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Original article

Prevalence of *Listeria* species in raw vegetables sold in Burdur province

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Abstract

In this research, a total of 90 raw vegetables (spinach, broccoli, lettuce, parsley, arugula, purslane, garden cress, scallions, and mushrooms) were used as materials, each of the vegetables included 10 samples obtained from different producers and district bazaars in Burdur province. *Listeria* spp. was detected in 9 (10%) of a total of 90 vegetable samples. However, none of the 9 isolated *Listeria* spp. from this study is classified as *L. monocytogenes*. Antibiotic susceptibility testing by disc diffusion method showed that 100% of the isolates were susceptible to ampicillin and penicillin G while the highest resistance has been found against meropenem and erythromycin (88.88%), and trimethoprim-sulfamethoxazole (66.66%). In conclusion, raw vegetables are considered to pose a hazard to food safety and public health due to *Listeria* species contamination.

Keywords *food safety, Listeria spp., PCR, vegetables*

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Introduction

People's eating habits have changed as their interest in healthy living has increased, and their demand for green-leafy vegetables and ready-to-eat salads prepared from vegetables has increased. From a nutritional point of view, vegetables are important low-calorie foods rich in vitamins, minerals, antioxidants, fibers, and bioactive compounds [1]. Leafy vegetables such as lettuce, spinach, and cabbage contain nutrients that help protect against heart disease, stroke, and cancer [2]. Vegetables, besides having significant health benefits, play an important role in the transmission of foodborne pathogens such as *E. coli* O157, Norovirus, *Salmonella, Listeria*, and *Cyclospora* [2].

Listeria is a genus of bacteria that belongs to the Listeriaceae family and has 21 species [3]. *Listeria* species can survive for long periods in various conditions such as low temperature (0-4^{o}C), low water activity (<0.9), high salt (10-40%), and a wide pH range (4.1-9.6) [4, 5]. *Listeria* spp. is widely available in various environments due to its resistance to harsh environmental conditions. In particular, *L. monocytogenes* is the most important foodborne pathogenic microorganism among the *Listeria* species [6]. *L. monocytogenes* causes listeriosis, an invasive disease leading to meningitis, septicemia, miscarriage, or infection of newborns, as well as a noninvasive disease with flu-like symptoms [7]. Susceptible populations such as adults aged 65 years and older, children under 5 years of age, individuals with weakened immune systems, and pregnant women are more likely to contract the disease [2, 6, 7]. Although there are relatively small number of the reported listeriosis outbreaks, the mortality rate after *L. monocytogenes* infection is high, reaching 20% to 30% in the U.S. [8], and it is recognized as a major public health problem [9]. Besides *L. monocytogenes*, other *Listeria* spp. species are also reported to be virulent [6]. *L. ivanovii* and *L. monocytogenes* remain the most important species that cause listeriosis in animals and humans [10]. In fact, *L. innocua* is thought to also be virulent [10].

Several studies have reported that *Listeria* species isolated from various foodstuff, animals, humans, and the environment carry virulence genes and show resistance to many antibiotics [4, 9, 11, 12, 13, 14]. Most *Listeria* species were found to be resistant to ampicillin, rifampin, penicillin G, tetracycline, clindamycin, cephalothin, and ceftriaxone [13]. The widespread and uncontrolled use of antibiotics and their presence in the environment and in foodstuffs cause an increase in bacteria and genes resistant to antibiotics, and this poses a risk for consumer health. In this perspective, monitoring of antibiotic resistance in *Listeria* species is a necessity [15].

Foodborne pathogenic microorganisms are ubiquitous in different stages of vegetables from production to consumption. Microorganisms can enter the food chain through insects, manure, water, dust, soil, decay of vegetation, and contaminate fresh food products [7, 16]. Foodborne diseases associated with fresh vegetables have been reported to increase over the past three decades. The reasons for these increases include livestock husbandry close to the vegetable production areas, the use of animal waste and waste-contaminated waters for irrigation in the fields without any treatment, the ability of microorganisms to remain on the product for long periods of time, an increase in the number of immunocompromised persons and an increase in vegetable consumption [7].

In terms of the quantity and diversity of vegetable species cultivated, Turkey ranks high among the world's countries. Vegetable production is carried out in the form of open field and greenhouse production depending on the ecological conditions. Open field vegetable growing is carried out for table consumption and industrial production in all regions of Turkey in the form of small family-owned enterprises without any protection measures in the fields [17]. Fresh raw vegetables, especially ready-to-eat salads, are consumed raw without applying treatments that provide microbial inhibition [7]. To ensure food safety, vegetables are stored in cold warehouse facilities and washed with antimicrobial containing water solutions such as chlorine in commercial applications [16, 18]. However, it is reported that chlorine has limited antimicrobial activity if used at permissible levels, and its excessive use is associated with the production of potentially toxic substances (trihalomethanes, haloacetic acids) [19]. In addition, washing leafy vegetables is not enough to destroy microorganisms. Microorganisms adhere to the surface of the leaves and enter into them. Therefore, the raw consumption of vegetables is a major cause of foodborne illness [2]. The existence of *Listeria* species, especially *L. monocytogenes* in vegetables can pose serious health risks.

Materials and Methods

Samples

Raw vegetable samples were collected between April and July 2021 by random sampling method from different producers and district bazaars in Burdur province in the Mediterranean Region of Turkey. In this research, a total of 90 raw vegetables were used as materials, 10 samples were collected for each vegetable that include spinach (*Spinacia oleracea*), broccoli (*Brassica oleracea italica*), lettuce (*Lactuca sativa*), parsley (*Petroselinum crispum*), arugula (*Eruca vesicaria*), purslane (*Portulaca oleracea*), cress (*Lepidi-*

um sativum), scallions (*Allium fistulosum*) and mushrooms (*Agaricus bisporus*). Fresh vegetable samples were placed in sterile bags and brought to the laboratory under cold chain and analyzed within 24 hours.

Detection of *Listeria* spp. in Vegetable Samples by Cultural Method

Isolation and identification of *Listeria* species in this study were done according to ISO 11290-1:2017 standard [20]. For analysis, 25 g vegetable samples were taken in a sterile stomacher bag, 225 mL of Half Fraser broth (Oxoid CM0895) was added to it and homogenized for two minutes in a stomacher device (IUL Masticator) and incubated for 25±1 hours at 30±1°C. At the end of the incubation period, 0.1 mL was taken from the culture and added to the second selective liquid enriched culture medium, Fraser broth (Oxoid CM0895), and incubated for 24±2 hours at 37±1°C. The pre-enrichment culture obtained because of the incubation was inoculated on two selective mediums. First, it was inoculated onto chromogenic *Listeria* agar base (Merck 1.00427.0500) and incubated for 48±2 hours at 37°C. Blue-green colonies that were surrounded by an opaque halo and reproduce in the medium were considered typical *L. monocytogenes* colonies, and non-opaque blue-green colonies were considered *Listeria* spp. However, some strains of *L. monocytogenes* that are exposed to stress conditions, especially acid stress, may show a very weak halo or do not form a halo at all. Oxford agar (Oxoid CM0856) was cultured as the second selective medium and incubated for 24±2 hours at 37±1°C. The formation of a blackish-green brown-black-zoned collapse-centered colony with a diameter of 2-3 mm onto Oxford selective agar was evaluated as *L. monocytogenes* colonies. Suspicious colonies reproducing on selective medium were inoculated into non-selective Tryptone Soy Agar (TSA-YE) containing 0.6% Yeast Extract and incubated for 24±3 hours at 37±1°C. Later, the suspicious *Listeria* isolates were analyzed in terms of Gram staining, catalase, oxidase reaction, β-hemolysis, typical umbrella motility in SIM medium (Oxoid CM 435), H_2S production, indole formation, carbohydrate tests (dextrose, maltose, mannitol, rhamnose, xylose, and sorbitol) [12, 21].

Confirmation of *Listeria* spp. by PCR

Genomic DNA Extraction

Genomic DNA isolation was done using the GeneJET Genomic DNA Purification Kit according to the manufacturer's instructions (Thermo Scientific, K0721).

PCR Analysis

PCR identification of *Listeria* isolates detected as suspicious by the cultural method was performed using different primer combinations derived from the *iap* gene that is specific for *Listeria* species [22, 23, 24]. In addition, the *flaA* (363 bp) gene, which is effective in the adhesion of flagella to the surface, and the *luxS* (208 bp) gene, which plays a positive role in biofilm formation, were examined. The genes used in PCR analysis and their primary sequences are given in Table 1.

PCR Amplification and Electrophoresis

Three µL of the extracted DNA was added to each of the PCR tubes. Six µL ddH_2O (sterile nuclease-free water), 0.5 µL forward primer, 0.5 µL reverse primer and 10 µL Ruby Taq Master (2x) Mix (Jena Bioscience, Germany) containing Taq polymerase, nucleotides (dATP, dCTP, dGTP, dTTP), KCl, $(NH4)_2SO_4$), $MgCl_2$, red stain, density reagent, enhancer and stabilizing additives were added to complete them to 20 µL. They were mixed by pipetting and the PCR mixture was brought to the ready-to-use state.

Listeria spp. isolates identified by bacteriological methods were tested by conventional PCR assay. For *Listeria* spp. identification, *iap* gene was amplified using primer

Table 1 - The genes and primer sequences used in PCR analysis.

Target gene	Primer sequences (5' → 3')	Product size (bp)	Reference
iap	MonoA F: CAAACTGCTAACACAGCTACT monoB R: GCACTTGAATTGCTGTTATTG	371	A. Bubert & al., 1992 [22]
iap	UnilisA F: GCTACAGCTGGGATTGCGGT Lis1B R: TTATACGCGACCGAAGCCAA	~1400	A. Bubert & al., 1997 [23]
iap	Iva1 F: CTACTCAAGCGCAAGCGGCAC Lis1B R: TTATACGCGACCGAAGCCAAC	1112	A. Bubert & al., 1999 [24]
iap	Sel1 F: TACACAAGCGGCTCCTGCTCAAC Lis1B R: TTATACGCGACCGAAGCCAAC	1099	A. Bubert & al., 1999 [24]
iap	Wel1 F: CCCTACTGCTCCAAAAGCAGCG Lis1B R: TTATACGCGACCGAAGCCAAC	1048	A. Bubert & al., 1999 [24]
iap	Ino2 F: 5-ACTAGCACTCCAGTTGTTAAAC Lis1B R: 5-TTATACGCGACCGAAGCCAAC	1017	A. Bubert & al., 1999 [24]
luxS	F: GGAAATGCCAGCGCTACACTCTTT R: ATTGCATGCAGGAACTTCTGTCGC	208	S.R. Warke & al., 2017 [26]
flaA	F: GCGCAAGAACGTTTAGCATCTGGT R: TTGAGTAGCAGCACCTGTAGCAGT	363	S.R. Warke & al., 2017 [26]

pairs UnilisA and LislB. The PCR conditions were initial denaturation at 94°C for 3 minutes, then 30 cycles of denaturation for 1 minute at 94°C, annealing at 56°C for 45 seconds, extension at 72°C for 45 seconds, and at the end of these processes, the amplicons were kept at +4ºC until the next stage [23]. The amplification of the MonoA and MonoB primer pair was performed in the conditions of initial denaturation for 4 minutes at 94ºC, then 30 cycles of denaturation for 45 seconds at 94ºC, annealing for 30 seconds at 55ºC, extension for 10 minutes at 72ºC, and at the end of these operations, the amplicons were kept at 4ºC until the next stage [22]. In the identification of other *Listeria* species, for Ino2 and Lis1B primer pairs a 45-second 30-cycle denaturation of the at 94ºC, annealing for 60 seconds at 62ºC, and a 45-second extension at 72°C were performed. For Sel1-Lis1B, Wel1-Lis1B, and Iva1-Lis 1B primer pairs, 30 cycles of denaturation for 30 seconds at 95ºC, 30 seconds of annealing at 62°C, and 90 seconds of extension at 72°C were performed [25]. For *luxS* (263 BP) and *flaA* (363 bp) pathogenicity genes, PCR conditions were an initial denaturation for 2 minutes at 94°C, then 30-second 35-cycle at 94ºC, annealing for 30 seconds at 58ºC, and extension for 7 minutes at 72ºC, and the amplicons were kept at +4ºC until the next stage [26]. After amplification, 1.5% agarose gel containing 1xTAE buffer was prepared (Prona Agarose, Biomax) and mixed with 10 mg/mL Ethidium Bromide (SNP Biotechnology). Then, DNA Marker (GeneRuler 100 bp DNA Ladder, Thermo Scientific), positive control (*L. monocytogenes* ATCC 7644), negative control (distilled water), and sample amplicons were loaded into the wells in the gel. Electrophoresis was performed for 1 hour at a current of 100 volts in the tank (Nyx Technik Voltronyx-V37, Taiwan). Then, the band formations were imaged through the UV-transilluminator (T12621D, Taiwan).

Antibiotics Susceptibility Test

Antimicrobial susceptibility tests of the isolates were carried out by disc diffusion method [27]. *Listeria* spp. isolates were incubated in TSA (Oxoid CM 131) for 24 hours at 37°C. The cultures obtained after incubation were taken with loops and suspended in sterile tubes containing 5 mL of 0.85% physiological saline solution and adjusted to 0.50 McFarland (10^8 CFU/mL) turbitide with a McFarland densitometer device (Biosan, Lithuania). Thereafter, the prepared suspension was inoculated onto Mueller Hinton agar (MHA) (Oxoid 337) containing 5% defibrinated horse blood and 20 mg/L β-NAD (Sigma-Aldrich, N6522) by streaking the sterile swab over the surface, and then the antibiotic discs were placed. The following antibiotic discs (Bioanalyse, Türkiye) were used: ampicillin (AM, 10 µg), penicillin G (P, 10 units), erythromycin (E, 15 µg), meropenem (MEM, 10 µg), and trimethoprim sulfamethoxazole (STX, 25 µg). Antibiotic discs were incubated at 35 ±1°C for 18 ±2 hours in 5% CO_2, and inhibition halos were measured. Interpretation of antibiotic susceptibility was determined in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing [28] for erythromycin, meropenem, trimethoprim sulfamethoxazole (*L. monocytogenes*), and the Clinical and Laboratory Standards Institute [29] for ampicillin and penicillin G (*Enterococcus* spp.). *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25293 were used as control cultures for the disk diffusion assay. Multidrug resistant isolates were defined as those which exhibited resistance to three or more antimicrobial classes of antimicrobial tested [30].

Statistical Analyses

The statistical analyses were performed Minitab for Windows Version Release 16.1. (Minitab Inc., 2010). The chi-square test was used to assess the differences between proportions at a significance level of 0.05.

Results

Nine (10%) of the 90 samples analyzed by the cultural method were presumed to be *Listeria* spp. Later, it was confirmed by PCR analysis that 9 of 9 isolates (100%) carried

Table 2 - Prevalence of *Listeria* spp. in raw vegetables.

Samples	n	Positive *Listeria* spp. n (%)	*Listeria* spp. positive isolates n					Isolates n
			L. monocytogenes	*L. ivanovii*	*L. seeligeri*	*L. welshimeri*	*L. innocua*	
Parsley	10	1(10)	0	0	1	0	0	1
Spinach	10	2(20)	0	2	1	1	1	5
Scallion	10	4(40)	0	0	1	2	2	5
Lettuce	10	0	0	0	1	0	0	1
Purslane	10	0	0	0	1	0	0	1
Cress	10	1 (10)	0	0	1	0	0	1
Arugula	10	1 (10)	0	1	1	1	1	4
Broccoli	10	0	0	0	1	0	0	1
Mushroom	10	0	0	0	1	0	0	1
Total	90	9 (10)	0	3	9	4	4	20

n: number of samples analyzed, a: At least one or more species were isolated from one positive sample.

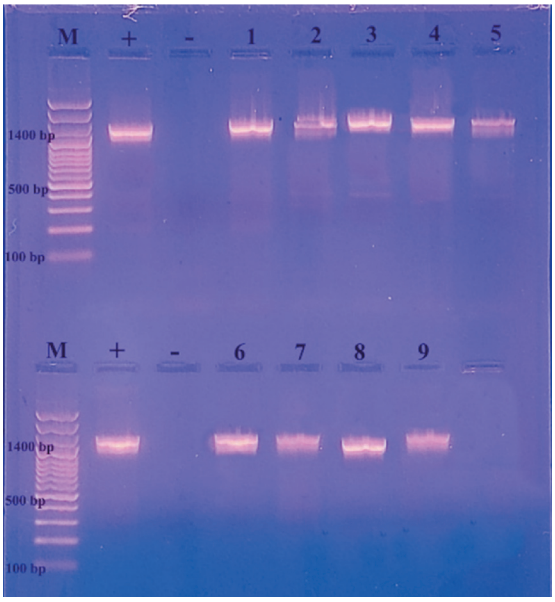

Figure 1 - Genus-specific identification of *Listeria* spp. isolates by PCR with the primer pairs UnilisA-Lis1B. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Line +: positive control (*L. monocytogenes* ATTC 7644), Line -: negative control (distilled water), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula.

Listeria spp genes. Also, *Listeria* spp. was detected in 2 of spinach (20%), 1 of parsley (10%), 4 of scallions (40%), 1 of cress (10%), and 1 of arugula (10%). However, *Listeria* species were not isolated in broccoli, mushrooms, lettuce, and purslane samples. The results on the prevalence of *Listeria* spp. in the analyzed sampled vegetable are given in Table 2, and the molecular identification of *Listeria* species by specific *iap* gene primers is shown in Figures 1-2. There was no statistically significant association between the type of vegetable surveyed and the presence of *Listeria* spp. (χ^2 = 4.659; p > 0.05).

Multiplex PCR of *luxS* and *flaA* genes revealed that 4 of 9 isolates (1 spinach, 3 scallions) harbored only *flaA* gene, whilst *luxS* and *flaA* genes were detected in 3 samples (spinach, parsley, scallion) and these two genes were found to be absent in 2 vegetable samples (cress, arugula), the results being shown in Figure 3.

Antimicrobial resistance profiles of 9 isolates confirmed by PCR as *Listeria* spp. were examined. The antimicrobial resistance of *Listeria* spp. isolates is shown in Table 3. In the present study, there was no significant association between the different *Listeria* spp. isolates in terms of antibiotic resistance (χ^2 = 6.750; p > 0.05). All 9 isolates (100%) were found to be susceptible to ampicillin and penicillin G. It was determined that 8 (88.88%) of 9 isolates analyzed were resistant to meropenem and erythromycin, and 6 (66.66%) were resistant to trimethoprim sulfamethoxazole. Also, multiple antibiotic resistance profiles were determined in 5 (55.55%) of *Listeria* spp. isolates.

Discussion

The *iap* (invasion-associated protein) gene, which is common to all members of the *Listeria* genus, encodes the p60 protein and is an important marker in PCR-based analyses of *Listeria* spp. [24]. *Listeria* spp. was detected in 9 (10%) of the total 90 vegetable samples obtained from the local district bazaar of Burdur province. *L. monocytogenes* was not detected in any of the vegetable samples. However, in this study, other *Listeria* species were isolated alone or in combined forms in raw vegetable samples (Figure 2). In this study, *L. seeligeri* was detected in all vegetable samples. *L. seeligeri* is reported to survive longer as a result of using xylose derived from cellulose which is abundant in the soil [31]. Although *L. seeligeri* is reported as a hemolytic but non-pathogenic bacterium, it has rarely been reported to cause acute purulent meningitis in a healthy adult person. In addition, *L. seeligeri* is reported to be a heterogeneous species in terms of pathogenicity and may contain strains that cause life-threatening diseases in humans [32]. In this study, *L. ivanovii* was isolated in 2 scallions and 1 arugula, a total of 3 vegetable samples, also *L. welshimeri* and *L. innocua* were isolated in 4 samples including 1 spinach, 2 scallions, and 1 arugula. *L. ivanovii* is a major problem in ruminants [33] as it causes abortion, stillbirths, and encephalitis in ruminants, but, sporadic cases of listeriosis caused by *L. ivanovii* have been reported in people, especially in immunocompromised people [34, 35]. Similarly, although *L. welshimeri* and *L. innocua* are not considered dangerous to human life, they are

Table 3 - Antibiotic resistance profiles of *Listeria* spp. isolated from raw vegetable samples.

Antimicrobial agent	*Listeria* spp. isolates (n=9)	
	S n (%)	R n (%)
AM,10 µg	9 (100)	0 (0)
P, 10U	9 (100)	0 (0)
MEM, 10 µg	1 (11.11)	8 (88.88)
STX, 25 µg	3 (33.33)	6 (66.66)
E, 15 µg	1 (11.11)	8 (88.88)

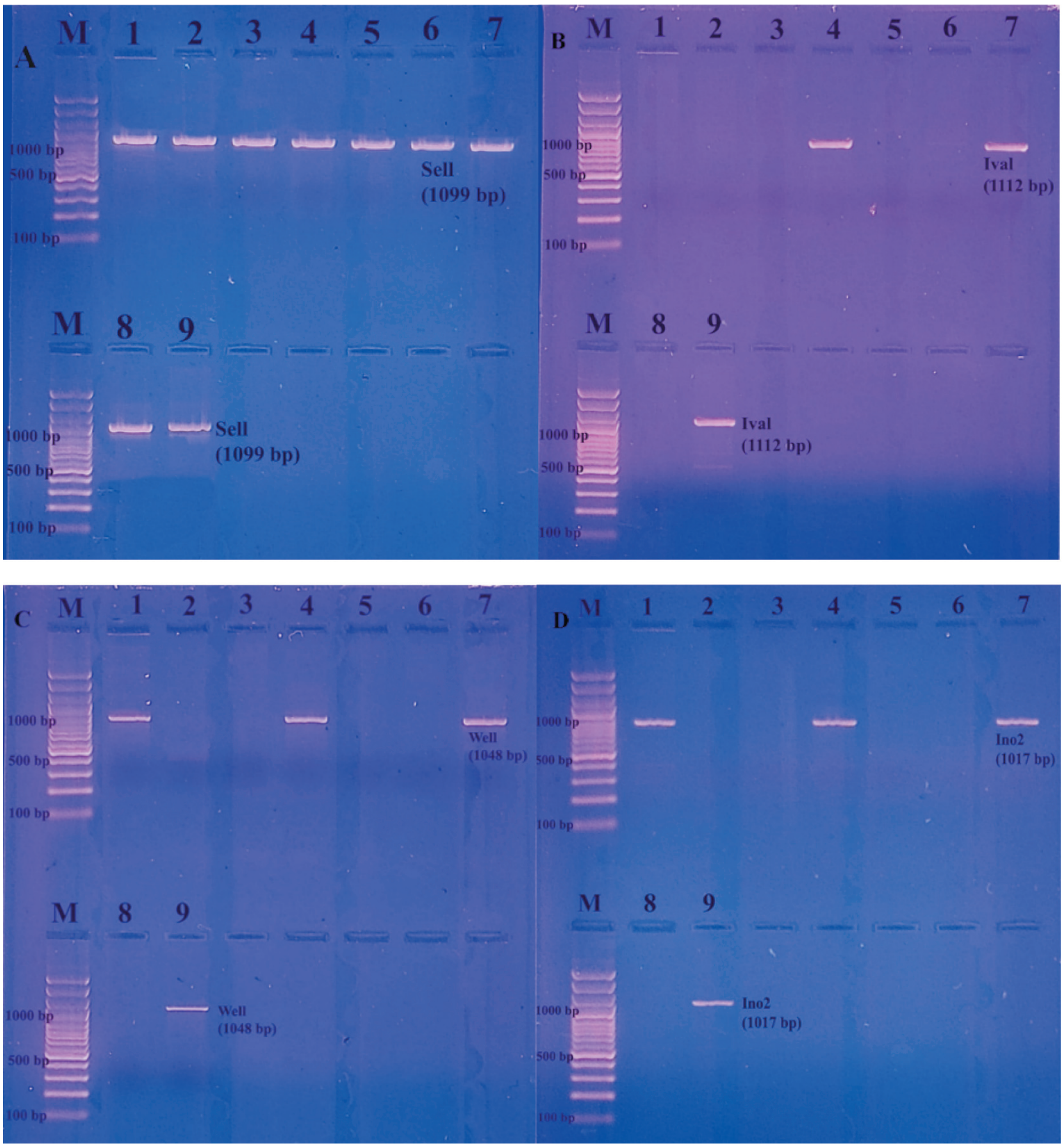

Figure 2 - Specific identification of *L. seeligeri* (A), *L. ivanovii* (B), *L. welshimeri* (C), and *L. innocua* (D) by PCR with primer pairs Sel1-Lis1B, Iva -Lis1B, Wel1-Lis1B, and Ino2-Lis1B, respectively. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula

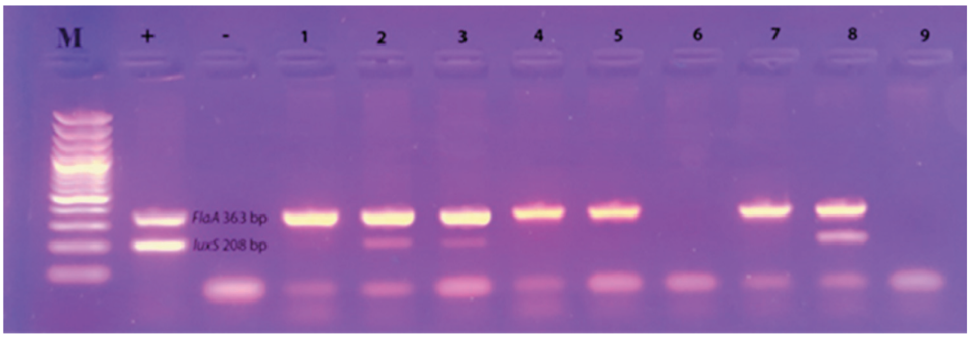

Figure 3 - Electrophorese image of luxS (208 bp) and flaA (363 bp) gene *Listeria* spp. by PCR. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Line +: positive control (*L. monocytogenes* ATTC 7644), Line -: negative control (distilled water), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula

predicted to pose a potential threat sometimes to people due to reported human cases [10, 36].

Although *L. monocytogenes* was not detected in any of the raw vegetable samples analyzed in this study, *flaA* and *luxS* genes were detected in the isolated *Listeria* spp. (Figure 3). All *Listeria* species use flagella to provide mobility in *in vitro* environments, though they are mobile at 20-25°C and immobile at 37°C. Flagella are critical for both surface adhesion and subsequent biofilm formation and are associated with virulence [12, 37, 38]. *Listeria* species can cause cross-contamination in food from contacted surfaces in the environment due to their ability to adhere to surfaces and create biofilms using peritric flagella [39]. In addition, the *flaA* gene encoding the Flagellin A protein is also used in the genotypic identification of *Listeria* species [40]. The *luxS* gene encodes an enzyme called S-ribosylhomocysteinase. This enzyme catalyzes the hydrolysis of S-ribosylhomocysteine to homocysteine and 4,5-dihydroxy-2,3-pentadione (DPD) and acts as a precursor to Autoinducer-2 (AI-2) [11]. In addition to being present in many gram-positive and gram-negative bacteria, it is responsible for pathogenesis, motility, and biofilm formation [41]. Although, *L. monocytogenes* was not detected in raw vegetables is a positive result for public health, the other *Listeria* species may pose health risks.

There are very few studies conducted on the presence of *Listeria* species in vegetables in Turkey. According to these studies, S. Lee & al. (2007) [42] detected *Listeria* spp. in 15 (40.5%), and *L. monocytogenes* in 3 (8.1%) of 37 frozen peppers in Bursa province. In addition, they detected *Listeria* spp. in 2 frozen strawberries (100%) and 1 frozen Brussels sprouts (100%). *Listeria* spp. was not detected in frozen tomato, pea, and scallion samples. S.A. Aytac & al. (2010) [43] analyzed a total of 164 leafy vegetable samples (8 basils, 15 dills, 20 cresses, 16 cabbage, 12 lettuces, 19 mint, 19 parsleys, 18 purslanes, 1 radish, 20 arugulas, 14 scallions, and 2 spinach) grown in the Ankara city. While *L. monocytogenes* was not detected in radish, spinach, and scallion samples, but detected in 14 samples (3 basils, 1 dill, 1 cress, 2 cabbage, 1 lettuce, 1 mint, 2 parsleys, 1 purslane, and 2 arugulas). R. Kara & al. (2019) [44] determined *L. monocytogenes* in 1 (1.43%) of 70 fresh lettuce samples collected from grocery stores and bazaars in Afyonkarahisar province. In contrast to the research results conducted by S. Lee & al. (2007) [42], S.A. Aytac & al. (2010) [43], and R. Kara & al. (2019) [44] *L. monocytogenes* was not isolated in green vegetable samples in this study. The variations in the prevalence of *L. monocytogenes* in vegetables are reported to be related with differences in seasonal, geographical and contamination exposure levels [3]. The inability to detect *L. monocytogenes* in raw vegetable samples in this study is probably due to the proper production process of the vegetables.

Studies in various countries, also stated that vegetables were contaminated with *Listeria* species at different rates. *L. monocytogenes* was detected in 2.5% of 120 packaged lettuce samples in Australia [45], in 13 (0.34%) of 5379 samples of freshly cut vegetables sold at markets in Canada [46]. *L. monocytogenes* was not detected in ready-to-eat vegetables sold in supermarkets in Portugal [47, 48]. D.K. Sonı & al. (2014) [48] isolated *L. monocytogenes* in 20 (10%) of 200 vegetable samples and in 10 (5%) of soil samples in India. V.V. Byrne & al., 2016 [16] isolated *L. monocytogenes* in a total of 4 (3.0%) samples, including 1 (2.22%) of 45 raw vegetables and 3 (5.56%) of 54 ready-to-eat vegetables (for salads) in Brazil. A.M. Gonı & al. (2016) [49] detected *Listeria* spp. in 84 (21%) of a total of 405 vegetable samples consisting of 16 types of vegetables (green amaranth, red amaranth, coriander, water spinach, winged bean, small water pepper, basil, lettuce, mint, scallion, gotu kola (pegaga), ulam raja, cucumber, mustard flowers, watercress, water celery) collected in Malaysia. *L. monocytogenes* were found in 69 (28.28%) of cabbages, 22 (9.02%) of carrots, 57 (23.36%) of cucumbers, 48 (19.67%) of lettuce, and 48 (19.67%) of tomatoes (19.67%) in Nigeria by T.A. Ajayeoba & al. (2016) [50].

While the *L. monocytogenes* ratio was 4.2% in the total of food samples in Ireland, it was determined as 3.8% in environmental samples [51]. M. Moravkova & al. (2017) [52] detected *L. monocytogenes* in 2 (2.1%) samples out of 97 by standard culture analysis, 4 (4.1%) samples by combined culture analysis, and 1 (1.9%) sample by PCR technique among 175 green-leafy vegetable and salad samples. In West Virginia, K. Lı & al. (2017) [53] detected *Listeria* spp. in 50% of the samples after analyzing 212 fresh products including tomatoes, green peppers, cucumbers, melons, and spinach, and *L. monocytogenes* in 3.78 % of the samples following identification analyses using PCR. M. Chen & al. (2018) [54] analyzed a total of 665 mushrooms in China, 237 of which were packaged and 428 of which were not packaged, and detected *L. monocytogenes* in 141 (21.2%) of fresh mushroom samples. I. Kljujev & al. (2018) [55] reported *Listeria* spp. in 25.58% of 43 vegetable samples (16 tomatoes, 13 sweet peppers, 2 cabbages, 1 hot pepper, 1 cucumber, 5 potatoes, 4 carrots, and 1 parsley), while *L. monocytogenes* was detected only in 1 (0.43%) carrot sample in the central Serbian Region. E.O. Kyere & al. (2020) [56] detected *L. monocytogenes* in 7 (11.666%) of 60 packaged vegetable samples, while 40 non-packaged vegetables did not find *L. monocytogenes* in New Zealand. A. Samad & al. (2020) [57] determined *L. monocytogenes* in 2 (2%) of the 100 fresh salads, but *L. monocytogenes* was not detected in 100 fresh vegetable samples in Pakistan. The findings obtained as a result of this research were found to be lower when compared with other studies [49, 55]. *L. monocytogenes* was not isolated from the samples collected in the present study, and is similar to the finding of J. Campos & al., 2013 [47], E.O. Kyere & al. (2020) [56], and A. Samad & al. (2020) [57]. *Listeria* spp. quantities in the data obtained by V.V. Byrne & al., 2016 [16], E.A. Szabo & al., 2000 [45], Health Canada, 2011 [46], D.K. Sonı & al. (2014) [48], T.A. Ajayeoba & al. (2016) [50], D. Leong & al., 2017 [51], M. Moravkova & al. (2017) [52], K. Lı & al. (2017) [53], M. Chen & al. (2018) [54], E.O. Kyere & al. (2020) [56], and A. Samad & al. (2020) [57] were found to be higher than the data presented in this study. The fact that the results of the research are different from the results of other research is thought to be caused by seasonal factors, geographical location, sample differences, raw material production and storage conditions, personnel hygiene, cross-contamination during transportation and sales, and differences in analysis methods.

The prevalence of antibiotic resistance, especially multiple antibiotic resistances, in the *Listeria* spp. is reported to be regularly rising [58]. In current study, it has been determined that *Listeria* spp. isolates are resistant to meropenem, trimethoprim-sulfamethoxazole, and erythromycin. The highest resistance has been found against meropenem and erythromycin (Table 3). Also, multidrug resistance, i.e., resistance to three or more antimicrobial classes, was observed in all *Listeria* spp. isolates. This situation suggests that it may cause public health problems for consumers. On the other hand, S. Stonsaovapak & M. Boonyaratanakornkıt (2010) [59] determined that *Listeria* spp. were resistant to penicillin, but sensitive to ampicillin, and sulfamethoxazole. L.M. Bılung & al., 2018 [13] reported that *Listeria* spp. isolated from vegetables are resistant to ampicillin, penicillin G, meropenem, and trimethoprim-sulfamethoxazole. *L. welshimeri, L. grayi, L. murrayi* and *L. innocua* cultures were reported to be sensitive to natural, semi-synthetic penicillins [14]. G. Cufaoglu & al. (2021) [60] reported the most resistant antibiotic was sulfamethoxazole (97.3%) and the less resistant antibiotic was meropenem (5.8%) in Turkey. Studies on the determination of antibiotic resistance of *Listeri*a species in vegetables and fruits are insufficient. In this study, the determination of the presence, virulence genes, and antibiotic susceptibility of *Listeria* species in the raw vegetable samples, it will contribute to other studies and epidemiological monitoring.

Conclusion

Vegetables are usually produced in insufficiently hygienic conditions and are sold wet in bazaars. In addition, it is consumed raw without any heat treatment or added as a mixture in ready-to-eat food products such as salads. Therefore, there may be serious risks in terms of the presence of *Listeria* spp. in vegetables. In this study, the fact that *L. monocytogenes* was not isolated from raw vegetables is considered as a positive result in terms of public health. However, it was concluded that detection of other *Listeria* species, *luxS* and *flaA* virulence genes, and multidrug resistance was observed in all *Listeria* spp. isolates in all of vegetables would pose a risk to public health. Also, it is considered that carrying out this research in different regions and with different vegetable species would be appropriate for determining the prevalence and virulence characteristics of *Listeria* species. To reduce contamination of vegetables at all stages from the field to the table, good agricultural practices and good hygiene practices should be carefully carried out to increase product safety in the cultivation, harvesting, classification, packaging, and distribution of fresh products.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

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