PAPER

OCCURRENCE OF *LISTERIA* SPP. AND ANTIBIOTIC RESISTANCE PROFILES OF *LISTERIA MONOCYTOGENES* FROM RAW MEAT AT RETAIL IN TURKEY

P. ŞANLIBABA*[1], B. UYMAZ TEZEL[2], G.A. ÇAKMAK[3], R. KESKİN[4] and M. AKÇELİK[5]

[1]Ankara University, Engineering Faculty, Department of Food Engineering, 50th Year Settlement, 06830 Gölbaşı, Ankara, Turkey

[2]Çanakkale Onsekiz Mart University, Bayramiç Vocational School, Food Technology Program, 17700 Bayramiç, Çanakkale, Turkey

[3]Akdeniz University, Department of Food Engineering, Faculty of Engineering, 07058 Antalya, Turkey

[4]Department of Food Engineering, Engineering Faculty, Ankara University, 50th Year Settlement, 06830 Gölbaşı, Ankara, Turkey

[5]Ankara University, Faculty of Science, Department of Biology, 06100 Ankara, Turkey

*Corresponding author: sanlibab@ankara.edu.tr

ABSTRACT

A total of 190 raw meat samples were collected in Ankara to examine the presence of *Listeria* spp. and *L. monocytogenes* and its antibiotic resistance. Of the examined samples, 57 were positive for *Listeria* spp. and among them, 23 were identified as *L. monocytogenes*. Among *L. monocytogenes* strains, 86.96% of isolates were positive for the presence of the *hly*A gene. All *L. monocytogenes* strains were resistant to ampicillin, fosfomycin, nalidixic acid, linezolid, and clindamycin. Multi-drug resistance was observed in 73.91% *L. monocytogenes* strains. In conclusion, the presence of *L. monocytogenes* in raw meat may be indicative of poor hygiene or cross-contamination.

Keywords: *Listeria monocytogenes*, prevalence, antibiotic resistance, raw meat

1. INTRODUCTION

Listeria spp. are Gram-positive, facultatively anaerobic, non-spore forming, rod-shaped bacteria with low G + C content and motile at 10–25°C (NAYAK *et al*., 2015; CORONEO *et al*., 2016). So far, twenty-one species of the genus have been identified (NCBI, 2019). Of them, *Listeria monocytogenes* has been known as the main causative agent of listeriosis in human and other mammals since the 1920s, while *L. ivanovii* is an animal pathogen and rarely infects humans (DOYLE *et al*., 2001; FALLAH *et al*., 2012). The overall rate of listeriosis in humans is low but it can be lethal for high-risk groups like pregnant women, unborn or newly delivered infants, elderly people, severe underlying disease conditions like immune-suppression, organ transplants, patients undergoing treatment for cancer, and AIDS (KHEN *et al*., 2015; ABAY *et al*., 2017; DU *et al*., 2017).

It is difficult to control of contamination by *L. monocytogenes* as it can grow at refrigeration temperature, low oxygen levels, high salt concentration (20% w/v), wide pH values ranging from 4.3 to 9.6, low water content, and hypoxic conditions (ALMEIDA *et al*., 2013; DU *et al*., 2017; KURPAS *et al*., 2018; NOLL *et al*., 2018). This pathogen is also able to survive in vacuum-packed food and modified atmospheres (LAMBERTZ *et al*., 2012). *L. monocytogenes* is a widely distributed in the environment such as soil, contaminated silage, feces of some animals, and in non-treated water. Therefore, it can easily contaminate food products of both animal and plant origin (PESAVENTO *et al*., 2010; ŞANLIBABA *et al*., 2018a). *L. monocytogenes* is also commonly found in food processing environments and various food items including ready-to-eat (RTE) foods, milk and dairy products, meat and its products, unwashed raw vegetables, seafood products, and poultry products (FALLAH *et al*., 2012). *L. monocytogenes* is of particular concern in raw, undercooked or RTE foodstuffs because processed foods are easily contaminated with raw foods in the food-processing environment or at homes (AL-NABUSI *et al*., 2015; OYELAMI *et al*., 2018). Raw meat, such as red meat and chicken, may become contaminated with *L. monocytogenes* either environmentally or during shipping and prolonged storage, particularly if they are stored at above 4°C. The additional handling of raw meats at the retail also results in the transmission of *L. monocytogenes* to raw meats mainly via slicing, weighing, and packaging (KOVACEVIC *et al*., 2012; KURPAS *et al*., 2018). The main concern in raw meat is that it contaminated with *L. monocytogenes* can infect processed foods (PESAVENTO *et al*., 2010).

Antimicrobial resistance (AMR) in foodborne pathogens, especially multidrug resistance, is a great concern for human and animal health at both national and international levels (ALONSO-HERNANDO *et al*., 2012; DU *et al*., 2017). It has been reported that about 33000 deaths occur each year in the European Union due to AMR food-borne pathogens (ANON., 2019a). *L. monocytogenes* is naturally susceptible to a wide range of antibiotics that act on Gram-positive bacteria (GOMEZ *et al*., 2014; WANG *et al*., 2015; BYRNE *et al*., 2016; MOHAMED *et al*., 2016). However, since the first documented report on multi-drug resistant *L. monocytogenes* strain isolated from a patient with meningoencephalitis in France in 1988 (WANG *et al*., 2013; NOLL *et al*., 2018), many strains isolated from food and environmental and clinical samples have shown resistance to one or more antibiotics used for treating listeriosis (ŞANLIBABA *et al*., 2018a). The use of antimicrobials in veterinary medicine is the main cause of the development of AMR foodborne bacterial pathogens including *L. monocytogenes*, as AMR pathogens can easily be transported from animal to human via food consumptions (CONTER *et al*., 2009). The genes responsible for antibiotic resistance could be transferred through movable genetic elements such as conjugative transposons, mobilizable plasmids, and self-transferable plasmids to other foodborne bacteria in the gastrointestinal tract. In *Listeria* spp., efflux pumps have also been reported

as the resistant mechanism (LUNGU *et al*., 2011). *Enterococcus* spp. and *Staphylococcus* spp. serve as a reservoir of resistance genes for *L. monocytogenes* (NATRATILOVA *et al*., 2004). Many studies have been focused on the prevalence of *L. monocytogenes* from raw meat and their antibiotic resistance from the different parts of the world (DIMIC *et al*., 2010; PESAVENTO *et al*., 2010; INDRAWATTANA *et al*., 2011; OSAILI *et al*., 2011; GOMEZ *et al*., 2014; AL-NABUSI *et al*., 2015). However, most of the earlier studies carried out in Turkey (AKPOLAT, 2004; YÜCEL *et al*., 2005; CEYLAN *et al*., 2008; EROL and AYAZ, 2011; DOGRUER *et al*., 2015; ABAY *et al*., 2017; KOCAMAN and SARIMEHMETOĞLU, 2017; ŞANLIBABA *et al*., 2018a; ŞANLIBABA *et al*., 2018b) have been focused on RTE foods, dairy products, vegetables, cooked meat, and poultry. The information on the occurrence of *Listeria* spp., particularly *L. monocytogenes* strains isolated from raw meat and their antimicrobial resistance, is limited. Therefore, the primary aim of this study was: i) to determine the incidence of *Listeria* spp., particularly *L. monocytogenes*, in raw meat samples (chicken meat and red meat) sold in Ankara, Turkey, ii) to identify the isolated strains by phenotypic and genotypic methods, and iii) to assess resistance in the isolated strains against 34 different antibiotics used for treating listeriosis.

2. MATERIALS AND METHODS

2.1. Collection of samples

A total of 190 raw meat samples were randomly purchased from various supermarkets and butchers in the capital city of Turkey over the period of January to April 2018. Sampling locations were randomly selected. The samples were collected only once from each place. These samples consisted of: 1) 80 samples of red meat (minced beef, sliced lamb, and meat cubes) and, 2) 110 samples of chicken meat (legs and wings). All of the samples were non-frozen and kept at refrigeration condition in the retail outlets. While chicken samples analyzed were prepackaged including vacuum and normal atmosphere of packaging, red meat samples were non-packaged. All of the samples were checked for expiry dates and transported to the laboratory under aseptic and refrigerated conditions (+4°C) on the sampling day.

2.2. Isolation and identification of *Listeria* spp.

The two-stage enrichment method, described by the International Organization for Standardization (EN ISO, 11290-1), was used for isolation and identification of *Listeria* spp. Briefly, 25 g of each sample was aseptically weighed and mixed with 225 mL half strength Fraser broth (Merck™, Germany) containing selective supplements as the first enrichment culture in a stomacher bag and homogenized in a stomacher (Seward 400, USA) for 2 min. The homogenized sample was incubated at 30±1°C for 24±2 h. Thereafter, 0.1 mL of pre-enriched Fraser broth was inoculated into 10 mL of full-strength Fraser broth containing selective supplements for second enrichment culture and incubated at 37°C for 48±2 h. After the enrichment procedure, a loopful each of the half- and full-strength Fraser broths was plated on the chromogenic *Listeria* agar (ALOA Agar) (Merck™, Germany) and polymixin acriflavine lithium chloride ceftazidime aesculin mannitol (PALCAM) agar (Merck™, Germany). The plates were incubated at 37°C for 24–48 h. The light blue colonies surrounded by an opaque halo on ALOA agar and gray-green colonies surrounded by

diffuse black zone on PALCAM agar were considered to be of *Listeria* spp. Five presumptive colonies were picked up and further purified on Tryptic Soy agar supplemented with 0.6% of yeast extract (TSA-YE) (Sigma™, Germany) as a non-selective medium. Subsequently, the pinpoint colonies of TSA-YE were subjected to identification procedures, which included Gram's staining, catalase reactions, oxidase tests, carbohydrate utilization, CAMP tests with *S. aureus* and *R. equi*, and motility at 20-25°C.
The isolated *Listeria* species and the reference strains used in this study were inoculated on Tryptic Soy Broth supplemented with 0.6% of yeast extract (TSB-YE) (Sigma™, Germany) and Brain Heart Infusion (BHI) broth (Merck™, Germany) and incubated at 35°C for 24 h. All of the strains used in this study were stored at –20°C with 30% (v/v) glycerol (Merck™, Germany) throughout the study period. The reference strains were obtained from the culture collection of Food Microbiology Laboratory, Department of Food Engineering, Ankara University, Ankara, Turkey.

2.3. Molecular identification of *Listeria* spp. and *L. monocytogenes*

The isolates were subjected to polymerase chain reaction (PCR) analysis to confirm their identity as *Listeria* spp. The genomic DNA of the strains grown at 35°C overnight in TSB-YE was extracted using a genomic DNA extraction kit (Thermo Fisher Scientific™), according to the manufacturer's instructions. The following reference strains were used: *L. monocytogenes* ATCC7644, *Listeria innocua* ATCC12612, *Listeria seeligeri* SLCC3945, *Listeria welshimeri* ATCC35897. The primer pairs designated as U1 (5'–CAGCMGCCGCGGTAATWC–3') and LI1 (5'–CTCCATAAAGGTGACCCT–3'), amplifying a 938-bp region in the 16S rRNA gene sequence of the *Listeri*a genus, were used (USMAN *et al*., 2016). In order to detect the presence of the *hly*A gene, an additional PCR was performed, with DG69 (5'–GTGCCGCCAAGAAAAGGTTA–3') and DG74 (5'-CGCCACACTTGAGATAT–3') as primers specifically amplifying a 636-bp fragment of the *hly*A gene (FALLAH *et al*., 2013). A standardized PCR protocol was followed for the bacterial lysates, in a final volume of 50 μL reaction mixture containing 5 μL PCR buffer, 1 μL 2 mM dNTP mix, 1 μL of each forward and reverse primers, 34.75 μL of sterile distilled water, 0.25 μL of Tag DNA polymerase, 4 μL of 25 mM $MgCl_2$ and 3 μL of the DNA template (BLAIOTTA *et al*., 2002). The PCR amplification was performed in a programmed thermocycler (Techne TC–512, Staffordshire, UK). The PCR conditions were as follows: an initial hold of 2 min at 95°C, followed by 35 cycles each of 45 s denaturation at 95°C, 45 s annealing at 55°C and 2 min extension at 72°C, followed by a final extension for 7 min at 72°C and hold at 4°C. The PCR products were electrophoresed on 1% agarose gels and then stained with ethidium bromide solution (0.5 μg/mL). An O'Gene Ruler™ 10000 bp DNA molecular weight ladder (Fermentas™, Finland) was used as a standard. The gels were visualized under UV light using a Kodak Gel Logic 200 Imaging System (Kodak, USA). Amplified PCR fragments were purified by the PCR purification kit (Thermo Fisher Scientific™) and were sequenced by REFGEN Biotechnology (Ankara, Turkey). Basic local alignment search tool BLAST) was used to compare the sequences against the nucleotide database in National Centre for Biotechnology Information (NCBI) (www. ncbi.nlm.nih.gov.tr/BLAST).

2.4. Antibiotic resistance of *L. monocytogenes* strains

Resistance to different antibiotics was determined for all *Listeria* isolates by the disc diffusion method using Mueller-Hinton agar (Merck™, Germany) as the medium, containing 0.5% defibrinated sheep blood, as described by the Clinical and Laboratory Standards Institute (CLSI) (2011). The selected antibiotics are the ones, commonly used in veterinary and human medicine against listeriosis. The following antibiotic discs were used: penicillin G (10 μg/disc), oxacillin (1 μg/disc), cefotaxime (30 μg/disc), fosfomycin (50 μg/disc), cephalothin (30 μg/disc), furazolidone (50 μg/disc), piperacillin (30 μg/disc), cefuroxime (30 μg/disc), cefoxitin (30 μg/disc), ampicillin (10 μg/disc), amoxicillin/clavulanic acid (20/10 μg/disc), erythromycin (15 μg/disc), clarithromycin (15 μg/disc), tetracycline (30 μg/disc), tigecycline (15 μg/disc), moxifloxacin (5 μg/disc), neomycin (10 μg/disc), ciprofloxacin (5 μg/disc), enrofloxacin (5 μg/disc), levofloxacin (5 μg/disc), nalidixic acid (30 μg/disc), linezolid (30 μg/disc), kanamycin (30 μg/disc), streptomycin (300 μg/disc), gentamicin (120 μg/disc), vancomycin (30 μg/disc), teicoplanin (30 μg/disc), meropenem (10 μg/disc), imipenem (10 μg/disc), clindamycin (2 μg/disc), trimethoprim (5 μg/disc), trimethoprim/sulfamethoxazole (1.25/23.75 μg/disc), chloramphenicol (30 μg/disc), and rifampicin (5 μg/disc). After the incubation at 35°C for 24 h, the diameters of the inhibition zones around each disc were measured. On the basis of the comparative results, the strains were categorized as susceptible, intermediate, or resistant as per the criteria established by CLSI (2011). The breakpoints given for *Staphylococcus* species were used to determine the antibiotic resistance profile of *L. monocytogenes*, as currently there are no resistance criteria given in the CLSI guidelines for *Listeria* spp. (WANG *et al*., 2013; KHEN *et al*., 2015; DU *et al*., 2017; KUAN *et al*., 2017). *E. coli* ATCC25922, *S. aureus* ATCC6538, and *L. monocytogenes* ATCC7644 were used as quality control strains.

2.5. Dendogram construction method

The sequences were aligned with the Multiple Sequence Alignment by CLUSTALW and neighbor-joining method was used for phylogenetic tree.

2.6. Statistical analysis

All statistical analyses were carried out using SPSS 16 package. The analysis of one-way variance (ANOVA) followed by Tukey's test was applied to determine the differences in the prevalence of *Listeria* spp. between the red meat and chicken samples, and also between the antibiotic resistance of *L. monocytogenes* strains. The statistical significance was set at $p<0.05$.

3. RESULTS

3.1. Occurrence and incidence of *Listeria* spp. and *L. monocytogenes*

A total of 190 samples were examined for the presence of *Listeria* spp. using a two-step selective enrichment method as recommended by EN ISO 11290-1. The occurrence of *Listeria* spp. and *L. monocytogenes* in raw meat samples marketed in Turkey is presented in Table 1. *Listeria* spp. was detected in 57 (30.00%) of the samples. The 16S rRNA sequence

analysis indicated *L. monocytogenes* (12.10%; 23/190) to be the most prevalent in the raw meat samples, followed by *L. innocua* (11.05%; 21/190), *L. welshimeri* (6.31%; 12/190), and *L. seeligeri* (0.52%; 1/190). Using neighbour joining method, phylogenetic relationships of 57 *Listeria* spp. were allowed to group into two main clusters. Cluster 1 was composed of 1 isolate. Fifty-six isolates were belonged to cluster 2 (Fig. 1). In addition, *L. monocytogenes* strains were also screened for the virulence-associated *hly*A gene. Among the 23 *L. monocytogenes* strains, only 20 (86.96%) were positive for the presence of the *hly*A gene. LP6, LP16, and LP54 strains of *L. monocytogenes*, isolated from raw chicken samples, were identified as atypical strains since they were devoid of the *hly*A gene.

The prevalence of *Listeria* spp. in the raw chicken meat samples was as follows: 14.54% (16/110) for *L. monocytogenes*, 11.81% (13/110) for *L. innocua*, 6.36% (7/110) for *L. welshimeri*, and 0.90% (1/110) for *L. seeligeri*. On the other hand, the prevalence in the raw red meat samples was as follows: 10.00% (8/80) for *L. innocua*, 8.75% (7/80) for *L. monocytogenes*, and 6.25% (5/80) for *L. welshimeri*.

The samples of raw chicken had the highest occurrence of *Listeria* spp. (33.63%, 37/190), followed by red meat (25.00%, 20/190). In the prevalence of *L. monocytogenes* was significantly higher ($p<0.05$) in the raw chicken meat than that in the raw red meat.

Table 1. Prevalence of *Listeria* species in raw meat samples.

Food Samples	Number of Samples (n)	Number of Positive Samples n (%)				
		***Listeria* spp.**	***L. monocytogenes***	***L. innocua***	***L. welshimeri***	***L. seeligeri***
Raw red meat	80	20 (25.00)	7 (8.75)	8 (10.00)	5 (6.25)	0 (–)[a]
Raw chicken meat	110	37 (33.63)	16 (14.54)	13 (11.81)	7 (6.36)	1 (0.90)
Totals	190	57 (30.00)	23 (12.10)	21 (11.05)	12 (6.31)	1 (0.52)

[a] Not detected.

3.2. Antibiotic resistance of *L. monocytogenes* strains

The antimicrobial resistance of the 23 *L. monocytogenes* strains against 34 different antibiotics was examined using the disk diffusion method according to CLSI (2011) (Table 2). Of the *L. monocytogenes* isolates, 23 were resistant to ampicillin, fosfomycin, nalidixic acid, linezolid, and clindamycin. Frequent resistance was seen against piperacillin (86.96%, 20/23), oxacillin (82.61%, 19/23), kanamycin (82.61%, 19/23), neomycin (78.26%, 18/23), penicillin G (73.92%, 17/23), amoxicillin/clavulanic acid (73.92%, 17/23), levofloxacin (73.92%, 17/23), teicoplanin (73.92%, 17/23), moxifloxacin (69.57%, 16/23), ciprofloxacin (69.57%, 16/23), and furazolidone (52.17%, 12/23). Furthermore, resistance to enrofloxacin (47.83%, 11/23), rifampicin (17.39%, 4/23), streptomycin (17.39%, 4/23), tigecycline (13.04%, 3/23), cefuroxime (13.04%, 3/23), cephalothin (13.04%, 3/23), trimethoprim/sulfamethoxazole (13.04%, 3/23), and cefotaxime (8.69%, 2/23) was also observed. At least one strain was resistant against either of tetracycline, gentamicin, and meropenem (4.35%). In contrast, all strains were susceptible to cefoxitin, erythromycin, clarithromycin, vancomycin, imipenem, trimethoprim, and chloramphenicol. The differences between the antibiotic resistance of *L. monocytogenes* strains were not found to be statistical significance ($p > 0.05$).

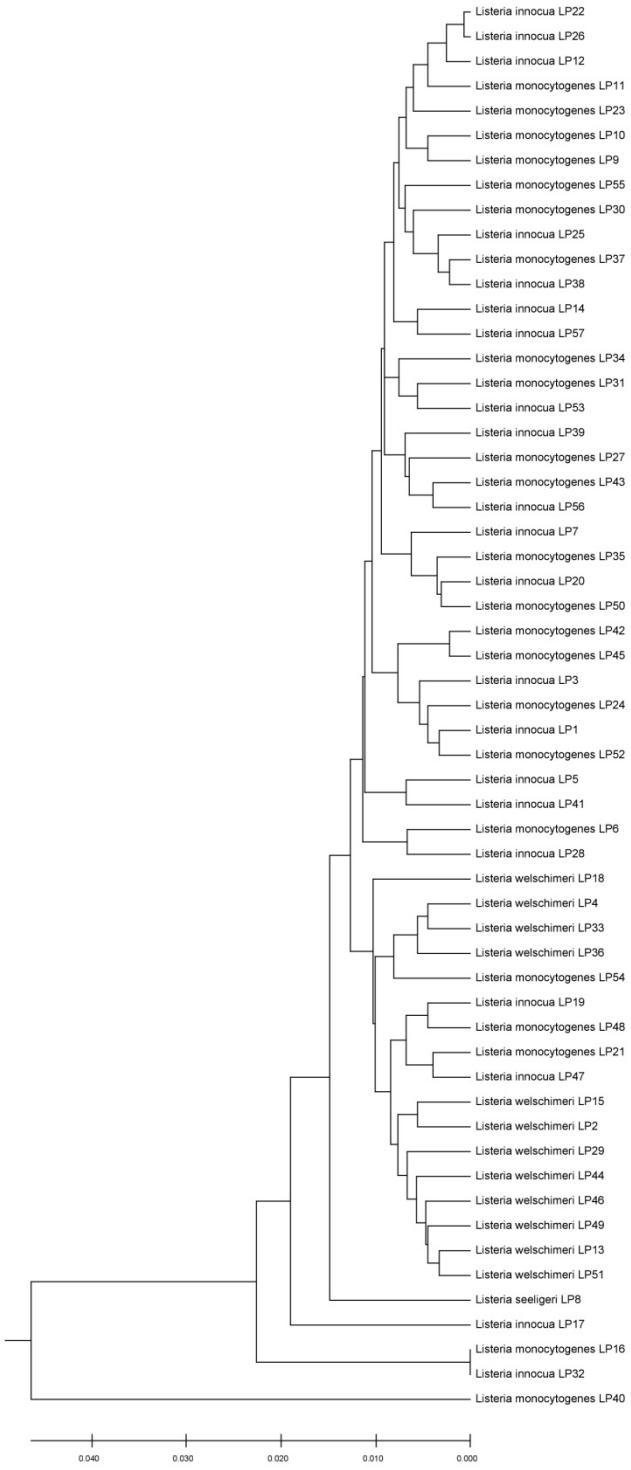

Figure 1. Dendrogram showing the evolutionary relationships among *Listeria* isolates based on the 16S rRNA sequence analysis.

Table 2. Antibiotic susceptibility and resistance (%) of *L. monocytogenes* strains isolated from raw red meat and chicken meat samples.

Antimicrobial Agent[a]	*L. monocytogenes* Strains																	
	RAW RED MEAT (n: 7)						RAW CHICKEN MEAT (n:16)						TOTALS (n:23)					
	S[b]		I[b]		R[b]		S[b]		I[b]		R[b]		S[b]		I[b]		R[b]	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Penicillins																		
Penicillin G	-[c]	-	2	28.57	5	71.43	2	12.50	2	12.50	12	75.00	2	8.69	4	17.39	17	73.92
Oxacillin	-	-	1	14.29	6	85.71	-	-	3	18.75	13	81.25	-	-	4	17.39	19	82.61
Ampicillin	-	-	-	-	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00
Amoxicillin/clavulanic acid	1	14.29	2	28.57	4	57.14	2	12.50	1	6.25	13	81.25	3	13.04	3	13.04	17	73.92
Piperacillin	2	28.57	-	-	5	71.43	1	6.25	-	-	15	93.75	3	13.04	-	-	20	86.96
Cephalosporins																		
Cephalothin	4	57.14	1	14.29	2	28.57	15	93.75	-	-	1	6.25	19	82.61	1	4.35	3	13.04
Cefotaxime	5	71.43	2	28.57	-	-	13	81.25	1	6.25	2	12.50	18	78.27	2	13.04	2	8.69
Cefuroxime	6	85.71	-	-	1	14.29	14	87.50	-	-	2	12.50	20	86.96	-	-	3	13.04
Cefoxitin	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00	-	-	-	-
Macrolides																		
Erythromycin	6	85.71	1	14.29	-	-	10	62.50	6	37.50	-	-	16	69.57	7	30.43	-	-
Clarithromycin	5	71.43	2	28.57	-	-	14	87.50	2	12.50	-	-	19	82.61	4	17.39	-	-
Tetracyclines																		
Tetracycline	5	71.43	1	14.29	1	14.29	14	87.50	2	12.50	-	-	19	82.61	3	13.04	1	4.35
Tigecycline	6	85.71	-	-	1	14.29	14	87.50	-	-	2	12.50	20	86.96	-	-	3	13.04
Quinolones																		
Ciprofloxacin	-	-	2	28.57	5	71.43	2	12.50	3	18.75	11	68.75	2	8.69	5	21.74	16	69.57
Levofloxacin	1	14.29	2	28.57	4	57.14	1	6.25	2	12.50	13	81.25	2	8.69	4	17.39	17	73.92
Nalidixic acid	-	-	-	-	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00
Moxifloxacin	-	-	3	42.86	4	57.14	4	25.00	-	-	12	75.00	4	17.39	3	13.04	16	69.57
Enrofloxacin	4	57.14	2	28.57	1	14.29	3	18.75	3	18.75	10	62.50	7	30.43	5	21.74	11	47.83
Monurol																		
Fosfomycin	-	-	-	-	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00

[a]The diameters of the zones were compared with the diameters of the Clinic Laboratory Standards Institute (CLSI 2011).

[b]S : Susceptible, I : Intermediately resistant, R : Resistant.

[c]Not detected.

Table 2. Antibiotic susceptibility and resistance (%) of *L. monocytogenes* strains isolated from raw red meat and chicken meat samples (Continued).

Antimicrobial Agent[a]	*L. monocytogenes* Strains																	
	RAW RED MEAT (n: 7)						RAW CHICKEN MEAT (n:16)						TOTALS (n:23)					
	S[b]		I[b]		R[b]		S[b]		I[b]		R[b]		S[b]		I[b]		R[b]	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Oxazolidinones																		
Linezolid	-	-	-	-	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00
Aminoglycosides																		
Kanamycin	-	-	1	14.29	6	85.71	-	-	3	18.75	13	81.25	-	-	4	17.39	19	82.61
Streptomycin	4	57.14	2	28.57	1	14.29	12	75.00	1	6.25	3	18.75	16	69.57	3	13.04	4	17.39
Gentamicin	5	71.43	2	28.57	-	-	10	62.50	5	31.25	1	6.25	15	65.22	7	30.43	1	4.35
Neomycin	1	14.29	-	-	6	85.71	4	25.00	-	-	12	75.00	5	21.74	-	-	18	78.26
Glycopeptides																		
Vancomycin	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00	-	-	-	-
Teicoplanin	-	-	2	28.57	5	71.43	-	-	4	25.00	12	75.00	-	-	6	26.08	17	73.92
Carbapenems																		
Meropenem	5	71.43	1	14.29	1	14.29	11	68.75	5	31.25	-	-	16	69.57	6	26.08	1	4.35
Imipenem	7	100.00	-	-	-	-	15	93.75	1	6.25	-	-	22	95.65	1	4.35	-	-
Lincosamides																		
Clindamycin	-	-	-	-	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00
Sulfonamides/trimethoprim																		
Trimethoprim	4	57.14	3	42.86	-	-	14	87.50	2	12.50	-	-	18	78.26	5	21.74	-	-
Trimethoprim/Sulfamethoxazole	5	71.43	1	14.29	1	14.29	10	62.50	4	25.00	2	12.50	15	65.22	5	21.74	3	13.04
Amphenicol																		
Chloramphenicol	7	100.00	-	-	-	-	16	100.00	-	-	-	-	23	100.00	-	-	-	-
Rifamycins																		
Rifampicin	2	28.57	3	42.86	2	28.57	9	56.25	5	31.25	2	12.50	11	47.83	8	34.78	4	17.39
Nitrofurans																		
Furazolidone	2	28.57	-	-	5	71.43	8	50.00	1	6.25	7	43.75	10	43.48	1	4.35	12	52.17

The diameters of the zones were compared with the diameters of the Clinic Laboratory Standards Institute (CLSI 2011).

[b]S : Susceptible, I : Intermediately resistant, R : Resistant.

[c]Not detected.

Multi-drug resistance, i.e., resistance to three or more antimicrobial agents, was observed in 73.91% (17/23) *L. monocytogenes* strains. While 69.56% (16/23) of the *L. monocytogenes* strains were resistant to four antibiotics, and 60.86% (14/23) of the isolates were resistant to five antibiotics. On the whole, 13 of 23 (56.52%) *L. monocytogenes* strains showed resistance to more than six antibiotics.

4. DISCUSSION

4.1. Prevalence of *Listeria* spp. and *L. monocytogenes* from raw meat samples

As a result of its psychotropic nature, *L. monocytogenes* is of great concern to the meat industry. *L. monocytogenes* contamination can occur in raw meat during processing and storage (ABAY *et al*., 2017). This initial contamination can spread, propagate, and increase during further processing of meat (YANG *et al*., 2017). Normally, only cooked meat is eaten, thus the existence of *L. monocytogenes* in raw meat could be problematic only if the meat is eaten raw or insufficiently cooked. We should not underestimate the risk of listeriosis in this type of food. There is no criterion for routine microbiological testing of raw meat for the presence of *L. monocytogenes* in Turkey (ANON., 2019b). Among the 190 samples tested, 57 were positive for *Listeri*a spp. and the isolation rate of *L. monocytogenes* from raw meat samples (12.10%) was higher than our expectations. The presence of *L. monocytogenes* in raw meat may be attributed to: i) fecal contamination during evisceration, ii) food handlers, and iii) cross-contamination during processing, transportation or marketing (AL-NABUSI *et al*., 2015; GOMEZ *et al*., 2015). This study was also aimed to detect the presence of the *hly*A gene in *L. monocytogenes* strains. This gene is one of the most virulent factors associated with *L. monocytogenes* and essential for intracellular infection (MORENO *et al*., 2014; USMAN *et al*., 2016). It was interesting to note that this virulence gene was detected in 86.96% of the *L. monocytogenes* strains isolated from the raw chicken samples and the rest 13.04% were devoid of it. These strains were named as atypical strains. The presence of this gene in the pathogens thriving on meat suggests a serious risk to human health (ZEINALI *et al*., 2015). To the best of our knowledge, this is the first report on the isolation of atypical *L. monocytogenes* strains, devoid of the *hly*A gene from foods in Turkey. Our results are consistent with those reported by KAUR *et al*. (2007), OSAILI *et al*. (2011), MORENO *et al*. (2014), USMAN *et al*. (2016) and ZEINALI *et al*. (2015), who identified some atypical *L. monocytogenes* strains from food. The *hly*A gene has an important role in the invasion process of *L. monocytogenes* (ZEINALI *et al*. 2015). The occurrence of some mutations alternating the genes responsible for pathogenesis may be the reason of the absence of *hly*A. This may explain why some species do not cause infections. Further studies are needed to determine the presence of other virulence genes in these strains.

The overall incidence of *Listeria* spp. in all raw meat samples was 30%, which is higher than that reported in Italy (21.4%) (PESAVENTO *et al*., 2010), but lower than that documented in Japan (58.7%) (INDRAWATTANA *et al*., 2011), Iran (34.7%) (FALLAH *et al*., 2012), Canada (70%) (AL-NABUSI *et al*., 2015), and Nigeria (80%) (OYELAMI *et al*., 2018). Our study showed that the overall incidence of *L. monocytogenes* in raw meat was 12.10%. The prevalence of *L. monocytogenes* was higher in raw chicken meat (14.54%) than raw red meat (8.75%). The rate of *L. monocytogenes* contamination in raw meat, from different parts of the world, was found to be 23.3% in Morocco (ENNAJI *et al*., 2008), 15.4% in Japan (INDRAWATTANA *et al*., 2011), 18.2% in Jordan (OSAILI *et al*., 2011), 14.1% in

Iran (FALLAH *et al*., 2012), 12% in China (WANG *et al*., 2013), 43.8% in Canada (AL-NABUSI *et al*., 2015), and 28% in Nigeria (OYELAMI *et al*., 2018). These differences suggest that *L. monocytogenes* contamination rates may be affected by geographical location, weather conditions, environmental conditions, the actions of food handlers, monitoring studies and isolation methods. In previous studies carried out in Turkey (AKPOLAT, 2004; YÜCEL *et al*., 2005; CEYLAN *et al*., 2008; EROL and AYAZ, 2011; DOGRUER *et al*., 2015; KOCAMAN and SARIMEHMETOĞLU, 2017), the contamination rate of *L. monocytogenes* in raw meat samples was found between 4.7% and 32.76%, which is partially consistent with our study. Related to these past results, we observed no increase in the incidence of *L. monocytogenes* in raw meats from Turkey. The reason for this consistency in the incidence of *L. monocytogenes* in raw meats may be the compliance with the Good Manufacturing Practices (GMPs), and Hazard Analysis and Critical Control Point (HACCP) systems.

4.2. Antibiotic resistance in *L. monocytogenes* strains

Earlier, *L. monocytogenes* was considered to be susceptible to a wide range of antibiotics (LUNGU *et al*., 2011; ALONSO-HERNANDO *et al*., 2012). However, there has been an increasing number of reports about *L. monocytogenes* strains resistant to one or more antibiotics from food, clinical, and environmental products since 1988 (GOMEZ *et al*., 2014; WANG *et al*., 2015).

Many strains of *L. monocytogenes* have been reported to be completely or partly resistant to fluoroquinolones, cephalosporins, aztreonam, pipemidic acid, fosfomycin, and macrolides, and other antibiotics, especially those of the third and fourth generations (RUIZ-BOLIVAR *et al*., 2011; WANG *et al*., 2013; GOMEZ *et al*., 2014; BRYNE *et al*., 2016). In the current study, the antimicrobial resistance tests of *L. monocytogenes* strains revealed that all of the *L. monocytogenes* strains were resistant to nalidixic acid, fosfomycin, ampicillin, linezolid, and clindamycin. We observed that 69.57%, 73.92%, 69.57% and 47.83 of *L. monocytogenes* strains were resistant to ciprofloxacin, levofloxacin, moxifloxacin, and enrofloxacin, respectively, which belong to the fluoroquinolones class of antibiotics. These high rates of resistance against fluoroquinolones could be ascribed to the frequent use of these antibiotics in animal feeds to treat infections (FALLAH *et al*., 2012; WANG *et al*., 2015). However, unexpectedly our isolates of *L. monocytogenes* showed low resistance to cephalothin (13.04%), cefuroxime (13.04%), and cefotaxime (8.69%), and all of them were susceptible to cefoxitin (100%).

The members of penicillin group antibiotics include ampicillin, oxacillin, penicillin G, amoxicillin, and piperacillin. They are the most active β-lactam compounds that inhibit the synthesis of bacterial cell wall peptidoglycan (ETABU and ARIKEKPAR, 2016). *L. monocytogenes* is naturally susceptible to β-lactams (LUNGU *et al*., 2011; BYRNE *et al*., 2016). Clinicians usually treat human listeriosis with the standard antibiotic therapy that includes high doses of penicillin, ampicillin, and amoxicillin alone or combined with gentamicin (KORSAK *et al*., 2012; GOMEZ *et al*., 2014; SHI *et al*., 2015; OLAIMAT *et al*., 2018). In the present study, *L. monocytogenes* strains showed high resistance to ampicillin (100%), piperacillin (86.96%), oxacillin (82.61%), penicillin G (73.92%), and amoxicillin/clavulanic acid (73.92%). This finding is highly significant as far as the treatment of human listeriosis is concerned. However, our results were not similar to the previous studies conducted in Turkey (TERZI *et al*., 2015; KOCAMAN and SARIMEHMETOĞLU, 2017), which reported low resistance to these antibiotics. Thus our observations are of great medical concern. Trimethoprim alone or combined with

sulfamethoxazole is generally used in the case of allergy to beta-lactams and rifampin, erythromycin, vancomycin, linezolid, and meropenem can also be used as possible alternatives (AL-NABUSI *et al*., 2015; ŞANLIBABA *et al*. 2018a). It is worth noting that our results showed that resistance to trimethoprim/sulfamethoxazole (13.04%) and meropenem (4.35%) was found to be low in this study. In addition, resistance to trimethoprim was not observed in this study, in line with WANG *et al*. (2015), OBAIDAT *et al*. (2015), and KUAN *et al*. (2017).

Clindamycin interferes with bacterial protein synthesis in a similar way to erythromycin and chloramphenicol (SHI *et al*., 2015). Therefore, owing to the similar mode of action, a cross-resistance among clindamycin, erythromycin, and chloramphenicol can sometimes be detected (RUIZ-BOLIVAR *et al*., 2011; MORENO *et al*., 2014). In this study, no resistance to chloramphenicol and erythromycin was observed; this concords with the findings of YÜCEL *et al*. (2005), ENNAJI *et al*. (2008), and CHEN *et al*. (2010). In contrast, resistance to clindamycin was 100%. This, to the best of our knowledge, seems to be the first report from Turkey showing the cross-resistance of these antibiotics. These results also concord with the theory of a specific enzyme that inactivates clindamycin, as previously reported for *Staphylococcus* spp. (RUIZ-BOLIVAR *et al*., 2011; MORENO *et al*., 2014).

Rifampin is the main antibiotic used in the treatment against *Mycobacterium tuberculosis* and Gram-positive bacteria (RUIZ-BOLIVAR *et al*., 2011). It has also been recommended to treat listeriosis (OLAIMAT *et al*., 2018). Fortunately, the resistance rate (17.39%), found in our work, does not seem to be alarming and is in agreement with FALLAH *et al*. (2013). Vancomycin is one of the last therapeutic options for the treatment of human listeriosis especially in case of bacteremia and endocarditis (OBAIDAT *et al*., 2015). Fortunately, all *L. monocytogenes* strains were found susceptible to the vancomycin in accordance with the results of RAHIMI *et al*. (2010), OKADA *et al*. (2011), WANG *et al*. (2015), AL-NABUSI *et al*. (2015), and BRYNE *et al*. (2016). The resistance of the isolates against vancomycin is a contrary finding to that obtained by IEREN *et al*. (2013) and FALLAH *et al*. (2013). Tetracycline and tigecycline are the members of tetracyclines, whose target of antimicrobial activity in bacteria is the ribosome (ETEBU and ARIKEKPAR, 2016). Tetracycline resistance has most frequently been reported in *Listeria* spp. of different origins (PESAVENTO *et al*., 2010; FALLAH *et al*., 2012; KORSAK *et al*., 2012). This might have arisen due to the extensive and prolonged use of these antimicrobials in human medicine and as growth promoters in animals (BRYNE *et al*., 2016). In contrast, these results could not be confirmed by us, because only one and two of the examined *L. monocytogenes* strains were resistant to tetracycline and tigecycline, respectively. The rare occurrence of resistance to tetracycline (4.35%) and tigecycline (13.04%) is in accordance with RAHIMI *et al*. (2010), AL-NABUSI *et al*. (2015), and NOLL *et al*. (2018). Tetracyclines are not used as the first drug of choice for listeriosis treatment and their use is also not recommended in children and pregnant women (RUIZ-BOLIVAR *et al*., 2011). While *L. monocytogenes* strains showed resistance to kanamycin (82.61%) and neomycin (78.26%), low resistance to streptomycin (17.39%) and gentamicin (4.35%) was also observed. The high frequency of resistance to kanamycin and neomycin was unexpected and might be in part due to the excessive use of these antibiotics in veterinary medicine in Turkey. These results are in agreement with ALONSO-HERNANDO *et al*. (2012) and SHI *et al*. (2015). However, some authors have reported high sensitivity of *L. monocytogenes* to kanamycin (OKADA *et al*., 2011; WANG *et al*., 2013; JAMALI *et al*., 2015; WU *et al*., 2015). In the present study, there was no significant association between the different *L. monocytogenes* strains in terms of antibiotic resistance ($p< 0.05$).

The prevalence of multidrug resistance *L. monocytogenes* strains isolated from raw meat was 73.91% (data not shown). The multi-resistance patterns reported in other countries are as follows: 16.4% in Iran (RAHIMI *et al*., 2010), 48% in Colombia (RUIZ-BOLIVAR *et al*., 2011), 64.3% in Nigeria (IEREN *et al*., 2013), 2.9% in Spain (GOMEZ *et al*., 2014), 21.25% in China (SHI *et al*., 2015), 21% in Germany (NOLL *et al*., 2018), and 81% in Malaysia (KUAN *et al*., 2017). These differences among multidrug resistance in *L. monocytogenes* strains could result from the differences in the use of antimicrobials at the regional level.
The result of our study suggested that the overall incidence of antibiotic resistance in *L. monocytogenes* is alarming. Further, the high resistance rate observed against antibiotics commonly used to treat listeriosis, such as penicillin, oxacillin, ampicillin, piperacillin, and amoxicillin/clavulanic acid, is also a great concern.

5. CONCLUSIONS

Our results regarding the occurrence of *L. monocytogenes* in raw meat and the presence of virulence-associated genes in the strains indicate an alarming situation to the public health. The counts of *L. monocytogenes* in raw meat at the retail level are crucial for contaminating with cooked foods. Retail centers must be controlled legally monitored. This study is the first report of *hly*A negative *L. monocytogenes* strains isolated from food in Turkey. In this study, we have demonstrated that all *L. monocytogenes* strains were resistant to ampicillin, fosfomycin, nalidixic acid, linezolid, and clindamycin against Gram-positive bacteria. The controlled use of antibiotics for therapeutic purposes may be important to limit the emergence of resistant *L. monocytogenes* strains in the world.

ACKNOWLEDGMENTS

This work was supported by the Ankara University Scientific Research Projects Coordination Unit (Project number 15B0443010).

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Paper Received June 10, 2019 Accepted November 19, 2019