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 healthier food, and it is observed that people prefer organic...
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 slaughter-house 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 serototyping 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), 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...
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). Datas 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; Elmalı 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. [34] and Maung et al. [35] reported 1/2b dominance in samples of chicken meats. In addition, Ayaz and Erol [36] 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. [37] reported that 351 L. monocytogenes isolates obtained from various foods were highly resistant to ampicillin, erythromycin, penicillin, and tetracycline. Davis and Jackson [38] 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. [39] 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. [40] 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 et al. [41] 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 et al. [42] reported that L. monocytogenes isolates 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.\cite{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. Filioussis et al.\cite{44} 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.\cite{45} 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.\cite{46} 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.\cite{47} 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.\cite{48} 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 \cite{49}. 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 Courvalin \cite{50} reported that plasmid pIP501, which is responsible for the resistance of chloramphenicol, macrolide, lincomamide and streptogramin was found in Streptococcus agalactiae, and can be transferred to L. monocytogenes under in-vitro conditions. Similarly, Biavasco et al.\cite{51} 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.

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**REFERENCES**

1. 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


8. EFSA (European Food Safety Authority) and EEC (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, 14:6434, 2016


