Original Article

Isolation of Listeria monocytogenes from Meat and Dairy Products

Ashraf Haj Hosseini¹, *Anousheh Sharifan¹, Akram Tabatabaee²

¹Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran; ²Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran.

Received Feb 06, 2016; accepted May 03, 2016

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 prfA 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.

*Correspondence: Anousheh Sharifan

Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Daneshgah Blvd, Hesarak, Tehran, Iran, 1477893855.

Email: anousheh.sharifan123@yahoo.com

Tel: +98 (21) 44867154 **Fax:** +98 (21) 66460700

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, umbrellashaped 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 prfA 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	TT	Dh	Acid production		
	Hemolysis	Phosphatidylinositol phosphatase	L-Rhamnose	D-Xylose	
L. monocytogenes	+	+	+	-	
L. ivanovii	+	+	-	+	
L. seeligeri	+	-	-	+	
L. innocua	-	-	${f v}$	-	
L. welshimeri	-	-	V	+	
L. grayi	-	-	\mathbf{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			
Type odd samples	No. of analyzed sample	No.	%		
Meat products	98	12	32.4		
Milk products	84	25	67.6		
Total	182	37	100		

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*.

ACKNOWLEDGEMENT

This study was supported by Shahd Ar Caspian Company.

CONFLICT OF INTEREST

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

REFERENCES

- 1. Perrin M, Bemer M, Delamare C. Fatal case of *Listeria innocua* bacteremia. J Clin Microbiol. 2003; 41 (11): 5308-9.
- 2. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. J Med Microbiol. 2006; 55 (Pt 6): 645-59.
- 3. FAO/WHO. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Microbiological risk assessment series 5. 2004.
- 4. Sleator RD, Gahan CG, Hill C. A postgenomic appraisal of osmotolerance in *Listeria monocytogenes*. Appl Environ Microbiol. 2003; 69 (1): 1-9.
- 5. Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. FEMS Microbiol Lett. 2005; 243 (2): 373-8.
- Endang P, Radu S, Ismail A, Kqueen Cheah Y, Maurice Bilung L. Characterization of *Listeria monocytogenes* Isolated from Chicken Meat: Evidence of Conjugal Transfer of Plasmidmediated Resistance to Antibiotic. J Anim Vet Adv. 2003; 2 (4): 237-46.
- 7. AbdEl-Malek AM, Ali SFH, Moemen RH, Mohamed A, Elsayh KI. Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*. Vet World. 2010; 3 (8): 353-9.
- 8. Jeyaletchumi P, Tunung R, Margaret SP, Son R, Farinazleen MG, Cheah YK. Detection of *Listeria monocytogenes* in foods. Int Food Res J. 2010; 17: 1-11.
- 9. Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med. 1985; 312 (7): 404-7.
- 10. Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Isolation and PCR amplification of a species-specific oxidoreductase-coding gene region in *Listeria grayi*. Can J Microbiol. 2005; 51 (1): 95-8.

- 11. Becker B, Schuler S, Lohneis M, Sabrowski A, Curtis GD, Holzapfel WH. Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1. Int J Food Microbiol. 2006; 109 (1): 127-31.
- 12. The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. Balows A, Hans, Trüper HG, Dworkin M, Harder W, Schleifer KH, editors. 2nd ed., Springer-Verlag, New York. 1992.
- 13. Vitas AI, Garcia-Jalon VA. Occurrence of Listeria monocytogenes in fresh and processed foods in Navarra (Spain). Int J Food Microbiol. 2004; 90 (3): 349-56.
- 14. Jami S, Jamshidi A, Khanzadi S. The presence of *Listeria monocytogenes* in raw milk samples in Mashhad, Iran. Iran J Vet Res. 2011; 11 (4): 363-7.
- 15. El Marnissi B, Bennani L, Cohen N, Lalami AE, Belkhou R. Presence of *Listeria monocytogenes* in raw milk and traditional dairy products marketed in the north-central region of Morocco. Afr J Food Sci. 2013; 7 (5): 87-91.
- 16. Molla B, Yilma R, Alemayehu D. Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop J Health Dev. 2004; 18 (3): 208-12.
- 17. Jamali H, Chai LC, Thong KL. Detection and isolation of Listeria spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. Food Control. 2013; 32 (1): 19-24.
- 18. Panahi Dorcheh M, Sohrabi R, Salajegheh M. Prevalence of Listeria species in retail quail products from Isfahan, Iran. J Vet Med Anim Health. 2013; 5 (1): 16-9.
- 19. Boubendir A, Hamidechi MA, Mostakim M, El Abed S, Ibnsouda Koraichi S. Incidence de Listeria spp. et autres bactéries psychrotrophes dans le lait cru bovin dans le Nord Est Algérien. Revue Med Vet. 2011; 162 (5): 265-9 [In French].
- 20. Kosek-Paszkowska K, Bania J, Bystron J, Molenda J, Czerw M. Occurrence of Listeria sp. in raw poultry meat and poultry meat products. Bull Vet Inst Pulawy. 2005; 49: 219-22.
- Adzitey F, Huda N. Listeria monocytogenes in foods: incidences and possible control measures. Afr J Microbiol Res. 2010; 4 (25): 2848-55.
- 22. Ning P, Guo K, Cheng L, Xu L, Zhang C, Cui H, Cheng Y, Xu R, Liu W, Lv Q, Cao W, Zhang Y. Pilot survey of raw whole milk in China for *Listeria monocytogenes* using PCR. Food Control. 2013; 31 (1): 176-9
- 23. Husu JR. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. J Vet Med, Series B. 1990; 37 (1-10): 276-82.