

## Research Article

# Prevalence and Antibiotic Resistance of *Listeria monocytogenes* Isolated from Ready-to-Eat Foods in Turkey

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The aim of the present study was the determination of the prevalence and antibiotic resistance of *L. monocytogenes* in ready-to-eat (RTE) foods in Ankara, Turkey. In order to detect and isolate *L. monocytogenes* from 201 RTE food samples, the EN ISO 11290:1 method was used. All isolates were identified using the polymerase chain reaction. The strains were also confirmed by the detection of the hemolysin gene (*hlyA*). The overall prevalence of *L. monocytogenes* was 8.5% among the food samples. Seventeen *L. monocytogenes* strains were examined by the disk diffusion assay for their resistance to 23 antibiotics. All strains were susceptible to erythromycin, clarithromycin, streptomycin, gentamicin, vancomycin, imipenem, trimethoprim, and chloramphenicol, while all strains were resistant to nalidixic acid, ampicillin, penicillin G, linezolid, and clindamycin. The higher resistance was found against oxacillin (94.1%), kanamycin (76.5%), levofloxacin (70.6%), and teicoplanin (64.7%), followed by amoxicillin/clavulanic acid (53.0%), rifampicin (47.1%), and ciprofloxacin (35.3%). A lower incidence of resistance was observed against tetracycline (5.9%), meropenem (5.9%), and trimethoprim/sulfamethoxazole (17.7%). All isolates were multidrug resistant showing resistance to at least three antibiotic classes. High *L. monocytogenes* prevalence among analyzed RTE foods represents a high risk for public health. Our findings show a high prevalence of *L. monocytogenes* in RTE foods in Turkey. More effective control strategies for *L. monocytogenes* are needed to reduce both prevalence and resistance of *L. monocytogenes* in Turkish RTE foods.

## 1. Introduction

*Listeria* species include Gram-positive, facultatively anaerobic, psychrotrophic, rod-shaped, non-spore-forming bacteria [1]. Currently, the genus *Listeria* contains eighteen different species [2], of which *L. monocytogenes* and *L. ivanovii* are pathogenic, and the former is the major causative agent of listeriosis in human and other mammals [3]. Although the incidence of listeriosis is rarely encountered compared to illnesses caused by other food-borne pathogens such as *Salmonella* spp., *Escherichia coli*, or *Campylobacter jejuni*, it can be lethal for newborns, the elderly, immunocompromised individuals, and pregnant women [4, 5].

*L. monocytogenes* is highly prevalent in clinical and food samples due to its ability to grow over a wide range of temperature including refrigeration temperatures and pH as low as 4.4, in high salinity (40% w/v), low water content, and hypoxic conditions [5, 6]. *L. monocytogenes* is often found in soil, contaminated silage, and nontreated water and therefore can easily contaminate food products of both animal and plant origin [7]. An important source of *L. monocytogenes* infection is the consumption of processed ready-to-eat (RTE) foods [4, 8], which provide a suitable medium for *L. monocytogenes* growth due to their long shelf life (one or more weeks) and low-temperature storage until consumption [9]. Furthermore, since RTE foods are consumed

without further cooking, contaminating *L. monocytogenes* remain viable [10]. Many RTE food-borne cases of listeriosis have been reported from all over the world. In March 2018, in South Africa, 978 people were infected with listeriosis, resulting in 674 hospitalizations and 183 deaths [11]. Another outbreak of listeriosis (20 cases) was reported in Australia by the World Health Organization (WHO) between January and April 2017, which resulted in the death of 35% of the patients [12]. European Food Safety Authority (EFSA) reported 1,763 confirmed cases of listeriosis from 27 member states, resulting in 191 deaths in 2013 [13]. In 2017, the Centers for Disease Control and Prevention (CDC) reported eight cases of listeriosis, after raw milk cheese consumption, resulting in two deaths [14]. In Turkey, food-borne listeriosis outbreaks have not been documented regularly, since they have not reached epidemic levels [15]. Although it is commonly detected in animals such as sheep and cattle, some food-borne listeriosis outbreaks have been reported in newborns and in cancer patients undergoing chemotherapy [16, 17]. There are several regulations concerning the acceptable microbiological level of *L. monocytogenes* in RTE foods. In the United States, it is 0 CFU/g of *L. monocytogenes* per 25 g of the food sample. However, according to the European Commission (EC) regulations no. 2073/2005 and no. 1441/2007, the number of *L. monocytogenes* should be less than 100 CFU/g in RTE products at the time of consumption. According to the Regulation 2073/2005, RTE foods able to support the growth of *L. monocytogenes* must not present in 25 g of the sample before the food has left the immediate control of the food industry. Its population must not exceed the limit of 100 CFU/g throughout the shelf life [18, 19]. In Turkey, the Turkish Food Codex has stipulated that *L. monocytogenes* must not be detected at all in the RTE foods [20].

Since the isolation of the first multidrug-resistant *L. monocytogenes* strain in France in 1988, several strains isolated from food and environmental and clinical samples have shown resistance to one or more antibiotics [5, 21, 22]. The levels of resistance vary among the strains and are also influenced by antimicrobial use in humans and animals and geographical differences [23]. *L. monocytogenes* is usually susceptible to a wide range of antibiotics used against Gram-positive bacteria. However, most strains of *L. monocytogenes* are naturally resistant to the currently used third and fourth generations fluoroquinolones and cephalosporins [22]. Genes conferring resistance to these drugs can successfully be transferred through movable genetic elements such as transposons and plasmids to other pathogenic bacteria. Common sources of resistance genes for *L. monocytogenes* are *Enterococcus* spp. and *Staphylococcus* spp. [24]. Moreover, increased exposure to biocides may select strains with high resistance to clinically relevant antibiotics [25]. The transmission of the resistant strains to humans via contaminated food products may have a serious impact on public health [21, 26].

So far very limited information is available on the prevalence of *L. monocytogenes* in RTE foods and their antimicrobial resistance in Turkey. Therefore, the objectives of this study were to (a) determine the prevalence of *L.*

*monocytogenes* in RTE foods, (b) identify the isolated strains by phenotypic and genotypic methods, and (c) determine the resistance of these strains against 23 antibiotics. According to our knowledge, this is the first detailed study that explores the prevalence of *L. monocytogenes* in RTE foods in Turkey.

## 2. Materials and Methods

**2.1. Sampling.** Between March 2017 and June 2017, a total of 201 RTE food samples were randomly purchased from various supermarkets, restaurants, weekly open bazaars, and street hawkers in Ankara, Turkey. The RTE foods were classified into eleven categories, as shown in Table 1. The foods sold by the street hawkers were fresh and unpackaged, while those sold at the supermarkets were packaged and nonfrozen. All RTE foods taken from restaurants were high heat-treated products, except for salads and raw lettuce. The samples were checked for expiry dates and transported to the laboratory under aseptic and refrigerated conditions (+4°C) on the sampling day and processed immediately.

**2.2. Isolation and Identification of *Listeria monocytogenes*.** The International Organization for Standardization (EN ISO, 11290–1) procedure with two-stage enrichment was used in this study. Briefly, 25 g of each food sample was first inoculated into 225 mL half Fraser broth (Merck™, Germany) for initial selective enrichment and homogenized in a stomacher (Seward 400, USA). After incubation at  $30 \pm 1^\circ\text{C}$  for  $24 \pm 2$  h, 0.1 mL of the broth culture was inoculated into 10 mL full-strength Fraser broth for second enrichment and cultured at  $37^\circ\text{C}$  for  $48 \pm 2$  h. A loopful each of the half- and full-strength Fraser broths were plated on the chromogenic *Listeria* agar Ottaviani and Agosti (ALOA agar) (Merck™, Germany) and polymyxin acriflavine lithium chloride cef-tazidime aesculin mannitol (PALCAM) agar (Merck™, Germany), and the plates were incubated at  $37^\circ\text{C}$  for 24–48 h. Five typical colonies each from ALOA and PALCAM agar were restreaked on tryptic soy agar supplemented with 0.6% of yeast extract (TSA-YE) (Sigma™, Germany) as a nonselective medium and incubated at  $37^\circ\text{C}$  for 24–48 h. The colonies from TSA-YE were verified by Gram staining, catalase reactions, oxidase tests, carbohydrate utilization, CAMP tests, and motility at 20–25°C.

**2.3. Bacterial Strains and Culturing.** *Listeria* species isolated in this study and some reference strains (*L. monocytogenes* ATCC7644, *L. innocua* ATCC12612, *L. seeligeri* SLCC3945, *L. welshimeri* ATCC35897, *Escherichia coli* ATCC25922, and *Staphylococcus aureus* ATCC6538) were inoculated on tryptic soy broth supplemented with 0.6% of yeast extract (TSB-YE) (Sigma™, Germany) and brain-heart infusion (BHI) broth (Merck™, Germany). The bacteria were grown at  $35^\circ\text{C}$  for 24 h. The initial isolates from the food samples were stored at  $-20^\circ\text{C}$  with 30% (v/v) glycerol (Merck™, Germany). The reference strains were obtained from the culture collection of Food

TABLE 1: Prevalence of *Listeria* species in RTE foods.

Product	Number of samples	Number of positive samples (%)				
		<i>Listeria</i> spp.	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. welshimeri</i>	<i>L. seeligeri</i>
Cooked red meat product	44	14 (31.8)	7 (15.9)	4 (9.1)	2 (4.6)	1 (2.3)
Cooked chicken product	38	9 (23.7)	4 (10.5)	3 (7.9)	2 (5.3)	0 (-)
Seafood product	25	7 (28.0)	3 (12.0)	2 (8.0)	2 (8.0)	0 (-)
Vegetarian product	15	1 (6.7)	0 (-)	1 (6.7)	0 (-)	0 (-)
Raw lettuce	15	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Fruit salad	13	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Egg-based product	12	1 (8.3)	0 (-)	0 (-)	1 (8.3)	0 (-)
Vegetable salad	11	4 (36.4)	2 (18.2)	1 (9.1)	1 (9.1)	0 (-)
Dairy product	10	3 (30.0)	1 (10.0)	2 (20.0)	0 (-)	0 (-)
Mayonnaise-based deli salad	10	2 (20.0)	0 (-)	1 (10.0)	1 (10.0)	0 (-)
Desserts with milk	8	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Total	201	41 (20.4)	17 (8.5)	14 (7.0)	9 (4.5)	1 (0.5)
CI 95%		11.4–21.2%	5.08–12.81%	3.67–12.65%	3.40–6.38%	0.201–2.914%

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**2.4. Molecular Identification.** Following the biochemical tests, the presumptive isolates were confirmed by polymerase chain reaction (PCR) analysis. Genomic DNA was extracted from the bacterial cells grown at 35°C overnight in TSB-YE using a genomic DNA extraction kit (Thermo Fisher Scientific™), as per the manufacturer's instructions. The primer pairs designated as U1 (5'-CAGCMGCCGCGGTAATWC-3') and L11 (5'-CTCCATAAAGGTGACCCT-3') were used to amplify a 938 bp region in the 16S rRNA gene of the *Listeria* genus [27]. In addition, the primer pairs F: 5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' and R: 5'-GCAACGTATCCTCCAGAGTGATCG-3' were used to amplify a 456 bp region of the *hlyA* gene of *L. monocytogenes* isolates [28]. Each 50 µL PCR mixture contained 5 µL PCR buffer, 1 µL 2 mM dNTP mix, 1 µL of each primer, 34.75 µL sterile distilled water, 0.25 µL Taq DNA polymerase, 4 µL of 25 mM MgCl<sub>2</sub>, and 3 µL DNA template [29]. PCR amplification was carried out in a programmed ThermoCycler (Techne TC-512, Staffordshire, UK) with the following conditions: (1) initial hold of 2 min at 95°C, (2) denaturation step at 95°C/45 s, annealing at 55°C/45 s, extension at 72°C/2 min, and (3) final extension step at 72°C/7 min. The PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide solution, and visualized under a UV illuminator (SYNGENE, Biosystems UK). The size of amplified fragments was determined by comparing with an O'Gene Ruler™ 10000 bp DNA ladder (Thermo Scientific™).

**2.5. Antibiotic Resistance Test.** *L. monocytogenes* strains were tested for their resistance to different antibiotics with the disk diffusion method on Mueller-Hinton agar (Merck™, Germany) containing 0.5% defibrinated sheep blood, as described by the Clinical and Laboratory Standards Institute (CLSI) [30]. Penicillin G (10 µg/disc), oxacillin (1 µg/disc),

ampicillin (10 µg/disc), amoxicillin/clavulanic acid (20/10 µg/disc), erythromycin (15 µg/disc), clarithromycin (15 µg/disc), tetracycline (30 µg/disc), ciprofloxacin (5 µg/disc), levofloxacin (5 µg/disc), nalidixic acid (30 µg/disc), linezolid (30 µg/disc), kanamycin (30 µg/disc), streptomycin (300 µg/disc), gentamicin (120 µg/disc), vancomycin (30 µg/disc), teicoplanin (30 µg/disc), meropenem (10 µg/disc), imipenem (10 µg/disc), clindamycin (2 µg/disc), trimethoprim (5 µg/disc), trimethoprim/sulfamethoxazole (1.25/23.75 µg/disc), chloramphenicol (30 µg/disc), and rifampicin (5 µg/disc) were used. These antibiotics were chosen for using or preferring in the veterinary and human medicine for treatment of listeriosis. After 24 h of incubation, the zones of inhibition were measured (mm), and the strains were categorized as susceptible, intermediate, or resistant to specific antibiotics as per the criteria of CLSI [30]. The breakpoints of *Staphylococcus* species resistance were considered since no resistance criteria exist in the CLSI guidelines for *Listeria* susceptibility testing [31, 32]. *E. coli* ATCC25922, *S. aureus* ATCC6538, and *L. monocytogenes* ATCC7644 were used as reference strains.

**2.6. Statistical Analysis.** All statistical analyses were carried out using SPSS 16 package. The analysis of one-way variance (ANOVA) followed by Tukey's test was applied to determine the differences between the antibiotic resistance of *L. monocytogenes* strains. Statistical significance was set at  $p < 0.05$ . 95% CI values were carried out using the Bonett method.

**2.7. Nucleotide Sequence Accession Numbers.** The nucleotide sequences of the 16S rRNA genes from 41 isolates of the present study were submitted and deposited to the GenBank.

**2.8. Dendrogram Construction Method.** The sequences were aligned with the multiple sequence alignment by CLUSTALW, and the neighbour-joining method was used for the phylogenetic tree.

### 3. Results

**3.1. Detection of *L. monocytogenes* in RTE Foods.** The presence of *Listeria* was identified in 41 (20.4%) of the 201 RTE food samples (Table 1). The 16S rRNA sequence analysis indicated the highest prevalence of *L. monocytogenes* (8.5%, 17/201) in the food samples, followed by *L. innocua* (7.0%, 14/201), *L. welshimeri* (4.5%, 9/201), and *L. seeligeri* (0.5%, 1/201). The analysis of the 16S rRNA sequence allowed separating the *Listeria* spp. isolates in six clusters. Cluster 1 was composed of 29 isolates. Eight isolates belonged to cluster 2. Finally, groups 3, 4, 5, and 6 included only one strain (Figure 1). Seventeen *L. monocytogenes* isolates were also screened for the virulence-associated *hlyA* gene, and all strains showed a positive result. Using the neighbour-joining method, phylogenetic relationships of 17 *L. monocytogenes* were allowed to group into two main clusters. Cluster 1 was composed of 15 isolates. Two isolates belonged to cluster 2 (Figure 2). *L. monocytogenes* was most frequently isolated from the cooked red meat products (41.2%, 7/17), followed by cooked chicken products (23.5%, 4/17), seafood products (17.6%, 3/17), vegetable salads (11.8%, 2/17), and dairy products (5.9%, 1/17). However, it was not detected in the samples from the other six categories of RTE foods including mayonnaise-based deli salads, desserts with milk, egg-based products, vegetarian products, fruit salads, and raw lettuce.

**3.2. Antibiotic Resistance.** The results of antibiotic resistance tests according to CLSI [30] are summarized in Table 2. All 17 *L. monocytogenes* strains were resistant to nalidixic acid, ampicillin, penicillin G, linezolid, and clindamycin. Frequent resistance was seen against oxacillin (94.1%, 16/17), kanamycin (76.5%, 13/17), levofloxacin (70.6%, 12/17), and teicoplanin (64.7%, 11/17). Furthermore, resistance to amoxicillin/clavulanic acid (53.0%, 9/17), rifampicin (47.1%, 8/17), ciprofloxacin (35.3%, 6/17), and trimethoprim/sulfamethoxazole (17.7%, 3/17) was also observed. Finally, one strain showed resistance against tetracycline and meropenem (5.9%). In contrast, all strains were susceptible to erythromycin, clarithromycin, streptomycin, gentamicin, vancomycin, imipenem, trimethoprim, and chloramphenicol.

Multidrug resistance, i.e., resistance to three or more antimicrobial agents, was observed in all *L. monocytogenes* strains. However, only one isolate (5.9%) was resistant to six antibiotics, and two (11.8%) of the isolates were resistant to seven antibiotics. On the whole, 14 of 17 (82.8%) *L. monocytogenes* strains showed resistance to more than seven antibiotics.

### 4. Discussion

**4.1. Prevalence of *L. monocytogenes*.** The overall prevalence of *L. monocytogenes* was 8.5% and was the most frequently isolated species from the products of animal origin. These products usually require several processing steps before consumption and may be contaminated with *L. monocytogenes* at the postprocessing stages, thereby reflecting

poor hygienic practices in the preparation of these foods at the retail level. Previous studies in Turkey [9, 10, 33] have reported occurrence of *L. monocytogenes* in RTE foods between 4% and 6.2%. Our findings showed higher rate of occurrence than that reported in previous studies in Turkey. The isolation rate of *L. monocytogenes* strains in this study was consistent with that reported in other countries, i.e., 6.25% in Ethiopia [3], 9.5% in Italy [6], 6.1% in Northern Greece [19], 8.1% in Greece [34], 6.2% in Spain [35], 6% in Canada [36], 6.87% in China [37], and 7.5% in Thailand [38]. Other studies have reported significantly higher isolation rates of *L. monocytogenes* in RTE foods, such as 18.2% in Jordan [4], 24.4% in Amman [5], 11.19% in Uruguay [8], 11.4% in Malaysia [39], 22.72% in Spain [40], and 32.3% in Iran [41].

*L. monocytogenes* was isolated from 41.2% of the RTE cooked red meat products in our study, which is also a higher rate than that reported by Lambert et al. [7] (1.2%), Yücel et al. [33] (6.4%), Shi et al. [37] (6.5%), Gomez et al. [40] (27.9%), and Iannetti et al. [42] (1.66%). The prevalence of *L. monocytogenes* among the RTE cooked chicken products was 23.5%, which was also higher than that reported in previous studies, which ranged from 3.5% to 18.2% [4, 18, 21, 33, 39]. The prevalence of *L. monocytogenes* in the RTE seafood products in our study was 17.6%, similar to that reported in Estonia (16.8%) [43], higher than that reported in Ethiopia (6%), Northern Greece (6.1%), the United Kingdom (6.74%), Columbia (5%), and Malaysia (6.7%) [3, 19, 36, 39, 44] and lower than the prevalence documented in Belgium (23.9%) [45] and Jordan (31.5%) [46]. We may speculate that the reason for the high prevalence of *L. monocytogenes* in the RTE cooked red meat, chicken, and seafood products could be due to (a) inadequate heat treatment, (b) inadequate physical separation between the raw and cooked food areas, (c) poor sanitation, or (d) cross contamination during processing and handling. Vegetable salads were the only raw products contaminated by *L. monocytogenes* in our study, with a prevalence of 11.8%. The isolation rate of *L. monocytogenes* in the vegetable salads was reported as 5.56% in Brazil [22], 25.8% in China [37], 4.1% in Nigeria [47], 22.5% in Malaysia [48], and 4.18% in Spain [49]. The presence of *L. monocytogenes* in the vegetable salads could be attributed to cross contamination and may occur during harvesting from the equipment, transport containers, or human handling. We did not detect *L. monocytogenes* in mayonnaise-based deli salads, desserts with milk, egg-based products, vegetarian products, fruit salads, and raw lettuce. *L. monocytogenes* can be controlled in these products by the appropriate implementation of good manufacturing practices (GMPs) and hazard analysis and critical control point (HACCP) systems.

**4.2. Antibiotic Resistance.** An increasing number of reports have been accumulated concerning the isolation of *L. monocytogenes* strains resistant to one or more antibiotics from food products, since 1988 [50]. Ampicillin, oxacillin, and penicillin are the most active  $\beta$ -lactams that inhibit the synthesis of bacterial cell wall peptidoglycan [51]. *L.*

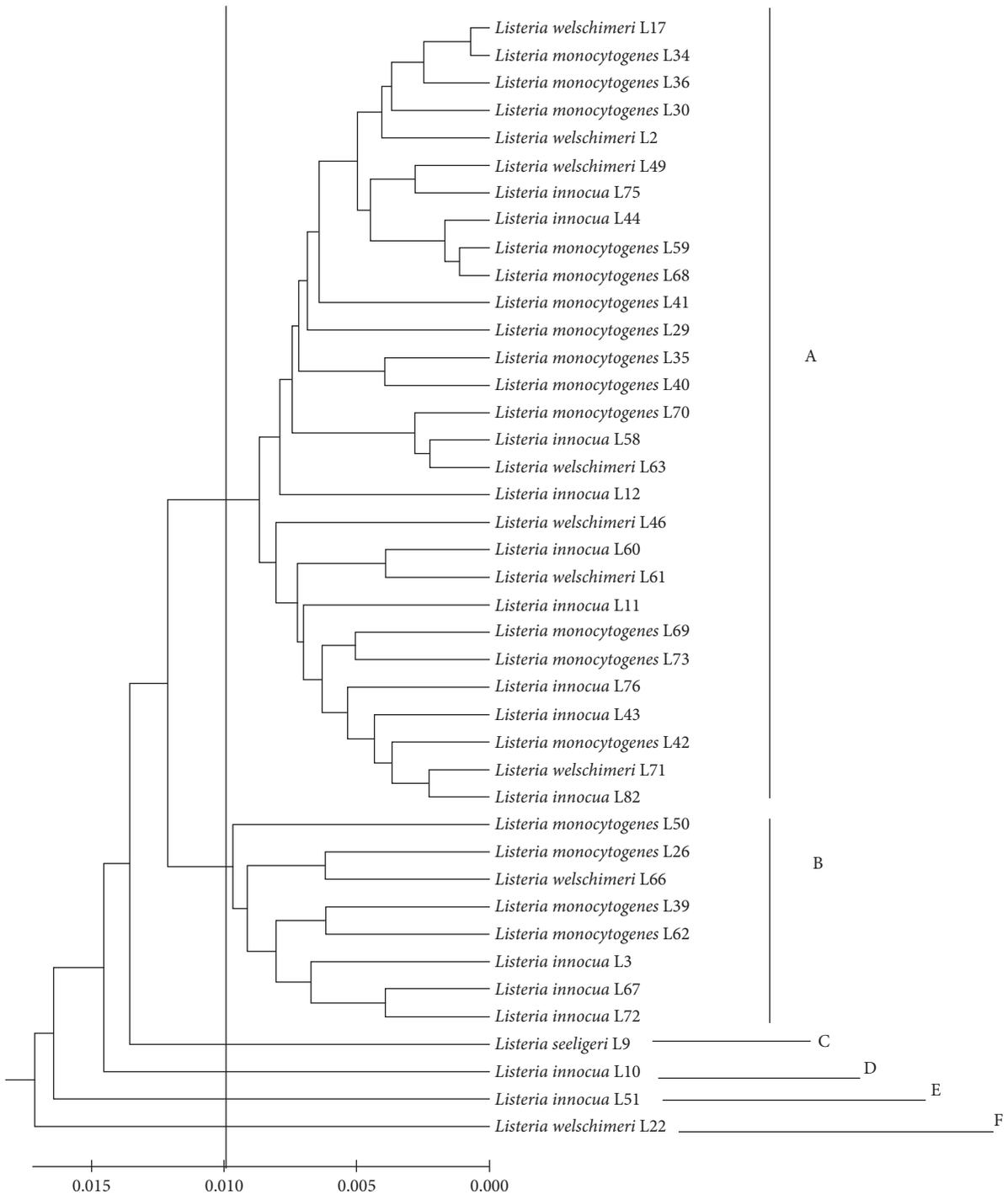


FIGURE 1: Dendrogram showing the evolutionary relationships among *Listeria* isolates based on the 16S rRNA sequence analysis.

*monocytogenes* is naturally susceptible to  $\beta$ -lactams, and the standard antibiotic therapy for human listeriosis includes penicillin/ampicillin alone or combined with an aminoglycoside (gentamicin) [5]. In the present study, all *L. monocytogenes* isolates from RTE foods displayed resistance to the  $\beta$ -lactam antibiotics, which is highly significant as far as the treatment of human listeriosis. The amoxicillin/clavulanic acid resistance was 53.0%, which was higher than that reported by Ruiz-Bolivar et al. [24] but

lower than that reported by Obaidat et al. [46] and Ennaji et al. [52]. Clavulanic acid is a nonantibiotic compound that can inhibit the beta-lactamase enzyme, thereby prolonging the antibacterial activity of amoxicillin [53]. The high level of resistance to oxacillin (94.1%) observed in this study was similar to the findings of Khen et al. [31], Ieren et al. [47], and Gomez et al. [50]. Only one isolate of *L. monocytogenes* was resistant to tetracycline (5.9%), which was significantly a lower rate than that reported by Garedew et al. [3] (37.5%),

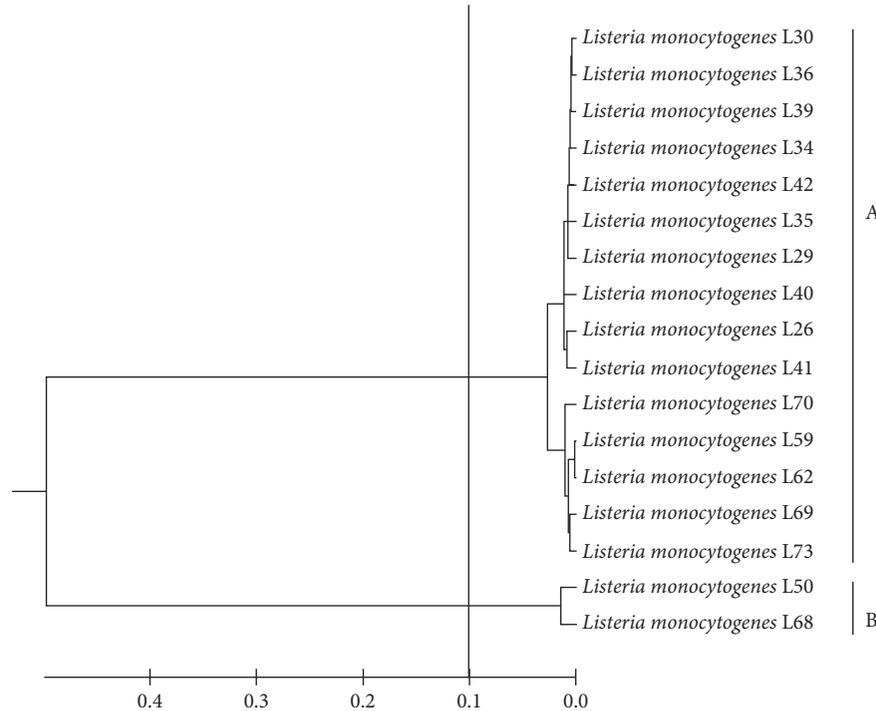


FIGURE 2: Dendrogram showing the evolutionary relationships among *L. monocytogenes* strains with the *hlyA* gene region.

Fallah et al. [21] (34.7%), Wang et al. [26] (26.9%), Jamali et al. [41] (27.9%), and Obaidat et al. [46] (64.4%). Tetracycline is not the primary drug for listeriosis treatment and is also not recommended for children and pregnant women [24]. All *L. monocytogenes* strains used in this study were resistant to nalidixic acid, which was similar to that reported by Ennaji et al. [52]. The resistance levels against ciprofloxacin (35.3%) were higher in our study compared to the findings of Wilson et al. [54] (2%), Kuan et al. [55] (5.2%), and Noll et al. [56] (9.7%). However, several studies have reported 100% susceptibility of *L. monocytogenes* to ciprofloxacin [4, 5, 22, 31, 50, 57]. Since ciprofloxacin is not routinely used as a treatment option in listeriosis [54], the resistance of *L. monocytogenes* strains against this antibiotic is noncritical. We also observed 100% resistance to linezolid, which belongs to the oxazolidinones class of antibiotics that have a broad-spectrum activity against Gram-positive bacteria. It binds to the 23S rRNA and disrupts the docking of the aminoacyl-tRNA in the A site of the ribosome and thus inhibits the delivery of peptides and the subsequent elongation of the polypeptide chain [51]. Contradictory to the findings of Wang et al. [26] and Shi et al. [37], none of the strains isolated in this study showed resistance against the aminoglycosides streptomycin and gentamicin that inhibit protein synthesis in bacteria by binding to one of the ribosomal subunits. However, 76.5% of the *L. monocytogenes* strains were resistant to kanamycin, in contrast to that reported by Al-Nabulsi et al. [5] (6.7%), Jamali et al. [41] (4.8%), and Obaidat et al. [46] (0%). Although none of the *L. monocytogenes* strains were resistant to the glycopeptide vancomycin, 64.7% were resistant to teicoplanin, another antibiotic of the same class. Glycopeptides prevent cross-

linking of peptidoglycan chains and inhibit cell wall synthesis. Vancomycin is used in treating *L. monocytogenes* bacteremia and endocarditis [46], and many studies [3, 5, 26] have reported the susceptibility of *L. monocytogenes* to vancomycin. However, other studies have reported 50% [10], 74.8% [46], 57.1% [47], and 20.9% [58] prevalence of vancomycin-resistant *L. monocytogenes* strains. Only one strain was found to be resistant to meropenem (5.9%) in our study, while none of the strains were resistant to imipenem. Meropenem has not been used in treating listeriosis in humans so far, and thus only a few studies have investigated meropenem resistance levels in *L. monocytogenes* strains [24, 55]. Therefore, our results would be important as a reference in any future study on meropenem resistance among *L. monocytogenes* strains. Clindamycin, erythromycin, and chloramphenicol inhibit protein synthesis by preferential binding to the 50S subunit of the bacterial ribosome. Although none of the strains were resistant to erythromycin and chloramphenicol in this study, 100% resistance was observed against clindamycin. Cross-resistance is frequently observed among clindamycins [24, 59] and is attributed to the enzyme modifying the structure of clindamycin, which inactivates the antibiotic action, as previously reported by Ruiz-Bolivar et al. [24] and Jamali et al. [41]. All *L. monocytogenes* strains were susceptible to trimethoprim in our study, in accordance with the reports of Osaili et al. [4] and Ruiz-Bolivar et al. [24]. Trimethoprim is used for patients with penicillin sensitivity [46], and trimethoprim/sulfamethoxazole is generally used for the treatment of listeriosis in the patients allergic to penicillin [22]. In our study, 17.7% of the strains were resistant to trimethoprim/sulfamethoxazole. Although Ennaji

TABLE 2: Antibiotic susceptibility and resistance (%) of *L. monocytogenes* strains isolated from RTE foods.

Antimicrobial agent <sup>a</sup>	<i>Listeria monocytogenes</i> strains (n:17)					
	S		I		R	
	n	%	n	%	n	%
Penicillin G	— <sup>c</sup>	—	—	—	17	100
Oxacillin	—	—	1	5.9	16	94.1
Ampicillin	—	—	—	—	17	100
Amoxicillin/clavulanic acid	8	47.0	—	—	9	53.0
Erythromycin	—	—	17	100	—	—
Clarithromycin	8	47.0	9	53.0	—	—
Tetracycline	16	94.1	—	—	1	5.9
Ciprofloxacin	—	—	11	64.7	6	35.3
Levofloxacin	1	5.9	4	23.5	12	70.6
Nalidixic acid	—	—	—	—	17	100
Linezolid	—	—	—	—	17	100
Kanamycin	—	—	4	23.5	13	76.5
Streptomycin	14	82.4	3	17.6	—	—
Gentamicin	12	70.6	5	29.4	—	—
Vancomycin	17	100	—	—	—	—
Teicoplanin	1	5.9	5	29.4	11	64.7
Meropenem	16	94.1	—	—	1	5.9
Imipenem	17	100	—	—	—	—
Clindamycin	—	—	—	—	17	100
Trimethoprim	10	58.8	7	41.2	—	—
Trimethoprim/sulfamethoxazole	10	58.8	4	23.5	3	17.7
Chloramphenicol	17	100	—	—	—	—
Rifampicin	1	5.8	8	47.1	8	47.1

<sup>a</sup>The diameters of the zones were compared with the diameters of the Clinic Laboratory Standards Institute (CLSI 2011). <sup>b</sup>S, susceptible; I, intermediate resistant; R, resistant. <sup>c</sup>Not detected.

et al. [52], Obaidat et al. [46], Fallah et al. [58], and Chen et al. [59] reported lower resistance to trimethoprim/sulfamethoxazole than our findings, Ieren et al. [47] reported higher incidence (57.1%). Rifampicin is a wide-spectrum antibiotic that acts against Gram-positive bacteria by inhibiting the RNA polymerase  $\beta$  subunit. It is commonly used against *Mycobacterium tuberculosis* when multidrug resistance is not detected [24]. The rifampicin resistance in our study was 47.1%, which is higher than that reported in previous studies: 41% [24], 3.9% [26], 4% [31], 10% [37], 0.72% [58], and 5.2% [60]. The higher levels of rifampicin resistance seen in our study could be clinically relevant.

In the present study, there was no significant association between the different *L. monocytogenes* strains in terms of antibiotic resistance ( $p > 0.05$ ). To our knowledge, this is the first study in Turkey that reports 100% prevalence of multidrug resistance among *L. monocytogenes* strains isolated from RTE foods. In contrast to our findings, Chen et al. [59] did not determine multidrug resistance in *L. monocytogenes* strains. The geographical distribution of multi-resistance patterns are as follows: 48.8% in Colombia [24], 60.2% in Iran [21], 57.7% in China [26], 95.2% in India [46], 64.3% in Nigeria [47], and 21% in Germany [56]. These differences may result from the regional use of antimicrobials.

## 5. Conclusions

Our findings showed a high prevalence of *L. monocytogenes* in RTE foods, which needs to be controlled in order to reduce the risk of listeriosis. Increasing antibiotic resistance among *L. monocytogenes* strains has been detected worldwide. This study is the first one to report multiple-drug resistance in *L. monocytogenes* strains isolated from RTE foods in Turkey, which is a public health concern. Our findings also show that RTE foods could be a reservoir for harboring multidrug-resistant *L. monocytogenes* strains.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this work.

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