TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2015) 39: 211-217
© TÜBİTAK
doi:10.3906/vet-1407-15

Serotyping and antibiotic susceptibility of *Listeria monocytogenes* isolated from ready-to-eat foods in Samsun, Turkey

Göknur TERZİ, Ali GÜCÜKOĞLU, Özgür ÇADIRCI, Tolga UYANIK*, Mustafa ALİŞARLI
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

Received: 04.07.2014 • **Accepted:** 10.11.2014 • **Published Online:** 01.04.2015 • **Printed:** 30.04.2015

Abstract: The objective of the present study was to assess the presence of *Listeria monocytogenes* and to investigate its serotyping and antibiotic resistance in ready-to-eat (RTE) foods. A total of 100 RTE foods, including 25 stuffed mussels, 25 ezme samples (a Turkish-style tomato dip/condiment), 25 fried spiced livers, and 25 mayonnaise-based salads were obtained from retail shops and supermarkets in Samsun, Turkey. Samples were analyzed using the standard procedure EN ISO 11290-1 and isolates of *L. monocytogenes* were confirmed for the presence of the hemolysin gene (*hylA*) by polymerase chain reaction. *L. monocytogenes* was identified in 1 of 25 (4%) mayonnaise-based salads as serotype 4b, 2 of 25 (8%) ezme samples as serotype 1/2a, and 1 of 25 (4%) fried spiced livers as serotype 4b. None of the stuffed mussel samples were found to be contaminated with *L. monocytogenes*. Antibiotic resistance profiles of *L. monocytogenes* isolates were detected by the disk diffusion method. Eight antibiotics disks were selected for resistance tests. One of the 4 isolates of *L. monocytogenes* was resistant to oxytetracycline and 1 isolate was resistant to vancomycin. In conclusion, the presence of *L. monocytogenes* serotypes 4b and 1/2a in RTE foods is of significant concern for public health as these serotypes are predominant serotypes that can cause listeriosis in humans.

Key words: *Listeria monocytogenes*, ready-to-eat food, serotyping, antibiotic susceptibility, polymerase chain reaction

1. Introduction

Listeria monocytogenes is a gram-positive foodborne pathogen that causes listeriosis, leading to septicemia, encephalitis, meningitis, and gastroenteritis, particularly in pregnant women, newborns, the elderly, and immunosuppressed individuals (1).

L. monocytogenes is widely distributed in nature. The organism is commonly found in silage, soil, sewage, fertilizer, vegetable matter, and many foods including cabbage, coleslaw, raw milk and dairy products, meat, poultry, and their products (2). The other important source of *L. monocytogenes* infection is consumption of ready-to-eat (RTE) foods such as cooked meats, desserts, sandwiches, cheese from either raw or pasteurized milk, and fish products. These foods are not cooked or reheated before serving. Therefore *L. monocytogenes* can survive and grow under refrigerated conditions in packaged RTE foods (3,4). Several outbreaks of listeriosis in the United States in 1998–2008 as indicated by the Centers for Disease Control and Prevention were associated with the consumption of RTE foods. It was reported that 359 people were affected in 24 confirmed listeriosis outbreaks, resulting in 215 hospitalizations and 38 deaths (5).

There is little information on the prevalence and contamination levels of *L. monocytogenes* in RTE foods in Turkey. RTE foods such as Turkish-style tomato dip/condiment (ezme), stuffed mussels, fried spiced liver, and mayonnaise-based salads are frequently consumed in Turkey. Ingredients and preparation methods of these RTE foods are summarized in Table 1.

Conventional bacteriological methods used for the identification of *L. monocytogenes* are not always reliable and are often time-consuming and laborious. Thus, more reliable, rapid, and cost-effective molecular techniques such as polymerase chain reaction (PCR)-based methods have been developed for the detection of these pathogens in food (6). The *hly* gene encoding hemolysin listeriolysin O (LLO), a pore-forming exotoxin with hemolytic activity, is an important virulence factor for the specific detection of *L. monocytogenes* (7).

The increased use of antibiotics for therapeutic purposes in animals and humans has led to the development of antibiotic resistance, an important public health concern (8). Studies have shown the existence of *L. monocytogenes* strains that are resistant to one or more antibiotics such as nalidixic acid, oxacillin, tetracycline, gentamicin,

* Correspondence: goknurt@omu.edu.tr

Table 1. Ingredients and preparation methods of RTE foods sampled for *Listeria* spp.

RTE foods	Ingredients	Making of product
Ezme (Turkish-style tomato dip/condiment)	Tomatoes, red pepper, onion, garlic, parsley, pomegranate syrup, lemon juice, tomato paste, red pepper paste, chili pepper, fresh mint, olive oil, salt.	Lemon juice, olive oil, tomato, and red pepper paste are mixed. Generally consumed alongside cooked meat.
Stuffed mussels	Mussels, white long-grain rice, parsley, onion, pine nuts, raisins, olive oil, sugar, dried mint, salt, ground black pepper, lemon juice.	All the ingredients are mixed, except mussels and parsley, for raw stuffing. Then shells are filled with raw stuffing and boiled for 30 min in water.
Fried spiced liver	Liver (mutton, lamb, or bovine), flour, salt, ground red pepper. Garnish: olive oil, onions, parsley, salt.	Cubed livers are mixed with salt and ground red pepper, then dipped into flour and fried in vegetable oil.
Mayonnaise-based salad	Boiled potatoes, carrots, boiled peas, salami, boiled egg, mayonnaise, salt and pepper.	Boiled potatoes and carrots are cubed and mixed with boiled peas, salami, boiled egg, mayonnaise, and salt and pepper.

penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulfonamide, trimethoprim, and rifampicin. Therefore, showing the existence of antibiotic resistance in *L. monocytogenes* strains in food is important (8,9).

The objectives of this study were to investigate the presence of *L. monocytogenes* in RTE foods, to confirm the presence of the *hly* gene, to serotype the isolates, and to determine the antibiotic resistance profiles of these isolates.

2. Materials and methods

2.1. Ready-to-eat food samples

A total of 100 RTE foods, traditional Turkish cold dishes including 25 stuffed mussels, 25 ezme samples (a Turkish-style tomato dip/condiment), 25 fried spiced livers, and 25 mayonnaise-based salads were collected from retail shops, weekly open bazaars, and supermarkets in May and June 2012 in Samsun, Turkey. All samples were immediately transported to the laboratory in a refrigerated box at 4 °C and analyses were performed within 2 h.

2.2. Isolation and identification of *L. monocytogenes*

All samples were analyzed for the presence of *L. monocytogenes* using the standard procedure, EN ISO 11290-1 (10). Briefly, 25 g of each sample was preenriched with 225 mL of half-Fraser broth (Merck, Darmstadt, Germany) and homogenized in a stomacher (Stomacher Lab-Blender 400, UK) for 2 min. The homogenates were then incubated at 30 °C for 24 h. After incubation, 0.1 mL from this primary enrichment medium was transferred to 10 mL of Fraser broth (secondary enrichment medium) (Merck, Germany) and incubated at 37 °C for 48 h. A loopful of enrichment culture was streaked onto Oxford *Listeria* Selective Agar (Merck) and the inoculated plates were incubated at 37 °C for 48 h. Up to 5 suspected *Listeria* colonies, with a black halo and a sunken center, were picked from the plate and streaked onto a trypticase soy agar (Merck) plate with 0.6% yeast extract and incubated at 37 °C for 48 h. Suspected colonies were verified by Gram staining, catalase reactions, oxidase tests, CAMP tests, motility at 20–25 °C, Methyl Red-Voges Proskauer (MR-VP) reactions, nitrate reduction, and the production of acids from rhamnose, xylose, and mannitol for the identification of *L. monocytogenes* (11).

2.3. PCR amplification

Genomic DNA was extracted from isolates using the Chelex-100 (Sigma, USA) resin-based technique (12). PCR amplifications were performed as described previously by Aznar and Alarcon (13). The primers targeting the hemolysin gene (*hlyA*) (LMA-5'-CGGAGGTTCCGCAAAAGATG-3' and LMB-5'-CCTCCAGAGTGATCGATGTT-3') were designed according to Mengaud et al. (14). PCR was performed in a volume of 50 µL containing 1X PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl) (Sigma), 1.5 mM $MgCl_2$ (Sigma), 0.1 mM of each dNTP (Sigma), 0.5 U of Taq polymerase (Sigma), 1 µM (each) of *hylA* primers, and 5 µL of template DNA. The amplification conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s with a final extension at 72 °C for 5 min. Reactions were performed in a thermal cycler (Bio-Rad MJ Mini-PTC-1148, Singapore). The PCR products were separated on 1.5% agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) (Sigma) and stained with ethidium bromide at 0.5 µg/mL. Electrophoresis was carried out at 100 V for 1 h (Bio-Rad Power Pac-Basic, Bio-Rad electrophoresis tank, Wide Mini). The PCR products were visualized under UV illumination (Wise-UV-Wuv-L50, Korea) (Figure 1).

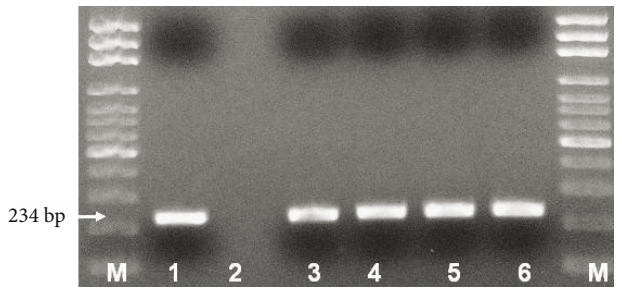

Figure 1. Agarose gel electrophoresis of PCR-amplified DNA from the *hlya* gene of *L. monocytogenes* isolated from RTE foods. M: 100-bp DNA ladder; Lane 1: *L. monocytogenes* reference strain ATCC7644; Lane 2: negative control; Lanes 3–6: *hylA* gene-positive strains isolated from RTE foods.

2.4. Serotype identification by multiplex PCR

Serogroup and serovar determinations were performed by multiplex PCR according to the method described by Doumith et al. (15) using the primers lmo0737 (906 bp), lmo1118 (691 bp), ORF2819 (471 bp), and ORF2110 (597 bp) (Alpha DNA, Canada). The details of the primer sequences are shown in Table 2. Initially, PCR conditions were optimized by using varying concentrations of the reagents. PCR was performed in a 50-µL reaction mixture containing 1X PCR buffer (Sigma); 2 mM $MgCl_2$ (Sigma); 0.2 mM of each dNTP (Sigma); 2 U of Taq polymerase (Sigma); 1 µM each of lmo0737, ORF2819, and ORF2110 primers; 1.5 µM of lmo1118 primer; and 5 µL of template DNA. PCR cycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, annealing at 53 °C for 75 s, and extension at 72° C for 75 s with a final extension at 72 °C for 7 min. After electrophoresis, strains that produced PCR products of the following sizes were identified: 691 bp identified as serotype 1/2a (or 3a), 471 bp identified as serotype 1/2b (or 3b), 691 to 906 bp identified as serotype 1/2c (or 3c), and 471 to 597 bp identified as serotype 4b (or 4d or 4e) (Figure 2).

2.5. Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar (Oxoid, UK). Eight antibiotic disks (Oxoid) were chosen as follows: amoxicillin/clavulanic acid (30 µg; AMC), ampicillin (10 µg; AMP), chloramphenicol (30 µg; C), erythromycin (15 mg; E), oxytetracycline (30 µg; O), penicillin G (10 units; P), tetracycline (30 µg; TE), and vancomycin (30 µg; VA). Fresh bacterial colonies were grown at 35 °C for 24 h in tryptic soy broth-yeast extract Merck). After incubation the turbidity level was adjusted to 0.5 McFarland with a compact benchtop densitometer (Biosan, DEN-1, Latvia), and then 1 mL of suspension was inoculated on Mueller-Hinton agar Oxoid) plates and spread uniformly. The plates were incubated at 35 °C for 18–24 h. The size of the inhibition zone around the antibiotic disks was measured, and the results were interpreted as susceptible, intermediate, or resistant according to the recommendations of the Clinical and Laboratory Standards Institute (16).

3. Results

Listeria spp. was detected in 16 (16%) samples, 4 of which (4%) were positive for *L. monocytogenes* from 100 RTE foods. As shown in Table 3, among the *Listeria* spp.-positive samples, 1 (4%) was from mayonnaise-based salads, 8 (32%) were from ezme, and 7 (28%) were from fried spiced livers. No *Listeria* spp. strain was isolated from stuffed mussel samples. A total of 30 isolates were obtained from 16 RTE foods that were positive for *Listeria* spp. All isolates were confirmed for the *hylA* gene by PCR analysis (Figure 1). Only 4 isolates were positive for *L. monocytogenes*. Among the *L. monocytogenes*-positive samples, 1 (4%) was from a mayonnaise-based salad, 2 (8%) were from ezme, and 1 (4%) was from fried spiced livers.

The serotype distribution of *L. monocytogenes* isolates was determined by multiplex PCR. Among 4 *L. monocytogenes* isolates, 2 of them were identified as 4b (or 4d, 4e) and the others as 1/2a (or 3a) (Table 4) (Figure 2).

Table 2. List of *L. monocytogenes* genes and primers used in the PCR assay.

Gene	Primer sequence (5'-3')	Product size (bp)	Serovar specificity
lmo0737	F-AGGGCTTCAAGGACTTACCC R-ACGATTTCTGCTTGCCATTC	691	Serovars 1/2a, 1/2c, 3a, and 3c
lmo1118	F-AGGGGTCTTAAATCCTGGAA R-CGGCTTGTTCGGCATACTTA	906	Serovars 1/2c and 3c
ORF2819	F-AGCAAAATGCCAAAACTCGT R-CATCACTAAAGCCTCCCATTG	471	Serovars 1/2b, 3b, 4b, 4d, and 4e
ORF2110	F-AGTGGACAATTGATTGGTGAA R-CATCCATCCCTTACTTTGGAC	597	Serovars 4b, 4d, and 4e

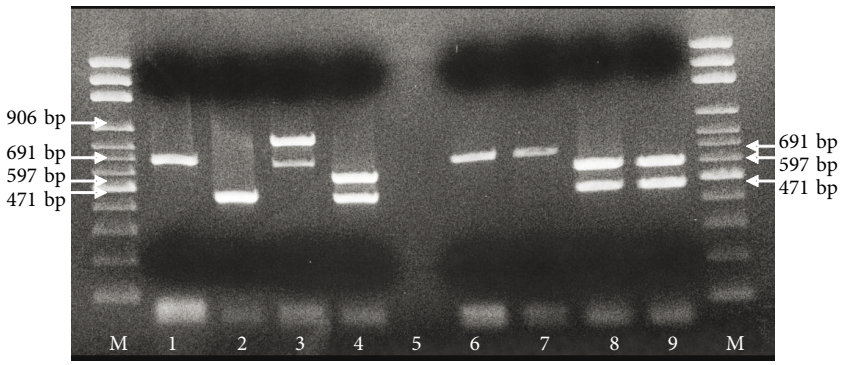

Figure 2. Serotype identification of *L. monocytogenes* isolates by multiplex PCR. M: 100-bp DNA ladder, Lane 1: *L. monocytogenes* reference strain serotype 1/2a, 691 bp (*L. monocytogenes* RSKK 471); Lane 2: *L. monocytogenes* reference strain serotype 1/2b, 471 bp (*L. monocytogenes* RSKK 472); Lane 3: *L. monocytogenes* reference strain serotype 1/2c, 691–906 bp (*L. monocytogenes* ATCC 7644); Lane 4: *L. monocytogenes* reference strain serotype 4b, 471–597 bp (*L. monocytogenes* RSKK 475); Lane 5: negative control (no DNA); Lanes 6 and 7: serotype 1/2a isolate (ezme sample), Lane 8: serotype 4b isolate (mayonnaise-based salad sample), Lane 9: serotype 4b isolate (fried spiced liver sample).

L. monocytogenes isolates were sensitive to penicillin G (100%), tetracycline (100%), oxytetracycline (75%), amoxicillin/clavulanic acid (75%), chloramphenicol (50%), and vancomycin (50%); intermediate resistance was found to ampicillin (100%), erythromycin (100%), chloramphenicol (50%), vancomycin (25%), and amoxicillin/clavulanic acid (25%) and resistance was found to oxytetracycline (25%) and vancomycin (25%). One *L. monocytogenes* serotype 1/2a isolate from an ezme sample was found to be resistant to oxytetracycline, whereas 1 *L. monocytogenes* serotype 4b isolate from a mayonnaise-based salad was found resistant to vancomycin.

4. Discussion

The results indicate *Listeria* spp. (16%) and *L. monocytogenes* (4%) contaminations of 4 different RTE food samples. The results are in agreement with those of Kovacevic et al. (17), who reported *Listeria* spp. (10%) and *L. monocytogenes* (5%) contaminations in RTE fish samples in British Colombia, Canada. A previous study in Turkey by Şireli and Gücükoğlu (18) reported *Listeria* spp. (13%) and *L. monocytogenes* (10%) contaminations in RTE food samples including mayonnaise-based salad, fried meatballs, fried livers, rice-stuffed mussels, and green salads.

In the present study, the incidence of *L. monocytogenes* was 4% in mayonnaise-based salads. The results of our study differ from those of Di Pinto et al. (19), who found *L. monocytogenes* in 27% of mayonnaise-based deli salads in Bari, Italy. The possible reason for the difference between our findings and those of Di Pinto et al. (19) may be that the mayonnaise-based deli salads were prepared and packaged at large-scale industrial food processing plants, and possibly due in part to contaminated raw materials

Table 3. Occurrence of *L. monocytogenes* isolates and serotypes in RTE foods.

Type and number of samples	No. of *Listeria* spp. detected by conventional methods		No. of *L. monocytogenes* detected by PCR		Serotypes
	Sample	Isolate	Sample	Isolate	
Mayonnaise-based salad (n = 25)	1 (4%)	2	1 (4%)	1	4b (4d, 4e)
Ezme (Turkish-style tomato dip/condiment) (n = 25)	8 (32%)	15	2 (8%)	2	1/2a (3a), 1/2a (3a)
Fried spiced liver (n = 25)	7 (28%)	13	1 (4%)	1	4b (4d, 4e)
Stuffed mussels (n = 25)	-	-	-	-	-
Total (n = 100)	16 (16%)	30	4 (4%)	4	

Table 4. Antimicrobial resistance profiles of *L. monocytogenes* isolates from RTE foods.

Antibiotic disks	Serotypes					
	1/2a (or 3a) n = 2			4b (or 4d, 4e) n = 2		
	R	I	S	R	I	S
Amoxicillin/clavulanic acid (30 µg)	0	0	2	0	1	1
Ampicillin (10 µg)	0	2	0	0	2	0
Chloramphenicol (30 µg)	0	1	1	0	1	1
Erythromycin (15 µg)	0	2	0	0	2	0
Oxytetracycline (30 µg)	1	0	1	0	0	2
Penicillin G (10 µg)	0	0	2	0	0	2
Tetracycline (30 µg)	0	0	2	0	0	2
Vancomycin (30 µg)	0	1	1	1	1	0

S, Susceptible; I, intermediate; R, resistant.

and inadequate application of good manufacturing practices. Moreover, in our study, while *L. monocytogenes* was identified in ezme (8%) and fried spiced livers (4%), *L. monocytogenes* was not identified in any of the stuffed mussels. During the preparation of stuffed mussels, mussels are boiled in water for 30 min. This treatment might be effective for the inactivation of the bacteria in mussels. Unlike in the present study, Şireli and Gücükoğlu (18) isolated *Listeria* spp. from rice-stuffed mussel samples (5%). These investigators might have isolated *Listeria* spp. due to the creation of a protective barrier by the mussel shell or inappropriate application of heat treatment. A study conducted by Jamali et al. (20) reported that salads and vegetables had the highest prevalence (14.7%) of *L. monocytogenes*, followed by chicken and chicken products (13.2%), beverages (10%), eggs and egg products (9.5%), beef and beef products (6.7%), lunch boxes (6.7%), and seafood and seafood products (6.7%) in Malaysia. The differences observed in the incidences of *L. monocytogenes* could be due in part to regions, contents of the RTE food, differences in the technologies used during the food processing, and, most importantly, contamination of the raw materials.

Although 13 serotypes have been identified for *L. monocytogenes,* according to various studies, 1/2a, 1/2b, and 4b are frequently isolated from food samples; the most prevalent serotype isolated from foods is 1/2a. However, strains of serotype 4b are responsible for the most cases of human listeriosis (15,21). In the current study, *L. monocytogenes* isolates were serotyped as 1/2a (50%) and 4b (50%) from RTE foods. Sant'Ana et al. (22) reported that the major serotype isolated from RTE vegetables in Sao Paulo, Brazil was 4b, followed by 1/2b. On the other hand, Lambertz et al. (23) isolated *L. monocytogenes* 1/2a as the main serovar from RTE foods in Sweden. These studies indicate that *L. monocytogenes* serotypes isolated from RTE food samples are different in different countries and that isolation of *L. monocytogenes* serotype 4b from RTE foods should be considered as a significant risk factor for the human health.

In our study, serotypes 4b and 1/2a were equally prevalent serotypes in RTE foods. *L. monocytogenes* 4b serotype was found in fried spiced livers. This finding may be related to *L. monocytogenes* serovar 4b isolates, which tend to be more resistant to heat treatment at 60 °C than the 1/2a isolates, as reported by Buncic et al. (24). Moreover, the 4b serotype was also found in mayonnaise-based salad. In this case, the origin of *Listeria* contamination might be the egg in the mayonnaise. In addition, this finding may be related to the better growth ability of serotype 4b than serotype 1/2a under cold conditions. Buncic et al. (24) reported that, following cold storage, *L. monocytogenes* serotype 4b had a shorter lag phase and higher pathogenicity than 1/2a at body temperature (37 °C). On the other hand, *L. monocytogenes* 1/2a serotype was found in ezme. Inappropriate washing, poor hygiene, and remnants of fertilizer may lead to contamination with *Listeria* spp. Since *L. monocytogenes* is a psychrotrophic bacterium, it can proliferate to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigeration temperatures (1).

The result that all *L. monocytogenes* isolates were found highly sensitive to penicillin G (100%) is similar to the results of Ennaji et al. (25), who found 100% susceptibility for strains of *L. monocytogenes* isolated from meat and poultry. However, Marian et al. (26) indicated that *L. monocytogenes* isolates from raw and RTE foods were highly resistant to ampicillin and penicillin G (100%).

On the other hand, tetracycline was shown to have good activity against *L. monocytogenes* in our study. These results are in agreement with those reported previously by Altuntas et al. (27)

Previous studies have shown that *Listeria* spp., including *L. monocytogenes*, are resistant to various antibiotics such as phosphomycin, streptomycin (27), rifampin (18), sulfonamide, tetracycline, ciprofloxacin (21), clindamycin, streptomycin, amikacin (17), tilmicosin, and tetracycline (28). Although most researchers reported that strains of *Listeria* spp. originating from humans, food, or the environment are not resistant to vancomycin (29), in our study, the isolated strains of *L. monocytogenes* were resistant to oxytetracycline (25%, 1 isolate) and vancomycin (25%, 1 isolate). Moreover, no multiple resistance was observed in *L. monocytogenes*. Our results agree with those of Walsh et al. (30), who found that 2 *Listeria* spp. isolates were resistant to vancomycin in retail food.

In conclusion, *L. monocytogenes* serovars in RTE foods are of significant concern for public health, as these serotypes are the predominant serotypes that can cause listeriosis in humans. Thus, it is imperative that preventative measures including the implementation of good hygiene practice and good manufacturing practice should be applied during the preparation of RTE foods in addition to ensuring the cold chain from production to consumption.

Acknowledgments

This research was presented at the 5th National Veterinary Food Hygiene Congress, held on 3–6 April 2013 in Antalya, Turkey.

References

1. Lovett J. *Listeria monocytogenes*. In: Doyle MP, editor. Foodborne Bacterial Pathogens. New York, NY, USA: Marcel Dekker; 1989. pp 284–311.
2. Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991; 55: 476–511.
3. Ben Embarek PK. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. Int J Food Microbiol 1994; 23: 17–34.
4. Saulo AA. Ready-to-eat foods and listeriosis. Food Safety and Technology 2005; 14: 1–2.
5. Cartwright EJ, Jackson KA, Johnson SD, Graves LM, Silk BJ, Mahon BE. Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. Emerg Infect Dis 2013; 19: 1–9.
6. Rodriguez-Lázaro D, Hernández M, Scortti M, Esteve T, Vazquez-Boland JA, Pla M. Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by real-time PCR: assessment of *hly, iap,* and *lin02483* targets and ampliFluor technology. Appl Environ Microbiol 2004; 70: 1366–1377.
7. Schuerch DW, Wilson-Kubalek EM, Tweten RK. Molecular basis of listeriolysin O pH dependence. P Natl Acad Sci USA 2005; 102: 12537–12542.
8. Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet 1990; 335: 1422–1426.
9. Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. Food Control 2010; 21: 708–713.
10. International Organization for Standardization. EN ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. Part 1: Detection Method. Geneva, Switzerland: International Organization for Standardization; 1997.
11. Seeliger HPR, Jones D. *Listeria*. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG, editors. Bergey's Manual of Systematic Bacteriology, Vol. 2. 9th ed. Baltimore, MD, USA: Williams & Wilkins; 1986. pp. 1235–1245.
12. Amills M, Francino O, Jansa M, Sanchez A. Isolation of genomic DNA from milk samples by using Chelex resin. J Dairy Res 1997; 64: 231–238.
13. Aznar R, Alarcon B. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. J Appl Microbiol 2003; 95: 958–966.
14. Mengaud J, Vicente MF, Chenevert J, Pereira JM, Geoffroy C, Gicquel-Sanzey B, Baquero F, Perez-Diaz JC, Cossart P. Expression in *Escherichia coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes*. Infect Immun 1988; 56: 766–772.
15. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol 2004; 42: 3819–3822.
16. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2010.
17. Kovacevic J, Mesak LR, Allen KJ. Occurrence and characterization of *Listeria* spp. in ready-to-eat retail foods from Vancouver, British Columbia. Food Microbiol 2012; 30: 372–378.
18. Şireli TU, Gücükoğlu A. Prevalence and antibiotic resistance of *Listeria* spp. isolated from ready-to-eat foods in Ankara. Turk J Vet Anim Sci 2008; 32: 131–135.
19. Di Pinto A, Novello L, Montemurro F, Bonerba E, Tantillo G. Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in Southern Italy. New Microbiol 2010; 33: 249–252.
20. 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: 19–24.

21. Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA, Meng J. Characterization of *Listeria monocytogenes* isolated from retail foods. Int J Food Microbiol 2007; 113: 47–53.

22. Sant'Ana AS, Igarashi MC, Landgraf M, Destro MT, Franco BDGM. Prevalence, populations and pheno- and genotypic characteristics of *Listeria monocytogenes* isolated from ready-to-eat vegetables marketed in São Paulo, Brazil. Int J Food Microbiol 2012; 155: 1–9.

23. Lambertz ST, Nilsson C, Brådenmark A, Sylvén S, Johansson A, Jansson LM, Lindblad M. Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010. Int J Food Microbiol 2012; 160: 24–31.

24. Buncic S, Avery SM, Rocourt J, Dimitrijevic M. Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? Int J Food Microbiol 2001; 65: 201–212.

25. Ennaji H, Timinouni M, Ennaji MM, Hassar M, Cohen N. Characterization and antibiotic susceptibility of *Listeria monocytogenes* isolated from poultry and red meat in Morocco. Infect Drug Resist 2008; 1: 45–50.

26. Marian MN, Sharifah Aminah, SM, Zuraini MI, Son R, Maimunah M, Lee HY Wong WC, Elexson N. MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. Food Control 2012; 28: 309–314.

27. Altuntas EG, Kocan D, Cosansu S, Ayhan K, Juneja VK, Materon L. Antibiotic and bacteriocin sensitivity of *Listeria monocytogenes* strains isolated from different foods. Food Nutr Sci 2012; 3: 363–368.

28. Osaili TM, Alaboudi AR, Nesiar EA. Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. Food Control 2011; 22: 586–590.

29. Charpentier E, Courvalin P. Antibiotic resistance in *Listeria* spp. Antimicrob Agents Ch 1999; 43: 2103–2108.

30. Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell DA. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. J Appl Microbiol 2001; 90: 517–522.