The Determination of Presence of Listeria monocytogenes in Ground Meat Sold in Istanbul

Arda Alara ULUDAG[1], [iD] , Elif Ozlem ARSLAN AYDOGDU[2*] [iD] , Ayten KIMIRAN[2] [iD]

[1] Istanbul University, Institute of Graduate Studies in Science, Department of Biology, 34116, Istanbul, Turkey
[2] Istanbul Univertsiy, Faculty of Science, Department of Biology, Section of Fundamental and Industrial Microbiology, 34134, Istanbul, Turkey

Highlights
• Listeria monocytogenes contamination in minced meat was investigated.
• It was determined that 9% of the isolates were *Listeria monocytogenes*.
• The fact that the isolates exhibit antibiotic resistance, is a threat in terms of public health.
• Results revealed that the proposed method from standard protocol should be improved.

Abstract

Listeria monocytogenes that the most common human listeriosis agent is one of 21 species of the *Listeria* genera. In fact, the mortality rate of listeriosis is higher than that of the more common foodborne pathogens such as *Vibrio* species or *Salmonella* Enteritidis. This study was aimed to determine the *L. monocytogenes* contamination in different minced meat samples. In this study, 100 minced meat samples purchased from different butchers in 11 districts of Istanbul between December 2018 and November 2019 were examined for the presence of *L. monocytogenes*. Isolates were molecularly confirmed for the presence of the *iap* and *hlyA* gene regions. It was determined that 21 biochemically defined isolates were susceptible to tetracycline and ampicillin, while resistant to amoxicillin/clavulanic acid, penicillin, cefaclor, and vancomycin. In addition, it was determined that only 16 of these 21 isolates were *L. monocytogenes* in terms of *iap* and *hlyA* gene regions. *L. monocytogenes* incidence in minced meat sold in Istanbul is low (17%), we believe that these contamination rates indicate a significant risk to public health due to cross-contamination and raw consumption of minced meat.

1. INTRODUCTION

Listeria bacteria are Gram-positive, spore-free, and facultative anaerobe, 0.5 µm wide and 1-2 µm long, round-tipped, rod-shaped bacteria. It is also known that bacteria can form filamentous forms in some cultures [1,2]. This bacterium, which can live in the wide temperature and pH range (-0.4-45°C and 3.0-9.5, respectively), is resistant to freezing and dryness and can grow at salt concentrations up to 10% [3,4]. It is not surprising that these bacteria, which show high tolerance to environmental conditions, spread in various environments, and are isolated from almost any living and inanimate environment [5,6].

The listeriosis agent *L. monocytogenes* is an obligate intracellular pathogen. Commonly, elderly people, children, newborns, babies, pregnant women, and people with immune system disorders due to illness are in the risk group [7]. Although patients have flu-like symptoms such as nonspecific muscle and joint pain, tremor, fatigue, and headache, cases with gastroenteritis, meningitis, miscarriage, septicemia, encephalitis, and in some cases, death has also been reported [8-10]. *L. monocytogenes* has a higher mortality rate (30%) than enteric pathogens such as *Salmonella* Enteritidis and *Vibrio* [11,12].

The bacterium, which was first identified as a human pathogen in 1929 [13], was not detected to be transmitted through food until 1980 [14-20]. Nowadays, almost all cases of listeriosis are recognized as food-borne [12,13,21-23].

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*Corresponding author, e-mail: eoarslan@istanbul.edu.tr

Meat and meat-related products are known to have a significant role in foodborne infections. Fresh meat is not resistant to microbiological contamination and spoilage due to its chemical and physical properties [24]. When cases are observed, it is seen that meat products are associated with many major listeriosis outbreaks [17,25,26].

Minced meat provides a very suitable environment for the reproduction of microorganisms due to its structure. The minced meat has a risk to human health by due to microorganisms can spread all part of meat when it chopped and mixing [27,28]. Minced meat contaminated with these bacteria may cause listeriosis infection due to the bacteria's ability to reproduce at refrigerator temperature and application errors.

Studies, investigating the minced meat's microbial quality, showed that these minced meats pose a great risk for human health [29-34]. Due to excessive or incorrect use of antibiotics, resistance to antibiotics develops in bacteria, making the treatment process difficult in combating diseases and epidemics. However, the increase in travel for both commercial and touristic purposes cause the spread of resistant bacteria between countries and even continents [35]. Although listeriosis is a disease that can be treated using antibiotics, it is a dangerous disease with up to the 30% mortality rate [8,36]. As with some other bacteria, resistance properties can be spread among *Listeria* bacteria by the transfer of genes [37]. Recently, the treatment of listeriosis has been primarily administered with amoxicillin and ampicillin in combination with gentamicin [38,39]. Another drug used in the treatment of listeriosis is trimethoprim-sulfamethoxazole, which can easily enter cells and is used especially in penicillin-sensitive patients [40]. The current study was aimed at detecting the *L. monocytogenes* bacteria in minced meat samples sold in Istanbul, to determine the resistance they will show in a treatment-oriented intervention, and thus to determine the possible public health threat.

2. MATERIAL METHOD

2.1. Sample Collection

100 minced meat samples, from butchers in 11 different districts of Istanbul, were tested for the presence of *L. monocytogenes* between December 2018 and November 2019. In the experiments, 100 g of sample, which was supplied with the original package sold by the butcher, after being transported to the laboratory in a cold chain was analyzed without waiting.

2.2. Isolation and Identification

For the determination of *L. monocytogenes*, the USDA-FSIS culture method was used [41]. ALOA (*Listeria* Ottaviani and Agosti Agar; Conda, Spain) medium was used in the experiments as the USDA-FSIS recommended the use of a second selective medium in combination with MOX (Modified Oxford Agar; Biolife, Italy) medium.

After the 25 g sample was homogenised in University of Vermont (UVM) Broth (Biolife, Italy) with a stomacher device (IUL Instruments, Spain), it was incubated at 30 °C. One night later, a loopful of cultures were streaked onto MOX and ALOA, while 0.1 mL of the same culture was transferred to 10 mL of Fraser Broth (Biolife, Italy). At the 24–48-hour incubation period at 37 °C, blackening in Fraser Broth culture was accepted as *L. monocytogenes* suspicious. At the same time the cultures on MOX and ALOA media, incubated at 37 °C for 24 hours, was evaluated with respect to the typical *L. monocytogenes* colony morphology. Colonies of approximately 1 mm in size that developed in the MOX medium with a grey, slightly curved circumference that turned black due to esculin hydrolysis, and typical colonies that developed in the ALOA medium with a green, slightly curved, light-colored zone were considered suspicious for *L. monocytogenes* and were streaked onto TSA-YE (Tryptone Soya Yeast Extract Agar; Conda, Spain) medium. Pure cultures were obtained by incubating Petri dishes at 37 °C for 24 hours.

The non-spore-forming Gram-positive rod shapes, catalase-positive, oxidase-negative, and able to grow in TSA-YE medium isolates were accepted as suspicious for *L. monocytogenes*. The isolates were identified in terms of biochemical tests such as nitrate reduction test, movement in SIM medium, Voges Proskauer

(VP), Methyl Red (MR), carbohydrate fermentation tests (mannitol, xylose, ribose, rhamnose, methyl α-D-mannopyranoside), hemolysis in blood media, and Christe Atkins Munch Peterson (CAMP) tests.

2.3. PCR Amplification of *hlyA* and *iap* Genes

Genomic DNA extraction: DNA isolations of *L. monocytogenes* bacteria detected by traditional culture method were performed with the GeneAll Exgene™ Cell SV DNA isolation kit.

PCR amplification: In addition to biochemical identification, the existence of the genes of *iap* and *hlyA* were investigated by polymerase chain reaction. Primers used to detect *iap* and *hlyA* genes are given in Table 1. The mixture used in PCR was prepared with MyTaq™ Red DNA Polymerase (Bioline, Singapore). The template DNA of *L. monocytogenes* from Refik Saydam National Type Culture Collection (RSKK 475) and ultrapure water was used for control. Amplification of *iap* and *hlyA* gene was carried out in Thermal Cycler (Bio-Rad, USA) and programmed as 60 s initial denaturation at 95 °C; 30 cycles of denaturation at 95 °C for 15 s, annealing for 15 s at 62 °C, and extension for 10 s at 72 °C. The obtained PCR products were prepared with 1.2% agarose and operated for 90 minutes at 60 volt current with electrophoresis and visualized with UV imaging system (UVITEC Cambridge, UK) at 388 bp and 131 bp, respectively. A 100 bp DNA Ladder (Bioline, Singapore) was used to evaluate the band lengths of the products.

2.4.   Sequencing and Phylogenetic Analysis

The PCR method was applied to determine the *16S rRNA* gene region of the *L. monocytogenes* bacteria isolated from minced beef samples. Primers (1492R and 27F) were prepared as recommended by the manufacturer (Table 1). 25 µL PCR mixes were prepared, containing 0.25 µL of DNA polymerase, 1 µL template DNA, 1 µL of each primer, and 5 µL Reaction Buffer. The template DNA of *L. monocytogenes* from Refik Saydam National Type Culture Collection (RSKK 475) and ultrapure water was used for control. Amplification of the *16S rRNA* gene was performed as described in Section 2.3 with annealing at 53°C. The obtained PCR products were prepared with 1.2% agarose and operated for 90 minutes at 60 volt current with electrophoresis and visualized with a UV imaging system (UVITEC Cambridge, UK). The band lengths of the PCR products obtained were determined by using Bioline (Singapore) brand 100 bp DNA Ladder. The BLAST analyzes of *16S rRNA* sequences were obtained by using the National Center for Biotechnology Information (NCBI) database.

Table 1. Primers used in experiments

Primers	Sequence (5′ → 3′)	Product size (bp)	Reference
iap	ACAAGCTGCACCTGTTGCAG TGACAGCGTGTGTAGTAGCA	131	[42]
hlyA	GAATGTAAACTTCGGCGCAATCAG GCCGTCGATGATTTGAACTTCATC	388	[43]
1492R 27F	GGT TAC CTT GTT ACG ACT T AGA GTT TGA TCM TGG CTC AG	1500	[44]

2.5. Antibiotic Susceptibility of the Isolates

Antibiotic susceptibility assays were performed according to Kirby Bauer disk diffusion method [45]. The antibiotics included Gentamycin (10 µg), Vancomycin (30 µg), Amoxicillin-clavulanic acid (30 µg), Ampicillin (10 µg), Penicillin (10 U), Cefaclor (30 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Clarithromycin (15 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Trimethoprim-sulfamethoxazole (25 µg), Rifampicin (5 µg).

3.   RESULTS

3.1. Prevalence of *L. monocytogenes* in Samples

100 minced meat samples were analyzed with the USDA-FSIS method; *L. monocytogenes* suspect colonies selected from MOX and ALOA media (Figure 1) were examined in terms of Gram reaction, catalase, oxidase and MR-VP reaction, nitrate reduction, movement in SIM medium, carbohydrate fermentation (rhamnose, ribose, xylose, methyl α-D-mannopyranoside and mannitol), CAMP and hemolysis tests. 569 of the colonies grown on MOX and ALOA media were selected and 186 of them were evaluated as suspicious for *L. monocytogenes* considering Gram, oxidase, and catalase reactions. As a result of biochemical tests of the 186 suspicious isolate, 21 strains from 17 different ground beef samples were identified as *L. monocytogenes*.
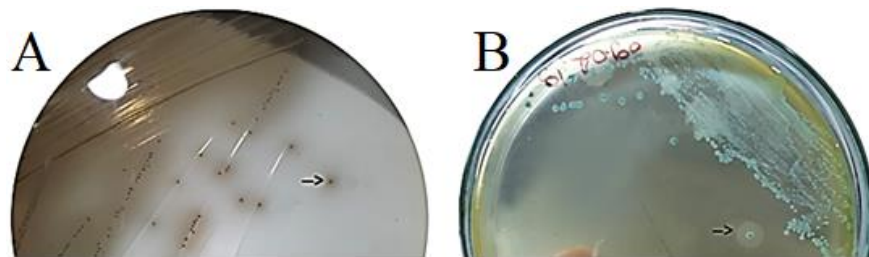

Figure 1. *The L. monocytogenes suspect colonies on MOX (A) and ALOA (B) media*

3.2. PCR Amplification of *hlyA* and *iap* Genes

16 of 21 strains isolated by traditional culture method and identified as *L. monocytogenes* by biochemical methods were named *Listeria* spp. because it was determined that had the *iap* gene region (Figure 2). In addition, it was determined that all of the 16 bacteria had *hlyA* gene region specific to *L. monocytogenes* by PCR method and were confirmed as *L. monocytogenes* (Figure 3).
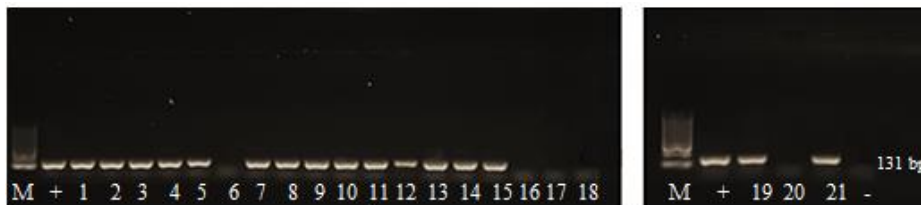

Figure 2. *Images of gel electrophoresis of the iap gene of L. monocytogenes isolates. A) M: 100 bp DNA marker. +: Positive control. 1: KA 26/2. 2: KA 11/1. 3: KA 45/1. 4: KA 11/2. 5: KA 12/1. 6: KA 14/1.7: KA 17/2. 8: KA 19/2. 9: KA 21/2. 10: KA 23/2. 11: KA 26/1. 12: KA 26/4.13: KA 30/2. 14: KA 30/3. 15: KA 55/2. 16: KA 69/2. 17: KA 72/1. 18: KA 79/2. -: Negative control. B) M: 100 bp DNA marker. +: Positive control. 19: KA 89/2. 20: KA 90/1. 21: KA 95/1. -: Negative control*

Figure 3. *Images of gel electrophoresis of the iap gene of L. monocytogenes isolates. A) M: 100 bp DNA marker. +: Positive control. 1: KA 26/2. 2: KA 11/1. 3: KA 45/1. 4: KA 11/2. 5: KA 12/1. 6: KA 14/1.7: KA 17/2. 8: KA 19/2. 9: KA 21/2. 10: KA 23/2. 11: KA 26/1. 12: KA 26/4.13: KA 30/2. 14: KA 30/3. 15: KA 55/2. 16: KA 69/2. 17: KA 72/1. 18: KA 79/2. -: Negative control. B) M: 100 bp DNA marker. +: Positive control. 19: KA 89/2. 20: KA 90/1. 21: KA 95/1. -: Negative control*

3.3. Sequencing and Phylogenetic Analysis

The *16S rRNA* sequences of the isolates were registered in GenBank under accession numbers MT633093 to MT633108.

3.4. Antimicrobial Susceptibility Testing

When the antibiotic resistance profiles of the isolated strains were evaluated, it was determined that six bacteria (KA 12/1, KA 19/2, KA 26/1, KA 26/4, KA 69/2, KA 95/1) were sensitive to all tested antibiotics (Table 2). Furthermore, all 21 L. monocytogenes bacteria were determined to be ampicillin and tetracycline resistant.

Table 2. *Antibiotic susceptibility of L. monocytogenes bacteria*

Strain code:	Antibiotic:												
	AMP	P	AMC	CEC	VA	AK	CN	CLR	TE	CIP	C	RD	SXT
KA 11/1	S	S	S	I	S	S	S	S	S	I	S	S	R
KA 11/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 12/1	S	S	S	S	S	S	S	S	S	S	S	S	S
KA 14/1	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 17/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 19/2	S	S	S	S	S	S	S	S	S	S	S	S	S
KA 21/2	S	S	R	R	S	S	S	S	S	S	R	S	S
KA 23/2	S	R	S	S	R	S	S	S	S	S	S	S	S
KA 26/1	S	S	S	S	S	S	S	S	S	S	S	S	S
KA 26/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 26/4	S	S	S	S	S	S	S	S	S	S	S	S	S
KA 30/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 30/3	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 45/1	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 55/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 69/2	S	S	S	S	S	S	S	S	S	S	S	S	S
KA 72/1	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 79/2	S	S	S	I	S	S	S	S	S	S	S	S	S
KA 89/2	S	S	R	I	S	S	S	S	S	I	S	S	S
KA 90/1	S	R	R	R	R	R	R	I	S	R	S	R	R
KA 95/1	S	S	S	S	S	S	S	S	S	S	S	S	S

AMP: Ampicillin, P: Penicillin, AMC: Amoxicillin/Clavulanic acid, CEC: Cefaclor, VA: Vancomycin, CN: Gentamicin, AK: Amikacin, CLR: Clarithromycin, CIP: Ciprofloxacin, TE: Tetracycline, C: Chloramphenicol, SXT: Trimethoprim-sulfamethoxazole, RD: Rifampicin, S: Susceptible, I: Intermediately resistant, R: Resistant.

4. DISCUSSION AND CONCLUSION

Due to its chemical and physical properties, fresh meat is an extremely favorable environment for the development and reproduction of microorganisms, whether pathogenic or not. It is known that minced meat, which can be consumed raw as well as in the content of fermented delicatessen products, which is not stored under appropriate conditions, contains *L. monocytogenes* bacteria causing meningitis, encephalitis, and listeriosis infection [46]. *L. monocytogenes* bacteria are a significant public health concern due to its capacity to proliferate at cold temperatures and a wide pH spectrum in contaminated foods, as well as their high pathogenicity. Especially when it comes to minced meat, it should be considered that bacteria will spread all over the food during the preparation of the food and will easily multiply. For these reasons, we aimed to determine the *L. monocytogenes* that is a causative agent of the important public health issue of listeriosis, the contamination rate of minced meats sold in Istanbul. In our study, firstly, bacteria suspected of *L. monocytogenes* were isolated by the USDA-FSIS method. In addition to the MOX medium, recommended according to the USDA-FSIS method, ALOA medium was used as the secondary solid medium to increase the isolation rates. When the selected enrichment medium was planted on the selective

solid medium, the quantity of *L. monocytogenes* bacteria isolated from the samples cultivated from the selective enrichment medium was observed to be greater.

A total of 16 bacteria from the ALOA and MOX media (five and 11 isolates, respectively). The results showed that A higher efficiency is obtained with ALOA medium compared to MOX medium. In other words, the enrichment step and the selection of the distinctive solid media were used are important for increasing the *L. monocytogenes* detection possibility from mincemeat. Similar to our study, in a study conducted in 2000 [47], in addition to PALCAM and Oxford media, ALOA was also used for the isolation of *L. monocytogenes*. In the study, Vlaemynck et al showed that with the detection of L. monocytogenes with the value of 86.1% the ALOA medium was more effective than Oxford/PALCAM (61.1%) medium.

In our study, *L. monocytogenes* suspicious bacteria isolated from MOX and ALOA selective solid media were examined in terms of biochemical tests. When the results were evaluated, it was determined that 21 strains obtained from 17 (17%) of 100 minced meat samples had similar phenotypic properties with *L. monocytogenes*. Our results correspond to the findings of studies investigating *L. monocytogenes* contamination of minced meat samples both in our country and in other countries. Guven and Patir [48], detected 35 *Listeria* spp. in 100 minced meat samples in their study conducted in the province of Elazığ and reported that 9% of samples had *L. monocytogenes*, 22% had *L. innocua*, and 4% had both species together. Berktas et al. [49] reported that they isolated *Listeria* strains from 73% of minced meat samples which were sold in butchers and supermarkets in Van, of which 11 (%15.07) were *L. monocytogenes*, 43 (%58.9) were *L. innocua*, four (%5.5) were *L. welshimeri*, four (%5.5) were *L. ivanovii*, five (%6.9) were *L. seeligeri* and six (%8.2) were *L. murrayi*. Akpolat et al. [50] examined 60 minced meat samples in their study in Diyarbakır and 5% of them were *L. monocytogenes*.

According to a study conducted in Mexico [51], *Listeria spp.* was obtained from 55 (62.5%) of 88 minced meat samples, while 16% of the isolates were found to be *L. monocytogenes* bacteria. De Simón et al. [52] examined 168 minced meat samples in Spain and found that 135 (80.3%) samples were found to be *Listeria* spp. and they determined that 29 (17.5%) of them were contaminated with *L. monocytogenes*, 112 (66.6%) with *L. innocua* and one (0.6%) with *L. welshimeri*. In the other research, similarly to our study, the percentage of *L. monocytogenes* in raw meats was reported to be 12.4% [53].

Some studies about the minced meat contaminated with *L. monocytogenes* found the isolation rate higher, unlike our findings. In our country, Sireli [54], Aydemir Atasever and Atasever [55], Ciftcioglu [56], Sireli and Erol [57] found that 32%, 24%, 32.4%, and 28%, respectively, of minced meat was contaminated with *L. monocytogenes*. In a study that 58% of 50 samples in Canada were contaminated with *L. monocytogenes* [58]. In a different study conducted in Canada, *L. monocytogenes* bacteria were detected in 77.3% and 100%, respectively, of minced beef and beef [59]. Likewise, in research carried out in Japan, *L. monocytogenes* was isolated from 60% of minced meat [60].

The results of the current study indicated that the rate of *L. monocytogenes* in samples was quite high. The high incidence of *Listeria* spp. in raw meats is due to faecal contamination during the evacuation of organs when slaughtering animals in slaughterhouses, food processing personnel, and cross-contamination during processing, shipping, or marketing [61-63]. Each part of the minced meat, which includes processes such as reducing, mixing and shredding, is heavily contaminated with microorganisms on the surface of the meat during processing [27,28]. Knives, soil, feces, manufacturing employees, and some equipment can all be responsible for contaminating meat and meat products [64,65]. The psychrophilic nature of *L. monocytogenes* bacteria is a serious concern for the meat industries. *L. monocytogenes* contamination that occurs during processing and storage in raw meat can spread and increase during meat processing [66,67].

In our study, when the PCR method was applied only 16 out of 21 strains, which were determined as *L. monocytogenes* by phenotypic methods, were found to be *L. monocytogenes*. *16S rRNA* sequences of 16 strains confirmed as *L. monocytogenes*, obtained because of sequence analysis, were identified, and recorded by the NCBI BLAST program based on the GenBank database.

Al-Nabulsi et al. [62] in their study about the determination of the contamination ratio of raw and processed meat by *L. monocytogenes*, detected that 13.7% and 50% of the processed and raw meat samples, respectively were contaminated. When the researchers confirmed by PCR of the isolates from processed and raw meat by culture method, they found that 87.5% and 92% of them were *L. monocytogenes*, respectively. In our study, it was observed that the number of *L. monocytogenes* isolates obtained by the traditional culture method was higher than that obtained by the PCR method. In a study [68] conducted to detection of *L. monocytogenes* contamination in raw meats, classical culture and PCR techniques were found to be compatible with each other in the samples examined. on the other hand, in only one sample, the researchers were able to detect *L. monocytogenes* with the PCR technique while they could not define by the culture method. Sanlibaba et al. [69] worked with 190 raw meat samples and 57 of them were identified as *Listeria* spp. by classical culture method and 23 of them were later identified as *L. monocytogenes* by PCR technique. When the obtained *L. monocytogenes* isolates were examined, it was found that 86.96% of them had the *hlyA* gene region. The results, in which the presence of *iap* and *hlyA* genes were examined after the classical culture method for the detection of *L. monocytogenes* in meat products, overlap with our study, which we advocate the necessity of confirming with PCR to complete the culture-based method.

Since the discovery of antibiotics, it is known that there has been a dramatic decrease in the rate of deaths due to infectious diseases. However, it has been predicted that microorganisms may gain resistance to these drugs and may encounter major problems in the treatment of the disease. The rigorous use of antibiotics in medicine is essential to control and reduce the prevalence of resistant organisms. In addition, antibiotic usage in veterinary medicine or livestock must be restricted cause of the fact that resistant bacteria can be transferred from food to humans or the environment. This situation involves two types of risks. The first is the spread of resistant pathogens, and the second is the transfer of resistance genes from non-pathogenic resistant bacteria to pathogenic species. Both conditions are of great importance as they cause diseases that are difficult to treat [70,71].

Studies have determined that it has been a correlation between the incidence of resistant pathogens and the existence of resistant food pathogens [72,73]. It is known that the resistance genes can be transferred from *L. monocytogenes* to other *Listeria* species, or non-pathogenic and pathogenic bacteria such as *S. aureus* and *Enterococcus spp.* [37, 74]. After the first resistant *L. monocytogenes* was identified in 1988 [75], due to the widespread or incorrect use of antibiotics, resistant strains of this bacterium continued to be detected at an increasing rate in cases of listeriosis [76-81]. The use of a combination of ampicillin or amoxicillin with gentamicin is currently the primary option in the treatment of human listeriosis [38,39]. Additionally, trimethoprim-sulfamethoxazolefor is used as the alternative treatment agent for penicillin-allergic patients [40].

In the current study, it was determined that bacteria identified as *L. monocytogenes* with biochemical methods were not resistant against all antibiotics used in the study. Three of the *L. monocytogenes* bacteria were found to be resistant to amoxicillin/clavulanic acid, two to penicillin, two to vancomycin, two to ciprofloxacin, and two to trimethoprim-sulfamethoxazole. The least resistance was found to be against amikacin, gentamicin, chloramphenicol, and rifampicin. No resistance was observed against ampicillin, clarithromycin, and tetracycline.

Yucel et al. [82] analyzed meat and meat products that 11% of 9 *L. monocytogenes* bacteria they isolated were resistant to kanamycin, 66% to trimethoprim-sulfamethoxazole, 100% to cephalothin and nalidixic acid; contrary to the results we obtained, they found that 66% of them were resistant to ampicillin. In a study of meat products and processing plants [83] examined the antibiotic susceptibilities of 206 *L. monocytogenes* bacteria, 100% were sensitive to chloramphenicol, 99.5% to tetracycline and ciprofloxacin, 96.1% to penicillin and 65% to clindamycin. They found that 100% was resistant to oxacillin, 35% to clindamycin, and 0.5% to tetracycline. The investigators determined that the isolates showed susceptibility against clarithromycin, tigecycline, imipenem, gentamicin, amoxicillin-clavulanic acid, ampicillin, levofloxacin, rifampicin, meropenem, linezolid, teicoplanin, trimethoprim-sulfamethoxazole, vancomycin, and moxifloxacin. Sanlibaba et al. [69], when they examined the antibiotic resistance properties of seven *L. monocytogenes* bacteria isolated from raw meat samples, they found that different from our results,

71.43% was resistant to penicillin, 71.43% to ciprofloxacin and 100% to ampicillin. It was found that the *L. monocytogenes* bacteria we isolated in our study were not resistant to tetracycline. Sanlibaba et al. [69], the rate of bacteria resistance to tetracycline was determined as 14.29%. It was also defined that the amoxicillin/clavulanic acid sensitivity ratio of isolates (14.29%) was lower than our study. It was determined that there was no isolate resistance to clarithromycin and the sensitivity rates against gentamicin (71.43%), vancomycin (100%), trimethoprim-sulfamethoxazole (71.43%), chloramphenicol (100%) antibiotics were similar to the results of our study. In a study conducted with raw and processed meat products, it was determined that 10% of *L. monocytogenes* bacteria isolated were resistant to tetracycline, 3% to streptomycin, 6.7% to kanamycin, 1.7% to doxycycline, and 5% to erythromycin. It was also found that all bacteria are sensitive to gentamicin, ampicillin, and vancomycin [62]. In a study by Camargo et al. [84], all of the isolated *L. monocytogenes* bacteria were sensitive to gentamicin, chloramphenicol, rifampicin, imipenem, vancomycin, erythromycin, penicillin, trimethoprim-sulfamethoxazole, and tetracycline, 52.5% were sensitive to clindamycin and 56.9% of them were found to be resistant to oxacillin. Dogruer et al. [85] found that six *L. monocytogenes* bacteria isolated from meat and meat products were sensitive to three antibiotics which are trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid, and vancomycin and resistant to clindamycin. In a study conducted with *L. monocytogenes* isolated from poultry, raw meat, and meat products, similarly to our findings, the bacteria showed to high susceptibility against chloramphenicol, amikacin, gentamicin, amoxicillin, trimethoprim-sulfamethoxazole, penicillin, and ampicillin [86]. Khen et al. [87] found that *L. monocytogenes* bacteria are sensitive to ampicillin (90%), clindamycin (36%), daptomycin (9%), erythromycin (100%), gentamicin (95%), oxacillin + 2% NaCl (16%), penicillin (96%), quinupristin/dalfopristin (92%), rifampicin (96%), tetracycline (99%) and vancomycin (97%). Unlike our results, Khen et al. [87] found that *L. monocytogenes* bacteria were 1% resistant to tetracycline but showed high resistance (99%) to ciprofloxacin.

When the antibiotic susceptibility test results were evaluated, it was found that the resistance profile of the *L. monocytogenes* bacteria we isolated against antibiotics was lower than in other studies. The low antibiotic resistance profile of the *L. monocytogenes* bacteria we isolated suggests that the source of minced meat samples studied was not exposed to antibiotics commonly used in animal husbandry before and that it was caused by the fact that *L. monocytogenes* bacteria did not encounter antibiotic-resistant bacteria and did not transfer antibiotic resistance gene.

In our study, we analyzed 100 minced meat samples from various districts of Istanbul using classical culture, biochemical, and molecular methods, and *L. monocytogenes* bacteria were found in 17% of them. This result made us think that it is a result of Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) in animal slaughterhouses and butchers.

To prevent *Listeria* contamination in foods, measures should be taken to prevent contamination in agricultural and livestock applications, and appropriate good manufacturing practices should be adhered to in processed foods. Our study data reveals that there is *L. monocytogenes* contamination, albeit at a low rate, in minced meat sold in Istanbul. The low antibiotic resistance of the isolates should not suggest that this contamination does not pose a serious public health problem. Detection of this organism, which should not be in food, is also an indication that the necessary hygiene and sanitation rules are not followed during the production stages.

It is recommended to frequently examine samples such as minced meat that can be consumed without heat treatment or cause cross-contamination in the kitchen environment for these bacteria, and to pay attention to hygiene and sanitation practices in slaughterhouses and butchers.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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