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PREVALENCE, SEROTYPE IDENTIFICATION BY MULTIPLEX POLYMERASE CHAIN REACTION AND ANTIMICROBIAL RESISTANCE PATTERNS OF *LISTERIA MONOCYTOGENES* ISOLATED FROM RETAIL FOODS

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ABSTRACT

In the present study, a total of 512 food samples composed of raw milk, dairy products, meat/meat products, chicken meat, seafood and raw vegetables were analyzed for the presence of *Listeria monocytogenes*. The results of the standard identification methods showed that 20 (3.9%) of the analyzed samples were found to harbor this pathogen. Further, 8.4% (13/155) of chicken meats, 0.9% (1/105) of meat/meat products and 13.6% (6/44) of fresh vegetables were contaminated with *L. monocytogenes*. Interestingly, only 18 of these isolates gave expected band size when they were subjected to molecular confirmation by polymerase chain reaction (PCR). Multiplex PCR serotyping of the strains revealed that 66.6% (12/18) of which belonged to serotype 1/2a (or 3a), 5.6% (1/18) to serotype 1/2b (or 3b, 7), 5.6% (1/18) to serotype 1/2c (or 3c) and 11.1% (2/18) to serotype 4b (or 4d, 4e). Two strains could not be serotyped by multiplex PCR. The strains were also evaluated by disk diffusion assay for their susceptibility to 15 commonly used antimicrobials. Antimicrobial resistance was most frequently observed for clindamycin (94.4%), followed by streptomycin and kanamycin (88.9%); penicillin (72.2%), tetracycline and gentamicin (66.7%); quinopristin/dalfopristin and erythromycin (61.1). Interestingly, 13 strains were resistant to more than five antibiotics. All strains were susceptible to linezolid, teicoplanin and vancomycin.

PRACTICAL APPLICATIONS

Listeria monocytogenes is one of the most important foodborne pathogens responsible for several outbreaks and cases of listeriosis in human. This study focused on the prevalence of *L. monocytogenes* in different raw and ready-to-eat foodstuffs, and serotype distribution among the isolates. Antibiotic resistance profiles of the isolates were also reported. Information and relief provided to consumers could help elaborate public health and food safety.

INTRODUCTION

The genus *Listeria*, which is a gram-positive, non–spore-forming bacterium, is composed of eight species: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. ivanovii*, *L. rocourtiae* and *L. marthii* (Keeratipibul and Techaruwichit 2012). Among these *Listeria* species, two are pathogenic: *L. monocytogenes* (pathogenic for human and animals) and *L. ivanovii* (pathogenic for animals) (Zunabovic *et al.* 2011).

L. monocytogenes is considered the causative agent for several outbreaks and cases of listeriosis in humans (Dalton *et al.* 1997; Lyytikainen *et al.* 2000; Knox *et al.* 2008). Although the frequency of listeriosis is relatively low, the higher mortality rate makes this bacterium an important human pathogen (Cocolin *et al.* 2005). It can cause serious

diseases, such as septicemia; meningitis; meningoencephalitis in immunocompromised individuals, newborns and the elderly; and abortion and stillbirth in pregnant women (Doumith *et al.* 2004; Aurora *et al.* 2009; Gambarin *et al.* 2012).

The main route of transmission to both humans and animals is believed to be through consumption of contaminated food or feed (Korsak *et al.* 2012). This pathogen has been isolated from a variety of food products. Meat (Jemmi *et al.* 2002; Miyasaki *et al.* 2009), poultry (Cetinkaya *et al.* 2004; Vitas and Garcia-Jalon 2004), raw milk and dairy (Pintado *et al.* 2005; Hamdi *et al.* 2007; Vanegas *et al.* 2009), seafood (Gudbjörnsdóttir *et al.* 2004; Pagadala *et al.* 2012) and vegetable products (Ponniah *et al.* 2010; Sant'Ana *et al.* 2012) have all been implicated as vehicles of listeriosis. *L. monocytogenes* is an important foodborne pathogen that is able to grow at refrigeration temperatures and survive in different environmental conditions (Borucki and Call 2003). This increases its possible danger notably in chilled ready-to-eat food products (Fallah *et al.* 2012).

Various methods, such as serotyping and phage typing, can be used to characterize *L. monocytogenes*. *L. monocytogenes* serotyping is based on the variation in the somatic (0) and flagellar (H) antigens allowing to one differentiate 13 different serotypes (Kerouanton *et al.* 2010). Among these serotypes, 1/2a, 1/2b, 1/2c and 4b are frequently isolated from clinical samples, whereas serotype 1/2a is the most prevalent serotype in food (Zhang *et al.* 2007). Routine analysis of *L. monocytogenes* by serotyping with traditional agglutination methods is effective, but problems regarding the availability of commercial antisera and occasional nontypeability of isolates may cause difficulties (Kerouanton *et al.* 2010). Recently, multiplex polymerase chain reaction (PCR) assays have been developed to identify four major *L. monocytogenes* serotypes (Aurora *et al.* 2009; Doijad *et al.* 2011; Erol and Ayaz 2011).

Antimicrobial resistance has been considered as a worldwide public health problem in recent decades (Fallah *et al.* 2012). One of the consequences of the excessive use of antimicrobials as therapeutics or growth promoters in animal husbandry is the risk of dissemination of antibiotic-resistant bacteria, including resistant *L. monocytogenes* into the environment and their transfer to humans through the food chain (Conter *et al.* 2009; Harakeh *et al.* 2009).

The aim of this study was to determine the occurrence of *L. monocytogenes* in various types of foods commercialized in different localizations from Southern Marmara region of Turkey and to characterize *L. monocytogenes* strains through serotyping using the multiplex-PCR method. Furthermore, susceptibility/resistance of isolates to various antimicrobials would provide background data on the distribution of this character among the isolates from the region.

MATERIALS AND METHODS

Sample Collection

Between September 2009 and September 2010, a total of 512 raw and ready-to-eat food samples, including 196 dairy origin (42 raw milk, 140 cheese, 11 butter, 2 yoghurt, 1 cream), 105 meat and meat products (52 minced meats, 20 steaks, 10 salamis, 12 sausages, 9 meatballs, 2 hams), 155 chicken meats, 12 seafoods and 44 raw fresh vegetables (leafy greens), were taken from different retail markets (15 markets) and local bazaars (12 bazaars) in Southern Marmara region of Turkey over a period 1 year. The samples were collected during summer ($n = 200$), fall ($n = 128$), winter ($n = 100$) and spring ($n = 84$). They were transported to the laboratory under refrigerated conditions and analyzed on the same day.

Isolation and Identification of *L. Monocytogenes*

For the isolation of *L. monocytogenes*, 25 g of food samples was homogenized with 225 mL of Listeria enrichment broth base (LEB) (Oxoid CM862, Oxoid, Basingstoke, Hampshire, U.K.) supplemented with Listeria selective enrichment supplement (Oxoid SR141) using a Stomacher Lab-Blender (Model 400, Seward, Hampshire, U.K.) for 2 min and then incubated at 37C for 24 h. A loopful from each enrichment solution was streaked on Listeria selective agar (LSA) (Oxoid CM856) supplemented with Listeria selective supplement (Oxoid SR140). After incubation at 37C for 24–48 h, presumptive *L. monocytogenes* colonies (gray-black in color with a black halo) were subjected to a standard series of biochemical tests, such as gram staining, catalase and oxidase reaction, motility, carbohydrate fermentation (mannitol, rhamnose, xylose, ribose, dextrose), β-hemolysis on 5% sheep blood agar, nitrate reduction, urea hydrolysis, methyl red, and Voges–Proskauer and CAMP tests. Presumptive positive results were also tested on the RAPID'LMono chromogenic agar (Bio-Rad, Marne la Coquette, France) by plating them on the enriched LEB directly for confirmative purposes.

PCR Confirmation of Strains and Multiplex-PCR for Serotyping

The genomic DNA was extracted according to Doumith *et al.* (2004). Briefly, five bacterial colonies grown on LSA were picked up by means of a sterilized toothpick and emulsified in 50 μL of sodium dodecyl sulfate (0.25%)–0.05 N NaOH solution. After incubating at 99C for 15 min, 100 μL of molecular biology grade H_2O was added to the mixture and 2 μL of which was used as DNA template.

For PCR confirmation of strains, the primers PCRGO and PCRDO (Alpha DNA, Montreal, Canada) designed on *hlyA* (LLO-listeriolysin O) gene by Bohnert *et al.* (1992) were used. The PCR assay was performed with a final volume of 25 µL of PCR buffer containing 0.50 µM each of primers, 1.5 mM $MgCl_2$ (Bioron Diagnostics, Ludwigshafen, Germany), 200 µM each of dNTPs (New England Biolabs, Ankara, Turkey), 1 U Taq DNA polymerase (Bioron) and DNA. Thermal cycling (Runik SCM 96G) was carried out with an initial denaturation step at 94C for 5 min, then 30 cycles of denaturation at 94C for 1 min, annealing at 65C for 1 min and an extension step at 72C for 2 min.

Multiplex-PCR assay to serotype *L. monocytogenes* strains was performed as described by Doumith *et al.* (2004). As positive control, reference strains ATCC 19111 (serotype 1/2a), N7144 (serotype 1/2b), ATCC 7,644 (serotype 1/2c) and RSKK 475 (serotype 4b) were used. The primer sets were added at the following final concentration: 1 µM for lmo0737, ORF2819 and ORF2110, and 1.5 µM for lmo1118 (Alpha DNA). The PCR assay was performed with a final volume of 50 µL of PCR buffer containing template DNA, primers, 1.5 mM $MgCl_2$, 200 µM each of dNTPs and 1 U Taq DNA polymerase. Thermal cycling (Runik SCM 96G) was carried out with an initial denaturation step at 94C for 5 min, followed by 35 cycles of denaturation at 94C for 24 s, annealing at 53C for 1.15 min, an extension step at 72C for 1.15 min and a final extension at 72C for 7 min. Amplified PCR fragments were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide (Sigma, Istanbul, Turkey) and visualized using Bio-Rad Gel Doc 2000TM imaging system (Bio-Rad Laboratories, Segrate, Milan, Italy).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing of PCR-confirmed *L. monocytogenes* colonies was performed by the Kirby-Bauer disk diffusion method using Mueller-Hinton agar (Oxoid CM337), according to the Clinical and Laboratory Standards Institute (CLSI 2006). The following antibiotics at the specified concentration per disc were used: ampicillin 10 µg, cefotaxime 30 µg, chloramphenicol 30 µg, clindamycin 2 µg, ciprofloxacin 5 µg, erythromycin 15 µg, gentamicin 10 µg, kanamycin 30 µg, linezolid 30 µg, penicillin 10 U, quinupristin/dalfopristin 15 µg, streptomycin 10 µg, tetracycline 30 µg, teicoplanin 30 µg and vancomycin 30 µg (Oxoid). To determine the antimicrobial susceptibility, the diameter of the inhibition zone around each antimicrobial impregnated disk was measured after an incubation period of 24–48 h at 37C. Each isolate was classified as resistant, intermediate or susceptible according to the guidelines of CLSI. *Escherichia coli* ATCC 35218 was used as the control strain.

RESULTS

Out of the total 512 food samples analyzed, 20 (3.9%) were found to be positive for *L. monocytogenes* according to the standard culture method, which is based on biochemical identification of suspected colonies on Oxford agar plates. On the Rapid L'Mono agar, 19 of the 20 isolates produced the blue halo colonies characteristic of *L. monocytogenes*. The prevalence data obtained with respect to the identification methods are represented in Table 1. The prevalence of *L. monocytogenes* contamination was 8.4% (13/155) in chicken meats, 0.9% (1/105) in meat and meat products, and 13.6% (6/44) in raw fresh vegetables. On the contrary, the pathogen was not isolated from raw milk, dairy products and seafood samples. The molecular confirmation of strains by PCR using specific primers revealed that, although two isolates identified as *L. monocytogenes* by standard testes did not give any band at the expected size even by different independent PCR assays, suggesting that they might be misidentified by standard techniques. By PCR results, overall positivity decreased to 3.5% (18/512) (Table 1).

The serotyping results of reference *L. monocytogenes* strains and those recovered from the samples as determined by multiplex-PCR assay are presented in Table 2. Out of 18 *L. monocytogenes* strains, 12 (66.6%) were identified as serotype 1/2a (or 3a), 1 (5.6%) as serotype 1/2b (or 3b,7), 1 (5.6%) as serotype 1/2c (or 3c) and 2 (11.1%) as serotype 4b (or 4d, 4e) (Fig. 1). Interestingly, two strains did not give any band either by multiplex PCR or by single PCR assays when the primers were used separately. These strains were

TABLE 1. PREVALENCE OF *LISTERIA MONOCYTOGENES* IN THE SAMPLES ACCORDING TO THE IDENTIFICATION METHODS

Type of samples	No. of samples	No. of positive samples for *L. monocytogenes* (%)		
		Biochemical tests	Rapid L'Mono	PCR
Chicken	155	13 (8.4)	13 (8.4)	12 (7.7)
Meat/meat products	105	1 (0.9)	1 (0.9)	1 (0.9)
Raw vegetables	44	6 (13.6)	5 (11.4)	5 (11.4)
Milk/dairy products	196	0	0	0
Seafood	12	0	0	0
Total	512	20 (3.9)	19 (3.7)	18 (3.5)

PCR, polymerase chain reaction.

TABLE 2. SEROTYPES OF *LISTERIA MONOCYTOGENES* ISOLATES OBTAINED FROM FOOD SAMPLES

Isolate No.	Source	Serotype
Standard	ATCC 19111	1/2a
Standard	N7144	1/2b
Standard	ATCC 7644	1/2c
Standard	RSKK 475	4b
6	Chicken meat	4b, 4d, 4e
30	Chicken meat	1/2a, 3a
32	Chicken meat	–*
33	Chicken meat	1/2a, 3a
34	Chicken meat	1/2a, 3a
35	Chicken meat	1/2a, 3a
36	Chicken meat	1/2a, 3a
37	Chicken meat	1/2a, 3a
42	Chicken meat	1/2a, 3a
43	Chicken meat	1/2c, 3c
49	Chicken meat	1/2a, 3a
55	Chicken meat	1/2a, 3a
31	Steak	1/2a, 3a
16	Vegetable	1/2a, 3a
54	Vegetable	1/2a, 3a
65	Vegetable	–*
66	Vegetable	4b, 4d, 4e
67	Vegetable	1/2b, 3b, 7

* Not serotyped.

then reconfirmed as *L. monocytogenes* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (data not shown).

Antimicrobial susceptibility profiles of 18 PCR-confirmed and serotyped strains were evaluated by the disk diffusion method on 15 antibiotics (Table 3). Seventeen (94.4%) of the strains exhibited resistance to clindamycin, 16 strains (88.9%) to streptomycin, 16 strains (88.9%) to kanamycin, 13 strains (72.2%) to penicillin, 12 strains (66.7%) to tetracycline, 12 strains (66.7%) to gentamycin, 11 strains (61.1%) to quinupristin/dalfopristin, and 11 strains (61.1%) to erythromycin. None of the strains showed resistance neither to ciprofloxacin nor chloramphenicol; however, some of the strains had only intermediate level of resistance to these two antibiotics. All of the strains were susceptible to linezolid, teicoplanin and vancomycin. All strains displayed multiple drug resistance (resistance to ≥2 antibiotics); as shown in Table 4, one isolate was resistant to two antibiotics, one strain to four antibiotics, three strains to five antibiotics, two strains to six antibiotics, six strains to seven antibiotics and five strains to eight antibiotics, indicating that multiple antibiotic resistance is common among the isolated strains.

DISCUSSION

L. monocytogenes is responsible for human and animal listeriosis (Filiousis *et al.* 2009). Ingestion of contaminated foods is considered to be the primary source of infection for human listeriosis cases (Doumith *et al.* 2004).

The results of the present study showed that 3.9% (20/512) of the samples analyzed were contaminated with *L. monocytogenes* according to the standard culture method. The prevalence of *L. monocytogenes* in raw chicken meats was 8.4% (13/155), followed by 13.6% (6/44) in raw fresh vegetables and 0.9% (1/105) in meat and meat products. *L. monocytogenes* had previously been isolated from a variety of foods. The rates of contamination of poultry by *L. monocytogenes* recovered in our study are close or above the incidences reported by some other groups (Cetinkaya *et al.* 2004; Vitas and Garcia-Jalon 2004; Osaili *et al.* 2011; Fallah *et al.* 2012). *L. monocytogenes* can cause the contamination of poultry during the slaughtering and processing phases (Aury *et al.* 2011) or at the farm level (Fallah *et al.* 2012). The prevalence of *L. monocytogenes* in vegetables was 13.6% in the present study. These findings are lower than the results of Ponniah *et al.* (2010), who reported overall incidence of 22.5% for *L. monocytogenes* in vegetables. However, our results were much higher than those (0.6–3.1%) reported by Sant'Ana *et al.* (2012) and Yan *et al.* (2010). Previous works conducted on meat and meat products in several countries found the prevalence rates ranging from 6.3 to 42% for this pathogen (Soyutemiz *et al.* 2000; Filiousis *et al.* 2009; Miyasaki *et al.* 2009; Yan *et al.* 2010). In comparison, much lower rate (0.9%) of contamination of meat by *L. monocytogenes* was observed in our study.

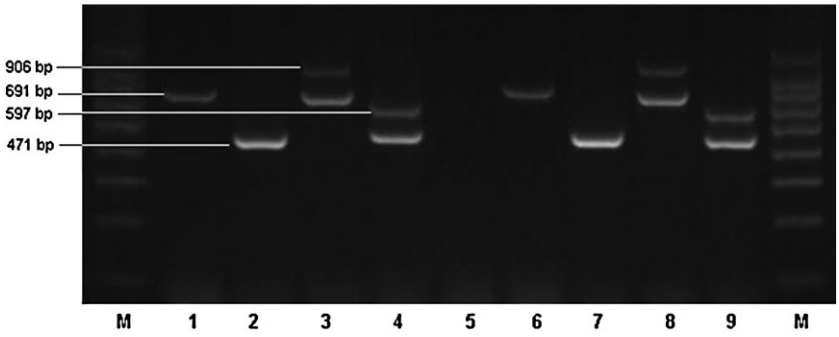

FIG. 1. SEROTYPING OF *L. MONOCYTOGENES* STRAINS/ISOLATES WITH MULTIPLEX PCR ASSAY

M: 100 bp DNA ladder; lane 1: *L. monocytogenes* ATCC 19111 serotype 1/2a; lane 2: *L. monocytogenes* N7144 serotype 1/2b; lane 3: *L. monocytogenes* ATCC 7644 serotype 1/2c; lane 4, *L. monocytogenes* RSKK 475 serotype 4b; lane 5: negative control; lane 6: isolate 30 (serotype 1/2a); lane 7: isolate 67 (serotype 1/2b); lane 8: isolate 43 (serotype 1/2c); lane 9: isolate 6 (serotype 4b).

TABLE 3. ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *LISTERIA MONOCYTOGENES* STRAINS

Strain No.	Source	Susceptibility profiles for antibiotics tested														
		TE	LZD	QD	TEC	VA	AMP	P	CIP	CTX	E	C	DA	K	CN	S
6	Chicken	S	S	IM	S	S	S	R*	S	IM†	IM	S‡	R	R	R	R
30	Chicken	S	S	R	S	S	R	R	S	IM	IM	S	R	R	R	R
32	Chicken	R	S	R	S	S	R	S	S	S	IM	S	R	R	R	R
33	Chicken	R	S	R	S	S	S	R	IM	IM	R	IM	R	R	R	R
34	Chicken	R	S	R	S	S	S	R	S	S	R	IM	R	R	IM	R
35	Chicken	R	S	R	S	S	S	R	S	IM	IM	S	R	R	IM	R
36	Chicken	S	S	S	S	S	S	S	S	S	IM	S	R	R	R	R
37	Chicken	R	S	S	S	S	S	R	S	S	R	S	R	R	R	R
42	Chicken	R	S	R	S	S	S	R	S	S	R	S	R	R	R	R
43	Chicken	R	S	R	S	S	S	R	S	S	R	S	R	R	IM	R
49	Chicken	R	S	R	S	S	S	R	S	S	R	S	R	R	R	R
55	Chicken	S	S	S	S	S	S	R	IM	IM	R	S	R	R	IM	R
31	Steak	S	S	S	S	S	R	S	IM	IM	R	S	R	R	R	R
16	Vegetable	R	S	R	S	S	S	R	S	IM	R	S	R	R	R	R
54	Vegetable	R	S	R	S	S	S	R	IM	IM	R	S	R	R	R	R
65	Vegetable	R	S	R	S	S	R	S	IM	IM	S	S	S	S	S	S
66	Vegetable	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S
67	Vegetable	R	S	S	S	S	S	R	S	IM	R	S	R	R	R	R

* Resistant.

† Intermediate susceptible.

‡ Susceptible.

AMP, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; DA, clindamycin; E, erythromycin; K, kanamycin; LZD, linezolid; P, penicillin G; QD, quinupristin/dalfopristin; S, streptomycin; TE, tetracycline; TEC, teicoplanin; VA, vancomycin.

The pathogen was not isolated from raw milk, dairy products and seafood samples. Earlier studies reported the contamination with *L. monocytogenes* of raw milk (Hamdi *et al.* 2007; Mahmoodi 2010), cheese (Pintado *et al.* 2005; Filiousis *et al.* 2009) and seafood (Yan *et al.* 2010; Pagadala *et al.* 2012). Recently, Fallah *et al.* (2013) reported the presence of *L. monocytogenes* in 16 (4.83%) of 331 raw seafood samples in Iran, and also the higher prevalence of the pathogen in freshwater fish (11.4%) compared with those in seawater fish (1.80%) and shrimp (1.69%). In our study, seafood samples were obtained from Black Sea and Marmara Sea but not from freshwaters which would be the possible explanation for the absence of the pathogen in seafood.

TABLE 4. NUMBER AND PERCENTAGE OF INTERMEDIATE OR RESISTANT STRAINS* OF *LISTERIA MONOCYTOGENES* TO ANTIMICROBIAL AGENTS

Antibiotics	Intermediate	Resistant
Tetracycline	0 (0%)	12 (66.7%)
Quinupristin/dalfopristin	1 (5.6%)	11 (61.1%)
Ampicillin	0 (0%)	4 (22.2%)
Penicillin	0 (0%)	13 (72.2%)
Ciprofloxacin	5 (27.8%)	0 (0%)
Cefotaxime	10 (55.6%)	1 (5.6%)
Erythromycin	5 (27.8%)	11 (61.1%)
Chloramphenicol	2 (11.1%)	0 (0%)
Clindamycin	0 (0%)	17 (94.4%)
Kanamycin	0 (0%)	16 (88.9%)
Gentamicin	4 (22.2%)	12 (66.7%)
Streptomycin	0 (0%)	16 (88.9%)

* All strains were susceptible to linezolid, teicoplanin and vancomycin.

In the present investigation, the results obtained by the standard conventional bacteriological method are of controversy with those obtained by the PCR technique. Eighteen out of 20 isolates were confirmed to be positive for *L. monocytogenes* by PCR amplification of the *hly* gene. Other studies also reported diverse results related to the characterization of *L. monocytogenes* using molecular and conventional methods (Miyasaki *et al.* 2009; Vanegas *et al.* 2009; Gambarin *et al.* 2012). Miyasaki *et al.* (2009) reported PCR identification of *L. monocytogenes* in 29% of fresh pork sausage samples, while the positivity for the pathogen was 42% by the ISO method.

Multiplex-PCR assay was used for serotyping of 18 PCR-confirmed strains of *L. monocytogenes*. As shown in Table 2, 66.6% (12/18) of the strains belonged to serotype 1/2a (or 3a), followed by 5.6% (1/18) to serotype 1/2b (or 3b, 7), 5.6% (1/18) to serotype 1/2c (or 3c) and 11.1% (2/18) to serotype 4b (or 4d, 4e). However, two of the isolates tested were not serotyped; this could probably be linked to the limitations of the technique used. It should be noticed that two serotypes (4a and 4c) are not recognized by PCR serotyping approach (Doumith *et al.* 2004). Large

variability among *L. monocytogenes* serovars is in agreement with the previous results obtained by multiplex-PCR serotyping for *L. monocytogenes* strains from different types of food in India (Aurora *et al*. 2009), Turkey (Erol and Ayaz 2011), Poland (Korsak *et al*. 2012) and the U.S.A. (Zhang *et al*. 2007). Hamdi *et al*. (2007) notified that all of *L. monocytohenes* isolates from milk samples belonged to the PCR-group IVb, corresponding to serovars 4b, 4d and 4e. However, Miyasaki *et al*. (2009) suggested that *L. monocytogenes* strains from fresh pork sausage in Brazil were identified as uncommon serotypes 4a and 4c using multiplex-PCR assay. Although 13 serovars of *L. monocytogenes* have been described, 3 three serotypes (1/2a, 1/2b and 4b) account for the majority of clinical cases (Borucki and Call 2003; De Santis *et al*. 2007). The results of this study demonstrated that *L. monocytogenes* 1/2a (or 3a) was the most frequently isolated serotype from the samples (66.6%). Furthermore, these serotypes were found to be the most prevalent in chicken samples. In our study, two strains were found to belong to serogroup 4b, 4d or 4e (potentially serotype 4b), which is the serotype of *L. monocytogenes* most commonly causing human listeriosis (Zhang *et al*. 2007).

As determined by disk diffusion method, *L. monocytogenes* strains showed resistance to clindamycin (94.4%), streptomycin (94.4%), kanamycin (88.9%), penicillin (72.2%), tetracycline (66.7%), gentamicin (66.7%), quinupristin/dalfopristin and erythromycin (61.1%). Resistance to a number of antibiotics, including streptomycin, penicillin, tetracycline, gentamicin, erythromycin and ampicillin, has also been observed among *L. monocytogenes* strains isolated from various food products in northern China (Yan *et al*. 2010). The prevalence of clindamycin resistance was remarkably high (94.4%) among our strains. Contrary results were found by Filiousis *et al*. (2009), who reported isolation of clindamycin susceptible strains from raw meat, meat products and cheese in Greece. Resistance to clindamycin may be linked to the excessive use of this drug in veterinary and human medicine (Harakeh *et al*. 2009). Relatively high level of resistance to gentamicin and erythromycin was noticed in the present study; however, previously published several studies reported high level of susceptibility among *L. monocytogenes* strains to these two antimicrobials (Conter *et al*. 2009; Harakeh *et al*. 2009; Osaili *et al*. 2011; Fallah *et al*. 2012; Korsak *et al*. 2012). Most (66.7%) of the examined strains displayed resistance to tetracycline. Tetracycline resistance was also demonstrated by Fallah *et al*. (2012) in Iran and by Yan *et al*. (2010) in China. A contrary result was reported by Harakeh *et al*. (2009), showing that all *L. monocytogenes* strains from Lebanese dairy-based food products in Lebanon were susceptible to tetracycline. The results of our survey signified that 72.2, 61.1 and 22.2% of the tested 18 *L. monocytogenes* strains were resistant to penicillin, erythromycin and ampicillin, respectively. Ampicillin or penicillin antimicrobials are generally used alone, or in combination with gentamicin, in the treatment of human listeriosis. Erythromycin is also used to treat pregnant women diagnosed with listeriosis (Alonso-Hernando *et al*. 2012). Antimicrobial resistance was not observed against ciprofloxacin or chloramphenicol; only five and two of the strains had intermediate resistance to these two antibiotics, respectively. However, other researchers (Conter *et al*. 2009; Yan *et al*. 2010; Pagadala *et al*. 2012) isolated ciprofloxacin-resistant *L. monocytogenes* strains from food sources. All strains were susceptible to linezolid, teicoplanin and vancomycin. These results are in agreement with those previously reported by Filiousis *et al*. (2009) for vancomycin or linezolid, and by Conter *et al*. (2009) for teicoplanin. However, vancomycin-resistant strains of *L. monocytogenes* from a variety of food were reported by some authors (Harakeh *et al*. 2009; Yan *et al*. 2010). Importantly, all of the strains isolated in this work showed resistance to multiple antimicrobials; thus, 13 strains showed resistance to more than five antibiotics, indicating wide distribution of multidrug resistance among strains.

CONCLUSION

The results of this survey indicate that retail food products, including chicken meat, meat and raw vegetable may serve as vehicles of *L. monocytogenes* strains. In addition, the presence of the serotypes more commonly associated with human clinical cases, including 1/2a, 1/2b and 4b in foods, and the multiple resistance of strains to antimicrobials commonly used to treat human listeriosis could be a potential health hazard for consumers. Moreover, data obtained are of primary importance to control the risks of *L. monocytogenes* and to improve background on antibiotic resistance among the strains.

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