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Research Article

Presence and Antibiotic Resistance of *Listeria monocytogenes* in Raw Milk and Dairy Products [1]

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Abstract

In this study, the presence of *Listeria monocytogenes* in raw milk and milk products produced from raw milk by traditional method, such as cheese and butter using cultural and molecular techniques and the antibiotic resistances were investigated. Isolation was performed using the FDA technique. Microbact 12L test kit was used for biochemical identification. Genotyping of isolates was done by traditional PCR method. Antibiotic resistance of isolates was determined by standard disk diffusion method. *Listeria* spp. was detected in 16 (5.3%) of the total 300 food samples according to the biochemical identification results. The highest prevalence of *Listeria* spp. was in raw milk samples (10%), followed by cheese samples (4%) and butter samples (2%). All 36 *Listeria* spp. isolates which were obtained from biochemical identification were tested for *L. monocytogenes* by PCR, and 15 (41.6%) of them were confirmed as *L. monocytogenes*. According to the antibiotic resistance test results of *L. monocytogenes* strains, four (26.7%) isolates were resistant to at least one antibiotic while only one (6.7%) had multiple antibiotic resistance. The highest resistance was found against trimethoprim-sulfamethoxazole. It has been determined that *L. monocytogenes* isolates had the same degree resistance against penicillin G, meropenem, amikacin and vancomycin. In conclusion, the presence of *L. monocytogenes* which has been detected in dairy products made from raw milk and in raw milk itself may constitute a potential risk for public health.

Çiğ Süt ve Süt Ürünlerinde *Listeria monocytogenes* Varlığı ve Antibiyotik Direnci

Öz

Bu çalışmada, çiğ süt ve geleneksel yöntemlerle çiğ sütten üretilen süt ürünlerinde (peynir ve tereyağ) kültürel ve moleküler teknikler kullanılarak *Listeria monocytogenes* varlığı ve antibiyotik direnci araştırıldı. İzolasyon FDA tekniğine göre yapıldı. Biyokimyasal identifikasyon için Microbact 12L test kiti kullanıldı. İzolatların genetik olarak tanımlanması klasik PCR yöntemi ile yapıldı. İzolatların antibiyotik direnci standart disk difüzyon yöntemi ile belirlendi. Biyokimyasal identifikasyon sonuçlarına göre toplamda 300 gıda örneğinin 16'sında (5.3%) *Listeria* spp. tespit edildi. *Listeria* spp. en yüksek çiğ süt örneklerinde bulunurken (%10), bunu peynir örnekleri (%4) ve (%2) tereyağı örnekleri izledi. Biyokimyasal test sonuçlarına göre elde edilen 36 adet *Listeria* spp. izolatının tümü *L monocytogenes* yönünden PCR ile test edildi ve 15'i (%41.6) *L. monocytogenes* olarak doğrulandı. *L monocytogenes* izolatlarının antibiyotik direnç test sonuçlarında izolatların dördü (%26.7) en az bir antibiyotiğe dirençli iken biri (%6.7) çoklu antibiyotik dirence sahipti. En yüksek direnç trimetoprim-sülfametoksazole karşı bulundu. *L. monocytogenes* izolatlarının penisilin G, meropenem, amikasin ve vankomisine karşı aynı oranda dirençli olduğu tespit edildi. Sonuç olarak, çiğ süt ve çiğ sütten yapılmış süt ürünlerinde tespit edilen *L. monocytogenes* varlığı halk sağlığı için potansiyel bir risk oluşturabilir.

Anahtar sözcükler: *Süt, Süt ürünü, L. monocytogenes, Listeria spp., Antibiyotik direnci*

INTRODUCTION

Listeria genus is a Gram-positive, spore-free and facultative anaerobic [1]. There are ten known pathogenic and non-pathogenic species. These include *Listeria monocytogenes*, *Listeria innocua, Listeria welshimeri, Listeria grayi, Listeria*

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seeligeri, Listeria ivanovii, Listeria marthii, Listeria rocourtiae, Listeria weihenstephanensis and *Listeria fleischmannii*. Among these, *L. monocytogenes* is the primary pathogenic species for humans and causes serious diseases [1,2].

Listeria monocytogenes is an important pathogen originating from food, which has emerged in the second half of the 20th century. *Listeria* species are commonly found in raw and unprocessed food products. Listeriosis has high mortality and morbidity rate, and is caused by various food products including soft cheese, deli meats and vegetable products [3]. *L. monocytogenes* can be found in a wide spectrum of raw and processed products. *Listeria spp*. contamination has been detected in milk and dairy products, beef, pork, meat and meat products such as fermented sausage, fresh products like radish and cabbage and in seafood and fish [4]. This bacterial infection poses a serious threat for vulnerable individuals including immunocompromised patients, newborns, elderly people and pregnant women [5]. Generally, non-invasive gastrointestinal disease is seen in individuals with strong immune system. However, invasive listeriosis infections cause septicemia, meningitis infections or abortion in pregnant women in immunocompromised individuals [6].

Antibiotic resistance is a significant problem worldwide as many forms of resistance spread rapidly. A minimum of 2 million people are infected with serious diseases caused by resistant bacteria each year in the U.S. and at least 23.000 people die as a direct result of infections caused by these antibiotic-resistant bacteria [7]. Currently, the treatment for human listeriosis is ampicillin or penicillin G combined with gentamicin. Trimethoprim-sulfamethoxazole, vancomycin and erythromycin are second choice to treat listeriosis in pregnant women [8].

In a study conducted in Turkey by Kevenk and Terzi Gülel [9], the presence of *L. monocytogenes* in a total of 210 raw milk and milk samples was investigated, and in five (5%) milk, one (5%) white cheese, three (30%) kuymak cheese, three (30%) cokelek, two (20%) farm cheese samples *Listeria monocytogenes* were positive while in kashar cheese, butter and ice cream *Listeria monocytogenes* were not detectible. Sağun et al.[10] has reported that six of the raw milk samples (2.40%) were *Listeria spp.* positive, and three (1.20%) of them were *L. monocytogenes*, one (0.40%) was *L. innocua* and one (0.40%) was found to be *L. welshimeri* positive. While 13 of the herb cheese samples (5.11%) were *Listeria spp*. positive, 10 (3.93%) of these isolated strains were also *L. monocytogenes*, one (0.39%) was *L. ivanovii*, one (0.39%) was *L. innocua* and one (0.39%) was *L. welshimeri* positive. Soyutemiz et al.[11] and Erol and Şireli [12] detected 3% and 5% *L. monocytogenes* from the raw milk, respectively. In another study, Arslan and Özdemir [13] have reported that they detected 9.2% of *L. monocytogenes* in white cheese samples.

It is estimated that 1600 people suffer from *L. monocytogenes* and 1.500 of them are hospitalized and 260 deaths are reported every year in the United States [14]. The incidence of listeriosis has been reported as 633 person among total population in Turkey of 68 million for 2003 [15]. Majority of listeriosis epidemics have been reported to occur due to the consumption of contaminating milk and dairy products [1,9,16]. About half of the reported listeriosis outbreaks in Europe have been associated with dairy products and the cause of outbreaks is mostly attributed to consumption of raw or unpasteurized milk products. Outbreaks from non-pasteurized soft cheeses in Switzerland between 1983 and 1987, from unpasteurized milks in Austria in 1986, and from Brie-type cheese in 1995 which was produced from unpasteurized milk in France put forth the risks of soft cheese consumption [17].

In this study, the presence of *L. monocytogenes* in cheese and butter produced from raw milk by traditional methods and its resistance against some antibiotics was determined.

MATERIAL and METHODS

Sampling

In this study, a total of 300 samples, including 100 samples of raw milk, 100 white cheese and 100 butter samples were obtained from different retail stores in Kars between October 2012 and March 2013. White cheese and butter samples are produced from raw milk in the traditional way which are offered for sale without any packaging. The samples were put in sterile bags and delivered to the laboratory as soon as possible under cold chain and were analyzed immediately.

Isolation of Listeria Species

For the isolation of *Listeria* species from the samples, the FDA (Food and Drug Administration) method as recommended by Hitchins [18] was used. According to this method, 25 g/25 mL sample was homogenized in 225 mL Buffered Listeria Enrichment Broth (BLEB, Oxoid, CM 897 Basingstoke, UK) medium and incubated at 30°C for 4 h. Subsequently, selective supplement (Listeria Selective Enrichment Supplement, SR0141) was added and incubation was completed to a total of 48 h. This pre-enrichment culture was inoculated to Listeria Selective Agar Base (LSA, Oxoid CM0856, Basingstoke, UK) medium consisting of Listeria Selective Supplement (Oxoid SR0140) by streak plate technique. The LSA plates were incubated at 37°C for 48-72 h. Blackish green brown colonies 2-3 mm in diameter surrounded by a black zone and having a sunken center which grew in the medium following the incubation were evaluated as suspected *Listeria* colonies. *Listeria* suspected colonies were inoculated by the streak plate method on Tryptone Soya Yeast Extract Agar (TSYEA) prepared as 30 g/L CASO Broth (Merck 1.05459), 6 g/L yeast extract (Merck 1.03753) and 15 g/L agar (Merck 1.01613) for

purification and identification procedures, and incubated at 30-37°C for 24 h.

Identification of Listeria Species

For the identification of the isolated strains, Gram-staining, oxidase (Bactident Oxidase, Merck) and catalase tests were applied to colonies growing in TSYEA. The Microbact™ 12L (MB1128A, Oxoid, Basingstoke, UK) test kit was used in order to identify Gram-positive bacil and coccobacil, oxidase negative and catalase positive colonies [19] on the basis of the species [20]. Each colony was incubated at 37°C for 24 h in a nutrient broth followed by transfer to each well in the 100 µL strain test kit. After incubation for 4-24 h, test results were recorded in the assessment form. The reaction results obtained were compared with the table provided in the kit. The results were assessed in the computer-aided identification program of Microbact 12L. As a positive control, *L. monocytogenes* ATCC 7644 strain was used in biochemical tests.

Genetic Identification of the Isolates

DNA extraction kit (QIAGEN Qlamp DNA mini kit-51304) was used for the genomic DNA extraction from the isolates. The extraction procedure was performed in accordance with the recommendations of the manufacturing company. For the amplification of the extracted DNAs, the method recommended by Aznar and Alarcon [21] was used in a modified way. The target gene for *L. monocytogenes* was selected as *hyl*A, and LM1: CCTAAGACGCCAATCGAA and LM2: AAGCGCTTGCAACTGCTC primers were used [22]. For PCR, master mix PCR buffer (10 mmol/L, Tris-HCl, pH 8.8, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.1% Triton X-100) was prepared in a total volume of 25 µL with 1 µmol/L of each primer (Methabion, Germany), 100 µmol/L of each dNTP (Sigma), 1 U of *Taq* DNA Polymerase (Sigma D1806) and 5 ng DNA/µL. Thermal conditions for PCR were applied in TProfessional Basic Thermocycler (Biometra, Model TProfessional Basic Gradient) device as initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 1 min, synthesis (extension) at 72°C for 1 min and final extension at 72°C for 5 min. 0.5 X TBE (Tris-Borate-EDTA) buffer at 1% agarose gel was used in order to observe the PCR products. *L. monocytogenes* (ATCC 7644) was used as positive control and the *hlyA* gene was visualized at 702 bp under UV illumination.

Antibiotic Resistance of the Isolates

Antimicrobial susceptibility tests of the isolates were carried out by disc diffusion method according to the Clinical and Laboratory Standards Institute [23,24]. After the isolates were activated, inoculation onto a TSA medium was performed. A suspension was prepared directly in physiological saline solution from single colonies. The density of the suspension was adjusted in a turbidity equivalent to the McFarland 0.5 standard. Inoculation was conducted on Mueller-Hinton agar containing 5% sheep blood by streaking the sterile swab over the surface. Four antibiotic discs were placed on each plate. The plates were incubated at 35°C for 24 h. After the incubation, inhibition zones on the plates were measured and recorded as resistant, intermediate and susceptible. *Staphylococcus aureus* ATCC 25293 was used as a quality control standard. Antimicrobial susceptibility test results were evaluated according to the intervals recommended by CLSI for *Staphylococcus spp* and by EUCAST for *L. monocytogenes* [23-25]. The following antibiotics were used for antimicrobial susceptibility tests: amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamycin (10 µg), meropenem (10 µg), penicillin G (10 U), rifampin (5 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), vancomycin (30 µg).

RESULTS

Isolation and Identification of Listeria spp.

Thirty-six *Listeria spp.* were isolated from a total of 300 foods having milk and milk products. As a results of the biochemical tests on these isolates using the Microbact 12 L, 22 (61.1%) *L. monocytogenes*, four (11.1%) *L. seeligeri*,

Table 1. *Identification and verification of Listeria spp isolates obtained from raw milk and dairy products*

Type of Sample	Microbact 12L							PCR	
	No. *Listeria spp*. Positive Samples	No. *Listeria spp*. Positive Isolates	*L. monocytogenes*	*L. seeligeri*	*L. ivanovii*	*L. welshimeri*	*L. innocua*	No. *L. monocytogenes* Positive Samples	No. *L. monocytogenes* Positive Isolates
Raw milk (n=100)	10 (10%)	26	16	4	5	1	-	6 (6%)	8
White cheese (n=100)	4 (4%)	8	5	-	2	-	1	3 (3%)	5
Butter (n=100)	2 (2%)	2	1	-	1	-	-	2 (2%)	2
Total (n=300)	16 (5.3%)	36	22	4	8	1	1	11 (3.7%)	15

Table 2. *Antibiotic resistance of Listeria monocytogenes isolates obtained*

Antimicrobial Agent	*Listeria monocytogenes* (%) n=15		
	R	I	S
[a]Amikacin	1 (6.7)	2 (13.3)	12 (80)
[b]Ampicillin	0 (0)	0 (0)	15 (100)
[a]Cefotaxime	0 (0)	1 (6.7)	14 (93.3)
[a]Chloramphenicol	0 (0)	0 (0)	15 (100)
[a]Ciprofloxacin	0 (0)	1 (6.7)	14 (93.3)
[b]Erythromycin	0 (0)	0 (0)	15 (100)
[a]Gentamycin	0 (0)	0 (0)	15 (100)
[b]Meropenem	1 (6.7)	0 (0)	14 (93.3)
[a]Penicillin G	1 (6.7)	0 (0)	14 (93.3)
[a]Rifampin	0 (0)	0 (0)	15 (100)
[a]Streptomycin	0 (0)	3 (20)	12 (80)
[a]Tetracycline	0 (0)	0 (0)	15 (100)
[b]SXT	4 (26.7)	0 (0)	11 (73.3)
[a]Vancomycin	1 (6.7)	0 (0)	14 (93.3)

** SXT: Trimethoprim-Sulfamethoxazole R: Resistant I: Intermediate S: Susceptible; [a] Results were evaluated for Staphylococcus spp. according to the intervals recommended by CLSI 2012, 2014; [b] Results were evaluated for L. monocytogenes according to the intervals recommended by EUCAST 2015*

12L were tested genetically for *L. monocytogenes* by PCR. *Fig. 1* shows the band obtained by PCR. Of the 36 isolates examined, 15 (41.6%) were identified as *L. monocytogenes*. According to the PCR results, 11 (3.7%) out of total 300 foodstuff *L. monocytogenes* were detected. The test results of *L. monocytogenes* using PCR were given *(Table 1)*.

Antibiotic Resistance

Antimicrobial resistance profiles of 15 isolates confirmed by PCR as *L. monocytogenes* were examined. Of the 15 *L. monocytogenes* positive isolates, four (26.7%) of them were resistant to only one antibiotic while one (6.7%) were resistant to more than one antibiotic, and 10 (66.6%) were not resistant to any antibiotics. The highest resistance was against trimethoprim-sulfamethoxazole. It has been determined that *L. monocytogenes* isolates had the same degree resistance against penicillin G, meropenem, amikacin and vancomycin. The antimicrobial resistance of *L. monocytogenes* isolates was shown in *(Table 2)*.

DISCUSSION

It has been demonstrated that milk and dairy products are responsible for approximately 50% of listeriosis epidemics and various sporadic cases in Europe [3,17,26,27]. In this study,

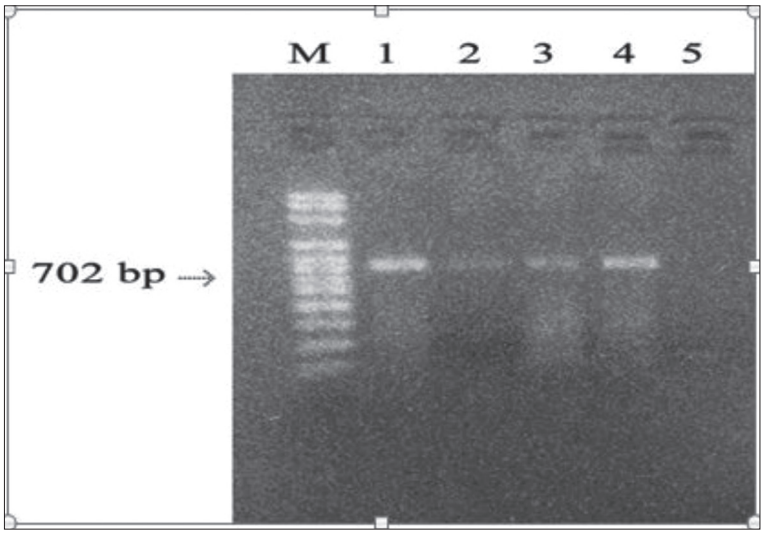

Fig 1. Amplification products obtained through PCR. Lane M: 100-bp DNA ladder, Lane 1-3: *Listeria monocytogenes* PCR amplification products, Lane 4: Positive control for *Listeria monocytogenes*, Lane 5: DNA free PCR mixture for *Listeria monocytogenes* negative control

eight (22.2%) *L. ivanovii*, one (2.7%) *L. welshimeri* and one (2.7%) was identified as *L. innocua*. When distribution of the isolates in term of foods was examined, 16 of the 300 samples (5.3%) were found to be positive for *Listeria spp. Listeria spp*. were isolated the highest from raw milk samples (10%), followed by cheese (4%) and butter samples (2%). Identification of *Listeria spp.* isolates obtained from raw milk and dairy products was shown *(Table 1)*.

Identification of Listeria monocytogenes by Molecular Techniques

A total of 36 *Listeria spp.* isolates identified by Microbat

L. monocytogenes was found to be positive in 6% of the raw milk samples. In partial consistency with our findings, Kevenk and Terzi Gülel [9], Erol and Şireli [12] and Jamali et al.[1] reported that they isolated *L. monocytogenes* from raw milk at the rates of 5%, 5% and 5.4% respectively. In contrast, Gebretsadik et al.[28] and Usman et al.[29] reported isolation of *L. monocytogenes* from raw milk at higher rates than our results revealed, i.e. at 13% and 8.3% respectively. On the other hand, Sağun et al.[10], Soyutemiz et al.[11], Rahimi et al.[16] Aygün and Pehlivanlar [30], Mahmodii [31], Shamloo et al.[32], Ünlü et al.[33] and Osman et al.[34] reported isolation of *L. monocytogenes* at the rates of 1.2%, 3%,

1.1%, 0%, 1.7-3.3%, 4.39%, 4% and 2% respectively. These results are lower than our findings.

Aygün and Pehlivanlar [30] and Mahmodii [31] reported isolation of *L. monocytogenes* from white cheese at the rates of 2.35% and 3.3-6.7% respectively (at two different production sites). Our study also determined that 3% of the white cheese samples were *L. monocytogenes* positive which is a finding close to those of the above-mentioned researchers. On the other hand, Kevenk and Terzi Gülel [9], Arslan and Özdemir [13], Rahimi et al.[16], Akkaya and Alişarlı [35], and Gülmez and Güven [36] reported isolation of *L. monocytogenes* at the rates of 5%, 9.2%, 15%, 6% and 5% respectively. These findings are higher than ours. On the other hand, Shamloo et al.[32] reported no isolation of *L. monocytogenes* in traditionally manufactured cheese samples. Karadal and Yıldırım [37] reported isolation of *L. monocytogenes* at a rate of 1% from both white cheese and tulum cheese made from raw milk by using traditional methods. These findings are lower than ours. It is observed that there are differences between the findings obtained in our study and the findings reported by other researchers. The reasons behind such difference may be the contamination level of the milk used in cheese production, environmental and seasonal differences, the differences in the traditional cheese production techniques and secondary contamination.

In our study, it was determined that 2% of the butter samples were contaminated with *L. monocytogenes*. Rahimi et al.[16] reported isolation of *L. monocytogenes* in 4% of the traditional butter samples. This result was higher than our findings. As for the findings of other researchers, Kevenk and Terzi Gülel [9], Aygün and Pehlivanlar [30], Shamloo et al.[32] and Rahimi et al.[38] reported that none of the butter samples were identified with *L. monocytogenes*. Our findings are higher than those of the above-mentioned researchers. This is considered to have resulted from the differences in the butter production techniques and especially lack of pasteurisation process. Other reasons may be cross contamination of the samples, inattentiveness to cold storage conditions during storage and marketing, and poor hygiene.

The number of *Listeria spp.* found in food products may vary depending on the type of food tested. It has been reported that the detection of *L. monocytogenes* in complex food matrices such as cheese may be more difficult than milk [16]. Thus, the present study revealed the highest level of *L. monocytogenes* contamination in raw milk followed by white cheese and butter. It is observed that various studies have obtained different results in similar products. This may be due to the isolation and identification methods used.

In general, *L. monocytogenes* is susceptible to those antimicrobials that are effective against Gram-positive bacteria whereas antimicrobial resistance against these bacteria has been reported in isolates obtained from food producing animals, food processing environments and food [6]. In this study, it has been detected that *L. monocytogenes* isolates are resistant to trimethoprim-sulfamethoxazole, penicillin G, meropenem, amikacin and vancomycine. The highest resistance has been found against trimethoprim-sulfamethoxazole. In parallel to our findings, Jamali et al.[1], Usman et al.[29], Osman et al.[34] and Harakeh et al.[39] reported resistance to trimethoprim-sulfamethoxazole. On the other hand, Karadal and Yıldırım [37] and Mackiw et al.[40] indicated no resistance to trimethoprim-sulfamethoxazole. Many researchers [1,9,13,16,29,34,38,39] reported resistance to penicillin G. In parallel to these results, our study also determined resistance to penicillin G. On the other hand, Karadal and Yıldırım [37] and Conter et al.[41] reported no resistance to penicillin G. Aydın et al.[42] reported that *L. monocytogenes* strains of food origin are resistant to meropenem, penicillin G and trimethoprim-sulfamethoxazole. In parallel to these findings, this study also determined resistance to the three antibiotics indicated. In this study, resistance to amikacin has been detected. Some researchers reported resistance to amikacin in *L. monocytogenes* isolates of food origin in parallel to our findings [43]. In our study, *L. monocytogenes* isolate with vancomycine resistance has been found. Our findings are consistent with the findings of certain researchers [9,39,41]. However, some researchers [1,16,34,38] reported that there was no vancomycine resistance. *L. monocytogenes* isolates can develop resistance by acquiring mobile genetic components such as mobilizable plasmids and conjugative transposons. Further, mutational events in chromosomal genes may play a role in giving *Listeria spp*. resistance [39].

Many variables including the number and rate of saprophytic microorganisms available in the environment, geographic regions and infected food affect genetic variety and the resistance of *L. monocytogenes* serotypes to antibiotics which in turn lead to different research results [44]. The present study also involves results which are different from those of some other researchers.

In conclusion, this study has revealed the presence of *L. monocytogenes* in raw milk and dairy products made with raw milk in the region. It has been concluded that this may constitute a potential risk to public health and that there is a need for enhancing the safety of milk and dairy products. Implementing good food and good manufacturing practices is extremely important for consumer health. It is essential to take the necessary hygienic measures during the entire process starting from the production of milk and dairy products until their consumption. In order to provide product safety, it is primarily recommended that pasteurasation conditions are fulfilled, cross contamination is prevented, cold storage conditions are complied with and especially unpackaged products are kept under inspection.

Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

1. Jamali H, Paydar M, Ismail S, Looi CY, Wong WF, Radmehr B, Abedini A: Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets. *BMC Microbiol,* 15, 144, 2015. DOI: 10.1186/s12866-015-0476-7

2. Beale DJ, Morrison PD, Palombo EA: Detection of *Listeria* in milk using nontargeted metabolic profiling of *Listeria monocytogenes*: A proof-of-concept application. *Food Control,* 42, 343-346, 2014. DOI: 10.1016/j.foodcont.2014.01.022

3. Schlech WF: Foodborne listeriosis. *Clin Infect Dis*, 31 (3): 770-775, 2000. DOI: 10.1086/314008

4. **Rocourt J, Cossart P:** *Listeria monocytogenes*. **In,** Doyle MP, Buechat LR, Montville TJ (Eds): Food Microbiology-Fundamentals and Frontiers. 337-352, DC, ASM Press, Washington, 1997.

5. Liu D: Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol,* 55, 645-659, 2006. DOI: 10.1099/jmm. 0.46495-0

6. Allen KJ, Wałecka-Zacharska E, Chen JC, Kosek-Paszkowska K, Devlieghere F, Van Meervenne EV, Osek J, Wieczorek K, Bania J: *Listeria monocytogenes*-An examination of food chain factors potentially contributing to antimicrobial resistance. *Food Microbiol*, 54, 178-189, 2016. DOI: 10.1016/j.fm.2014.08.006

7. Frieden T: Antibiotic resistance threats in the United States. U.S. Department of health and human services. Centers for disease control and prevention. https://www.cdc.gov/drugresistance/threat-report-2013/index.html; *Accessed:* 13 March 2018.

8. Wang XM, Lü XF, Yin L, Liu HF, Zhang WJ, Si W, Yu SY, Shao ML, Liu SG: Occurrence and antimicrobial susceptibility of *Listeria monocytogenes* isolates from retail raw foods. *Food Control,* 32 (1): 153-158, 2013. DOI: 10.1016/j.foodcont.2012.11.032

9. Kevenk TO, Terzi Gulel G: Prevalence, antimicrobial resistance and serotype distribution of *Listeria monocytogenes* isolated from raw milk and dairy products. *J Food Safety*, 36, 11-18, 2016. DOI: 10.1111/jfs.12208

10. Sağun E, Sancak YC, İşleyici Ö, Ekici K: The presence and prevalence of *Listeria* species in milk and herby cheese in and around Van. *Turk J Vet Anim Sci*, 25, 15-19, 2001.

11. Soyutemiz E, Çetinkaya F, Özakın C, Gedikoğlu S: Presence of *Listeria monocytogenes* in raw milk samples from West Anatolia. *Turk J Infect*, 15, 5-9, 2001.

12. Erol I, Şireli UT: 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, 2002.

13. Arslan S, Özdemir F: Prevalence and antimicrobial resistance of *Listeria spp*. in homemade white cheese. *Food Control*, 19 (4): 360-363, 2008. DOI: 10.1016/j.foodcont. 2007.04.009

14. Centers for Disease Control and Prevention (CDC): Centers for Disease Control and Prevention National Enteric Disease Surveillance. Surveillance System Overview: The *Listeria* Initiative. February 2016. http://www.cdc.gov/listeria/surveillance.html; *Accessed:* 21 February 2018.

15. Anonymous: Statistics by country for Listeriosis, Incidence (annual) of Listeriosis, http://www.rightdiagnosis.com/l/listeriosis/stats-country.htm; *Accesed:* 21 February, 2018.

16. Rahimi E, Ameri M, Momtaz H: Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control,* 21 (11): 1448-1452. 2010. DOI: 10.1016/j.foodcont.2010.03.014

17. Lundén J, Tolvanen R, Korkeala H: Human Listeriosis outbreaks linked to dairy products in Europe. *JDS*, 87, E6-E12, 2004. DOI: 10.3168/jds.S0022-0302(04)70056-9

18. Hitchins AD: *Listeria monocytogenes*. **In,** FDA Bacteriological Analytical Manual. 7th edn., 148, Arlington VA: AOAC Int, 1992.

19. Harrigan WF: Laboratory Methods in Food Microbiology. 3rd edn., 1-532, Academic Press, California, USA, 1998.

20. Murtiningsih S, Cox JM: Evaluation of the Serobact™ and Microbact™ systems for the detection and identification of *Listeria* spp. *Food Control,* 8 (4): 205-210, 1997. DOI: 10.1016/S0956-7135(97)00042-X

21. Aznar R, Alarcon B: PCR detection of *Listeria monocytogenes*: A study of multiple factors affecting sensitivity. *J Appl Microbiol,* 95 (5): 958-966, 2003. DOI: 10.1046/j.1365-2672.2003.02066.x

22. Border PM, Howard JJ, Plastow GS, Siggens KW: Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Lett Appl Microbiol,* 11 (3): 158-162, 1990. DOI: 10.1111/j.1472-765X.1990.tb00149.x

23. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing; twenty second informational supplement (Vol. 32, No. 3). Wayne, PA, USA: Clinical and Laboratory Standards Institute, 2012. M100-S22.

24. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing; twenty forth informational supplement (Vol. 34, No. 1). Wayne, PA, USA: Clinical and Laboratory Standards Institute, 2014. M100-S24.

25. European Committee on Antimicrobial Susceptibility Testing (EUCAST): Break point tables for interpretation of MIC and zone diameter. Version 5.0, valid from 2015-01-01.

26. De Buyser ML, Dufour B, Maire M, Lafarge V: Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *Int J Food Microbiol,* 67, 1-17, 2001. DOI: 10.1016/S0168-1605(01)00443-3

27. Parisi A, Latorre L, Fraccalvieri R, Miccolupo A, Normanno G, Caruso M, Santagada G: Occurrence of *Listeria* spp. in dairy plants in Southern Italy and molecular subtyping of isolates using AFLP. *Food Control,* 29, 91-97, 2013. DOI: 10.1016/j.foodcont. 2012.05.036

28. Gebretsadik S, Kassa T, Alemayehu H, Huruy K, Kebede N: Isolation and characterization of *Listeria monocytogenes* and other *Listeria* species in foods of animal origin in Addis Ababa, Ethiopia. *J Infect Publ Health,* 4, 22-29, 2011. DOI: 10.1016/j.jiph.2010.10.002

29. Usman UB, Kwaga JKP, Kabir J, Olonitola OS: Isolation and antimicrobial susceptability of *Listeria monocytogenes* from raw milk and milk products in Northern Kaduna State, Nigeria. *J Appl Environ Microbiol*, 4 (3): 46-54, 2016. DOI: 10.12691/jaem-4-3-1

30. Aygün O, Pehlivanlar S: *Listeria spp*. in raw milk and dairy products in Antakya, Turkey. *Food Control,* 17, 676-679, 2006. DOI: 10.1016/j.foodcont.2005.09.014

31. Mahmoodi MM: Occurrence of *Listeria monocytogenes* in raw milk and dairy products in Noorabad, Iran. *J Anim Vet Adv*, 9, 16-19, 2010. DOI: 10.3923/javaa.2010.16.19

32. Shamloo E, Jalali M, Mirlohi, M, Madani G, Metcalf D, Merasi MR: Prevalence of *Listeria* species in raw milk and traditional dairy products in Isfahan, Iran. *Int J Env Health Eng,* 4 (1): 1-5, 2015. DOI: 10.4103/2277-9183.150384

33. Vardar Ünlü G, Ünlü M, Bakıcı MZ: Incıdence of *Listeria spp*. from raw milk in Sivas. *Tr J Med Sci*, 28, 389-392, 1998.

34. Osman KM, Samir A, Abo-Shama UH, Mohamed EH, Orabi A, Zolnikov T: Determination of virulence and antibiotic resistance pattern of biofilm producing *Listeria* species isolated from retail raw milk. *BMC Microbiol,*16, 263, 2016. DOI: 10.1186/s12866-016-0880-7

35. Akkaya L, Alişarlı M: Afyonkarahisar'da tüketime sunulan peynirlerde *Listeria monocytogenes* ve *Salmonella* spp. varlığının belirlenmesi. *YYÜ Vet*

Fak Derg, 17 (1-2): 87-91, 2006.

36. Gülmez M, Güven A: Investigetion of *Campylobacter*, *Salmonella* and *Listeria* spp. from Turkish white and çeçil cheese. *Kafkas Univ Vet Fak Derg,* 7 (2): 155-161, 2001.

37. Karadal F, Yıldırım Y: Antimicrobial susceptibility and serotype distribution of *Listeria monocytogenes* isolates obtained from raw milk cheese samples sold in Niğde. *Ankara Üniv Vet Fak Derg,* 61, 255-260, 2014.

38. Rahimi E, Momtaz H, Sharifzadeh A, Behzadnia A, Ashtari MS, Esfahani ZS, Riahi M, Momeni M: Prevalence and antimicrobial resistance of *Listeria* species isolated from traditional dairy products in Chahar Mahal & Bakhtiyari, Iran. *Bulgarian J Vet Med,* 15 (2): 115-122, 2012.

39. Harakeh S, Saleh I, Zouhairi O, Baydoun, E, Barbour E, Alwan N: Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. *Sci Total Environ,* 407 (13): 4022-4027, 2009. DOI: 10.1016/j.scitotenv.2009.04.010

40. Mackiw E, Modzelewska M, Maka L, Sciezynska H, Pawłowska K, Postupolski J, Korsak D: Antimicrobial resistance profiles of *Listeria monocytogenes* isolated from ready-to-eat products in Poland in 2007-2011. *Food Control,* 59, 7-11, 2016. DOI: 10.1016/j.foodcont. 2015.05.011

41. Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianier A: Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiol,* 128 (3): 497-500, 2009. DOI: 10.1016/j.ijfoodmicro.2008.10.018

42. Aydın A, Sudağıdan M, Çoban A: Gıda kaynaklı *Listeria monocytogenes* suşlarında antibiyotik ve ağır metal direnci. *7. Veteriner Gıda Hijyeni Kongresi,* 4-8 Ekim, Kuşadası, İzmir, Turkey, 2017.

43. Wang K, Ye K, Zhu Y, Huang Y, Wang G, Wang H, Zhou G: Prevalence, antimicrobial resistance and genetic diversity of *Listeria monocytogenes* isolated from chilled pork in Nanjing, China. *LWT - Food Sci Technol*, 64, 905-910, 2015. DOI: 10.1016/j.lwt.2015.06.015

44. Dümen E, Issa G, İkiz S, Bağcıgil F, Özgür Y, Kahraman T, Ergin S, Yeşil O: Determining existance and antibiotic susceptibility status of *Listeria monocytogenes* isolated from dairy products, serological and moleculer typing of the isolates. *Kafkas Univ Vet Fak Derg,* 17 (Suppl.-A): S111-S119, 2011. DOI: 10.9775/kvfd. 2010.3632