laboratory in adequate conditions adjacent to an ice pack in less than 4 to 6 h.

Isolation and identification

The genus isolation was carried out as stated in the standard method (ISO 11290-1: 1996), with slight modifications (ISO, 1996). Twenty-five g or 25 ml of each sample was added to the Erlenmeyer flask containing 225 ml of mixed yeast extract and *Listeria* enrichment broth (HiMedia, Mumbai, India). Each culture was placed in an incubator at 30 °C for 24 h, and then 1 ml from Erlenmeyer flask and 9 ml of Fraser broth base containing Fraser supplements (HiMedia, Mumbai, India) were transferred into a glass tube and placed in an incubator for 24 h at 35 °C. After that, three loops of each tube was cultured on Polymyxin Acriflavin Lithium chloride Ceftazidime

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Aesculin Mannitol agar (PALCAM agar; Merck, Darmstadt, Germany), and placed in an incubator at 35 °C for 48 h. Three suspected colonies (black colonies with black sunken centers) were cultivated on blood agar from each plate and placed in an incubator at 35 °C for 24 h. In the following, complementary diagnostic tests such as catalase, Gram staining, motility at 22 °C, and Methyl Red and Voges-Proskauer (MR-VP) tests were performed.

DNA extraction and Polymerase Chain Reaction (PCR)

The bacteria were incubated for a night in 10 ml Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India) at 37 °C. The DNA extraction for bacteria were carried out by using bacterial DNA extraction kit (Cinnagen, Tehran, Iran), based on the manufacturer instructions. The quantity and quality of each DNA sample were evaluated at a wavelength of 260 and 280 nm by spectrometry methods (Nanodrop 1,000; Thermo Scientific, Wilmington, DE, USA) and also by visualization on the gel electrophoresis. The extracted DNA was kept at -20 °C freezer. Sterile distilled water and *L. monocytogenes* ATCC 13932 were utilized as negative and positive controls in the PCR experiments, respectively.

To recognize the *Listeria* genus and species, particular primers (listed in Table 1) were employed by following the techniques detailed in studies by Doumith et al. (2004) and Ryu et al. (2013). The Multiplex PCR reactions were carried out in a volume of 50 µl containing 5 µl 10×PCR buffer (pH 9.0, 75 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, and 20 mM $[NH_4]_2SO_4$), 20 µM dNTPs (1 µl), 4 µl of a template DNA (around 100 ng), and 2 units of *Taq* DNA polymerase (Cinnagen, Tehran, Iran). Furthermore, in each PCR reaction, 0.2 µM prs-R primer, 0.2 µM of prs-F primer, 0.56 µM of lmo1030-F and lmo1030-R primers, 0.52 µM of liv22-228-F and liv22-228-R primers, and 1.36 µM of lseelin-R and lseelin-F primers were used to identify all species of *Listeria*, *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*, respectively (Table 1). For the purpose of running PCR program, an initial denaturation step at 94 °C for 3 min was performed, accompanied by 35 cycles for 15 s at 94 °C, 53 °C for 75 s, 72 °C for 50 s, and final extension cycle was carried out at 72 °C for 10 min.

Serogroups identification

A Multiplex PCR was also performed using different primers (Table 1), according to the study of Doumith et al. (2004), to identify four major serogroups of *L. monocytogenes*, serogroup IIa containing 1/2a serotype, serogroup IIb containing serotypes1/2b, serogroup IIc containing serotypes1/2c, and serogroup IVb containing serotype 4b (Doumith et al., 2004). Other serovars (3a, 3b, 3c, 4a, 4c, 4e, 4d, and 7) can have the same pattern in this PCR set and are indistinguishable from these four major serovars, as previously mentioned in Doumith's study (Doumith et al., 2004). However, these serovars are very infrequent in food; therefore, in this study, each pattern of the PCR classification method was considered for 1/2a, 1/2b, 1/2c, and 4b. The thermal program consisted of a step of 94 °C for 3 min, followed by 34 cycles of 94 °C for 30 s, 53 °C for 60 s, 72 °C for 50 s, and the final step was performed at 72 °C for 10 min.

Prevalence of L. monocytogenes virulence genes

The *L. monocytogenes* isolates were analyzed using a multiplex PCR technique to determine the prevalence of the *hlyA*, *actA*, *iap*, and *plcA* genes associated with virulence. The sequences of the primers (Furrer et al., 1991; Notermans et al., 1991; Paziak-Domańska et al., 1999; Suárez et al., 2001), which have been optimized as a multiplex PCR by Rawool et al. (2007), are shown in Table 1. The thermal program included 95 °C for 2 min as an initial step, followed by 35 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 90 s, and final step was carried out for 10 min at 72 °C. All PCR products were electrophoresed on agarose gel (1.5%) containing safe stain (0.5 mg/ml; Cinnagen, Tehran, Iran) for 60 min using 80 V and observed by UV transilluminator (BTS-20, Tokyo, Japan). As a molecular size marker, a 100-bp DNA ladder (100-1,000 bp, 100-1,500 bp, or 100-3,000 bp; Cinnagen, Tehran, Iran) was utilized.

Statistical analyses

SPSS software, version 16.1 (SPSS Inc., Chicago, IL, USA) was used to analyze data obtained in the current study. The frequency of data was calculated as the prevalence rate in each food item.

Results

Distribution of Listeria spp.

Out of 900 samples collected from various sources, 136 samples (15.1%) were *Listeria* positive. Table 2 shows the prevalence of different *Listeria* species in all food samples. Positive samples were found in all types of food, except for the yogurt samples. Among all isolated *Listeria* species, 52 samples (38.2%) were *L. monocytogenes*, 15 samples (11%) were *L. ivanovii*, and 10 samples (7.3%) were *L. seeligeri* (Figure 1 shows the related PCR products).

Prevalence of Listeria in meat products

Among various food samples in this study, the most contaminated samples were found to be beef samples (38.8%), followed by chicken samples (32.2%), which are shown in Table 2. The incidence of *L. monocytogenes* was 31.4% in beef meat sample, on the other hand, 42.8% of contaminated beef samples belonged to other *Listeria*

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species. However, for chicken samples, *L. monocytogenes* had the prevalence rate of 62%.

Prevalence of Listeria in milk and dairy products

Examination of milk and dairy products revealed 12.2 and 8.8% *Listeria* contamination for milk and traditional cheese, respectively, while no yogurt samples were found contaminated with *Listeria*. The main *Listeria* spp. was isolates from 45.4 and 75% from raw milk and traditional cheese, respectively (Table 2).

Prevalence of Listeria in fish and seafood products

The incidence of *Listeria* in fish and shrimp were 6.6 and 10%, respectively. In fish samples, half of the samples were contaminated by *L. monocytogenes* and the other half by *L. ivanovii*. In the case of shrimp, however, a large portion of positive samples (55%) were other *Listeria* species.

Prevalence of Listeria in RTE foods (Olivier salad) products

The analysis of Olivier salad showed that 5 out of 90 samples (5.5%) were *Listeria* positive and the main species was *L. monocytogenes* (Table 2).

Prevalence of Listeria in vegetables

Vegetables and salads are the most susceptible food types for contamination by *Listeria*. The incidence of *Listeria* in green vegetables and vegetable salad samples was high and was recorded 25.5 and 11.1%, respectively (Table 2).

L. monocytogenes serogrouping and virulence genes

Table 3 illustrates the prevalence ratios of different serovars of *L. monocytogenes* in food sources. The most detectable serovar in *L. monocytogenes* isolates was serovar 1/2a (serogroup IIa including 1/2a and 3a and considered as 1/2a) with the prevalence rate of 57.7%. Furthermore, the virulence genes evaluation showed that, the prevalence of *hlyA*, *iap*, *actA*, and *plcA* genes was 100% amongst all isolates of *L. monocytogenes* (Figure 2).

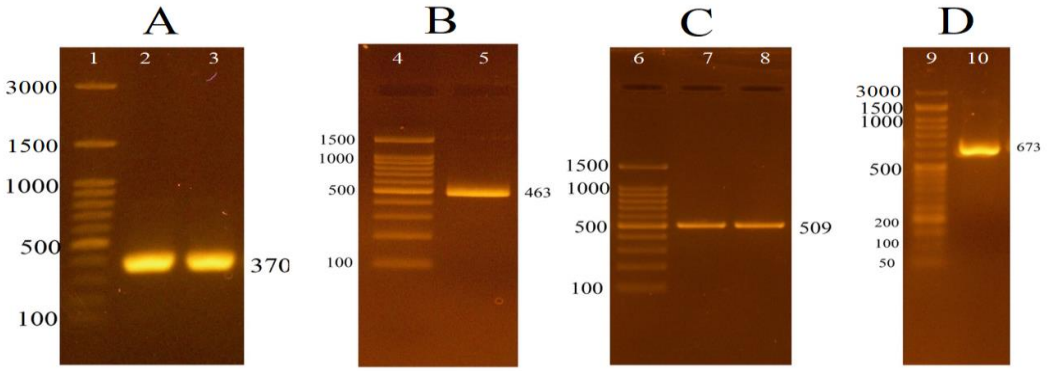


Figure 1: From left to right; the Polymerase Chain Reaction (PCR)amplifications of (A) *prs* gene (370 bp) representing *Listeria* genus (lane 2 and 3); (B) *namA* gene (463 bp) representing *L. ivanovii* isolates (lane 5); (C) *lmo1030* gene (509 bp) that represent *L. monocytogenes* isolates (lane 6 and 7); (D) *lmo0333* gene (673 bp) representing *L. seeligeri* isolates (lane 9). The first line in each gel electrophoresis (lane 1,4,6, and 9) shows the 100 bp-DNA ladder (100-1,000 bp,100-1,500 bp, or 100-3,000 bp, Cinnagen, Tehran, Iran)

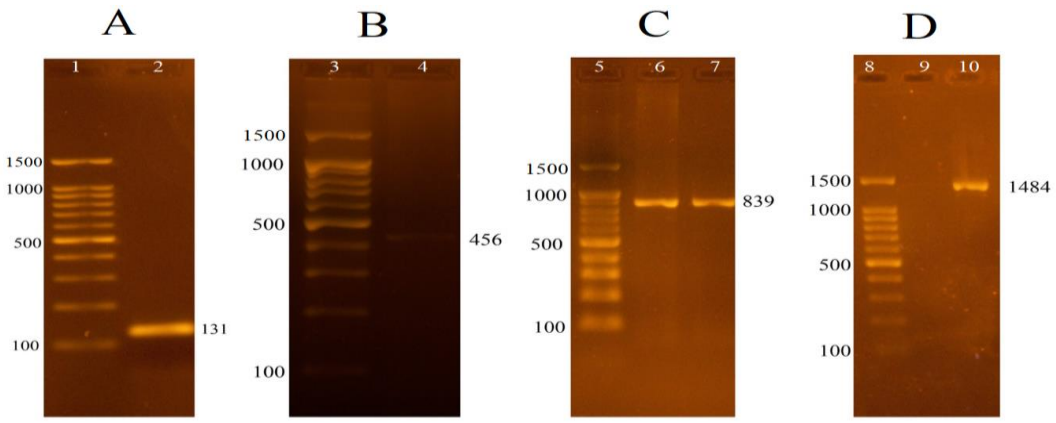


Figure 2: From left to right; Polymerase Chain Reaction (PCR)amplifications of the virulence genes of (A) *iap* (131 bp) presented in lane 2; (B) *hlyA* (456 bp) presented in lane 4; (C) *actA* (839 bp) presented in lane 6 and 7; and (D) *plcA* (1484) presented in lane 10. The first line in each gel electrophoresis (Lane 1, 3, 5, and 8) shows the 100bp-DNA ladder (100-1,500 bp, Cinnagen, Tehran, Iran)

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Table 1: Nucleotide sequences used as primers in this study for the detection of species of *Listeria*, serotyping, and virulence genes of *Listeria monocytogenes* isolated from food samples

Target	Target gene	Primers	Sequences (5- 3)	Product size (bp)	Reference
Listeria genus	*prs*	prs-F [a]	GCTGAAGAGATTGCGAAAGAAG	370	Doumith et al., 2004
		prs-R [b]	CAAAGAAACCTTGGATTTGCGG		
Listeria monocytogenes	*lmo1030*	lmo1030-F	GCTTGTATTCACTTGGATTTGTCTGG	509	Ryu et al., 2013
		lmo1030-R	ACCATCCGCATATCTCAGCCAACT		
Listeria ivanovii	*namA*	liv22-228-F	CGAATTCCTTATTCACTTGAGC	463	Ryu et al., 2013
		liv22-228-R	GGTGCTGCGAACTTAACTCA		
Listeria seeligeri	*lmo0333*	lseelin-F	GTACCTGCTGGGAGTACATA	673	Ryu et al., 2013
		lseelin-R	CTGTCTCCATATCCGTACAG		
1/2a [c]	*lmo0737*	lmo0737-F	AGGGCTTCAAGGACTTACCC	691	Doumith et al., 2004
		lmo0737-R	ACGATTTCTGCTTGCCATTC		
1/2c [d]	*lmo1118*	lmo1118-F	AGGGGTCTTAAATCCTGGAA	906	Doumith et al., 2004
		lmo1118-R	CGGCTTGTTCGGCATACTTA		
1/2b [e]	*ORF2819*	ORF2819-F	AGCAAAATGCCAAAACTCGT	471	Doumith et al., 2004
		ORF2819-R	CATCACTAAAGCCTCCCATTG		
4b [f]	*ORF2110*	ORF2110-F	AGTGGACAATTGATTGGTGAA	597	Doumith et al., 2004
		ORF2110-R	CATCCATCCCTTACTTTGGAC		
Virulence gene	*plcA*	plcA-F	CTGCTTGAGCGTTCATGTCTCATCCCCC	1484	Notermans et al., 1991
		plcA-R	ATGGGTTTCACTCTCCTTCTAC		
Virulence gene	*hlyA*	hlyA-F	GCAGTTGCAAGCGCTTGGAGTGAA	456	Paziak-Domańska et al., 1999
		hlyA-R	GCAACGTATCCTCCAGAGTGATCG		
Virulence gene	*actA*	actA-F	CGCCGCGGAAATTAAAAAAAGA	839	Suárez et al., 2001
		actA-R	ACGAAGGAACCGGGCTGCTAG		
Virulence gene	*iap*	iap-F	ACAAGCTGCACCTGTTGCAG	131	Furrer et al., 1991
		iap-R	TGACAGCGTGTGTAGTAGCA		

[a] Forward; [b] Reverse; [c] *L. Monocytogenes* serovars 1/2a, 1/2c, 3a, and 3c; [d] *L. Monocytogenes* serovars 1/2c and 3c; [e] *L. Monocytogenes* serovars 1/2b, 3b, 4b, 4d, and 4e; [f] *L. monocytogenes* serovars 4b, 4d, and 4e.

Table 2: The prevalence of *Listeria* species in different food samples

Biological origin (sample size)	*Listeria* species (%) of contaminated samples)	*Listeria monocytogenes* (%)	*Listeria ivanovii* (%)	*Listeria seeligeri* (%)	Other *Listeria* species (%)
Milk (90)	11 (12.2)	5 (45.4) *	0 (0) *	3 (27.2) *	3 (27.2) *
Traditional cheese (90)	8 (8.8)	6 (75)	2 (25)	0 (0)	0 (0)
Yogurt (90)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fish (90)	6 (6.6)	3 (50)	3 (50)	0 (0)	0 (0)
Shrimp (90)	9 (10)	3 (33.3)	1 (11.1)	0 (0)	5 (55.5)
Beef (90)	35 (38.8)	11 (31.4)	5 (14.2)	4 (11.4)	15 (42.8)
Chicken (90)	29 (32.2)	18 (62)	3 (10.3)	1 (3.4)	7 (24.1)
Green vegetables (90)	23 (25.5)	2 (8.6)	1 (4.3)	1 (4.3)	19 (82.6)
Vegetables salad (90)	10 (11.1)	1 (10)	0 (0)	1 (10)	8 (80)
Olivier salad (90)	5 (5.5)	3 (60)	0 (0)	0 (0)	2 (40)
Total (900)	136 (15.1)	52 (38.2)	15 (11)	10 (7.3)	59 (43.3)

* Parenthesis shows the positive samples percentage.

Table 3: The prevalence of different serovars of *Listeria monocytogenes* in food samples

Biological origin	Number of positive samples	Serovars * (%)			
		1/2a	1/2b	1/2c	4b
Milk	5	4 (80)	0 (0)	0 (0)	1 (20)
Traditional cheese	6	3 (50)	1 (16.7)	0 (0)	2 (33.3)
Yogurt	0	0 (0)	0 (0)	0 (0)	0 (0)
Fish	3	3 (100)	0 (0)	0 (0)	0 (0)
Shrimp	3	3 (100)	0 (0)	0 (0)	0 (0)
Beef	11	5 (45.4)	1 (9.1)	2 (18.2)	3 (27.3)
Chicken	18	10 (55.6)	3 (16.7)	3 (16.6)	2 (11.1)
Green vegetables	2	2 (100)	0 (0)	0 (0)	0 (0)
Vegetables salad	1	0 (0)	1 (100)	0 (0)	0 (0)
Olivier salad	3	0 (0)	0 (0)	1 (33.3)	2 (66.6)
Total (%)	52	30 (57.7)	6 (11.5)	6 (11.5)	10 (19.3)

*: Each serovars indicates the specific pattern in Polymerase Chain Reaction (PCR) and represents each serogroup; however, in this study we consider only the major serovars.

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Discussion

Amajoud et al. (2018) reported that *L. monocytogenes* has the ability to withstand harsh environmental conditions, allowing it to be present in various food types with varying characteristics. As a result, it is crucial to monitor regularly and maintain up-to-date information on *Listeria* prevalence. The present study data on the incidence of *Listeria* were in conformity with the results of the studies of Indrawattana et al. (2011) and Little et al. (2007), that reported *Listeria* contamination of 15.4 and 10.8%, respectively. However, in the present study, no sample was contaminated with two or more *Listeria* species, simultaneously. Overall, in various food samples surveyed in this study, the most contaminated samples were beef samples, followed by chicken, and vegetables. These findings were confirmed by others studies (Amajoud et al., 2018; Jalali and Abedi, 2008). Jalali and Abedi (2008) investigated the prevalence of *Listeria* spp. in several food types and reported that among them meat and meat products were the most contaminated products. Moreover, in accordance with the current study, Amajoud et al. (2018) found that most contaminated samples were bovine and chicken meat products.

Listeria contamination was commonly found in raw meat products and may be attributed to the presence of *Listeria* strains in farm and livestock feces. In this regard, contamination can be occurred in slaughterhouse through fecal contamination of carcass during evisceration, improper rinse process, transferring via food handlers, as well as cross contamination (Nightingale et al., 2004). This highlights the importance of following proper food safety guidelines during meat handling, preparation, and cooking to reduce the risk of *Listeria* infection.

Rahimi et al. (2010) reported a 9.3% contamination of *Listeria* spp. (55 out of 594 samples) in milk and dairy products, which is in compliance with the findings in the present study. Furthermore, *L. monocytogenes* contamination in raw milk samples of Portugal was reported 16.7% (Mena et al., 2004). Transmission of bacteria from the livestock to dairy products is one of the main ways of contamination, which emphasizes the importance of hygiene control during milking. Arslan and Özdemir (2020) reported 5% *Listeria* contamination for cheese samples, which is similar to the results of the current study. However, Jalali and Abedi (2008) reported no contamination of cheese in Iran. This may be due to the differences in sampling methods, as in the current study, traditional cheese samples were collected, which are usually produced in poor hygienic conditions. In accordance to the findings of the present study, negative contamination of yogurt samples with *Listeria* has been observed in previous studies (Dalzini et al., 2016; Rahimi et al., 2010). This might be due to the existence of Lactic Acid Bacteria (LAB) and/or low pH value of yogurt. LAB are capable of producing bacteriocins and organic acids such as lactic acid, which can prevent the growth of other bacteria in yogurt (Benkerroum et al., 2003). El Hag et al. (2021) analyzed 120 milk samples from dairy farms to detect *Listeria* spp. and reported that *L. monocytogenes* was the most frequent species, similar to the present result.

Poor-quality silage present in animal husbandries acts as a reservoir for *Listeria* spp. This silage has the potential to contaminate feed, animals, and their environment, including livestock milking systems. Hence, milk and dairy products, specifically raw milk, maybe contaminated with *Listeria*. Therefore, sufficient attention should be paid to hygiene and frequent disinfection of these systems.

Kramarenko et al. (2013) tested 371 fish samples and found that 8.8% of them were *Listeria* positive. Moreover, in the study by Fallah et al. (2013), the ratio of the *Listeria* contamination in 105 samples of freshwater fish in Iran was reported 11.4%. These two studies reported similar contamination levels for *Listeria* species in seafood samples analyzed in the current study. Most fish farms use freshwater of rivers that can be polluted with rural and/or factory sewage. Moreover, these farms use domestic animal feces as fertilizers, which are another source of contamination. Without a complete water treatment system, there will always be a risk of *Listeria* contamination and circulation in the mentioned fish farms. Moreover, Acciari et al. (2017) investigated 774 smoked salmon to examine the prevalence of *Listeria*. They observed that 20.2% of fish samples were contaminated and 3.3% of them contained more than 100 Colony Forming Unit (CFU)/mg of *Listeria*. In another study by Jalali and Abedi (2008), 73 samples of fish and shrimps were investigated and it was revealed that the most frequent incidence of *Listeria* was *L. innocua.* This was in disagreement with the findings of the present study. Studies have indicated that seafood and fish may be an underestimated threat for consumers. Furthermore, this risk becomes higher when they are consumed in raw form or prepared in the kitchen; thus, they may contaminate the contact surfaces, knives, and other kitchen utensils. Seafood is classified in the category of healthy food and the existence of *Listeria* spp. in them is alarming, since they might be handled in a kitchen without any disinfection and consumed as raw food in several cuisines.

The results of the current study for RTE food were in accordance with the study of Jalali and Abedi (2008). The RTE food types including Olivier salad are prone to contamination with pathogens such as *Listeria* (Shi et al., 2015). Koskar et al. (2019) evaluated 30,000 various RTE food samples and reported that 3.6% of samples were contaminated with *L. monocytogenes*, which is lower than

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the results of the current study. Certain types of RTE food, such as Olivier salad, are typically stored in the refrigerator and consumed without any further heating process. While many consumers appreciate this convenience, it is important to remember that these types of food may pose a potential threat to human health if not handled properly. High prevalence of *Listeria* in salads and vegetable food has been reported in several studies (Arslan and Özdemir, 2020; Kramarenko et al., 2013; Little et al., 2007). Little et al. (2007) conducted a study on 2,682 salad samples to isolate *Listeria*. They reported that *Listeria* spp. and *L. monocytogenes* were found 10.8 and 4.8%, respectively in mixed salad samples. Interestingly, according to the PCR analysis in the present study, most *Listeria* isolates obtained from green vegetables and vegetable salads did not belong to the three species of tested *Listeria* in this study. Major *Listeria* spp. found in green vegetables and vegetable salads (82.6 and 80%, respectively) were categorized as other *Listeria* species (Table 2). This difference compared to other positive samples in this study might be due to the contamination source of the plant samples. Naturally, the herbal products are in more contact with soil than the other samples. It has been shown that the soil may be the main source of some of the other species of *Listeria* such as *L. innocua* (Oliveira et al., 2011). Moreover, Arslan and Özdemir (2020) reported that *L. innocua* was the most frequent species in salad samples. Therefore, vegetables are susceptible to be contaminated by different species, compared to other food samples such as meat and dairy products.

The European Food Safety Authority (EFSA) reported that the most important serovars involved in human listeriosis are serovar 4b (34.3%) followed by 1/2a (6.4%) (EFSA and ECDC, 2015). However, in the current study, serotyping tests indicated that serogroup 1/2a (IIa) was the dominant serogroup followed by 4b (IVb) and 1/2b (IIb) serogroups, respectively. The study results were in accordance with the study of Kramarenko et al. (2013). Accordingly, Wieczorek and Osek (2017) examined 812 samples of cattle carcasses for isolation of *L. monocytogenes* serovars and demonstrated that 1/2a was the dominant serovar. The high incidence of these serovars (4b and 1/2a) is alarming and indicates the existence of a health risk problem for people consuming contaminated food with these types of *Listeria*.

The data obtained by virulence gene analysis in the present study are consistent with the study of Indrawattana et al. (2011), which reported that all the isolated *Listeria* carried *hlyA*, *plcA*, *iap* as well as *actA* genes. Similar results were also published by Momtaz and Yadollahi (2013). In general, virulence genes discovered in the present study, were involved in pathogenesis of human listeriosis and indicated the high pathogenicity of these isolates. Thus, contaminated food samples were capable of infecting consumers and could cause severe diseases.

In the current study, we investigated three species of *Listeria*, while 59 out of 136 (43.3%) *Listeria* positive food samples belonged to the other *Listeria* species; this was one of the main limitations of the present study. Herein, in this study, it has been revealed that all species of *Listeria* for traditional cheeses and fish samples were *L. monocytogenes* and *L. ivanovii* and there was no need to perform further tests to determine other *Listeria* spp. However, for green vegetables and vegetable salad samples, the main proportions were categorized as other *Listeria* species (82.6 and 80%, respectively), which needed further analysis to detect their species. It is suggested that in future studies, more species of *Listeria* should be investigated.

Conclusion

Monitoring food samples to find contamination with *Listeria* as well as analysis of virulence genes are important to prevent and/or reduce the incident of listeriosis. Considerable amounts of food samples (15.1%) were contaminated with *Listeria* and the most contaminated food was beef followed by chicken and green vegetables. Moreover, *L. monocytogenes* was more frequent compared to *L. ivanovii* and *L. seeligeri*. Most of the *L. monocytogenes* isolates were classified in 1/2a and 4b serogroups, known as the main cause of human listeriosis. Furthermore, *L. monocytogenes* isolates exhibited a 100% occurrence of numerous virulence genes, posing a significant risk to public health.

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Author contributions

M.R., H.S.-A., O.R.C., and S.S. designed the study; M.H., A.A., R.K., Z.M., and S.M.A.N. conducted the experimental work; M.R., H.S.-A., and O.R.C. analyzed the data; M.R., H.S.-A., and M.H. wrote the manuscript; all authors read and approved the final version of the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest

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