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

Ali GÜCÜKOĞLU 1,a Özgür ÇADIRCI 1,b Göknur TERZİ GÜLEL 1,c Tolga UYANIK 1,d Sibel KANAT 1,e

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

1 Ondokuz Mayıs University, Faculty of Veterinary Medicine Department of Food Hygiene and Technology, TR-55200 Kurupelit/Samsun - TURKEY

How to Cite This Article

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 Antibiotik Direnç Profilinin Belirlenmesi

Öz
Bu çalışmadada, 240 organik tavuk parça eti (80 but, 80 kanat, 80 derisiz-göğüs eti) *Listeria monocytogenes* varlığı yönünden analiz edildi. Analiz bulguları değerlendirdikten sonra; *L. monocytogenes* varlığı, 240 örnekden toplam 60 (25%) örnek olarak tespit edildi. **Serotyping** analiz sonucunda ise but örneklerinden 24 (30%), kanat örneklerinden 20 (25%), derisiz-göğüs eti örneklerinden 16 (20%) örnek *L. monocytogenes* tespit edildi. Bu sayının **antibiyotik direnç** analizi yaparak; 26 izolatın (27%) ampişilin dirençli olduğu tespit edildi. Diğer izolatlar sırısında ise meropenem, tetraksiklin, sülfametoksazol/trimetoprim, penilisin G, amoksikilin/klavulanik asit, vanomisin, oksitetrasiklin, eritromisin ve kloramfenikol dirençli izolat sayısını artırdı. Bu sayının **antibiotik direnç profilinin** belirlenmesi **26** (27%) izolatın ampişilin direnciyle karşılaştırıldığında; 26 izolatın **(27%)** ampişilin dirençli olduğu tespit edildi. Diğer izolatlar sırısında ise meropenem, tetraksiklin, sülfametoksazol/trimetoprim, penilisin G, amoksikilin/klavulanik asit, vanomisin, oksitetrasiklin, eritromisin ve kloramfenikol dirençli izolat sayısı sırasıyla; 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 tespit edildi. 

Anahtar sözcükler: *Listeria monocytogenes*, Organik tavuk, Serotip, mPCR, Antibiotik 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 foods.
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), ß-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 (Epsilometer 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

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.
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 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. However, multiple antibiotic resistance profiles were determined in 12 of L. monocytogenes isolates (Table 4, Table 5). Data including the MIC levels were mentioned in Table 6.

Table 1. Primer sequences used in the study

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer Sequence</th>
<th>PCR Product (bp)</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>hlyA</td>
<td>F:GAATGTAACCTCGGGCAATCARG:GGTGATGATTGTAACCTCATC</td>
<td>388</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>lmo0737</td>
<td>F:AGGGCTCAAGAAGCTTACCRAG:ATGTTCTGCGAATCC</td>
<td>691</td>
<td>1/2a, 1/2c, 3a, 3c</td>
</tr>
<tr>
<td>lmo1118</td>
<td>F:AGGGCTTAAATCCTGGAARG:CGGCTTGTCCGGCACTTA</td>
<td>906</td>
<td>1/2c, 3c</td>
</tr>
<tr>
<td>ORF2819</td>
<td>F:AGCAAAATGCCTGCAAACGTGRC:ATCAGAAATCCCTCCATTA</td>
<td>471</td>
<td>1/2b, 3b, 4b, 4e, 4d</td>
</tr>
<tr>
<td>ORF2110</td>
<td>F:AGTGGAATTTGATTGTTGAAAG:ATCCATTTACTTGGAC</td>
<td>597</td>
<td>4b, 4e, 4d</td>
</tr>
</tbody>
</table>

Table 2. Incidence of L. monocytogenes in organic poultry

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

Table 3. Serotype distribution of L. monocytogenes isolates

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Number of L. monocytogenes Positive Isolates Obtained by IMS-based Conventional Method</th>
<th>Number of L. monocytogenes Positive Isolates Verified by PCR (hlyA gene)</th>
<th>Distribution of L. monocytogenes Serotypes by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thigh (n: 80)</td>
<td>40</td>
<td>40</td>
<td>1/2a (3a) 1/2b (3b) 1/2c (3c) 4b (4d,4e)</td>
</tr>
<tr>
<td>Wing (n: 80)</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Breast (n: 80)</td>
<td>22</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Total (n: 240)</td>
<td>96</td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>

*Fig 1. PCR electrophoresis image of L. monocytogenes isolates: [M: 100 bp DNA ladder, lane 1: L. monocytogenes positive control (L. monocytogenes RS04 471), lane 2: negative control, lane 3-13: L. monocytogenes positive isolates]*

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 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. However, multiple antibiotic resistance profiles were determined in 12 of L. monocytogenes isolates (Table 4, Table 5). Data including the MIC levels were mentioned in Table 6.
DISCUSSION

From last few decades to present, studies on the presence of *L. monocytogenes* in poultry meat continue to be important in the worldwide. Unlike 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
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 400 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 [29].

In the present study, 71.8% of L. monocytogenes isolates were detected as serotype 1/2a. In other studies, Carvalho et al. [30] reported mostly 1/2a (94.6%) in chicken meat and chicken-meat processing environment, Oliveira et al. [31] identified 87% of the L. monocytogenes isolates as 1/2a in samples of chicken carcasses, and Zeinali et al. [32] 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 [33] 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.[43] 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. Fililoumis et al.[41] 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.[41] 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.[41] 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.[41] 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 enterococal and streptococcal plasmids and transposons that carrying antibiotic resistance genes were transferred to *Listeria* species by conjugation. Charpentier and Couvalin[42] 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.[42] 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 Mayis University with project number PYO.VET.1901.18.009.

**REFERENCES**

1. Fanatocci 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. Poul Sci, 86, 2245-2255, 2007. DOI: 10.1093/ps/86.10.2245