Polish Journal of Veterinary Sciences Vol. 27, No. 1 (2024), 13–23

DOI 10.24425/pjvs.2024.149329

Original article

Virulence properties of *Listeria monocytogenes* isolated from meat and meat contact surfaces in a slaughterhouse

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Abstract

Listeria monocytogenes is a ubiquitous microorganism that is isolated from a variety of sources such as soil, water, decaying vegetation, sewage, animal feeds, silage, farm environments and food-processing environments. This study aimed to determine the prevalence, serogroups, biofilm formation, virulence factor genes, and genetic relationships of *L. monocytogenes* strains isolated from beef meat and meat contact surfaces obtained from a slaughterhouse in Burdur, Turkey. In this study, a total of 179 beef meat and meat contact surface samples were analyzed for the presence of *L. monocytogenes* by polymerase chain reaction (PCR). Out of a total of 179 beef meat and meat contact surface samples, 83 (46.37%) were found to be contaminated with *L. monocytogenes*, with the highest incidence (53.01%) occurring in beef meat. In the present study, most of the isolated strains belonged to serogroups IIB and IVB (lineage I). The *L. monocytogenes* strain also contained *monoA-B, prfA, plcA, plcB, mpl, hlyA, actA, gtcA, dltA, Fri, flaA, InlA, InlC, InlJ*, and *iap* genes. Biofilm formation was not determined in the tested samples at pH 5.5 and different temperatures (4°C, 10°C, 25°C, and 37°C). However, strong biofilm formation was observed in 6.45% (2/31) of the strains at pH 7.0 after 48 h incubation at 37°C, and in 3.22% (1/31) of the strains at pH 7.0 after 48 h incubation at 4°C and 10°C.

Pulsed-field gel electrophoresis (PFGE) results showed that *L. monocytogenes* isolates were clonally related, and cross-contamination was present. In addition, PFGE results also revealed that *AscI* had more distinguishing power than the *ApaI* restriction enzyme. These results indicate that *L. monocytogenes* detected from meat and meat contact surfaces in the slaughterhouse pose a potential risk to public health.

Keywords: beef meat, biofilm, *Listeria monocytogenes*, PCR, PFGE, virulence genes

Introduction

Listeria is a genus of bacteria belonging to the family Listeriaceae, and has 17 known species. Only two of these species, *L. monocytogenes* and *L. ivanovii*, are accepted as pathogenic (Orsi and Wiedmann 2016). *L. monocytogenes* is a high-risk zoonotic pathogen that can be present in various environments (Oh et al. 2018). Its abundance in nature, ability to grow at refrigeration temperatures, low water activity, resistance against various environmental conditions such as high salt concentration, and ability to tolerate a wide spectrum of pH make it difficult to control (Ayaz and Cufaoglu 2016, Chen et al. 2019). Even though *L. monocytogenes* is present in food at a low rate, it leads to serious public health problems due to its high mortality rate and major economic losses in the food industry. Therefore, surveillance for *L. monocytogenes* in foodstuffs is of considerable importance for risk assessment (Orsi and Wiedmann 2016, Olaimat et al. 2018, Chen et al. 2019).

Pathogenic microorganism contamination of carcasses when slaughtering animals in slaughterhouses occurs by means of direct fecal material, contaminated skin during removal of viscera, contact of carcasses with one another, and with the equipment in the slaughterhouse (Costa et al. 2020). *L. monocytogenes* exists in the skin and intestines of several domestic and wild animals, with ruminants being its particularly significant natural reservoirs. Agents can spread not only from the feces of clinically affected animals but also via healthy carriers. For this reason, food of animal origin has become an important vehicle for the transmission of this microorganism to humans (Oevermann et al. 2010, Hellström 2011, Demaître et al. 2020). Farm settings, the environment around the slaughterhouses, and meat processing plantations can be frequently contaminated by *L. monocytogenes* (Cherifi et al. 2020). Despite hygiene and sanitation applications, it has been determined that some strains of *L. monocytogenes* can survive in the slaughterhouse, and resistant strains are responsible for recurrent food contaminations (Cherifi et al. 2020). On the other hand, biofilm is a durable form composed as a result of the adhesion of microorganisms to biotic surface such as human, animal, plant and abiotic surfaces such as walls, floors, floor drainage, conveyor belts, rubber, plastic, glass, and stainless steel (Mazaheri et al. 2020, Penesyan et al. 2021). A biofilm creates a protective barrier against various stress factors, disinfectants, and antimicrobials while providing easier access for bacteria to nutrients. Thus, it increases the likelihood of microorganism survival and cross-contamination (Andrade et al. 2020, Mazaheri et al. 2020).

The identification of virulence factor genes of *L. monocytogenes* is crucial to determine its pathogenesis. The virulence factors of *L. monocytogenes* include internalins that provide cell penetration (encoded by *inlA, inlC,* and *inlJ* genes) (Liu et al. 2007), hemolysin/ /listeriolysin O (encoded by *hlyA*), actin-based motility (encoded by *actA*), phosphatidylinositol phospholipase C (PI-PLC), which allows bacteria to escape from the vacuole (coded by *plcA)*, phospholipase C protein, which allows bacteria to spread from cell to cell (coded by *plcB*), invasion-associated protein (coded by *iap*) (Iglesias et al. 2017), a zinc-dependent metalloprotease that contributes to intracellular survival ability and is required to activate PC-PLC to initiate a new infection cycle (coded by *mpl)* (Poimenidou et al. 2018), cell wall teichoic acid glycosylation protein (coded by *gtcA*) (Promadej et al. 1999), virulence regulator protein (coded by *prfA*) (Soni et al. 2014), cytoplasmic D-alanine-D-alanylcarrier protein ligase (coded by *dltA*) (Abachin et al. 2002), flagellin protein (coded by *flaA*) (Liu et al. 2008), and the iron-binding ferritin-like protein that plays a protective role against peroxide stress (coded by *Fri* also known as *Dps*) (Dussurget et al. 2005).

Multiplex-PCR serotyping and PFGE are important tools for the characterization of *L. monocytogenes* isolates, and they are used to estimate possible risks for consumers (Neves et al. 2008). The *L. monocytogenes* serotypes are grouped into four genetic lineages (I, II, III, and IV), and molecular serogroups are divided into the following groupings: IIA, 1/2a or 3a; IIB, 1/2b, 3b or 7; IIC, 1/2c or 3c; IVA, 4a or 4c; and IVB, 4b, 4d or 4e (Doumith et al. 2004, Jennison et al. 2017). More than 90% of the strains extracted from the samples of food, animals and humans typically belong to the serotypes 1/2a, 1/2b, 1/2c, and 4b (Doumith et al. 2004). Serotype 4b is not generally found in food and has been reportedo be responsible for invasive listeriosis cases and 1/2a to be responsible for sporadic listeriosis epidemics. Serovar 1/2c strains are generally contained in food (Kayode et al. 2019). PFGE is accepted as the golden standard method used for molecular subtyping of *L. monocytogenes* owing to its repeatability and high-resolution power to identify the genetic relatedness of the strains (Graves et al. 2005, Neves et al. 2008, Yan et al. 2010).

Based on the above considerations, the aims of the present study were (i) to identify the presence of *L. monocytogenes* on the meat supplied from the slaughterhouse and the in contact with the surfaces meat, (ii) to determine the potential virulence gene content and biofilm formation of the isolates, and (iii) to characterize the serotypes and genetic diversity of *L. monocytogenes* strains.

Materials and Methods

Sampling

A total of 179 beef meat and meat contact surface samples supplied from a slaughterhouse in Burdur, Turkey were collected. Approximately 200 grams of meat samples (n = 83) were taken from the neck of cattle. Surface samples (n = 96) were taken with swabs from the animal slaughtering department (hands of personnel, knives, aprons, boots, offal tanks, walls, weighing platform, drain and carcass splitting saw), carcass shredding department (hands of personnel, aprons, boots, steel gloves, carcass shredding tables, knives, meat-bone conveyor belt, mincing machine and meat mallet), packaging department (meat hook trolleys and meat trolleys), and the cold storage (mince trolley, meat hook trolleys, walls and doors). The samples were collected in sterile plastic bags and analyzed within 24 hours after being transferred to the laboratory inside containers with ice.

Isolation of *L. monocytogenes*

The isolation and identification of *L. monocytogenes* were performed according to the ISO 11290-1:2017 method (ISO 2017). The suspicious *Listeria* isolates were analyzed in terms of Gram staining, catalase, oxidase reaction, methyl red and Voges-Proskauer tests, the hemolytic activity on blood agar, H_2S production, indole formation, typical umbrella motility in Sulfate, Indole, and Motility (SIM) Medium, and carbohydrate tests (dextrose, maltose, mannitol, rhamnose, xylose, and sorbitol) (Liu et al. 2008, Hitchins et al. 2022). *L. monocytogenes* ATCC 7644 strain was used as a positive control for all biochemical analyses. Isolated strains were kept in 20% (v/v) glycerol at -80°C for further analyses.

Identification and serotypes of *L. monocytogenes* strains

Bacterial genomic DNA was extracted from the cultures using the phenol/chloroform extraction and isopropanol precipitation method according to the procedure recommended by Liu et al. (2004). Briefly, the pellet was obtained by centrifugation from 500 µL of overnight bacteria at 10,000 rpm for 5 min. The pellet was dissolved in 300 µL 1 × TE (10 mM Tris-HCl and 1 mM EDTA pH 8.0) containing 2 mg/mL lysozyme (Sigma). After each tube had been incubated for 30 min at 37°C, 250 µL 10% (w/v) SDS (Applichem) and 25 µL 10 mg/mL proteinase K (Sigma) were added. Following the incubation at 56°C for 2 hours, 500 µL of phenol/chloroform/isoamyl alcohol (25:24:1) (Amresco) was added, and the tubes were centrifuged at 14,000 rpm for 15 min. The DNA samples were dried in a dry block thermostat (Biosan TDB-120). Finally, the extracted DNA samples were dissolved again in 1 × TE and stored at − 20°C for PCR tests.

PCR targeting the *monoAB* gene was performed to confirm suspected *L. monocytogenes*. MonoA (5'-CAAACTGCTAACACAGCTACT-3') and MonoB (5'-GCACTTGAATTGCTGTTATTG-3') primer couple, which is specific to all serotypes of *L. monocytogenes,* was used (Bubert et al. 1992, Bubert et al. 1997, Bubert et al. 1999). PCR amplification conditions were as follows: initial denaturation at 95°C for 5 min, 35 cycles consisting of denaturation at 95°C for 45 s, annealing at 53°C for 45 s, and extension at 72°C for 45 s; final extension at 72°C for 7 min. Additionally, all *L. monocytogenes* strains were serotyped using multiplex PCR as described by Doumith et al. (2004). For *lmo0737* (691bp)*, lmo1118* (906bp), *ORF2819* (471), *ORF2110* (597bp) and *prs* (370bp) genes, PCR conditions were an initial denaturation at 94°C for 5 min, 40 cycles at 94°C for 45 s, 53°C for 1.15 min, and 72°C for 1.15 min, and the final cycle was performed at 72°C for 7 min.

Determination of virulence factor genes

The presence of *prfA* and *mpl* (Nishibori et al. 1995)*, plcA* (Leimeister-Wachter et al. 1991)*, plcB* (Vasquez-Boland et al. 1992), *hlyA* (Furrer et al. 1991), *actA* (Jaradat et al. 2002), *dltA, gtcA,* and *iap* (Kyoui et al. 2014), *Fri* and *flaA* (Slama et al. 2013), *InlA, InlC*, and *InlJ* (Liu et al. 2007) genes in *L monocytogenes* strains was determined using PCR.

The reactions were carried out in a 25 µl volume containing 2.5 µL 10 × KCl buffer, 1.5 mM $MgCl_2$, 2.5 µL each of the four dNTPs (2 mM each), 0.2 µM each primer 0.75 U Taq DNA polymerase (Thermo), and 2 µL of the bacterial DNA template. Obtained PCR amplicons were resolved using 1−1.5% (w/v) agarose gel electrophoresis in 1 × TAE buffer. They were then visualized using SafeView™ Classic stain (Applied Biological Materials, Canada) in an Infinity Gel Imaging System (Vilber Lourmat, France).

Biofilm Formation

The biofilm forming abilities of *L. monocytogenes* strains were investigated quantitatively using the microplate technique (Sudagidan et al. 2008). All *L. monocytogenes* strains were grown in TSB at 37°C for 24 h. The grown culture was centrifuged and resuspended in TSB (pH 5.5 and pH 7.0). Each *L. monocytogenes* isolate (20 µL) was then added to 180 µL media (TSB pH 5.5 or pH 7.0) in 96-well tissue culture plates (Corning

Table 1. Incidence of *Listeria monocytogenes* in meat and meat contact surfaces.

Type of samples	n	No. of isolates for *L. monocytogenes* (%)
Beef meat	83	44 (53.01)
Meat contact surface samples	96	39 (40.63)
Total	179	83 (46.37)

n: Number of analyzed sample

Table 2. Serotype distribution of *Listeria monocytogenes* in meat and meat contact surface samples.

Type of samples	*lmo0737* serovars 1/2a,1/2c, 3a, and 3c	*lmo1118* serovars 1/2c, and 3c	*ORF2819* serovars 1/2b, 3b, 4b, 4d and 4e	*0RF2110* serovars 4b, 4d, and 4e	*prs* All *Listeria* spp.
Beef meat	43/83 (51.81%)	43/83 (51.81%)	43/83 (51.81%)	26/83 (31.33%)	45/83 (51.81%)
Meat contact surface samples	0/96 (0%)	7/96 (7.29%)	13/96 (13.54%)	0/96 (0%)	9/96 (9.38%)
Total	43/179 (24.02%)	50/179 (27.93%)	56/179 (31.28%)	26/179 (14.53%)	54/179 (30.17%)

Table 3. Virulence gene contents of *L. monocytogenes* strains.

Gene	No. of positives/No. of isolates for *L. monocytogenes* in total samples (%)
prfA	83/83(100%)
plcA	83/83(100%)
plcB	60/83 (72.29%)
mpl	83/83(100%)
hlyA	83/83(100%)
iap	32/83 (38.55%)
actA	83/83(100%)
gtcA	83/83(100%)
dltA	83/83(100%)
Fri	83/83(100%)
flaA	83/83(100%)
InlA, InlC, InlJ	83/83(100%)

Costar 3599, Lowell, USA). The plates were incubated at 4, 10, 25, and 37°C for 48 h. The content of each well was then removed and washed three times with 200 µL 0.9% NaCl. The attached bacteria were fixed for 15 min in 200 µL methanol. The plates were dried and stained with 200 µL crystal violet for 10 min, and the wells were rinsed under tap water. The stained bacteria were then dissolved using 200 µL 33% (v/v) acetic acid (Merck). The optical density (OD) of each well was measured using a microplate spectrophotometer (Epoch, BioTek, USA) at 590 nm.

Pulsed-Field Gel Electrophoresis

The genetic profile of *L. monocytogenes* isolates was subjected to DNA profiling using the restriction enzymes *Asc*I and *Apa*I (Thermo Fisher Scientific, USA). It was then identified by electrophoresis using the CH-DR II (Bio-Rad Inc, Hercules, CA, USA) system according to the PFGE PulseNet protocol (CDC 2017). After the digestion of DNA in the prepared plugs, they were subjected to electrophoresis in 1% (w/v) Pulse Field Certified Agarose (BioRad). The values of the electrophoretic parameters used were: initial running time, 4.0 s; final running time, 40.0 s; total time 22 h; angle, 120°; 6.0 V/cm; temperature, 14°C; ramp factor, linear. The PFGE gels were stained with EZ-Vision (Amresco Inc., USA) for 30 minutes after electrophoresis. They were then destained with deionized water twice for 15 min. Phylogenetic dendrograms were drawn using BioNumerics 7.6 (Applied Maths, Belgium) with the Unweighted Pair Group Method and Arithmetic Mean (UPGMA) method (Dice similarity coefficient, 1.5% band tolerance, 0.5% optimization and 80% degeneracy cutoff value).

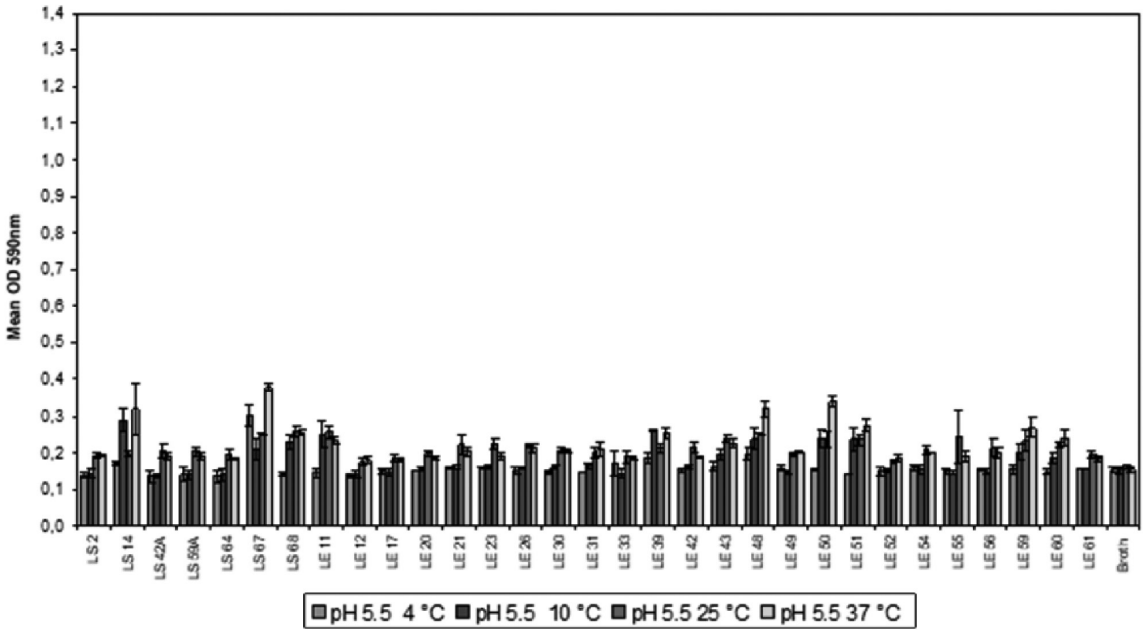

Fig. 1. Biofilm formation by *L. monocytogenes* strains at different temperatures in TSB pH 5.5.

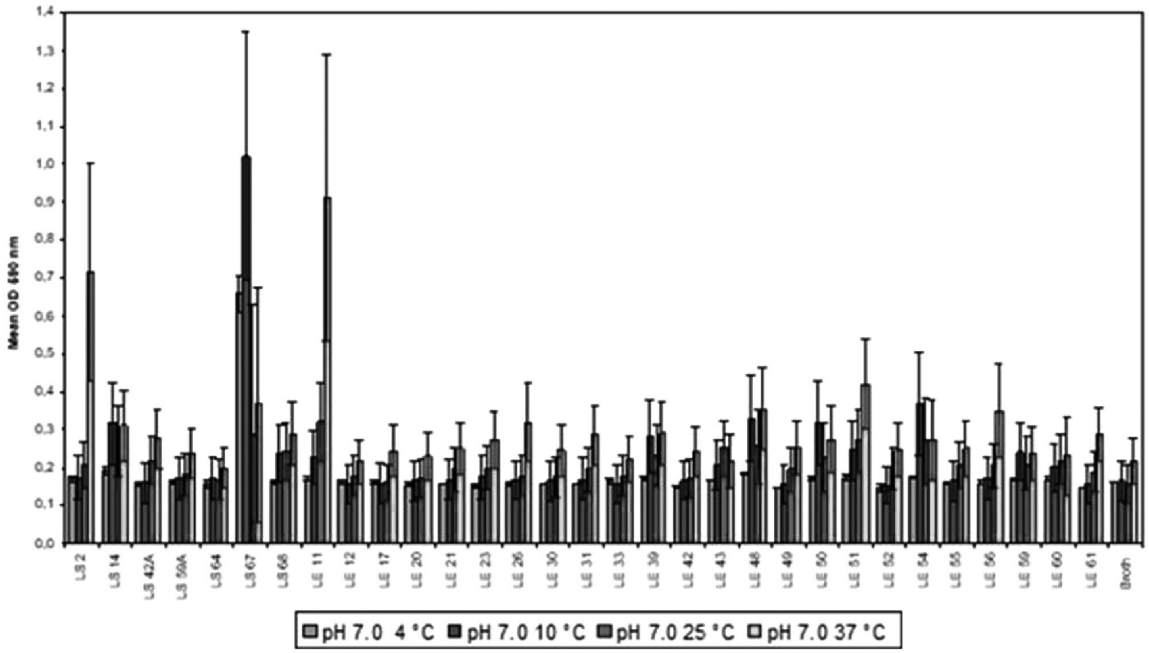

Fig. 2. Biofilm formation by *L. monocytogenes* strains at different temperatures in TSB pH 7.0.

Results

Out of 179 samples examined, 83 (46.37%) were found to be contaminated with *L. monocytogenes* (Table 1). In addition, the *L. monocytogenes* isolates from beef meat were identified in five serogroups (IIA, 1/2a or 3a; IIB, 1/2b, 3b or 7; IIC, 1/2c or 3c; IVA, 4a or 4c; and IVB, 4b, 4d or 4e), while the meat contact surface samples were identified in the three serogroups (IIB, 1/2b, 3b, or 7; IIC, 1/2c, 3c; IVA, 4a, or 4c) (Table 2). As a result of the PCR analysis, the *InlA, InlC, InlJ, prfA, plcA, mpl, hlyA, actA, gtcA, dltA, Fri,* and *flaA* (100%), *plcB* (72.29%), and *iap* genes (38.55%) were detected in *L. monocytogenes* strains (Table 3).

In this study, microplate test results revealed that strong biofilm formation was observed in 6.45% (2/31) of the strains at pH 7.0 after 48 h incubation at 37°C with an OD of 590 nm > 0.5, whereas biofilm formation was not determined after 48 h of incubation at pH 5.5. In the *L. monocytogenes* LS 67 strain, biofilm formation was observed at both 4°C and 10°C, but not at 25°C and 37°C (Fig. 1 and Fig. 2).

In the PFGE analysis, two restriction enzymes (*Asc*I

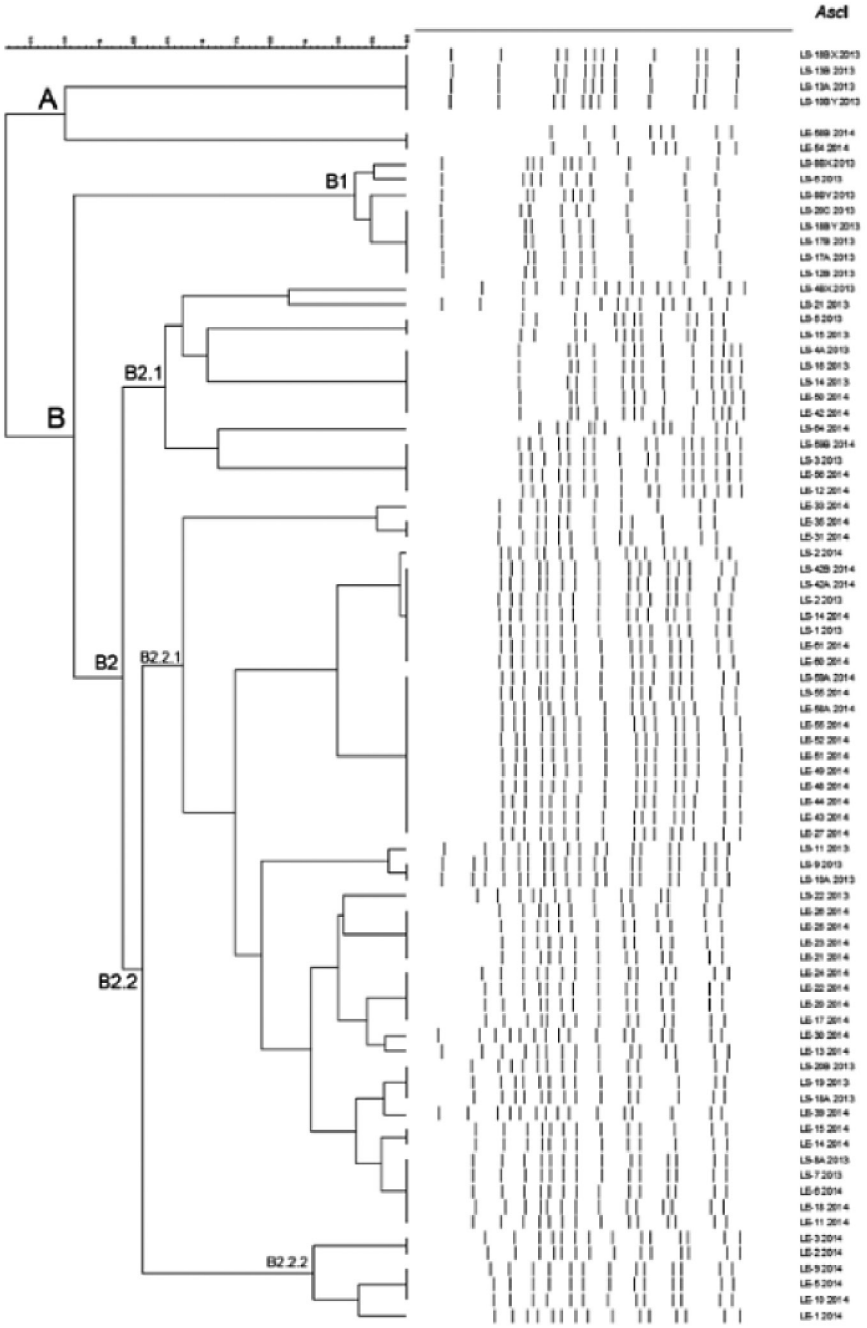

Fig. 3. Pulsed-Field Gel Electrophoresis (PFGE) profiles of *L. monocytogenes* isolates obtained with restriction enzyme *Asc*I.

and *Apa*I) were used to investigate the ASC genetic relatedness of *L. monocytogenes* strains. The band profiles of all strains obtained by *Asc*I showed two main groups (A and B) with 41.3% homology and 6 strains belonging to group A with 50% homology. The rest of the *L. monocytogenes* strains were placed in Group B (Fig. 3). The B1 and B2 groups were distinguished with 51.2% homology, and the homology between groups B2.1 and B2.2 was 58.5%. On the other hand, B2.2.1 and B2.2.2 groups were distinguished from each other with 61.3% homology. Some *L. monocytogenes* strains such as LS-18BX-2013, LS-13B-2013, LS-13A-2013 and LS-10BY-2013 showed 100% homology with indistinguishable band patterns (Fig. 3). In the case of *Apa*I restriction band patterns, the two main groups, A and B, were separated from each other with 40.4% homology. Two members of group A (LS-64-2014 and LE-54-2014) were found to be very different from the other isolated *L. monocytogenes* strains. Groups B1 and B2 were separated from each other with 52.3% homology (Fig. 4). PFGE results also showed that *Asc*I has more distinguishing power than the *Apa*I restriction enzyme.

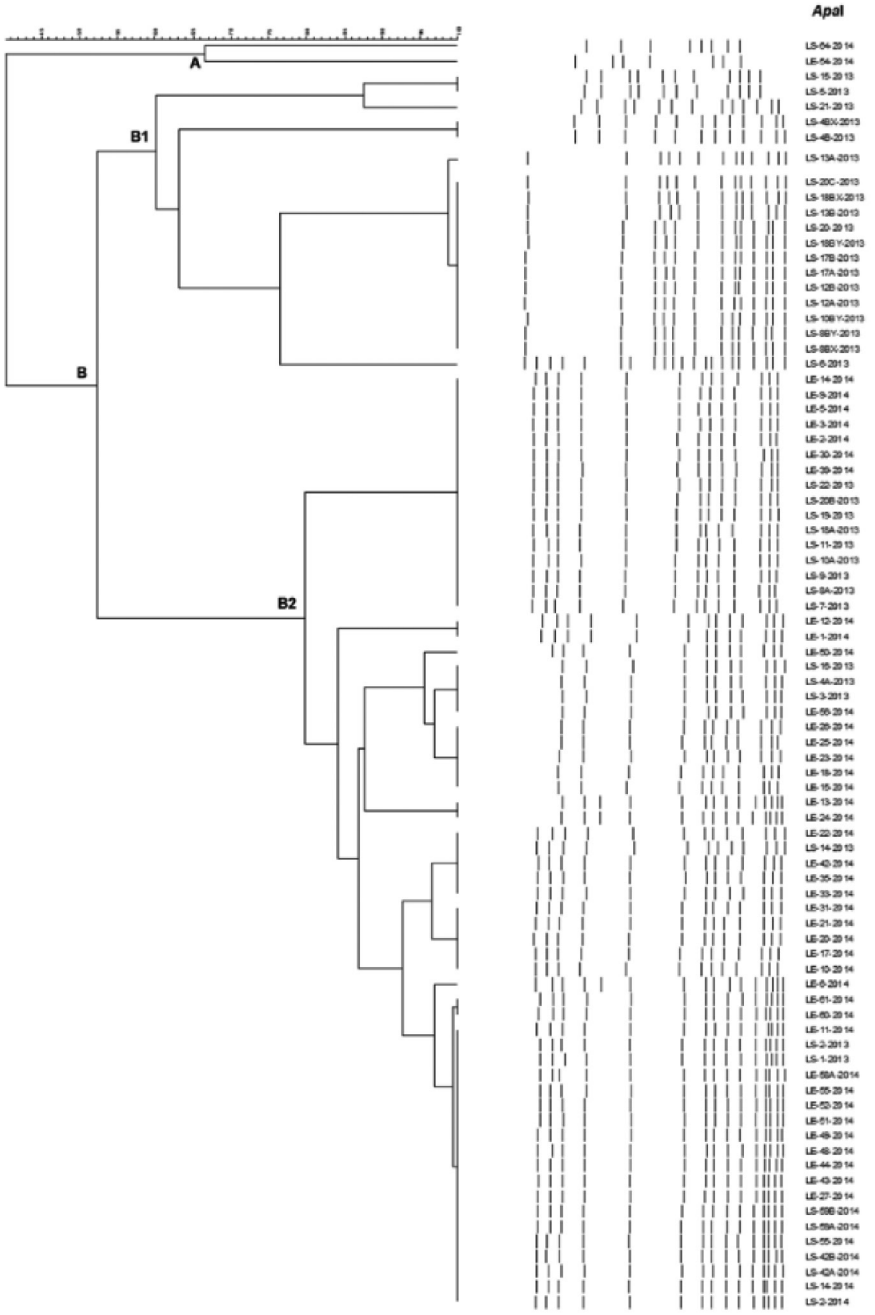

Fig. 4. PFGE profiles of *L. monocytogenes* isolates obtained with restriction enzyme *Apa*I.

Discussion

In this study, *L. monocytogenes* was isolated from beef meat, cutting knives, carcass splitting saws, weighing tables, hooks, carcass conveyor belts, walls, meat transport trolleys, carcass shredding tables and cold storage. However, *L. monocytogenes* was not isolated from staff hands, aprons and boots. It is reported that the slaughtering of animals, removal of cattle skin and carcass shredding, knives, knife sharpeners, and floors in slaughterhouses are the primary sources of microorganism contamination. Nevertheless, since *L. monocytogenes* is not isolated from the hands of the staff, it is stated that the contamination is not of human origin (Çadırcı et al. 2018). The prevalence of *L. monocytogenes* was investigated in beef meat and meat contact surfaces obtained from slaughterhouses worldwide and in Turkey. For example, Yucel et al. (2005) isolated *L. monocytogenes* in 4.7% (2/42) of raw minced meat, 5.2% (1/19) of raw beef and in 6.4% (2/28) of cooked red meat in Ankara, Turkey. Similarly, Arslan and Baytur (2019) found *L. monocytogenes* in 41.9% (26/62) of ground beef meat in Bolu, Turkey. Sahin et al. (2020) detected *L. monocytogenes* in 14.8% (8/54)

of beef samples and 9.6% (5/52) of sheep meat samples in Sivas, Turkey. On the other hand, Çadırcı et al. (2018) isolated *L. monocytogenes* in 1.6% (5/300) of samples from a slaughterhouse in Samsun, Turkey. It was isolated at 2.7% (1/36) from cattle carcass, 2.7% (1/36) from the blade, 2.7% (1/36) from the blade sharpener, 2.7% (1/24) from the floor, and 2.7% (1/36) from cowhide. Studies from other countries include the research by Teixeira et al. (2020) who reported the presence of *L. monocytogenes* in 12% (6/50) of beef samples in Brazil. Boukili et al. (2020) detected *L. monocytogenes* in 7.14% (10/140) of analyzed beef meat samples in Morocco. Zhang et al. (2021) determined that *L. monocytogenes* was present in 29.29% (70/239) of raw, intermediate and end meat products, and in 12.2% (29/239) of meat contact surface samples such as the floor, walls, transport units, pipes, trash can and weighing equipment, as well as the hands, clothes, and shoes of the handlers in Shanghai. Likewise, Papatzimos et al. (2022) isolated *L. monocytogenes* in 4.44% (2/45) of raw unprocessed meat, in 5.90% (1/17) of food handlers' hands, and in 5.11% (9/176) of environmental and processing surfaces in Greece. Jang et al. (2021) found *L. monocytogenes* in 0.7% (2/300) of raw beef, 28.6% (6/21) of gloves used in carcass splitting, 5.6% (1/18) of the splitting saw, and in 6.7% (1/15) of the drain zone in Korea. The amount of *L. monocytogenes* obtained as a result of the present research was found to be higher when compared to other studies. It has been concluded that the reasons for this high rate are that the cattle are in contact with the natural environment, they are fed with contaminated silage and are slaughtered and cut up in abattoirs which do not comply with hygiene regulations.

In the present study, serovars 1/2b, 3b, 4b, 4d, and 4e were determined in 51.81% of the isolates from the analyzed meat samples and in 13.54% of the isolates from the meat contact surfaces (Table 2). Most of the isolated strains belong to serogroup IIB and IVB (lineage I). It has been reported that most outbreaks of human listeriosis are associated with lineage I isolates, and lineage I strains are more represented among human isolates compared to lineage II strains. Lineage II strains are common in foods and in natural and farm environments, and they are also commonly isolated from animal listeriosis cases and sporadic human clinical cases (Orsi et al. 2011). The distribution of serogroups differed between beef meat and meat contact surface samples isolates. As a result of the serotyping conducted by Çadırcı et al. (2018), five isolates (serotype 1/2b or 3b) from one cattle carcass, five isolates (serotype 1/2b or 3b) from one knife, two isolates (serotype 4b or 4d, 4e) from one knife sharpener, five isolates (serotype 1/2a or 3a) from one floor sample, and one isolate (serotype 1/2a or 3a) from one cowhide were determined. Serotypes 1/2b and 1/2a were determined to be dominant in the samples obtained from the slaughterhouse, whilst serotype 1/2c was not encountered. Arslan and Baytur (2019) reported that 13 (50%), 12 (46.2%), and 1 (3.8%) of 26 isolates from ground beef were identified as serotypes 1/2c, 1/2a, and 3c, respectively. Papatzimos et al. (2022) found 11 strains belonging to serogroup IIa (1/2a and 3a) and one to IIc (1/2c and 3c) from a meat processing facility. Jang et al. (2021) found that 10 isolates from raw beef and the slaughterhouse environments belonged to 1/2a (66.7%), 1/2b (6.7%), 1/2c (26.7%), and 4b (6.7%) serotypes. The distribution of serotypes varies in different geographic regions (Arslan and Baytur 2019).

The virulence of *L. monocytogenes* is associated with the invasion of the host and the reproduction, loss, or function of the virulence genes (Li et al. 2022). In the present study, the *InlA, InlC, InlJ, prfA, plcA, mpl, hlyA, actA, gtcA, dltA, Fri* and *flaA* genes were detected in all *L. monocytogenes* strains, except the *plcB* and *iap* genes. Arslan and Baytur (2019) detected all virulence-related genes (*hlyA, actA, inlA, inlB, inlC, inlJ, prfA, plcA* and *iap*) in 100% of *L. monocytogenes* strains in ground beef meat. On the other hand, Papatzimos et al. (2022) found that *L. monocytogenes* strains isolated from 12 samples carried the *inlA, inlC, inlJ* and *plcA* genes, while 4 samples did not carry the *iap* gene. In addition, 8 of the 12 samples contained the *actA* and *hlyA* genes. It has been reported that differences in the prevalence of virulence genes may be related to the use of several PCR target fragments within the genes (Coban et al. 2019).

In this study, biofilm formation was not determined in the tested samples at pH 5.5 and different temperatures (4°C, 10°C, 25°C, and 37°C). However, strong biofilm formation was observed in 6.45% (2/31) of the strains at pH 7.0 after 48 h incubation at 37°C, and in 3.22% (1/31) of the starins at pH 7.0 after 48 h incubation at 4°C and 10°C. In addition, it was determined that *L. monocytogenes* isolated from carcass splitting saws, meat mallets, and cold storage walls had significantly higher biofilm forming ability. The biofilm formation abilities of *L. monocytogenes* strains isolated from meat, food processing equipment, and work surfaces have been investigated in various studies (Agostinho Davanzo et al. 2021, Jang et al. 2021, Papatzimos et al. 2022). Papatzimos et al. (2022) noted that 91.7% (11/12) of *L. monocytogenes* isolated from environmental and processing samples during production time showed strong biofilm formation ability. Jang et al. (2021) reported that *L. monocytogenes* isolates from splitting saws, drains and gloves showed significantly higher biofilm-forming ability. These findings

support the results of the present study. However, Agostinho Davanzo et al. (2021) detected that *L. monocytogenes* isolates formed weak biofilms in 11 (78.57%) of 14 isolates in a 24 hour incubation period at 37°C and in 9 out of 14 (64.3%) isolates in 168 hours incubation at 12°C from poultry slaughterhouses. The presence of nutrients on stainless steel and polytetrafluoroethylene substrates affects biofilm formation more than temperature (Chavant et al. 2002). Therefore, it is recommended that slaughterhouses take hygiene and sanitation measures to produce quality beef and to protect public health.

Dendrograms obtained from PFGE of *L. monocytogenes* are given in Fig. 3 and Fig. 4. Some *L. monocytogenes* strains (LS-18BX-2013, LS-13B-2013, LS-13A-2013 and LS-10BY-2013) showed 100% homology. In this study, PFGE results show that *L. monocytogenes* isolates are clonally related, and cross-contamination is present. PFGE results also showed that *AscI* has more distinguishing power than the *ApaI* restriction enzyme. Teixeira et al. (2020) determineted that the distance between processing plants was an important factor in genetic similarity between isolates, and genetic similarity decreased as the distance between plants increased.

In conclusion, the high rate of *L. monocytogenes* contamination on meat and surfaces that come into contact with meat is important for public health and trade. Therefore, appropriate hygiene conditions should be met to prevent *L. monocytogenes* contamination for human, animal and environmental health, a pre-processing checklist should be developed to identify high-risk areas in slaughterhouses, and food safety management systems should be followed.

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