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Original Article

Isolation of *Listeria monocytogenes* from Meat and Dairy Products

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Introduction: This study was intended to determine the presence and distribution of *Listeria monocytogenes* in various meat and dairy products from Qazvin Province by culture followed by biochemical and morphological assays. The identity of the isolates was further obtained by amplification of *prf*A gene in bacteria isolates. This gene is a transcriptional activator of virulence gene expression within the pathogenic *L. monocytogenes*. **Method:** In a cross-sectional design, a total of 182 different food samples were collected from different areas in Qazvin, Iran. Bacterial isolates were obtained by the cold enrichment method. DNA extraction from the pelleted cells was conducted and then *prfA* gene was amplified by conventional PCR. **Results:** As many as 37 (20.3%) food samples were positive for *Listeria* spp. including 21 (56.8%) *L. monocytogenes*, 7 (18.9%) *Listeria innocua*, 4 (10.8%) *Listeria welshimri,* 3 (8.1%), *Listeria seligeri*, and 2 (5.4%) *Listeria grayi*. None of the isolated specimen was *Listeria ivanovii*. The *PrfA* gene was amplified in all *L. monocytogenes* specimen. Moreover, PCR assay had high sensitivity and specificity for bacterial identification. **Conclusion:** To sum up, presence of *L. monocytogenes* in food samples was confirmed in this region, it was more frequent in milk specimen. In addition to common culture techniques, PCR assay showed higher sensitivity and specificity for *L. monocytogenes* detection in contaminated foods. *J Med Microbiol Infec Dis, 2014, 2 (4): 159-162.*

Keywords: *Listeria monocytogenes*, *PrfA*, PCR, Milk, Meat.

INTRODUCTION

Listeria spp. are Gram-positive and facultative anaerobic bacteria, with a low G+C content in their genetic material. The genus comprises six species, namely *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri,* and *Listeria grayi*. The only pathogenic species of this genus is *L. monocytogenes*, although there have been few reports on the capability of causing disease by *L. seeligeri*, *L. ivanovii* and *L. innocua* [1].

L. monocytogenes is an intracellular pathogen, it is referred as a main cause of human food-borne infections across the globe [2]. Food-borne listeriosis induced by *L. monocytogenes*, is a relatively rare, yet a serious condition with a greater mortality (20-30%) than other food-borne microbial pathogens, such as *Salmonella* spp. [3]. While *L. monocytogenes* commonly cause a mild gastroenteritis in healthy adults, it may be associated with high severity in susceptible individuals. Basically, *L. monocytogenes* frequently affects immunocompromised individuals, pregnant women, and elderly people. The signs and symptoms of *L. monocytogenes* infection range from flu-like illness to meningitis and septicaemia and it may lead to spontaneous abortion or listeriosis of the newborn [3].

Listeria species are tolerant to harsh circumstances including low pH, low temperature and high salt [4, 5]. Accordingly, they are able to grow in various soil environments, sewages, silages, water, effluents and various food items. Concurrent with globalization and growing popularity of manufactured ready-to-eat foods, much attention has been currently drawn to *L. monocytogenes*.

On the other hand, the huge amounts of reports on contamination in food industry products by pathogenic bacteria have raised a great public concern. *Listeria* species, *L. monocytogenes* in particular, have been recognized in different food sources such as meat and chicken [6]. The presence of *Listeria* spp. in meat is a serious threat for food safety since the organism can grow in both raw and cooked meat during refrigerated storage [7]. There have been some reports on successful isolation of the *Listeria* spp. across the globe. However, no thorough records on outbreaks of human listeriosis are available [8]. Over the past 25 years, listeriosis has been a health-threatening food-related disease leading to considerable number of deaths. There has been plenty of evidence regarding this issue that outbreaks of human listeriosis have been associated dairy products namely milk [9] and soft cheese [10]. Given the growing concern over *L. monocytogenes* infection as well as its potential adverse effects on the human's health, it seems a priority to conduct research on contamination of various food sources with this pathogenic agent in different parts of the country, including Qazvin Province. Therefore, the aims of this study were: (i) to specify the prevalence of *L. monocytogenes* in three different food categories; (ii) and to specify the performance of two pairs of primers for identifying *L. monocytogenes*.

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MATERIAL AND METHODS

Different samples were randomly selected from dairy products (raw milk, soft cheese, butter and yogurt, n=84) as well as raw and processed meat (beef, chicken, sausages, chicken nugget and burgers, n=98). They were directly transferred to the laboratory using an ice box. The International Standards Organization (ISO) 11290 method with a two-stage enrichment process, which inhibits competitive organisms, was utilized for isolation and identification of *L. monocytogenes* [11]. The samples were thawed overnight in refrigerator and then were aseptically and carefully separated from their casings and grounded completely by a sterile mortar. The isolation was initially carried out by the use of *Listeria* selective enrichment broth (LSEB) to increase the growth of *Listeria* spp. The LSEB base contained trypticase soy broth besides 0.6% yeast extract supplemented with *Listeria* selective supplement (Hi Media laboratories) including acriflavin-HCL (15 $mg.L^{-1}$), nalidixic acid (40 $mg.L^{-1}$), and cycloheximide (50 $mg.L^{-1}$). Amounts of 10 g of meat samples were aseptically mixed with LSEB (90 mL). For dairy products, 25 g of samples were aseptically mixed with 225 mL Brain Heart Infusion (BHI). All samples were incubated at 4°C for 7 to 20 days. A loopful of the homogenate was used to streak onto *Listeria* selective agar base (BHI and LSEB agar Media laboratories) followed by incubation at 37°C for 24 h. Identification of suspected colonies of *Listeria* was performed by various assays including Gram staining, catalase reaction, umbrella-shaped motility pattern, hemolysis on sheep blood agar, fermentation of mannitol, rhamnose, xylose, glucose and maltose, and MR-VP and CAMP tests, in accordance with Bergey's Manual of Systematic Bacteriology [12] as summarized in table 1. Cooling enrichment methods were performed for *Listeria* spp. isolation during four months as well.

DNA extraction and PCR assay. The obtained culture (10 mL) was cultivated to mid-log growth phase using Tryphosa Soya (TSY) broth for every *Listeria* isolates. Of which, 1 mL of cells were pelleted via centrifugation at 13.000×g for 5 min. The pellets were subsequently resuspended and centrifuged again at 12.500 ×g for 15 min. DNA was then extracted from the pelleted cells using the Wizard genomic DNA purification kit (Promega, USA) and stored at -20°C until used. Two primers, LIS-F and LIS-R, were used (Table 2) to amplify the *prf*A gene of *L. monocytogenes* as described by others [13]. The final 25 µL PCR reactions contained 2 µl of extracted DNA, 12.5 µl GoTaq Green Master Mix (Promega, USA), forward primer and reverse primers, 500 µM each, and Ultra-Pure DNase/RNase-Free distilled Water (Gibco, USA). The amplifications were performed in a thermocycler (Techne, Cyclogene) with pre-incubation at 95°C for 30 s, denaturation at 95°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 30 s , as well as final elongation at 72°C for 5 min.

PCR products were resolved through 1% agarose gels and stained using ethidium bromide. Detection was performed via a short-wavelength ultraviolet light source and photographed by an EDVOTEK Gel documentation system. The method adopted by Jami *et al.* was utilized to measure the sensitivity and specificity of the primers. Briefly, serial dilutions based on the overnight culture of *L. monocytogenese* in BHI broth were enumerated applying surface plating technique. As for the specificity of the primers, DNA extracted from various bacterial colonies was taken into account as the template for the m-PCR assay [14].

Table 1. Main laboratory tests for the differentiation of *Listeria* spp.

Species	Hemolysis	Phosphatidylinositol phosphatase	Acid production	
			L-Rhamnose	D-Xylose
L. monocytogenes	+	+	+	-
L. ivanovii	+	+	-	+
L. seeligeri	+	-	-	+
L. innocua	-	-	V	-
L. welshimeri	-	-	V	+
L. grayi	-	-	V	-

Rapid Method (PCR)

Table 2. Ologonucleotide sequences used for identification of *L. monocytogenes* by PCR

Target gene	Primer sequence (5' - 3')	Amplified fragment length	Reference
***prfA* gene**	LIS-F: TCA TCG ACG GCA ACC TCG G LIS-R: TGA GCA ACG TAT CCT CCA GAG T	217 bp	[13]

Table 3. Isolation rate of *Listeria* spp. in different food and human sample

Type odd samples	No. of analyzed sample	Positive sample	
		No.	%
Meat products	98	12	32.4
Milk products	84	25	67.6
Total	182	37	100

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RESULTS

Tables 3 and 4 presented data for the analysis of milk and meat products. As many as 37 (20.3%) food samples were positive for *Listeria* spp. including 21 (56.8%) *L. monocytogenes*, 7 (18.9%) *L. innocua*, 4 (10.8%) *L. welshimri*, 3 (8.1%), *L. seligeri*, and 2 (5.4%) *L. grayi*. None of the isolated specimen was *L. ivanovii*. The *PrfA* gene in *L. monocytogenes* was detected in all samples. Meat and chicken products were frequently contaminated with *L. monocytogenes*. The performance of the primers was confirmed by measuring their sensitivity and specificity. The findings indicated that these primers possess high sensitivity (96.9%) and specificity (100%) for the target sequence and merely for *Listeria* spp. (data not shown).

Table 4. Incidence of *Listeria* spp. in different food and human sample

Sample	*L. monocytogenes*		*L. innocua*		*L. ivanovil*		*L. weishimeri*		*L. seeligeri*		*L. grayi*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Meats (n=25)	1	4	1	4	--	--	1	4	--	--	--	--
Frozen chicken (n=20)	1	5	1	5	--	--	--	--	--	--	2	10
Roast (meat) steak (n=25)	2	8	1	4	--	--	--	--	1	4	--	--
Ham (n=10)	1	10	--	--	--	--	--	--	--	--	--	--
Milk (n=34)	4	11.8	1	2.9	--	--	1	2.9	--	--	--	--
Cheese (n=20)	9	45	2	10	--	--	2	10	1	5	--	--
Yogurt (10)	1	10	--	--	--	--	--	--	--	--	--	--
Cream (n=10)	2	20	1	10	--	--	--	--	1	10	--	--
Butter (n=10)	--	--	--	--	--	--	--	--	--	--	--	--
Total	21	56.8	7	18.9	--	--	4	10.8	3	8.1	2	5.4

DISCUSSION

The recurring occurrence of *L. monocytogenes* in food items including meat or dairy products may create formidable challenges on the part of customers. This is claimed that human infections foundationally arise from consuming contaminated foods which in turn, are associated with serious, if not life-threatening conditions of listeriosis [7, 14]. It has been indicated that the food borne transmission of *L. monocytogenes* is one of the pivotal causes behind such cases [10, 15].

Molla *et al.* revealed that raw meat products possessed a high level of contamination (50.6%) with *Listeria* spp. [16]. Jamali *et al.* reported that prevalence of *L. monocytogenes* in ready-to-eat food samples was 45 out of 396 [17]. It is assumed that the slaughter methods (evisceration), food handling practice, and food processing increase the probability of microbial contamination of *Listeria* spp. in meat products. Moreover, *Listeria* spp. widely spread out in the environment [13]. Panahi Dorcheh *et al.* found that the prevalence of *Listeria* spp. in meat, liver, and feces of quail were 15, 5, and 30%, respectively. More to the point, heart and kidney demonstrated to be free of *Listeria* spp. In their study, *L. innocua* showed the high frequency, even more than *Listeria* spp. [18]. El Marnissi *et al.* exhibited 5.90% as an overall prevalence of *L. monocytogenes* in raw milk [15]. Boubendir *et al.* observed a comparable occurrence in bovine raw milk from the North Eastern Algeria [19]. Jami *et al.* reported a lower contamination rate for milk samples in Mashhad, Iran [14]. Therefore, it was concluded that meat products have been presented to be at high risk for *L. monocytogenes* contamination rather than dairy ones. Furthermore, the current disparity of the prevalence depends on geographic location, sampling technique, detection procedure, types of media utilized, and hygienic production [14, 20].

L. innocua has shown to grow more rapidly than other pathogenic species in enrichment broth media, and it is more likely to overgrow *L. monocytogenes*. *L. innocua* holds the similar ecological niche with high incidence. In other words, *L. innocua* is capable of being contaminated by *L. monocytogenes* [21]. Lower records were found in china, where the prevalence rate was 0.23% to 1.2% [22]. As for common sources of *L. monocytogenes* in raw milk, following parameters are influential: milking processing, transportation, storage methods, infected cows, and silage quality [14, 23].

Akin to the study by Jami *et al.*, our study corroborated the sensitivity and the specificity of the applied primers for identification of *L. monocytogenes* [14]. Therefore, it is recommended that application of common culture techniques along PCR assay can considerably enhance the chance of detecting *L. monocytogenes* dairy and meat products.

This study revealed the presence of *L. monocytogenes* and other *Listeria* spp. in various dairy and meat products collected from different areas of Qazvin province, Iran. Moreover, the combination of culture methods and PCR analysis can afford to confirm whether the presumptive isolates were *L. monocytogenes.*

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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