Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

PREVALENCE, ANTIMICROBIAL RESISTANCE AND SEROTYPE DISTRIBUTION OF *LISTERIA MONOCYTOGENES* ISOLATED FROM RAW MILK AND DAIRY PRODUCTS

TAHSIN ONUR KEVENK[1,3] and GOKNUR TERZI GULEL[2]

[1]Veterinary Control Central Research Institute, 06020, Ankara Turkey
[2]Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Ondokuz Mayıs University, 55139, Samsun, Turkey

[3]Corresponding author.
TEL: +905357827930;
FAX: +903123211755;
EMAIL: o.kevenk@gmail.com

Received for Publication January 19, 2015
Accepted for Publication June 15, 2015

doi: 10.1111/jfs.12208

ABSTRACT

The objectives of study were to assess presence of *Listeria monocytogenes*, perform serotyping and investigate antibiotic resistance in raw milk and dairy products. A total of 210 milk and dairy products including white ($n = 20$) and kashar cheese ($n = 20$), ice cream ($n = 20$), butter ($n = 20$), cokelek ($n = 10$), kuymak ($n = 10$) and farm cheese ($n = 10$) were obtained from Samsun, Turkey. All samples were analyzed using an immunomagnetic separation-based culture technique and strains of *L. monocytogenes* were confirmed by presence of *hlyA* and *iap* genes by polymerase chain reaction (PCR). *L. monocytogenes* was identified in 5 of 100 (5%) milk samples, serotyped as 4b and 1/2b, and in 9 of 110 (8.2%) dairy products, serotyped as 1/2a, 1/2b and 1/2c. However, *L. monocytogenes* was not identified from butter, kashar and ice cream samples. The antibiotic susceptibility against ampicillin, amoxicillin/clavulanic acid, erythromycin, chloramphenicol, penicillin G, oxytetracycline, tetracycline and vancomycin was assessed by disc diffusion method. It was found that 15.3% of isolates were resistant to at least one drug and 36.5% were multidrug resistant. Among isolates, resistance to tetracycline was most commonly encountered (34.6%), followed by resistance to chloramphenicol (25%) and penicillin G (23%). In conclusion, our data also indicate that consuming raw and unpasteurised milk and dairy products could pose a risk of listeriosis in humans.

PRACTICAL APPLICATIONS

Although there are plenty of studies about the incidence of *L. monocytogenes* in raw milk and dairy products in Turkey, our study is the first one in Samsun (Middle Black Sea) Region about Listeria in milk and dairy products. Also in our study, we characterized some virulence genes and serotype distribution of *L. monocytogenes* by PCR. Finally, we performed antibiotic resistance tests of isolates to see possible public health threats because of using overabundant antibiotics. If we analyze all these work to see the potential risk assessments in this region, our study could be a leading study in near future.

INTRODUCTION

Listeria monocytogenes is an ubiquitous, motile, gram-positive, psychrotrophic, facultative intracellular microorganism that is capable of causing important foodborne disease as listeriosis. The bacterium can grow at refrigeration temperature and low pH (Rocourt and Cossart 1997; Swaminathan *et al.* 2007). Listeriosis is associated with the consumption of contaminated milk, soft or semi-soft cheese, under-cooked and ready-to-eat foods, unwashed raw vegetables and fruits (Oliver *et al.* 2005; Rahimi *et al.* 2010). Pregnant women, newborns, immunosuppressed patients and the elderly (>65 years old) are at the highest risk of listeriosis. The disease has a long incubation period and causes

abortions, premature births, meningitis, septicemia and has a high (20–30%) mortality rate (Gandhi and Chikindas 2007; Swaminathan *et al.* 2007).

L. monoyctogenes contains 13 serotypes with different virulence potential according to somatic (O factor) and flagella (H factor) antigens. Various studies have shown serotypes 4b, 1/2a, 1/2b and 1/2c are responsible for more than 98% of human listeriosis and serotype 4b has been related to the most recent outbreaks of listeriosis (Liu *et al.* 2006; Roberts *et al.* 2006).

Milk and dairy products are important sources of proteins, essential amino acids, calcium, phosphorus, riboflavin and vitamin A (Huth *et al.* 2006). In developing countries, commonly, smallholder farms produce milk and dairy products. This circumstance provides relatively quick returns for small-scale producers and is an important source of cash (FAO 2014). Several outbreaks of listeriosis have been caused by consumption of contaminated milk and dairy products (Dalton *et al.* 1997; MacDonald *et al.* 2005). In the U.S.A., between 2007 and 2011, 2,373 persons were infected with listeriosis and in total 386 deaths were reported to the CDC (CDC 2014). In 2011, 1,516 confirmed cases of listeriosis were reported in 28 EU countries (ECDC 2013).

The objectives of this study were (1) to investigate the presence of *L. monocytogenes* in raw milk and dairy products in Samsun (2) to confirm the presence of *hly* and *iap* genes using polymerase chain reaction (PCR), (3) to serotype the strains isolated by multiplex PCR, and (4) to determine the antibiotic resistance profiles of these isolates using the disc diffusion method.

MATERIALS AND METHODS

Bacterial Strains

L. monocytogenes ATCC 7644 (serotype 1/2c), *L. monocytogenes* RSKK 472 (serotype 1/2b), *L. monocytogenes* RSKK 471 (serotype 1/2a), *L. monocytogenes* RSKK 475 (serotype 4b), *S. aureus* ATCC 26923 and *R. equi* ATCC 33701 were used as positive controls.

Raw Milk and Dairy Product Samples

A total of 100 raw milk samples (50 from local bazaars and 50 from smallholders in the region) and 110 dairy products (20 white cheese, 20 kashar cheese, 20 ice cream, 20 butter, 10 cokelek, 10 kuymak cheese and 10 farm cheese from local bazaars, traditionally produced) were collected from Samsun, Turkey between November 2011 and April 2012. All samples were transported in an unbroken cold chain (4C) and analyzed immediately.

Traditional homemade cheeses used in our study such as kuymak cheese, cokelek and farm cheeses are often consumed in the Samsun region in the Middle Black Sea, Turkey. The farm cheeses were produced from raw milk. During the production, dairy will be kept overnight after milking and filtered without applying any heat treatment, then fermented at 28–32C. Then, the curd is cut into small pieces and heated to 36–38C. This clot is introduced into heated curd baskets or pasta strainers followed by filtration under its own weight without adding any weight. In the meantime, the curd takes the shape of the basket while straining. The cheese is turned over from time to time. The cheese is salted for a total of 15 days at 2-day intervals and is ready to be consumed after 1–2 months of maturation (Kinik *et al.* 1999; Kamber 2005; Unsal 2009; Cakmakci 2011). The production of Kuymak cheese is as follows: milk is heated to brewing temperature and fermented at 28–32C. After fermenting, coagulation is achieved by heating up to 40–45C; then, the clot is filtered through the mangle cloth. After filtration, the clot is acidified by keeping it at room temperature for 3–4 days. Then, the rancid cheese is heated in salted water at 55C for 3–5 min. The heated cheese is placed on a flat surface and, after cooling slightly, cut into strips. The cut pieces are hand-pulled by stretching to give the cheese its characteristic stringy appearance. After this stage, the cheese is salted lightly and stored (Kamber 2005; Unsal 2009; Cakmakci 2011). Another traditional cheese, cokelek cheese, is produced in the following way. Following milking, it is left to sour overnight. Then, the milk begins to precipitate at mild temperatures (40–45C). The resulting precipitate is filtered with filter cloths. The obtained residue is put in a hanging bag and allowed to drain thoroughly. Then, the remaining clot is removed from the bag, salted lightly and usually consumed fresh (Unsal 2009; Cakmakci 2011).

Immunomagnetic Separation-Based Cultivation Method

All samples were analyzed for the presence of *L. monocytogenes* using an IMS (Immunomagnetic Separation)-based EN ISO 11290-1 culture technique (Anonymous 1996). Briefly, 25 g/mL of sample was weighed and added to 225 mL of Half Fraser Broth (Merck 110398, Darmstadt, Germany), homogenized in a stomacher (Interscience Bagmixer 400, St Nom, France) for 2 min and incubated for pre-enrichment at 30C for 24 h. After incubation, IMS was performed according to the manufacturer's manual (Dynal 1996). Briefly, 20 µl of magnetic beads coated with a specific antibody against *Listeria* spp. (Dynabeads anti-*Listeria*, Dynal, Invitrogen, Oslo, Norway) was mixed with 1 mL of the pre-enrichment suspension and incubated at room temperature for 10 min with continuous mixing. The magnetic plate was inserted into the Dynal MPC-M. The beads were then concentrated onto the side of

the tube. Afterwards, 1 mL of PBS-Tween 20 (Sigma P3563, St. Louis, MO, U.S.A.) was added for washing the bead and bacteria complex. The washing steps were then repeated twice. At the end of the IMS procedure, 100 µL of PBS-Tween 20 was added to resuspend the beads. Then, 50 µL of the resuspended IMS mixture was plated onto Modified Oxford Agar (MOX, Merck 1.07004). The plates were then incubated at 35C for 24–48 h. Up to five typical suspected *Listeria* colonies, with a black halo and a sunken center, were selected from the MOX agar, streaked onto Tryptic Soy Agar (Merck 1.05458) with 0.6% yeast extract (Oxoid LP0021, Basingstoke, U.K.) and incubated at 37C for 24 h. All the isolates were subjected to standard identification and biochemical tests including Gram staining, catalase activity, oxidase reactivity, Christie Atkins Munch-Petersen (CAMP) test, motility at 20–25C, nitrate reduction and the production of acids from rhamnose, xylose and mannitol for the identification of *L. monocytogenes* (Hitchins 1998).

PCR Assay for the Confirmation of *L. monocytogenes* Isolates

Genomic DNA was extracted from isolates using Proteinase K (Sigma P2308) and a Chelex-100 (Bio-Rad, Hercules, CA, U.S.A.) resin-based method (Amills *et al.* 1997). The isolates of *L. monocytogenes* were confirmed by the presence of *hlyA* (Listeriolysin O; Bohnert *et al.* 1992) and *iap* (invasion-associated protein) gene-specific primers (Kohler *et al.* 1990; Table 1). PCR was performed in a final volume of 50 µL containing 1X PCR Buffer (Sigma D4545), 1.5 mM $MgCl_2$ (Sigma D4545), 0.1 mM dNTP (Sigma D7295), 0.5 U Taq-Polymerase (Sigma D4545), 1 µM of each primer and 5 µL of target DNA. The PCR reaction was performed in thermal cycler (Bio-Rad MJ Mini-PTC-1148) as follows: initial denaturation at 94C for 5 min, followed by 35 cycles at 94C for 30 s, annealing at 65C for 45 s for *hlyA* and 55C for 45 s for *iap*, extension at 72C for 45 s and final extension at 72C for 5 min (Kohler *et al.* 1990; Bohnert *et al.* 1992). The PCR products were separated on a 1.5% agarose gel in Tris-borate-EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) and stained with ethidium bromide at 0.5 µg/mL. Electrophoresis was carried out at 80 V for 1 h (BioRad Power Pac-Basic, Singapore; BioRad electrophoresis tank, Wide Mini, Singapore). The PCR products were visualized under UV illumination (Wise-UV-Wuv-L50, DAIHAN Scientific, Seoul, Korea). The *hlyA* gene was visualized at 388 bp and the *iap* gene was visualized at 131 bp (Fig. 2).

Multiplex PCR Assays for Serotyping of *L. monocytogenes* Isolates

Serotyping of *L. monocytogenes* isolates was performed by multiplex PCR according to the method reported by Doumith *et al.* (2004) using the primers *lmo0737* (906 bp), *lmo1118* (691 bp), *ORF2819* (471 bp), *ORF2110* (597 bp) and prs (370 bp; Alpha DNA, Québec, Canada). All the primers used in this study are displayed in Table 1. PCR was performed in a volume of 50 µL containing 1X PCR Buffer (Sigma D4545), 2 mM $MgCl_2$ (Sigma D4545), 0.2 mM dNTP (Sigma D7295), 2 U Taq-Polymerase (Sigma D4545), 1 µM each of the *Imo0737*, *ORF2819* and *ORF2110* primers, 1.5 µM of the *Imo1118* primers and 5 µL template DNA. The PCR cycling conditions were as follows: initial denaturation at 94C for 3 min, followed by 35 cycles at 94C for 40 s, annealing at 53C for 75 s, and extension at 72C for 75 s, followed by a final extension at 72C 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; Fig. 1).

TABLE 1. PRIMERS USED FOR CONFIRMATION AND SEROTYPING OF *L. MONOCYTOGENES* ISOLATES

Primer name	Primer sequence	Product size (bp)	Reference
hlyA	PCRGO: 5′ GAA TGT AAA CTT CGG CGC AAT CAG 3′ PCRDO: 5′ GCC GTC GAT GAT TTG AAC TTC ATC 3′	388	Bohnert *et al.* 1992
iap	IAP1: 5′ ACA AGC TGC ACC TGT TGC AG 3′ IAP2: 5′ TGA CAG CGT GTG TAG TAG CA 3′	131	Kohler *et al.* 1990
lmo0737	F: 5′ AGG GCT TCA AGG ACT TAC CC 3′ R: 5′ ACG ATT TCT GCT TGC CAT TC 3′	691	Doumith *et al.* 2004
lmo1118	F: 5′ AGG GGT CTT AAA TCC TGG AA 3′ R: 5′ CGG CTT GTT CGG CAT ACT TA 3′	906	Doumith *et al.* 2004
ORF2819	F: 5′ AGC AAA ATG CCA AAA CTC GT 3′ R: 5′ CAT CAC TAA AGC CTC CCA TTG 3′	471	Doumith *et al.* 2004
ORF2110	F: 5′ AGT GGA CAA TTG ATT GGT GAA 3′ R: 5′ CAT CCA TCC CTT ACT TTG GAC 3′	597	Doumith *et al.* 2004
prs	F: 5′ GCT GAA GAG ATT GCG AAA GAA G 3′ R: 5′ CAA AGA AAC CTT GGA TTT GCG G 3′	370	Doumith *et al.* 2004

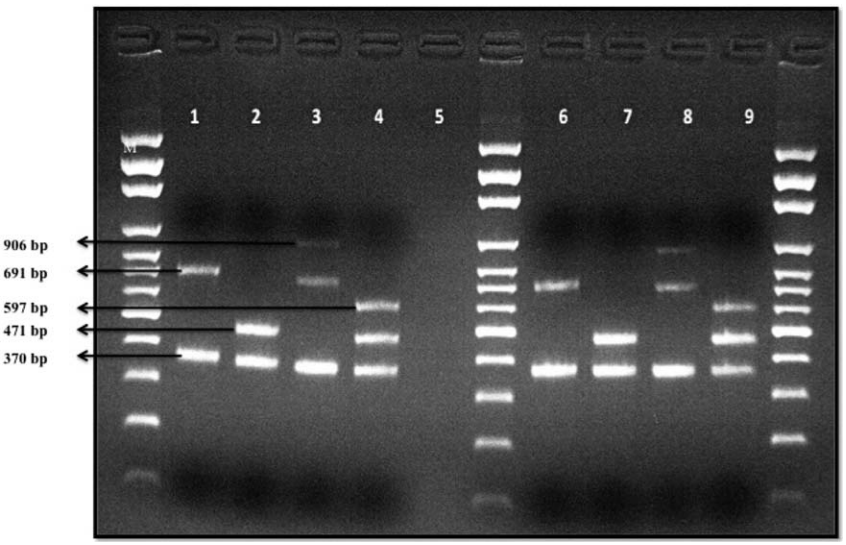

FIG. 1. SEROTYPE IDENTIFICATION OF *LISTERIA MONOCYTOGENES* ISOLATES FROM MILK AND DAIRY PRODUCTS BY MULTIPLEX PCR ASSAY
M: 100 bp marker, lane 1: positive control of *L. monocytogenes* 1/2A (RSKK 471); lane 2: positive control of *L. monocytogenes* 1/2B (RSKK 472); lane 3: positive control of *L. monocytogenes* 1/2C (ATCC 7644); lane 4: positive control of *L. monocytogenes* 4B (RSKK 475); lane 5: negative control; lane 6: serotype 1/2A isolate; lane 7: serotype 1/2B isolate; lane 8: serotype 1/2C isolate; lane 9: serotype 4B isolate.

Antibiotic Resistance Profiles of *L. monocytogenes* Isolates

Antibiotic susceptibility tests on the *L. monocytogenes* isolates were carried out with the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI – M45-A; Anonymous 2006) in Mueller-Hinton Agar (MHA, Oxoid CM 337). Eight antibiotics were chosen: amoxycillin/clavulanic acid (Oxoid CT0223), ampicillin (Oxoid CT0003B), chloramphenicol (Oxoid CT0013B), erythromycin (Oxoid CT0020B), oxytetracycline (Oxoid CT0041), penicillin G (Oxoid CT0043B), tetracycline (Oxoid CT0054B) and vancomycin (Oxoid CT0058B). Briefly, *L. monocytogenes* isolates were grown in tryptic soy broth (Merck 1.05459) with yeast extract (Oxoid LP0021) and incubated at 35C for 24 h. After incubation, the turbidity level of fresh colonies was adjusted to 0.5 McFarland with a compact benchtop densitometer (DEN-1 densitometer, Biosan, Riga, Latvia). Then, 1 mL of the suspension was spread onto MHA (Oxoid CM 337) plates uniformly. The plates were incubated at 35C for 18–24 h. The diameter of the inhibition zone around the discs was measured and the results were interpreted as susceptible, intermediate or resistant according to the recommendations of the Clinical and Laboratory Standards Institute (Anonymous 2006).

RESULTS

A total of 210 samples from raw milk and dairy products were analyzed for the presence of *L. monocytogenes*. The results are presented in Table 2. *Listeria* spp. were detected in 12 of 100 (12%) raw milk samples, five of which (5%) were positive for *L. monoctogenes. Listeria* spp. were isolated in 22 of 110 (20%) dairy product samples, nine of which (8%) were positive for *L. monocytogenes*. Among these dairy products, the highest prevalence of *L. monocytogenes* was found in cokelek and kuymak cheese (30%), followed by farm cheese (20%) and white cheese (5%). However, *L. monoytogenes* was not detected in kashar cheese, butter and ice cream samples. Using culture techniques, a total of 52 *L. monocytogenes* isolates were identified from the raw milk and dairy products. All isolates were confirmed for the *hylA* and *iap* genes by PCR as *L. monocytogenes*.

In the present study, 52 *L. monocytogenes* isolates were serotyped by multiplex PCR. Four different serotypes (1/2a, 1/2b, 1/2c and 4b) of *L. monocytogenes* were identified. As shown in Table 2, 20 *L. monocytogenes* isolates were identified as serotype 4b and five isolates were identified as 1/2b from raw milk samples. In dairy products, six isolates were serotyped as 1/2a, 15 isolates were serotyped as 1/2b and six isolates were serotyped as 1/2c. *L. monocytogenes* 4b and 1/2b were the dominant serotypes in all samples (Fig. 1).

The antimicrobial susceptibility of the 52 isolates of *L. monocytogenes* to the eight antimicrobial agents examined is shown in Table 3. According to disc diffusion tests, 8 of 52 isolates (15.3%) were resistant to only one drug, 19 of 52 (36.5%) were resistant to multiple drugs and the remaining 25 isolates did not show any resistance to antibiotics. Among the 52 isolates, resistance to tetracycline was most commonly encountered (34.6%), followed by resistance to chloramphenicol (25%) and penicillin G (23%).

DISCUSSION

In this study, the results indicate that 5% of raw milk was contaminated with *L. monocytogenes*. The results are in agreement with Kalorey *et al.* (2008), Van Kessel *et al.* (2004) and Erol and Sireli (2002) as they were also isolated

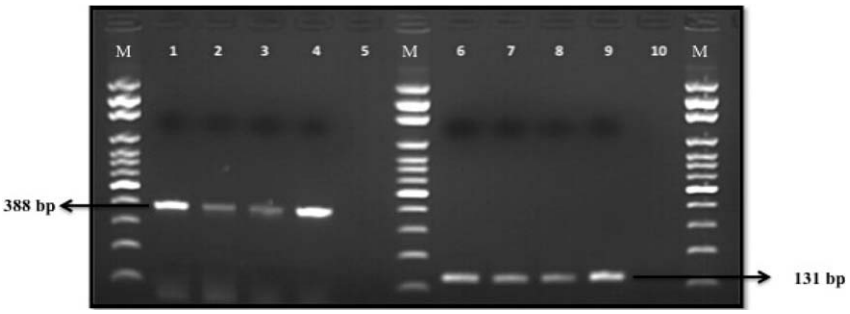

FIG. 2. ELECTROPHORESE IMAGE OF *Hlya* (388 BP) AND *Iap* (131 BP) GENE FROM MILK AND DAIRY PRODUCTS ISOLATES BY PCR
M: 100 bp DNA marker, lanes 1–6: positive control for *Hlya* gene (*L. monocytogenes* ATCC7644); lanes 2–4: *Hlya* gene positive *L. monocytogenes* isolate; lane 5: negative control; lanes 7–9: *Iap* gene positive *L. monocytogenes* isolate; lane 10: negative control.

L. monocytogenes from raw milk 5.1%, 4.9% and 5%, respectively. Contrary to this, higher than our results, Pak *et al.* (2002) isolated *L. monocytogenes* from raw milk 6.5%. On the other hand, Aygun and Pehlivanlar (2006), Jayarao *et al.* (2006), Sagun *et al.* (2001) and Soyutemiz *et al.* (2001) isolated lower percentages of *L. monocytogenes* than our results from raw milk 0%, 2.2%, 1.2% and 3%, respectively.

In the present study, *L. monocytogenes* was identified in one of 20 (5%) white cheese samples. Our findings show similarities with Kahraman *et al.* (2010), the prevalence of *L. monocytogenes* was 4.8% for 105 white cheese samples in Istanbul, Turkey. In another study, *L. monocytogenes* was isolated (6%; 20 of 333) from soft and semi-soft cheese samples in Sweden by Loncarevic *et al.* (1995). However, Aygun and Pehlivanlar (2006) isolated *L. monocytogenes* from only 2.35% of white cheese samples, which is a lower rate than in our findings. On the other hand, other studies had higher isolation rates than are reflected in our results, 9.2% and 13.6% reported by Arslan and Ozdemir (2008) and Almeida *et al.* (2013), respectively. The reason for these high rates may be due to inadequate pasteurization or cross-contamination of samples.

In the present study, *L. monocytogenes* could not be detected in kashar cheese, butter and ice cream samples. However, Kahraman *et al.* (2010) and Cagri-Mehmetoglu *et al.* (2011) isolated *L. monocytogenes* from kashar cheese at rates of 1.7% and 2.61%, respectively, which were higher

TABLE 2. PREVALENCE OF *L. MONOCYTOGENES* AND SEROTYPE DIVERSITY IN MILK AND DAIRY PRODUCTS

		No. of *Listeria* spp.		No. of *L. monocytogenes*		Serotypes of *L. monocytogenes*			
No. of samples		Sample	Isolate	Sample	Isolate	1/2a (or 3a)	1/2b (or 3b)	1/2c (or 3c)	4b (or 4d, 4e)
Milk	Raw milk (n = 100)	12 (12%)	60	5 (5%)	25	–	5 (9.6%)	–	20 (38%)
	White cheese (n = 20)	5 (25%)	25	1 (5%)	1	–	–	1 (1.9%)	–
Dairy product	Kashar cheese (n = 20)	0	0	0	0	–	–	–	–
	Butter (n = 20)	3 (15%)	15	0	0	–	–	–	–
	Ice cream (n = 20)	0	0	0	0	–	–	–	–
	Kuymak cheese (n = 10)	6 (60%)	30	3 (30%)	10	–	10 (19%)	–	–
	Cokelek (n = 10)	4 (40%)	20	3 (30%)	7	2 (3.8%)	5 (9.6%)		
	Farm cheese (n = 10)	4 (40%)	20	2 (20%)	9	4 (7.6%)	–	5 (9.6%)	–
	Total (n = 210)	34 (16%)	170	14 (6.6%)	52	6 (11.5%)	20 (38.4%)	6 (11.5%)	20 (38.4%)

TABLE 3. ANTIMICROBIAL RESISTANCE PROFILES OF *L. MONOCYTOGENES* ISOLATES FROM MILK AND DAIRY PRODUCTS (N = 52)

		No. of isolates (%)		
Antibiotics	Concentration μg/disc	Resistant	Intermediate	Susceptible
Amoxicillin/clavulanic acid	30	2 (3.8)	–	50 (96.2)
Ampicillin	10	5 (9.6)	6 (11.5)	41 (78.9)
Chloramphenicol	30	13 (25)	14 (26.9)	25 (48.1)
Erythromycin	15	5 (9.6)	10 (19.2)	37 (71.2)
Oxytetracycline	30	10 (19.2)	5 (9.6)	37 (71.2)
Penicillin G	10U	12 (23.0)	–	40 (77.0)
Tetracycline	30	18 (34.6)	2 (3.8)	32 (61.6)
Vancomycin	30	7 (13.4)	10 (19.3)	35 (67.3)

than those in our results. Also in contrast to our study, Erol and Sireli (2002) detected *L. monocytogenes* in 5% of butter samples and Rahimi *et al.* (2010) isolated *L. monocytogenes* from 2 of 40 (5%) ice cream samples. The reason for this might be cross-contamination of samples because of different storage and marketing conditions.

In our study, among the different kinds of traditional homemade cheeses tested, kuymak and cokelek cheese had the highest (30%) rate of *L. monocytogenes*, followed by farm cheese (20%). The reason for this high contamination rate might be the traditional homemade manufacturing techniques utilized. As a general rule, during homemade traditional cheese production, milk is only slightly heated up till it reaches fermentation temperature, without pasteurization. Similarly, *L. monocytogenes* isolation rates have also been high in other studies with traditional cheese. In studies with traditional homemade cheese samples, Guner and Telli (2011) found *L. monocytogenes* in Van herby cheese, carra cheese, Konya moldy cheese and Urfa Tulum at rates of 30%, 46.6%, 23.3% and 13.3%, respectively, in Turkey. On the other hand, Gulmez and Guven (2001) isolated *L. monocytogenes* in only 2.5% of cecil cheese samples in Kars, Turkey, a lower rate than in our findings. Colak *et al.* (2007) collected 250 tulum cheese samples from Istanbul, Turkey and 12 of them (4.8%) were positive for *L. monocytogenes*. Similarly, Manfreda *et al.* (2005) isolated *L. monocytogenes* in 4.8% of Gorgonzola cheeses in Italy.

In the present study, four different serotypes of *L. monocytogenes* (1/2a, 1/2b, 1/2c and 4b) were identified from milk and dairy products. The results indicate a higher prevalence of serotypes *L. monocytogenes* 4b and 1/2b (38%) in our study. Similarly, Hofer *et al.* (2000) detected *L. monocytogenes* 4b as the most frequent serotype from raw milk in Brazil and *L. monocytogenes* 1/2b was second in their study. Makino *et al.* (2005) found *L. monocytogenes* 1/2b was the dominant serotype in homemade cheeses from Japan. Pintado *et al.* (2005) isolated 4b, 1/2b and 1/2a serotypes in soft cheese at rates of 83.3%, 12.5% and 4.1%, respectively.

In the current study, some *L.monocytogenes* isolates were resistant to ampicillin, amoxycillin/clavulanic acid, erythromycin, chloramphenicol, penicillin G, oxytetracycline, tetracycline and vancomycin. The highest prevalence of resistance was for tetracycline (34.6%). Similar results have been reported by other authors (Charpentier *et al.* 1995; Jamali *et al.* 2013). Although most researchers have reported that strains of *Listeria* spp. are not resistant to vancomycin (Rahimi *et al.* 2012), in our study, significant numbers of isolated strains of *L. monocytogenes* were resistant to vancomycin (13.4 %, seven isolates). Our results agree with those of Conter *et al.* (2009) and Harakeh *et al.* (2009), who also found *Listeria* spp. isolates resistant to vancomycin in their studies. Because of transferable elements between bacteria, resistance quickly becomes more common. In several studies, transfer by conjugation, plasmids and transposons carrying antibiotic genes from other bacteria to *Listeria* and between species of *Listeria* have been reported (Charpentier and Courvalin 1999). Moreover, using antibiotics for prophylaxis and as growth stimulants is the major reason for antibiotic resistance (Van Duijkeren and Houwers 2000).

CONCLUSION

In conclusion, the presence of *L. monocytogenes* serotypes 4b and 1/2a in milk and dairy products 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, pasteurization techniques and good manufacturing practice should be applied during the preparation of milk and dairy products in addition to ensuring the cold chain during storage.

ACKNOWLEDGMENTS

This study was supported by Ondokuz Mayıs University, Samsun, Turkey, Scientific Research Project Programs (Project No: PYO.VET -1904.11.010) and this article was part of a PhD thesis.

REFERENCES

ALMEIDA, G., MAGALHÃES, R., CARNEIRO, L., SANTOS, I., SILVA, J., FERREIRA, V., HOGG, T. and TEIXEIRA, P. 2013. Foci of contamination of *Listeria monocytogenes* in different cheese processing plants. Int. J. Food Microbiol. *167*, 303–309.

AMILLS, M., FRANCINO, O., JANSA, M. and SANCHEZ, A. 1997. Isolation of genomic DNA from milk samples by using Chelex resin. J. Dairy Res. *64*, 231–238.

ANONYMOUS. 1996. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1: Detection Method, EN ISO 11290-1:1996.

ANONYMOUS. 2006. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline (M45-A). Clinical and Laboratory Standards Institute, Wayne, PA.

ARSLAN, S. and OZDEMIR, F. 2008. Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. Food Control *19*, 360–363.

AYGUN, O. and PEHLIVANLAR, S. 2006. *Listeria* spp. in raw milk and dairy products in Antakya, Turkey. Food Control *17*, 676–679.

BOHNERT, M., DILASSER, F., DALET, C., MENGAUD, J. and COSSART, P. 1992. Use of specific oligonucleotides for direct

enumeration of *Listeria monocytogenes* in food samples by colony hybridization and rapid detection by PCR. Res. Microbiol. *143*, 271–280.

CAGRI-MEHMETOGLU, A., YALDIRAK, G., BODUR, T., SIMSEK, M., BOZKIR, H. and EREN, N.M. 2011. Incidence of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in two kasar cheese processing environments. Food Control *22*, 762–766.

CAKMAKCI, S. 2011. Turkiye Peynirleri. In *Peynir Biliminin Temelleri. 1*. (A. Hayaloglu and B. Ozer, eds.) pp. 585–614, Sidas Medya, Baskı, Izmir.

CDC. 2014. Centers for Disease Control and Prevention National Enteric Disease Surveillance. http://www.cdc.gov/listeria/surveillance.html (accessed March 15, 2014).

CHARPENTIER, E. and COURVALIN, P. 1999. Antibiotic resistance in *Listeria* spp. Antimicrob. Agents Chemother. *43*(9), 2103–2108.

CHARPENTIER, E., GERBAUD, G., JACQUET, C., ROCOURT, J. and COURVALIN, P. 1995. Incidence of antibiotic resistance in Listeria species. J. Infect. Dis. *172*, 277–281.

COLAK, H., HAMPIKYAN, H., BINGOL, E. and ULUSOY, B. 2007. Prevalence of *L. monocytogenes* and *Salmonella* in tulum cheese. Food Control *18*, 576–579.

CONTER, M., PALUDI, D., ZANARDI, E., GHIDINI, S., VERGARA, A. and IANIERI, A. 2009. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. Int. J. Food Microbiol. *128*, 497–500.

DALTON, C.B., AUSTIN, C.C., SOBEL, J., HAYES, P.S., BIBB, W.F., GRAVES, L.M., SWAMINATHAN, B., PROCTOR, M.E. and GRIFFIN, P.M. 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N. Engl. J. Med. *336*(2), 100–105.

DOUMITH, M., BUCHRIESER, C., GLASER, P., JACQUET, C. and MARTIN, P. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol. *42*(8), 3819–3822.

DYNAL. 1996. Magnetic protein purification using Dynabeads. In *Cell Separation and protein purification. Technical Handbook*, 2nd Ed. (A.S. Dynal, ed.) pp. 118–150, Norway Printed, Oslo, Norway.

ECDC. 2013. European Centers for Disease Control. Surveillance Report: Annual epidemiological report. http://www.ecdc.europa.eu/en/publications/Publications/annual-epidemiological-report-2013.pdf (accessed March 25, 2014).

EROL, I. and SIRELI, T. 2002. Occurrence and contamination levels of *Listeria* spp. in milk and dairy products in Ankara. FEMS Symposium on the Versatility of Listeria Species, 10–11 October, Izmir, Turkey.

FAO. 2014. Food and Agriculture Organization Milk Production. Avaible from: http://www.fao.org/agriculture/dairy-gateway/milk-production/en/#.uqrwzjhwtdı. Accessed 2014 April 7.

GANDHI, M. and CHIKINDAS, M.L. 2007. *Listeria*: A foodborne pathogen that knows how to survive. Int. J. Food Microbiol. *113*, 1–15.

GULMEZ, M. and GUVEN, A. 2001. Investigation of Campylobacter, Salmonella and Listeria spp. from Turkish white and cecil cheese. Kafkas Univ. Vet. Fak. Derg. *7*(2), 155–161.

GUNER, A. and TELLI, N. 2011. A survey on the presence of *Listeria monocytogenes* in various semi-hard cheeses from different regions of Turkey. J. Anim. Vet. Adv. *10*(14), 1890–1894.

HARAKEH, S., SALEH, I., ZOUHAIRI, O., BAYDOUN, E., BARBOUR, E. and ALWAN, N. 2009. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy based products. Sci. Total Environ. *407*, 4022–4027.

HITCHINS, A.D. 1998. *Listeria monocytogenes*. In *Bacteriological Analytical Manual*, 8th Ed. (G.J. Jackson, ed.) pp. 1001–1013, AOAC International., Gaithersburg.

HOFER, E., RIBEIRO, R. and FEITOSA, D.P. 2000. Species and serovars of the genus *Listeria* isolated from different sources in Brazil from 1971 to 1997. Mem. Inst. Oswaldo Cruz *95*, 615–620.

HUTH, P.J., DIRIENZO, D.B. and MILLER, G.D. 2006. Major scientific advances with dairy foods in nutrition and health. J. Dairy Sci. *89*, 1207–1221.

JAMALI, H., RADMEHR, B. and THONG, K.L. 2013. Prevalence, characterisation, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. Food Control *34*, 121–125.

JAYARAO, B.M., DONALDSON, S.C., STRALEY, B.A., SAWANT, A., HEGDE, N.V. and BROWN, J.L. 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. J. Dairy Sci. *89*, 2451–2458.

KAHRAMAN, T., OZMEN, G., OZINAN, B. and GOKSOY, E.O. 2010. Prevalence of *Salmonella* spp. and *Listeria monocytogenes* in different cheese types produced in Turkey. Brit. Food J. *112*(11), 1230–1236.

KALOREY, D.R., WARKE, S.R., KURKURE, N.V., RAWOOL, D.B. and BARBUDDHE, S.B. 2008. *Listeria* species in bovine raw milk: A large survey of Central India. Food Control *19*(2), 109–112.

KAMBER, U. 2005. Geleneksel Anadolu Peynirleri. 1. Baskı, Ankara, Miki Matbaacılık.

KINIK, O., ERGULLU, E. and AKBULUT, N. 1999. A study on the production and some characterization of sepet cheese. Gıda *24*(3), 151–161.

KOHLER, S., LEIMEISTER-WACHTER, M., CHAKRABOTRY, T., LOTTSPEICH, F. and GOEBEL, W. 1990. The gene coding for protein p60 of *Listeria monocytogenes* and its use as a specific probe for *Listeria monocytogenes*. Infect. Immun. *58*(6), 1943–1950.

LIU, D., LAWRENCE, M.L., WIEDMANN, M., GORSKI, L., MANDRELL, R.E., AINSWORTH, A.J. and AUSTIN, F.W. 2006. *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied virulence potential. J. Clin. Microbiol. *44*, 4229–4233.

LONCAREVIC, S., DANIELSSON-THAM, M.L. and THAM, W. 1995. Occurrence of *Listeria monocytogenes* in soft and semi-soft cheeses in retail outlets in Sweden. Int. J. Food Microbiol. *26*, 245–250.

MACDONALD, P.D., WHITWAM, R.E., BOGGS, J.D., MACCORMACK, J.N., ANDERSON, K.L., REARDON, J.W., SAAH, J.R., GRAVES, L.M., HUNTER, S.B. and SOBEL, J. 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clin. Infect. Dis. *40*(5), 677–682.

MAKINO, S.I., KAWAMATO, K., TAKESHI, K., OKADA, Y., YAMASAKI, M., YAMAMOTO, S. and IGIMI, S. 2005. An outbreak of foodborne listeriosis due to cheese in Japan during 2001. Int. J. Food Microbiol. *104*(2), 189–196.

MANFREDA, G., DE CESARE, A., STELLA, S., COZZI, M. and CANTONI, C. 2005. Occurrence and ribotypes of *Listeria monocytogenes* in gorgonzola cheese. Int. J. Food Microbiol. *102*(3), 287–293.

OLIVER, S.P., JAYARAO, B.M. and ALMEIDA, R.A. 2005. Food-borne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathog. Dis. *2*, 115–129.

PAK, S.L., SPAHR, U., JEMMI, T. and SALMAN, M. 2002. Risk factors for *L. monocytogenes* contamination of dairy products in Switzerland, 1990–1999. Prev. Vet. Med. *53*, 55–65.

PINTADO, C.M.B.S., OLIVEIRA, A., PAMPULHA, M.E. and MASS, F. 2005. Prevalence and characterization of *Listeria monocytogenes* isolated from soft cheese. Food Microbiol. *22*, 79–85.

RAHIMI, E., AMERI, M. and MOMTAZ, H. 2010. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. Food Control *21*, 1448–1452.

RAHIMI, E., MOMTAZ, H., SHARIFZADEH, A., BEHZADNIA, A., ASHTARI, M.S., ZANDI ESFAHANI, S., RIAHI, M. and MOMENI, M. 2012. Prevalence and antimicrobial resistance of *Listeria* species isolated from traditional dairy products in Chahar Mahal & Bakhtiyari, Iran. BJVM *15*(2), 115–122.

ROBERTS, A., NIGHTINGALE, K., JEFFERS, G., FORTES, E., KONGO, J.M. and WIEDMANN, M. 2006. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. Microbiology *152*, 685–693.

ROCOURT, J. and COSSART, P. 1997. *Listeria monocytogenes*. In *Food Microbiology Fundamentals and Frontiers* (M.P. Doyle, L.R. Beuchat and T.J. Montville, eds.) pp. 337–352, American Society for Microbiology (ASM) Press., Washington DC.

SAGUN, E., SANCAK, Y.C., ISLEYICI, O. and EKICI, K. 2001. The presence and prevalence of *Listeria* species in milk and herby cheese in and around Van. Turkish J. Anim. Sci. *25*(1), 15–19.

SOYUTEMIZ, E., CETINKAYA, F., OZAKIN, C. and GEDIKOGLU, S. 2001. Presence of *Listeria monocytogenes* in raw milk samples from West Anatolia. Turk J. Infect. *15*(1), 5–9.

SWAMINATHAN, B., CABANES, D., ZHANG, W. and COSSART, P. 2007. *Listeria monocytogenes*. In *Food Microbiology Fundamentals and Frontiers*, 3rd Ed. (M.P. Doyle and L.R. Beuchat, eds.) pp. 457–491, American Society for Microbiology (ASM) Press., Washington DC.

UNSAL, A. 2009. *Süt Uyuyunca, Turkiye Peynirleri. 5*, Yapı Kredi Yayınları, Baskı, Istanbul.

VAN DUIJKEREN, E. and HOUWERS, D.J. 2000. A critical assessment of antimicrobial treatment in uncomplicated *Salmonella enteritis*. Vet. Microbiol. *73*, 61–73.

VAN KESSEL, J.S., KARNS, J.S., GORSKI, L., MCCLUSKEY, B.J. and PERDUE, M.L. 2004. Prevalence of *Salmonellae*, *Listeria monocytogenes* and fecal coliforms in bulk tank milk of US dairies. J. Dairy Sci. *87*, 2822–2830.