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Prevalence of Listeria Species and Serotyping of *Listeria monocytogenes* Bacteria Isolated from Seafood Samples

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LISTERIA species are well acknowledged as causal agents of human listeriosis with foodborne appearances. Seafoods are measured as the chief sources of Listeria spp. An existing survey was performed to apprise the incidence rate of Listeria spp. as well as distribution of pathogenic serotypes amongst the *Listeria monocytogenes* bacteria recovered from seafood samples. Three-hundred and fifty seafood samples were obtained and cultured. Listeria isolates were recognized using biochemical and PCR techniques. Distribution of 1/2a, 1/2b and 4b serotypes were examined using the PCR. Forty out of 350 (11.42%) samples harbored Listeria Spp. Incidence of *L. monocytogenes*, *L. ivanovii* and *L. seeligeri* were 62.50%, 20% and 7.50%, respectively. Incidence of Listeria spp. amongst the fish, shrimp, lobster and crab samples were 14%, 12%, 13% and 8%, respectively. Incidence of *L. monocytogenes* amongst the fish, shrimp, lobster and crab samples were 9%, 6%, 7% and 6%, respectively. Distribution of 1/2a, 1/2b and 4b serotypes were 48%, 28% and 8%, respectively. Discoveries disclosed the latent portion of seafoods as sources of Listeria spp. and alerted to health personnel regarding the requirement for appropriate management and cooking in persons that have recognized high-risk of developing food poisoning.

Keywords, *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, Serotypes, Seafood.

Introduction

Amongst diverse kinds of foodborne pathogens such as *Staphylococcus aureus* (*S. aureus*), Salmonella species (spp.), *Helicobacter pylori* (*H. pylori*), *Escherichia coli* (*E. coli*), *Vibrio cholerae* (*V. cholerae*), *Toxoplasma gondii* (*T. gondii*), Yersinia spp. and Listeria spp., the last one has imperative position in seafood samples, exclusively fish, shrimp, lobster and crab [1-19].

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Listeria spp. are abundant, Gram-positive, facultative anaerobic, rod-shaped bacteria. *Listeria monocytogenes* (*L. monocytogenes*), *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* are the chief species of the genus Listeria [12]. Hemolytic species including *L. ivanovii*, *L. monocytogenes*, and *L. seeligeri*, are infrequently cause human infection. *L. seeligeri* and *L. ivanovii* are unusual reasons of human infection. They are hardly accountable for meningitis in non-immunocompromised patients [12]. Reversely, *L. seeligeri* and *L. ivanovii* have been labeled as rare causes of foodborne outbreaks in [12, 20, 21].

L. monocytogenes is one of the most renowned reasons of foodborne outbreaks owing to the consumption of contaminated food [12, 20, 21]. *L. monocytogenes* is chiefly a causal agent of listeriosis in human characterized by bacteremia, abortion, sepsis, meningitis and meningoencephalitis [12, 20, 21]. It is also able to survive in extensive assortments of ecological circumstances such as growing in refrigerator [12, 20, 21]. Predictable information discovered that around 3,000 cases of human listeriosis with nearly 20% morbidity rate have been happened per annum in the United States [12, 20, 21].

Listeria spp. have also the aptitude to grow in seafood samples. Epidemiological surveys have been disclosed the boost incidence of Listeria spp. in fish, shrimp, crab and lobster samples from around the world [12, 20, 21]. Numerous outbreaks of listeriosis have been perceived owing to the consumption of contaminated seafood products [12, 20, 21].

L. monocytogenes bacteria recovered from outbreaks of foodborne diseases and of human clinical infections habitually harbored three diverse kinds of serotypes including 1/2a, 1/2b, and 4b [20-22]. They have accounted for about 98% of the outbreaks of listeriosis [20-22]. Serotypes 1/2a and 1/2b accounted for lower than 50% of the *L. monocytogenes* recovered from food stuffs and environment. Otherwise, serotype 4b is measured as a chief cause of human listeriosis outbreaks [20-22].

Rendering an indistinct epidemiological features of Listeria spp. in seafood samples and owing to their boost standing, the existing survey was performed to measure incidence rate of Listeria spp. and molecular detection of serotypes of *L. monocytogenes* bacteria recovered from seafood samples in Iran.

Materials and Methods

Samples

From May to August 2017, a total of 350 fresh seafood including fish (*Scomberomorus commerson*) (n= 100), shrimp (*Penaeus indicus*) (n= 100), lobster (*Panulirus versicolor*) (n= 100) and crab (*Portunus segnis*) (n= 50) samples were caught from the Persian Gulf, Genaveh port, Iran. Seafood samples were fresh and displayed normal physicochemical (odor, color, density and pH) properties. Samples (dorsal muscles, 100 g) were transported in refrigerator (at 4°C) to laboratory. All samples were kept under refrigeration. All samples were fresh seafood without any processing stages.

Listeria spp. isolation and identification

ISO 11290 protocols were applied for isolation of Listeria spp. [20]. At that time, isolates were confirmed using distinctive biochemical examinations including Gram staining, catalase test, acid production from glucose, manitol, rhamnose, zylose, α-Methyl-D-mamoside, hydrolysis of esculin, motility test at 25 °C and 37 °C, nitrate reduction, and MR/VP tests [21]. Sheep blood agar media (7% sheep blood agar, Merck, Germany) was applied to examine the hemolysis activities of Listeria spp. Cultures were incubated at 37 °C for 24 h. *L. ivanovii* displays β-hemolysis, though *L. monocytogenes* and *L. seeligeri* display α-hemolysis activities. Christie, Atkins, Munch and Petersen (CAMP) test was applied to recognize diverse species. *S. aureus* ATCC 29213 and *Rhodococcus equi* (*R. equi* ATCC 14887) were applied as indicator organisms. Listeria bacteria with positive-CAMP reaction toward *S. aureus* were measured as *L. monocytogenes*, while those with positive-CAMP reaction toward *R. equi* were measured as *L. ivanovii*. Listeria isolates were also examined by the Phosphatidyiinositol-specific phospholipase C (PI-PLC) assay rendering the protocol described formerly [23]. PI-PLC activity was measured by assess the growth of Listeria isolates on L. mono differential agar (Merck, Germany). Phosphatidyiinositol-specific phospholipase C (PC-PLC) technique was applied for identification of Listeria isolates rendering protocol described beforehand [24]

DNA extraction

Confirmed colonies were sub cultured on nutrient broth media (Merck, Germany) and incubated at 37 °C for 24 h. Formerly, DNA extraction kit (Thermo Fisher Scientific, St. Leon-

Rot, Germany) was applied for DNA extraction. Guidelines of the producing factory were applied for this purpose. Quantity and quality of extracted DNA were examined using the NanoDrop device (Thermo Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

PCR confirmation of Listeria spp. and identification of serotypes of L. monocytogenes

Programmable PCR thermal cycler (Eppendrof Mastercycler 5330, Hamburg, Germany) was applied in PCR protocols [25, 26]. Negative control of PCR grade water was applied. Positive control of *L. monocytogenes* (ATCC 19115), *L. ivanovii* (ATCC 19119) and *L. seeligeri* (ATCC 35967) were also applied. Table 1 signifies the PCR circumstances applied for amplification of Listeria spp. and serotypes of the *L. monocytogenes*. PCR gel electrophoresis and gel visualization were performed rendering the protocols labeled formerly [25, 26].

Statistical examination

Microsoft Excel spreadsheet (version 15; Microsoft Corp., Redmond, WA, USA) was applied for classification of data obtained from tests. Statistical examination was performed using the SPSS statistical software (version 16; SPSS Inc., Chicago, USA). Statistical analysis was performed using the Chi-square and Fisher's exact two-tailed tests. P value ≤ 0.05 was determined as significant level.

Results

Table 2 characterizes the incidence of Listeria spp. amongst diverse kinds of samples. Forty out of 350 (11.42%) seafood samples harbored Listeria spp. Incidence of *L. monocytogenes*, *L. ivanovii* and *L. seeligeri* amongst Listeria spp. recovered from seafood samples were 62.50%, 20% and 7.50%, respectively. Ten percent of all examined isolates were related to other species of Listeria. Listeria spp. had the upper most incidence in fish (15%). *L. monocytogenes* and *L. ivanovii* had the upper most incidence in crab samples (75% and 25%, respectively). *L. seeligeri* had the upper most incidence in shrimp samples (8.33%). Statistical substantial variance was gotten amid kinds of samples and incidence of Listeria spp. ($P \leq 0.05$). Incidence of *L. monocytogenes* amongst the fish, shrimp, lobster and crab samples were 9%, 6%, 7% and 6%, respectively.

All the 18 *L. monocytogenes* isolates displayed the typical development of hemolytic zone with *S. aureus*. Also, all of the 25 *L. monocytogenes* isolates were found to be pathogenic by PI-PLC and PC-PLC. Table 3 characterizes the incidence of serotypes in the *L. monocytogenes* bacteria recovered from samples. Figure 1 exhibits the PCR gel electrophoresis of serotypes of *L. monocytogenes* bacteria. Incidence of 1/2a, 1/2b and 4b serotypes amid the *L. monocytogenes* bacteria were 48%, 28% and 8%, respectively. Serotypes of 16% of examined *L. monocytogenes* bacteria were related to other not examined serotypes. Statistical substantial variance was gotten amid kinds of samples and incidence of serotypes ($P \leq 0.05$).

Discussion

Listeriosis is one of the most imperative zoonotic diseases with universal spreading. Disease has extensive financial and public health positions. Maximum of the cases of human listeriosis chiefly happened owing to consumption of contaminated foodstuffs, exclusively seafoods [20].

An existing survey was performed to appraise the incidence rate of Listeria spp. and determination of serotypes of the *L. monocytogenes* in fish, shrimp, lobster and crab samples. *L. monocytogenes* was the most generally recovered bacteria from examined marine samples (25/350 (7.14%)). The second uppermost organism was *L. ivanovii* (8/350 (2.28%)). Incidence of *L. seeligeri* bacteria was 0.85% (3/350). Higher incidence of *L. monocytogenes* was also conveyed from India [27], Turkey [28] and Iran [29]. Since 1975, outbreaks of foodborne listeriosis have been described in most parts of the world except Asia, Africa and Latin America [30, 31]. Notwithstanding the boost clinical importance of an attendance of Listeria spp. in seafood samples, few researches have been conducted in this field in Iran [32, 33]. Rahimi et al. [34] described that the incidence of *L. monocytogenes* and *L. innocua* in frozen and fresh seafood samples were 1.9% and 5.7%, respectively. Zare et al. [33] stated the low incidence of *L. monocytogenes* in sea-food samples collected from Iran (1.4%). Akhondzadeh Basti et al. [32] described that the incidence of *L. monocytogenes* in smoked fish samples was 2.6%. Higher incidence of *L. monocytogenes* were conveyed by Miettinen and Wirtanen [35]. They exhibited that the incidence of *Listeria* spp. and *L. monocytogenes* in fresh fish samples were 35% and 14.6%, respectively. A Turkish investigation [36] discovered that the incidence of Listeria spp., *L. monocytogenes* and *L. murrayi* in examined seafood samples were 10.40%, 44.50% and 83.50%, respectively which was higher than our outcomes.

TABLE 1. PCR circumstances used for detection of Listeria spp. and also serotypes of *L. monocytogenes*.

Target genes	Primer sequence (5'-3')	Size of product (bp)	Volume of PCR reaction (50 µl)	PCR programs
L. monocytogenes	TTATACGCGACCGAAGCCAAC CAAACTGCTAACACAGCTACT	660	5 µL PCR buffer 10X	1 cycle, 95 ^{0C} ---------- 1 min.
L. ivanovii	TTATACGCGACCGAAGCCAAC CTACTCAAGCGCAAGCGGCAC	1100	1.5 mM $Mgcl_2$	30 cycles, 95 ^{0C} ---------- 15 s
L. seeligeri	TTATACGCGACCGAAGCCAAC TACACAAGCGGCTCCTGCTCAAC	1100	200 µM dNTP (Thermo Fisher Scientific, St. Leon-Rot, Germany) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany) 2.5 µL DNA template	58 ^{0C} ---------- 50 s 72 ^{0C} ---------- 48 s 1 cycle, 72 ^{0C} ---------- 5 min
L. monocytogenes serovar1/2a	AGGGCTTCAAGGACTTACCC ACGATTTCTGCTTGCCATTC	691	5 µL PCR buffer 10X	1 cycle, 95 ^{0C} ---------- 3 min.
L. monocytogenes serovar1/2b	AGCAAAATGCCAAAACTCGT CATCACTAAAGCCTCCCATTG	471	1.5 mM $Mgcl_2$	35 cycles, 95 ^{0C} ---------- 24 s
L. monocytogenes serovar 4b	AGTGGACAATTGATTGGTGAA CATCCATCCCTTACTTTGGAC	597	200 µM dNTP (Thermo Fisher Scientific, St. Leon-Rot, Germany) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany) 2.5 µL DNA template	53 ^{0C} ---------- 75 s 72 ^{0C} ---------- 75 s 1 cycle, 72 ^{0C} ---------- 7 min

TABLE 2. Incidence of Listeria spp. in examined seafood samples.

Kinds of samples	No. samples collected	No. Listeria spp. (%)					
		Listeria spp.	*L. monocytogenes*	*L. ivanovii*	*L. seeligeri*	*L. monocytogenes* + *L. ivanovii*	Other species
Fish	100	14 (14)	9 (64.28)	3 (21.42)	1 (7.14)	1 (7.14)	1 (7.14)
Shrimp	100	10 (12)	6 (60)	2 (20)	1 (10)	-	1 (10)
Lobster	100	12 (13)	7 (58.33)	2 (15.38)	1 (8.33)	1 (8.33)	2 (15.38)
Crab	50	4 (8)	3 (75)	1 (25)	-	-	-
Total	350	40 (11.42)	25 (62.50)	8 (20)	3 (7.50)	2 (5)	4 (10)

TABLE 3. Distribution of different serotypes in the *L. monocytogenes* bacteria recovered from different seafood samples.

Kinds of samples (No. positive)	Serotypes			
	1/2a	1/2b	4b	Other serotypes
Fish (9)	4 (50)	3 (33.33)	1 (12.50)	1 (12.50)
Shrimp (6)	3 (60)	2 (33.33)	-	1 (20)
Lobster (7)	3 (50)	1 (16.66)	1 (16.66)	2 (28.57)
Crab (3)	2 (66.66)	1 (33.33)	-	-
Total (25)	12 (48)	7 (28)	2 (8)	4 (16)

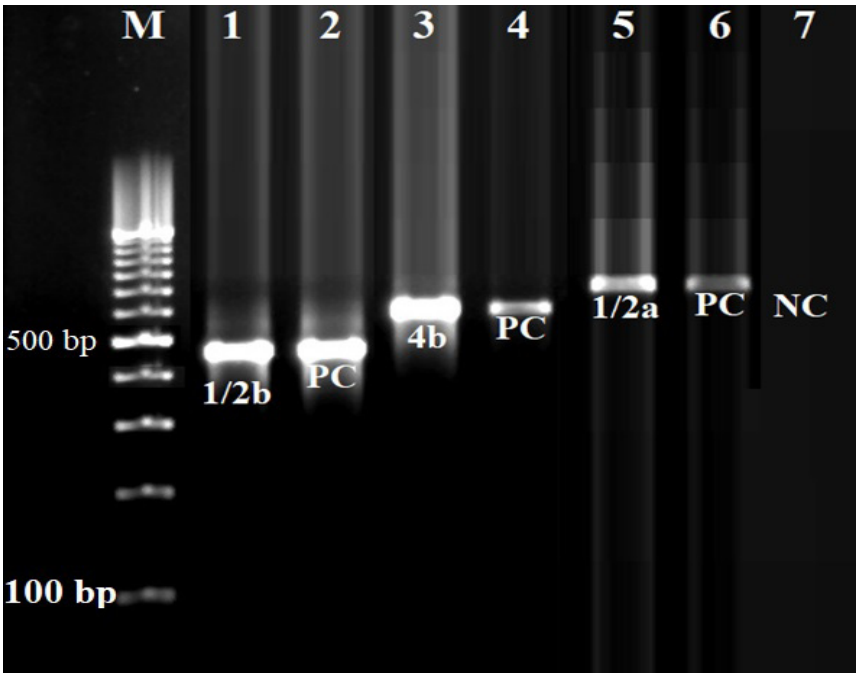

Fig. 1. PCR electrophoresis of serotypes of L. monocytogenes isolated from seafood samples. M, Ladder (100 bp), PC, Positive control; NC, Negative control; 1/2a (Lane 5, 691 bp), 1/2b (Lane 1, 471 bp) and 4b (Lane 3, 597 bp) serotypes were detected.

The mentioned variances in the incidence of Listeria spp. in seafood samples is owing to modifications in kinds of samples (shrimp, fish, oyster, crab, and lobster), sampling size, experiment method, sampling methods and geographical area of diverse reports.

The primary source of Listeria contamination is generally measured to be raw fish and seafood material. Because Listeria is generally originated in coastal waters and lake surface waters, fish captured or cultivated in these waters may harbor the bacterium. Nevertheless, fish that are purchased in bulk and repackaged before sale may be vulnerable to Listeria contamination. Existing data proposes that *L. monocytogenes* occurs obviously in freshwater fish and seafood from contaminated waters but is improbable to happen on fish samples from open seawaters. Samples of our survey were collected from the ports, harbors and sales centers of the Genaveh city. There were no strict hygienic conditions in the sites of samples collection. Another significant reason for the high incidence of Listeria spp. in seafood samples and exclusively fish samples of our investigation is the high presence of Listeria spp. in gills of caught fish. Gills are an outstanding place for bacteria even though this area is part of the immunological system. Presence of bacteria in gills can transmit to other parts of the fish. Close contact of lobster, shrimp and crab with fish is a likely cause for the boost incidence of Listeria spp. in these samples. Filter-feeding manner of seafood samples is another imperative reason for accumulation of pathogenic bacteria. Sellers mainly put fish, shrimp, lobster and crab out together. Consequently, contamination of one can disseminate to others.

The most generally determined serotypes in *L. monocytogenes* isolates of our research were 1/2a (48%), followed by 1/2b (28%) and 4b (8%). High incidence of 1/2a serotype amongst the *L. monocytogenes* bacteria recovered from seafood samples was also conveyed formerly [25, 26, 37]. A report from the National Reference Center of France disclosed that more than 95% of *L. monocytogenes* isolates harbored 1/2a, 1/2b, 1 /2c, and 4b serotypes [25]. Momtaz and Yadollahi [38] conveyed that the incidence of 4b, 1/2a and 1/2b serotypes in the *L. monocytogenes* bacteria of seafood samples were 66.66%, 5.55% and 27.77%, respectively. Braga et al. [39] specified that the incidence of 1/2a, 1/2b and 4b serotypes of the *L. monocytogenes* bacteria recovered from frozen food samples were 11.76%, 35.29% and 47.08%, respectively. The perceived variance may be owing to changes in the kinds of food examined in diverse researches. Likewise, Munoz [40] described that the most predominant serotypes recovered from 1424 food samples were 4b, 1/2b and 1/2a. Reversely, the most common serotypes recovered from ready to eat seafood samples in Japan [41] were 1/2a (47.6%), 1/2b (20.6%) and 4b (14.3%) which was parallel to our findings. Comparable findings have been conveyed from Poland [42], Ireland [43] and China [44].

Conclusions

To summarize, outcomes of the existing survey displayed a high range of contamination with Listeria spp. and exclusively *L. monocytogenes* in the fresh fish, shrimp, lobster and crab samples caught from the Genaveh, Iran. Listeria spp. had the upper most incidence in fish, while *L. monocytogenes* and *L. ivanovii* had the upper most incidence in crab samples (75% and 25%, respectively). *L. seeligeri* had the upper most incidence in shrimp samples (8.33%). The uppermost incidence was gotten for fish and shrimp samples. Findings also exhibited the higher incidence of 1/2a and 1/2b serotypes amongst the *L. monocytogenes* bacteria. Results display an imperative public health menace rendering the consumption of contaminated fish, shrimp, lobster and crab samples. The existing research highlights the imperative position of the seafood samples as a probable hazard factor for transmission of Listeria spp. into the human populaces. Nevertheless, supplementary researches are compulsory to gotten more epidemiological aspects of Listeria spp. in marine samples.

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Conflict of interest
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