

Serotyping and Antibiotic Resistance Profile of *Listeria monocytogenes* Isolated from Organic Chicken Meat ^[1]

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^[1] This study was supported by Ondokuz Mayıs University with project number PYO.VET.1901.18.009

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Article ID: KVFD-2019-23638 Received: 22.11.2019 Accepted: 22.03.2020 Published Online: 22.03.2020

How to Cite This Article

Gücükoğlu A, Çadircı Ö, Terzi Gülel G, Uyanık T, Kanat S: Serotyping and antibiotic resistance profile of *Listeria monocytogenes* isolated from organic chicken meat. *Kafkas Univ Vet Fak Derg*, 26 (4): 499-505, 2020. DOI: 10.9775/kvfd.2019.23638

Abstract

In this study, 240 organic chicken pieces (80 thighs, 80 wings, 80 skinless-breast meat) were analyzed for the presence of *Listeria monocytogenes*. Within the framework of the analysis findings; *L. monocytogenes* was detected in 60 (25%) of all 240 collected samples. In particular, *L. monocytogenes* was detected in 24 (30%) of 80 thigh samples, 20 (25%) of 80 wing samples and 16 (20%) of 80 skinless-breast meat samples. Serotyping distribution of 96 *L. monocytogenes* isolates determined as 71.8% serotype 1/2a, 21.9% serotype 1/2b, 4.2% serotype 4b and 2.1% serotype 1/2c. According to antibiotic resistance profile, 26 isolates (27%) were found to be resistant to ampicillin. The other isolates were found to be resistant to meropenem, tetracycline, sulfamethoxazole/trimethoprim, penicillin G, amoxicillin/clavulanic acid, vancomycin, oxytetracycline, erythromycin and chloramphenicol as 23 (23.9%), 14 (14.5%), 13 (13.5%), 12 (12.5%), 9 (9.3%), 7 (7.2%), 5 (5.2%), 4 (4.1%) and 3 (3.1%) respectively. Multiple antibiotic resistance profiles were determined in 12 of *L. monocytogenes* isolates. The findings of this study are thought to be unique data for serotyping studies that will help in revealing the epidemiology of *L. monocytogenes* in organic poultry meat, enterprises operating in food sector and diagnosis and treatment of listeriosis.

Keywords: *Listeria monocytogenes*, Organic chicken, Serotype, mPCR, Antibiotic resistance

Organik Tavuk Etlerinden İzole Edilen *Listeria monocytogenes* İzolatlarının Serotip ve Antibiyotik Direnç Profilinin Belirlenmesi

Öz

Bu çalışmada, 240 organik tavuk parça eti (80 but, 80 kanat, 80 derisiz-göğüs eti) *Listeria monocytogenes* varlığı yönünden analiz edildi. Analiz bulguları çerçevesinde; toplam 240 örneğin 60'ında (%25) *L. monocytogenes* saptandı. Bulgularının örnekler göre dağılımı incelendiğinde; but örneklerinin 24'ünde (24/80-%30), kanat örneklerinin 20'sinde (20/80-%25), derisiz göğüs eti örneklerinin ise 16'sında (16/80-%20) *L. monocytogenes* tespit edildi. Serotip dağılımında ise but örneklerinden elde edilen 40 *L. monocytogenes* izolatının 31'inin *L. monocytogenes* 1/2a, 6'sının *L. monocytogenes* 1/2b, 3'ünün *L. monocytogenes* 1/2c, 1'inin ise *L. monocytogenes* 4b serotipinde olduğu, kanat örneklerinden elde edilen 34 *L. monocytogenes* izolatının 24'ünün *L. monocytogenes* 1/2a, 9'unun *L. monocytogenes* 1/2b, 1'inin ise *L. monocytogenes* 4b serotipi olduğu, derisiz göğüs eti örneklerinden elde edilen 22 *L. monocytogenes* izolatının 14'ünün *L. monocytogenes* 1/2a, 6'sının *L. monocytogenes* 1/2b, 2'sinin ise *L. monocytogenes* 4b serotipi olduğu belirlendi. Antibiyotik direnç profiline bakıldığında; 26 izolat (%27) ampisiline dirençli bulunurken, meropenem, tetrasiklin, sülfametoksazol/trimetoprim, penisilin G, amoksisilin/klavulanik asit, vankomisin, oksitetrasiklin, eritromisin ve kloramfenikole karşı dirençli izolat sayısı sırası ile 23 (%23.9), 14 (%14.5), 13 (%13.5), 12 (%12.5), 9 (%9.3), 7 (%7.2), 5 (%5.2), 4 (%4.1) ve 3 (%3.1) olarak saptandı. *L. monocytogenes* izolatlarının 12'sinde ise çoklu antibiyotik direnç profili belirlendi. Sonuç olarak bu çalışmada tespit edilen bulguların *L. monocytogenes*'in organik kanatlı etlerinde epidemiyolojisini ortaya koyacak serotiplendirme çalışmalarına, gıda sektöründe faaliyet gösteren işletmelere ve listeriyozisin tanı ve tedavisinde özgün veri niteliğinde olacağı düşünülmektedir.

Anahtar sözcükler: *Listeria monocytogenes*, Organik tavuk, Serotip, mPCR, Antibiyotik direnç

INTRODUCTION

The awareness of balanced nutrition, which emerged after the second half of the twentieth century, has led to drastic

changes in people's lifestyles and food consumption. However, the increase in chronic diseases in recent years drove people's desire towards eating more reliable and healthier food, and it is observed that people prefer organic



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products in their nutrition ^[1,2]. “Organic”, “biological”, “biodynamic” and “agricultural ecological production” is a production system in which animal welfare comes to the fore in a controlled and certified manner with appropriate breeding techniques for the consumer mass demanding of high quality, healthy and risk-free products ^[3].

Besides the high nutritional value of poultry meat, it is an ideal environment for the development of saprophyte and pathogenic microorganisms as a result of the shredding and possible cross-contamination due to the technological processes that applied. Poultry meat is most commonly contaminated with pathogens such as *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, *Listeria* spp., *Yersinia enterocolitica*, *Aeromonas* spp. and *Clostridium perfringens* ^[4].

Several studies have focused on the comparison of conventional and organic production techniques in the presence of pathogens in poultry. Control of pathogens cannot be guaranteed due to the fact that poultry in organic breeding is more likely to be released in the open environment by free release, and there are restrictions on antimicrobial agents in feed and therapeutic use ^[5-7]. However, it is emphasized that contamination in poultry meat produced by both organic and conventional methods occurs through cross-contamination in the slaughterhouse and during the processing ^[8]. The presence and serotype distribution of *L. monocytogenes*, which is the most common cause of foodborne infections among pathogenic microorganisms in poultry meat, is of great importance. *L. monocytogenes* is one of the most emphasized microorganisms due to the occurrence of the sporadic or epidemic character of listeriosis in humans, especially through food of animal origin, and its presence as a common flora in the food production ^[9]. In various studies, it was reported that contamination with *L. monocytogenes* was mostly observed in slaughter-houses and in the processing of foodstuffs, and that the prevalence of *L. monocytogenes* increased after cutting by 70-100% compared to pre-slaughter ^[10].

This study was aimed to i) determine the incidence of *L. monocytogenes* in organic chicken piece meats by using classical culture and IMS techniques, ii) confirm the isolates by PCR, iii) make a serotyping of isolates by mPCR and iv) detect the resistance status of the obtained isolates to selected antibiotics.

MATERIAL and METHODS

In the study, 240 pieces of organic chicken meat (thigh, skinless-breast meat, wing) which were sold in Samsun province in packed form were used as material. Each month 80 samples were obtained between October and December of 2018. Samples were purchased at least 500 g and were brought to the laboratory under the cold chain as soon as possible.

Isolation and Identification of *Listeria monocytogenes*

The IMS-based culture technique recommended by ISO 11290-1 ^[11] and Dynal ^[12] was used for the isolation. 25 g of the samples were weighed under aseptic conditions and diluted with 225 mL of Half Fraser Broth (Oxoid-CM0895) and homogenized in the stomacher for 90 sec at medium speed and then incubated at 30°C for 24 h. Following pre-enrichment, 20 µL of the immunomagnetic microparticle solution (Dynabeads anti-*Listeria* 710.06) homogenized with vortex was placed into the 1.5 mL microcentrifuge tubes according to the manufacturer's instructions and placed into the Dynal magnetic particle port with the magnetic stick removed. Subsequently, 1 mL of homogenized pre-enriched in Half Fraser Broth was added and the ongoing steps were completed in accordance with the recommendations of the manufacturer. From the obtained 100 µL Dynabeads *Listeria* complex, 50 µL was streaked on MOX (Modified Oxford Agar, Oxoid-CM0856 + Modified *Listeria* Selective Supplement, Oxoid-SR0206) agar and plates were incubated at 35°C for 24-48 h. After incubation, up to 5 suspected colonies were selected from the plaques and these colonies were cultured into TSA-YE (Tryptic Soy Agar-Yeast Extract, Oxoid-CM131) for biochemical tests and plates were incubated at 30°C for 24 h. The colonies that breed in TSA-YE (Tryptic Soy Agar-Yeast Extract, Oxoid, CM131) were tested by using, respectively; Gram staining, catalase, oxidase activity in SIM medium (Sulphate Indole Motility Medium; Merck 5470), β-hemolysis and CAMP tests, sugar fermentation and nitrate reduction tests.

Verification of *Listeria monocytogenes* by PCR

DNA extraction of identified isolates was performed according to the boiling method. In addition, PCR protocol was designed using the primer sequences shown in [Table 1](#) designed by Bohnert et al. ^[13] and Doumith et al. ^[14] for PCR confirmation and serotyping. Electrophoresis of amplicons were performed in 2% agarose at 80 volts.

Antibiotic Resistance

Antibiotic resistance of the isolates were determined by the disc diffusion method on Mueller Hinton Agar (Oxoid, CM0337) based on the methods reported by CLSI ^[15] and EUCAST ^[16]. Besides, minimum inhibition concentration (MIC) of isolates resistant to various antibiotics were determined by Etest (Epsilon meter test) method.

RESULTS

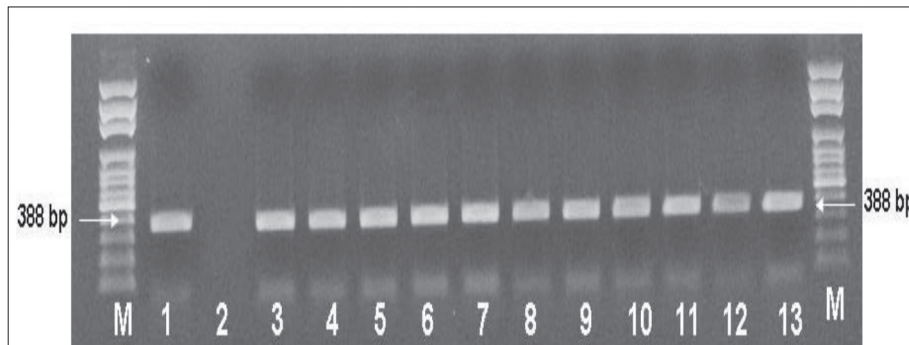
According to analysis, 60 (25%) of 240 samples were positive for *L. monocytogenes*. Distribution of isolates regarding to sample types was shown in [Table 2](#) and [Fig. 1](#). Consisting of a large amount of portion (71.8%), 1/2a was found to be the dominant serotype. Distribution of all serotypes according to sample type was shown in

Table 1. Primer sequences used in the study ^(13,14)

Target Gene	Primer Sequence	PCR Product (bp)	Serotype
<i>hlyA</i>	F:GAATGTAACTTCGGCGCAATCAG R:GCCGTCGATGATTGAACTTCATC	388	<i>L. monocytogenes</i>
<i>Imo0737</i>	F:AGGGCTTCAAGGACTTACCC R:ACGATTTCTGCTTGCCATTCC	691	1/2a, 1/2c, 3a, 3c
<i>Imo1118</i>	F:AGGGGTCTTAAATCCTGGAA R:CGGCTTGTTCCGCATACTTA	906	1/2c, 3c
<i>ORF2819</i>	F:AGCAAATGCCAAAACCTCGT R:CATCACTAAAGCTCCCATTTG	471	1/2b, 3b, 4b, 4e, 4d
<i>ORF2110</i>	F:AGTGGACAATTGATTGGTGAA R:CATCCATCCCTTACTTTGGAC	597	4b, 4e, 4d

Table 2. Incidence of *L. monocytogenes* in organic poultry

Sample	Number of Samples	Number of <i>L. monocytogenes</i> Positive Samples (%)	Number of <i>L. monocytogenes</i> Positive Isolates
Thigh	80	24 (30%)	40
Wing	80	20 (25%)	34
Breast	80	16 (20%)	22
Total	240	60 (25%)	96

**Fig 1.** PCR electrophoresis image of *L. monocytogenes* isolates: [M: 100 bp DNA ladder, lane 1: *L. monocytogenes* positive control (*L. monocytogenes* RSKK 471), lane 2: negative control, lane 3-13: *L. monocytogenes* positive isolates]**Table 3.** Serotype distribution of *L. monocytogenes* isolates

Number of Samples	Number of <i>L. monocytogenes</i> Positive Isolates Obtained by IMS-based Conventional Method	Number of <i>L. monocytogenes</i> Positive Isolates Verified by PCR (<i>hlyA</i> gene)	Distribution of <i>L. monocytogenes</i> Serotypes by PCR			
			1/2a (3a)	1/2b (3b)	1/2c (3c)	4b (4d,4e)
Thigh (n: 80)	40	40	31	6	2	1
Wing (n: 80)	34	34	24	9	-	1
Breast (n: 80)	22	22	14	6	-	2
Total (n: 240)	96	96	69	21	2	4

Table 3 and **Fig. 2.** The antibiotic resistance profile of our study revealed that 26 isolates (27%) were resistant to ampicillin. The other portion of the isolates were resistant to meropenem, tetracycline, sulfamethoxazole/trimethoprim, penicillin G, amoxicillin/clavulanic acid, vancomycin, oxytetracycline, erythromycin and chloram-

phenicol; as 23 (23.9%), 14 (14.5%), 13 (13.5%), 12 (12.5%), 9 (9.3%), 7 (7.2%), 5 (5.2%), 4 (4.1%) and 3 (3.1%) respectively. However, multiple antibiotic resistance profiles were determined in 12 of *L. monocytogenes* isolates (**Table 4**, **Table 5**). Datas including the MIC levels were mentioned in **Table 6**.

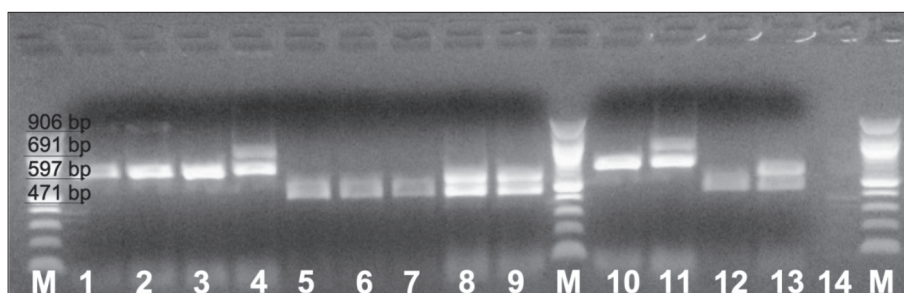


Fig 2. Multiplex PCR electrophoresis image of serotypes: [M: 100 bp DNA ladder, lane 1-3: *L. monocytogenes* serotype 1/2a, lane 4: *L. monocytogenes* serotype 1/2c, lane 5-7: *L. monocytogenes* serotype 1/2b, lane 8-9: *L. monocytogenes* serotype 4b, lane 10: *L. monocytogenes* serotype 1/2a positive control (*L. monocytogenes* RSKK 471), lane 11: *L. monocytogenes* 1/2c positive control (*L. monocytogenes* ATCC 7644), lane 12: *L. monocytogenes* serotype 1/2b positive control (*L. monocytogenes* RSKK 472), lane 13: *L. monocytogenes* serotype 4b positive control (*L. monocytogenes* RSKK 475), lane 14: negative control]

Antibiotic	Number of Resistant Isolates (%)
Ampicillin (2 µg)	26 (27%)
Meropenem (10 µg)	23 (23.9%)
Tetracycline (30 µg)	14 (14.5%)
Sulfamethoxazol/Trimethoprim (1.25/23.75 µg)	13 (13.5%)
Penicillin G (1U)	12 (12.5%)
Amoxicillin/Clavulanic acid (30 µg)	9 (9.3%)
Vancomycin (30 µg)	7 (7.2%)
Oxytetracycline (30 µg)	5 (5.2%)
Erythromycin (15 µg)	4 (4.1%)
Chloramphenicol (30 µg)	3 (3.1%)

Table 5. Phenotypic multiple antibiotic resistance profile of *L. monocytogenes*

Number of Antibiotics	Antibiotic Profile*	Serotype	Sample Origin
4	AMP, TE, VA, SXT	1/2a	Thigh
4	E, PG, MEM, SXT	4b	Thigh
4	AMC, TE, VA, SXT	1/2a	Thigh
4	C, OT, VA, MEM	1/2a	Wing
3	AMP, OT, MEM	4b	Wing
3	PG, TE, SXT	1/2a	Breast
3	AMC, TE, VA	1/2a	Thigh
3	AMP, TE, MEM	1/2b	Thigh
3	AMC, E, MEM	1/2a	Wing
3	AMP, E, TE	1/2b	Breast
3	AMP, TE, SXT	1/2a	Wing
3	AMP, MEM, SXT	1/2a	Wing

AMC: Amoxicillin/Clavulanic acid, AMP: Ampicillin, C: Chloramphenicol, E: Erythromycin, OT: Oxytetracycline, PG: Penicillin G, TE: Tetracycline, VA: Vancomycin, MEM: Meropenem, SXT: Sulfamethoxazole/Trimethoprim; * Only one of the same antibiotic group was evaluated

DISCUSSION

From last few decades to present, studies on the presence of *L. monocytogenes* in poultry meat continue to be

important in the worldwide. Unlikely to our findings, in many other studies a high-value prevalence was recorded, like Schafer et al.^[17], who detected *L. monocytogenes* in 8.64-44.19% of chicken meat samples; Rahmat et al.^[18], in

Table 6. MIC values of *L. monocytogenes* serotypes with multiple antibiotic resistance profiles

Serotype	1/2a	4b	1/2a	1/2a	4b	1/2a	1/2a	1/2b	1/2a	1/2b	1/2a	1/2a
Sample Origin	Thigh	Thigh	Thigh	Wing	Wing	Breast	Thigh	Thigh	Wing	Breast	Wing	Wing
Multiple Antibiotic Resistance Profile	AMP TE VA SXT	E PG MEMSXT	AMC TE VA SXT	C OT VA MEM	AMP OT MEM	PG TE SXT	AMC TE VA	AMP TE MEM	AMC E MEM	AMP E TE	AMP TE SXT	AMP MEM SXT
MIC (µg/mL)	AMC	-	-	16	-	-	-	16	-	16	-	-
	AMP	2	-	-	-	2	-	-	2	-	2	2
	C	-	-	-	32	-	-	-	-	-	-	-
	E	-	2	-	-	-	-	-	-	1.5	2	-
	OT	-	-	-	32	32	-	-	-	-	-	-
	PG	-	3	-	-	-	4	-	-	-	-	-
	TE	16	-	24	-	-	16	16	24	-	24	16
	VA	4	-	4	4	-	-	4	-	-	-	-
	MEM	-	0.5	-	0.5	0.5	-	-	0.75	0.75	-	-
SXT	0.064	0.064	0.064	-	-	0.125	-	-	-	-	0.094	0.064

AMC: Amoxicillin/Clavulanic acid, AMP: Ampicillin, C: Chloramphenicol, E: Erythromycin, OT: Oxytetracycline, PG: Penicillin G, TE: Tetracycline, VA: Vancomycin, MEM: Meropenem, SXT: Sulfamethoxazole /Trimethoprim

62.5% of 24 carcass samples; Weis^[19], in 62.5% of 8 chicken samples; Farber et al.^[20], who reported the presence of 50% *L. monocytogenes* in 16 chicken meat; Elmali et al.^[21], who detected *L. monocytogenes* in 45% of chicken wing meat samples. On the other hand, many others reported the presence of *L. monocytogenes* in proportions similar to the findings of our study, like Bailey et al.^[22] in 23% of 90 chicken carcasses; Rorvik et al.^[23] in 20% to 100% of chicken carcasses from 5 slaughterhouses. In literature review, some of the studies that reported the presence of *L. monocytogenes* at lower values than the results of our study were as follows: Alsheikh et al.^[24], 13.6% of the 250 ready-made chicken products; Alsheikh et al.^[25], 12.8% of the 500 frozen chicken samples; Genigeorgis et al.^[26] 12.5% of 160 chicken meats; Zeinali et al.^[27] 18% of 200 fresh chicken carcasses; Basaran Kahraman et al.^[28] 0% of 400 chicken carcasses. Although there were no studies on the presence of *L. monocytogenes* in organic poultry in Turkey, a limited number of studies are available in the literature. In a comparative study of *L. monocytogenes* in organic and conventional poultry, the contaminations levels of products were reported 49.1% to 41% respectively^[5].

In the present study, 71.8% of *L. monocytogenes* isolates were detected as serotype 1/2a. In other studies, Carvalho et al.^[29] reported mostly 1/2a (94.6%) in chicken meat and chicken-meat processing environment, Oliveira et al.^[30] identified 87% of the *L. monocytogenes* isolates as 1/2a in samples of chicken carcasses, and Zeinali et al.^[31] determined that 52.77% of *L. monocytogenes* 1/2a serotype were predominant in chicken carcasses, followed by 4a and 4c serotypes (27.77%) in Iran. Arslan and Baytur^[32] revealed 57.6% of *L. monocytogenes* strains isolated from chicken meat were 1/2a. In contrast, Zeinali

et al.^[33] and Maung et al.^[34] reported 1/2b dominance in samples of chicken meats. In addition, Ayaz and Erol^[35] identified 4b as the dominant serotype (51.4%) in samples of turkey meats. Serotype dominance appears to be different due to changes in animal species, geography and seasonal parameters.

In terms of antibiotic resistance, in parallel to our study, in Ireland, Walsh et al.^[36] reported that 351 *L. monocytogenes* isolates obtained from various foods were highly resistant to ampicillin, erythromycin, penicillin, and tetracycline. Davis and Jackson^[37] investigated the antimicrobial resistance properties of *L. monocytogenes* isolates from human, environmental and food origin in the United States using Sensititre® method and similar to our study, isolates were found to be resistant against ampicillin, penicillin G, erythromycin and tetracycline. Harakeh et al.^[38] revealed that 93.33% of *L. monocytogenes* isolates isolated from dairy products in Lebanon were resistant to oxacillin and 90% were resistant to penicillin. Similarly, Rahimi et al.^[39] reported that *L. monocytogenes* isolates isolated from milk and dairy products in Iran were resistant to various antibiotics such as nalidixic acid, ciprofloxacin, erythromycin, tetracycline, gentamicin, ampicillin, penicillin, and chloramphenicol. Researchers have linked this high resistance to genetic material transfers that may occur between different species and unconscious drug use. Bilir Ormanci et al.^[40], conducted the antibiotic resistance tests of *L. monocytogenes* isolates isolated from turkey meat by disk diffusion method and reported that the isolates they obtained were resistant to penicillin and ampicillin. Ayaz and Erol^[35] reported that *L. monocytogenes* isolated from turkey meats were resistant to penicillin and ampicillin and were resistant to erythromycin but they couldn't

detect resistance to tetracycline, chloramphenicol and vancomycin. As an emerging problem of this century, increasing resistance to multiple antibiotics complicates the treatment of infections. In the present study, 12.5% of the isolates were found to be resistant to at least two different antibiotic type and MIC values were determined. In comparison to other studies conducted, Lemes-Marques et al.^[41] determined the MIC values of 13 *L. monocytogenes* isolates obtained from patients with listeriosis in Brazil against ampicillin and vancomycin by microdilution method. They identified that the isolates were not resistant to vancomycin and ampicillin. In our study, also resistance to vancomycin was not detected. Filiouis et al.^[42] reported that one of the 30 *L. monocytogenes* isolates obtained from various foods in Greece was resistant to tetracycline and this MIC was determined to be 64 µg/mL. However, they reported that they could not detect any resistance to other antibiotics. Osaili et al.^[43] reported that 11% of *L. monocytogenes* isolates obtained from ready-to-eat chicken products in Jordan were resistant to tetracycline and MIC values were determined as 16 µg/mL. Conter et al.^[44] searched MIC values against 19 antibiotics including penicillin G, ampicillin, erythromycin, vancomycin and tetracycline by VITEK 2. The researchers reported that they could not detect any resistance to penicillin and erythromycin, but reported that 2% of their isolates were resistant to ampicillin and 0.8% to tetracycline and vancomycin. Yan et al.^[45] investigated the antibiotic resistance profiles of 70 *L. monocytogenes* isolates obtained from various foods in China by microdilution method and according to their findings, 14 isolates were resistant to tetracycline, 2 isolates to ampicillin, erythromycin and chloramphenicol, and 1 isolate was resistant to penicillin and vancomycin. Okada et al.^[46] mentioned in their study on 201 *L. monocytogenes* isolated from food, environment, animals, and humans in Japan, 31 of 32 isolates found to be resistant to chloramphenicol and had MIC values of 16 µg/mL and 1 isolate had MIC of 32 µg/mL. The researchers found that MIC of 1 isolate found to be resistant to oxytetracycline was 64 µg/mL. Despite the prohibition of antimicrobial use in organic poultry production, several studies have demonstrated that pathogenic and non-pathogenic bacteria have drug resistance properties. In the second half of the twentieth century, glycopeptide (vancomycin) resistance was not reported, but since the 1980s, staphylococci and enterococci suddenly developed resistance to vancomycin^[47]. In studies, it was determined that enterococcal and streptococcal plasmids and transposons that carrying antibiotic resistance genes were transferred to *Listeria* species by conjugation. Charpentier and Courvalin^[48] reported that plasmid pIP501, which is responsible for the resistance of chloramphenicol, macrolide, lincosamide and streptogramin was found in *Streptococcus agalactiae*, and can be transferred to *L. monocytogenes* under *in-vitro* conditions. Similarly, Biavasco et al.^[47] reported that the resistance gene from vancomycin-resistant Enterococci strains was transferred to *Listeria*

species. In our study, the resistance that we detected for different antibiotics can be attributed to the occurring of mutations in bacteria and to genetic material transfers caused by the interaction between bacteria.

ACKNOWLEDGMENTS

This study was supported by Ondokuz Mayıs University with project number PYO.VET.1901.18.009.

REFERENCES

- Fanatico AC, Pillai PB, Emmert JL, Owens CM:** Meat quality of slow and fast growing chicken genotypes fed low nutrient or standard diets and raised indoors or with outdoor access. *Poult Sci*, 86, 2245-2255, 2007. DOI: 10.1093/ps/86.10.2245
- Alali WQ, Thakur S, Berghaus RD, Martin MP, Gebreyes WA:** Prevalence and distribution of salmonella in organic and conventional broiler poultry farms. *Foodborne Pathog Dis*, 7, 1363-1371, 2010. DOI: 10.1089/fpd.2010.0566
- Food and Agriculture Organization of the United Nations (FAO):** Organic agriculture and the law, 2012.
- Rouger A, Tresse O, Zagorec M:** Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms*, 5:50, 2017. DOI: 10.3390/microorganisms5030050
- Miranda JM, Vazquez BI, Fente CA, Calo-Mata P, Cepeda A, Franco CM:** Comparison of antimicrobial resistance in *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* strains isolated from organic and conventional poultry meat. *J Food Prot*, 71, 2537-2542, 2008. DOI: 10.4315/0362-028x-71.12.2537
- Cui S, Ge B, Zheng J, Meng J:** Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Appl Environ Microbiol*, 71 (7): 4108-4111, 2005. DOI: 10.1128/AEM.71.7.4108-4111.2005
- Rosenquist H, Boysen L, Krogh AL, Jensen AN, Nauta M:** *Campylobacter* contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat. *Int J Food Microbiol*, 162 (3): 226-230, 2013. DOI: 10.1016/j.ijfoodmicro.2013.01.022
- EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control):** The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J*, 14:4634, 2016
- Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC:** A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*, 75, 1-13, 2017. DOI: 10.1016/j.foodcont.2016.12.016
- Jamshidi A, Zeinali T:** Significance and characteristics of *Listeria monocytogenes* in poultry products. *Int J Food Sci*, 2019:7835253, 2019. DOI: 10.1155/2019/7835253
- ISO 11290-1:** Microbiology of the Food Chain -Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 1: Detection Method. 2017.
- Dynal AS:** Cell Separation and Protein Purification, Technical Handbook. 2nd ed., Dynal AS. Norway Printed. 02 96, 1996.
- Bohnert M, Dilasser F, Dalet C, Mengaud J, Cossart P:** 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, 1992. DOI: 10.1016/0923-2508(92)90019-k
- Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P:** Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J Clin Microbiol*, 42 (8): 3819-3822, 2004. DOI: 10.1128/JCM.42.8.3819-3822.2004
- Clinical and Laboratory Standards Institute (CLSI):** Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed, CLSI Guideline M45. Wayne, PA; 2016.

- 16. The European Committee on Antimicrobial Susceptibility Testing (EUCAST):** Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 7.1, 2017.
- 17. Schafer DF, Steffens J, Barbosa J, Zeni J, Paroul N, Valduga E, Junges A, Backes GT, Cansian RL:** Monitoring of contamination sources of *Listeria monocytogenes* in a poultry slaughterhouse. *LWT- Food Sci Technol*, 86, 393-398, 2017. DOI: 10.1016/j.lwt.2017.08.024
- 18. Rahmat GR, Ibrahim A, Bakar FA:** Prevalence of *Listeria monocytogenes* in retail beef and poultry. *Pertanika*, 14 (3): 249-255, 1991.
- 19. Weis J:** Vorkommen von Listerien in Hackfleisch. *Tierarztl Umsch*, 52 (7): 456-458, 1989.
- 20. Farber JM, Sanders GW, Johnston MA:** A survey of various foods for the presence of *Listeria* species. *J Food Prot*, 52 (7): 456-458, 1989. DOI: 10.4315/0362-028X-52.7.456
- 21. Elmali M, Can HY, Yaman H:** Prevalence of *Listeria monocytogenes* in poultry meat. *Food Sci Technol*, 35 (4): 672-675, 2015. DOI: 10.1590/1678-457X.6808
- 22. Bailey JS, Fletcher DL, Cox NA:** Recovery and serotype distribution of *L. monocytogenes* from broiler chickens in southeastern United States. *J Food Prot*, 52 (3): 148-150, 1989. DOI: 10.4315/0362-028X-52.3.148
- 23. Rorvik LM, Aase B, Alvestad T, Caugant DA:** Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products. *J Appl Microbiol*, 94, 633-640, 2003. DOI: 10.1046/j.1365-2672.2003.01895.x
- 24. Alsheikh ADI, Mohammed GE, Abdalla MA:** Isolation and identification of *Listeria monocytogenes* from retail broiler chicken ready to eat meat products in Sudan. *Int J Anim Vet Adv*, 5 (1): 9-14, 2013. DOI: 10.19026/ijava.5.5570
- 25. Alsheikh ADI, Mohammed GE, Abdalla MA, Bakhiet AO:** First isolation and identification of *Listeria monocytogenes* isolated from frozen and shock frozen dressed broiler chicken in Sudan. *Br Microbiol Res J*, 4 (1): 28-38, 2014. DOI: 10.9734/BMRJ/2014/2449
- 26. Genigeorgis CA, Dutulescu D, Garayzabal JF:** Prevalence of *Listeria* spp. in poultry meat at supermarket and slaughterhouse level. *J Food Prot*, 52 (9): 618-624, 1989.
- 27. Zeinali T, Jamshidi A, Bassami M, Rad M:** Isolation and identification of *Listeria* spp. in chicken carcasses marketed in northeast of Iran. *Int Food Res J*, 24 (2): 881-887, 2017.
- 28. Basaran Kahraman B, Issa G, Kahraman T:** Prevalence, antimicrobial resistance and molecular characterization of *Salmonella* spp. and *Listeria monocytogenes* isolated from chicken carcass. *Kafkas Univ Vet Fak Derg*, 24 (5): 775-779, 2018. DOI: 10.9775/kvfd.2018.19754
- 29. Carvalho FT, Vieira BS, Vallim DC, Carvalho LA, Carvalho RCT, Pereira RCL, Figueiredo EES:** Genetic similarity, antibiotic resistance and disinfectant susceptibility of *Listeria monocytogenes* isolated from chicken meat and chicken-meat processing environment in Mato Grosso, Brazil. *LWT- Food Sci Technol*, 109, 77-82, 2019. DOI: 10.1016/j.lwt.2019.03.099
- 30. Oliveira TS, Varjão LM, da Silva LNN, Pereira, RCL, Hofer E, Vallim DC, Almeida RCC:** *Listeria monocytogenes* at chicken slaughterhouse: Occurrence, genetic relationship among isolates and evaluation of antimicrobial susceptibility. *Food Control*, 88, 131-138, 2018. DOI: 10.1016/j.foodcont.2018.01.015
- 31. Zeinali T, Jamshidi A, Bassami M, Rad M:** Serogroup identification and virulence gene characterization of *Listeria monocytogenes* isolated from chicken carcasses. *Iranian J Vet Sci Technol*, 7 (2): 9-19, 2015. DOI: 10.22067/veterinary.v7i2.43658
- 32. Arslan S, Baytur S:** Prevalence and antimicrobial resistance of *Listeria* species and subtyping and virulence factors of *Listeria monocytogenes* from retail meat. *J Food Saf*, 39:e12578, 2019. DOI: 10.1111/jfs.12578
- 33. Zeinali T, Jamshidi A, Rad M, Bassami M:** A comparison analysis of *Listeria monocytogenes* isolates recovered from chicken carcasses and human by using RAPD PCR. *Int J Clin Exp Med*, 8 (6): 10152-10157, 2015.
- 34. Maung AT, Mohammadi TN, Nakashima S, Liu P, Masuda Y, Honjoh K, Miyamoto T:** Antimicrobial resistance profiles of *Listeria monocytogenes* isolated from chicken meat in Fukuoka, Japan. *Int J Food Microbiol*, 304, 49-57, 2019. DOI: 10.1016/j.ijfoodmicro.2019.05.016
- 35. Erol I, Ayaz ND:** Serotype distribution of *Listeria monocytogenes* isolated from turkey meat by multiplex PCR in Turkey. *J Food Saf*, 31, 149-153, 2011. DOI: 10.1111/j.1745-4565.2010.00278.x
- 36. Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell DA:** Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J Appl Microbiol*, 90, 517-522, 2001. DOI: 10.1046/j.1365-2672.2001.01273.x
- 37. Davis JA, Jackson CR:** Comparative antimicrobial susceptibility of *Listeria monocytogenes*, *L. innocua* and *L. welshimeri*. *Microb Drug Resist*, 15, 27-32, 2009. DOI: 10.1089/mdr.2009.0863
- 38. Harakeh S, Saleh I, Zouhairi O, Baydoun E, Barbour E, Alwan N:** Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy based products. *Sci Total Environ*, 407, 4022-4027, 2009. DOI: 10.1016/j.scitotenv.2009.04.010
- 39. Rahimi E, Ameri M, Momtaz H:** Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control*, 21, 1448-1452, 2010. DOI: 10.1016/j.foodcont.2010.03.014
- 40. Bilir Ormanci FS, Erol I, Ayaz ND, Iseri O, Sariguzel D:** Immunomagnetic separation and PCR detection of *Listeria monocytogenes* in turkey meat and antibiotic resistance of the isolates. *Br Poult Sci*, 49 (5): 560-565, 2008. DOI: 10.1080/00071660802298328
- 41. Lemes-Marques EG, Cruz CD, Destro MT:** Phenotypic and genotypic characterization of *Listeria monocytogenes* clinical isolates from the south-western region of the state of Sao-Paolo, Brazil. *Braz J Microbiol*, 38, 287-292, 2007. DOI: 10.1590/s1517-83822007000200019
- 42. Filiouis G, Johansson A, Frey J, Perreten V:** Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. *Food Control*, 20, 314-317, 2009. DOI: 10.1016/j.foodcont.2008.05.018
- 43. Osaili TM, Alaboudi AR, Nesiari EA:** Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. *Food Control*, 22, 586-590, 2011. DOI: 10.1016/j.foodcont.2010.10.008
- 44. Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianieri V:** Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiol*, 128, 497-500, 2009. DOI: 10.1016/j.ijfoodmicro.2008.10.018
- 45. Yan H, Neogi SB, Mo Z, Guan W, Shen Z, Zhang S, Li L, Yamasaki S, Shi L, Zhong N:** Prevalence and characterization of antimicrobial resistance of foodborne *Listeria monocytogenes* isolates in Hebei province of Northern China, 2005-2007. *Int J Food Microbiol*, 144, 310-316, 2010. DOI: 10.1016/j.ijfoodmicro.2010.10.015
- 46. Okada Y, Okutani A, Suzuki H, Asakura H, Monden S, Nakama A, Maruyama T, Igimi S:** Antimicrobial susceptibilities of *Listeria monocytogenes* isolated in Japan. *J Vet Med Sci*, 73 (12): 1681-1684, 2011. DOI: 10.1292/jvms.11-0051
- 47. Biavasco F, Giovanetti E, Miele A, Vignaroli C, Facinelli B, Valardo PE:** In vitro conjugative transfer of VanA vancomycin resistance between Enterococci and *Listeria* of different species. *Eur J Clin Microbiol Infect Dis*, 15, 50-59, 1996. DOI: 10.1007/bf01586185
- 48. Charpentier E, Courvalin P:** Antibiotic resistance in *Listeria* spp. *Antimicrob Agents Chemother*, 43 (9): 2103-2108, 1999.