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Detection of the contamination sources of *Listeria monocytogenes* in pickled white cheese production process line and genotyping with the pulsed-field gel electrophoresis method

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Abstract: This study was conducted to determine the contamination sources, serotyping profiles, and antibiotic resistance patterns of *Listeria monocytogenes* isolated during the production of pickled white cheese. The genetic-relatedness of the isolates to EGD SLCC (5835) (1/2a, lineage II) and ATCC (13932) (4b, lineage I) reference strains was also determined with pulsed-field gel electrophoresis (PFGE) as a result of digestions with AscI and ApaI enzymes. Samples were collected from 16 different points in the production process of 4 different plants at 3 different times. Among the 192 samples examined, 17 (8.85%) were determined to be contaminated with *Listeria* spp. Three isolates (3.53%) obtained from raw milk, wall/ground, and press cases were identified as *L. monocytogenes* via the conventional culture method and confirmed by polymerase chain reaction. These isolates were found to belong to serotype 4b. According to antibiotic resistance testing against 10 antibiotics (ampicillin, gentamicin, erythromycin, tetracycline, chloramphenicol, cefalotin, streptomycin, vancomycin, penicillin, and sulfamethoxazole/trimethoprim), it was determined that isolates from raw milk and press cases were resistant to erythromycin. PPGE band patterns of the isolates displayed indistinguishable with AscI and 80%-94% homology with ApaI. The isolates were observed to display high homology to ATCC (13932) and lower homology to EGD SLCC (5835) obtained by both enzymes.

Key words: Antibiotic resistance, *Listeria monocytogenes*, PCR, PFGE, pickled white cheese, serotyping

1. Introduction

Listeria monocytogenes is a foodborne bacterial pathogen causing serious public health problems. *L. monocytogenes* can cause diseases in many tissues, organs, and systems apart from the intestinal system (1). Listeriosis is a result of consuming food contaminated with *L. monocytogenes* and has a high mortality rate, especially in predisposed individuals (2). The ubiquitous nature of the microorganism increases the possibility of food contamination (1,2). In listeriosis epidemics and the epidemiology of severe sporadic cases, consumption of pasteurized milk and soft cheese was important (2).

Pickled white cheese is a variety of a soft or semihard cheese and the leading type of cheese produced and consumed in Turkey (3). The production process of pickled white cheese consists of many stages and *L. monocytogenes* contaminations are possible in almost all production stages, such as milk processing machines, counters, separators, coagulum presses, press cases, and the personnel (4,5).

Linking the genetic-relatedness of *L. monocytogenes* isolated from the final product to one isolated from a particular phase of the production line is important in terms of determining contamination sources. This can be possible with the practice of genotyping. By genotyping, the phenotypical and serological relatedness of isolates can be explained, in addition to determination of contamination sources (6). Several molecular subtyping methods are available, such as random amplified polymorphic DNA, repetitive element polymerase chain reaction, and pulsed-field gel electrophoresis (PFGE); PFGE is generally considered the gold standard for subtyping because of its distinctiveness for foodborne and other kinds of bacteria (7,8).

A network and a database (PulseNet) were established by the Centers for Disease Control and Prevention (CDC) in order to enable quick detection and subtyping of the pathogenic bacteria. PFGE databases from PulseNet set standard methods in bacteria subtyping. Through use of these methods and the network, comparing PFGE profiles of foodborne pathogens has prompt results (8,9). Collection of routine analysis results and subtyping data of *L. monocytogenes* by laboratories that are members of the PulseNet database network is important to put forward the epidemiology of listeriosis (9).

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In this context and in order to contribute to listeriosis epidemiology, this study aimed to determine the contamination sources, serotypes, antibiotic resistance, and genetic-relatedness of *L. monocytogenes* isolates in the production line of pickled white cheese.

2. Material and methods

2.1. Sample collection

Samples were collected from 16 different production line points (raw milk, pasteurized milk, the milk in cheese tanks without rennet, coagulum, whey, curd, starter cultures, rennet, $CaCl_2$, internal surface of milk tanks, internal surface of cheese tanks, cheese cloth, press cases, brine, wall/ground, final product) at three different times at an interval of 1 month. For this purpose, four different cheese processing plants in Konya, Turkey, were used. A total of 192 samples (48 samples from each dairy plant) were examined for the presence of *L. monocytogenes*.

2.2. Isolation and identification of *L. monocytogenes*

Samples were collected aseptically under cold chain and analyzed within 4 h. Cultural isolation and identification of *L. monocytogenes* were carried out as suggested by the US Food and Drug Administration (10). After adding 225 mL of Listeria Enrichment Broth (Merck 1.11951; Kenilworth, NJ, USA) to 25 g of solid and liquid samples, they were taken into sterile homogenizer bags in aseptic conditions and then homogenized (Colworth Stomacher Lab-Blender 400; Seward Limited, Easting Close, UK) for 2 min. Preenrichment of the samples in Listeria Enrichment Broth was performed at 30 °C for 4 h. By adding Listeria Enrichment Broth selective supplement (Merck 1.11781), selective enrichment was carried out for 44 h. After enrichment, a loopful from each sample was streaked on Oxford Listeria Selective Agar Base (Merck 1.07004) and incubated in aerobic conditions at 35 °C for 48 h. In Oxford Agar, 5 presumptive colonies were plated onto Tryptone Soya Agar (TSA) (Oxoid-CM 131; Thermo Fisher Scientific, Waltham, MA, USA) containing 0.6 % Yeast Extract (YE) (Merck 1.03753) and incubated at 30 °C for 24 or 48 h. Presumptive colonies grown in TSA-YE were further analyzed to identify *Listeria* species with biochemical tests, including Gram staining, catalase reaction, indole and oxidase tests, utilization of urea, reduction of nitrate, H_2S, methyl red, the Voges–Proskauer test, hemolysis in blood agar, motility in Sulfate Indole Motility medium (Merck 1.05470), carbohydrate fermentation tests (rhamnose, dextrose, esculin, xylose, mannitol, and maltose), and the Christie–Atkins–Munch-Petersen test.

2.3. Molecular identification

DNA isolation of the isolates was carried out with a commercially available genomic DNA purification kit (Thermo Fisher Scientific). For molecular identification, the *hly* gene primer pair [primer A (5'- CAT TAG TGG AAA GAT GGA ATG-3') and primer B (5'-GTA TCC TCC AGA GTG ATC GA-3')] was used as stated by Gouws and Liedemann (11), which amplifies an area of 732 bp in length.

AccuPower Multiplex PCR premix (Bioneer; Daejeon, Korea) was used, containing Top DNA polymerase, a dNTP set, reaction buffer, $MgCl_2$, stabilizer, and tracking dye. One microliter of primers A and B, 2.5 µL of template DNA, and nuclease-free water were added to the PCR premix tubes and the PCR reaction was adjusted in a reaction mixture of 50 µL final volume.

Thermal cycler conditions were set as 10 min at 80 °C; first denaturation for 3 min at 94 °C; 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and primer extension for 30 s at 72 °C; and final extension for 2 min at 72 °C (11). The amplification products were detected by agarose gel (1.8%) electrophoresis performed at 90 V and 40 mA for 60 min (Owl EC300XL2 Compact Power Supply, Thermo Fisher). The gel was stained with ethidium bromide (E 7637; Sigma, St. Louis, MO, USA) and visualized under a UV transilluminator (DNR Bio-Imaging Systems MiniBISPro; Jerusalem, Israel).

2.4. Serotyping

Reference strain (ATCC 13932) and *L. monocytogenes* isolates were inoculated in Brain Heart Broth (Merck 1.10493) and incubated at 37 °C for 24 h. After incubation, they were transferred to Brain Heart Infusion Agar (BHIA, Merck 1.13825). Colonies grown in BHIA were transferred to 0.2% (w/v) sodium chloride. After suspensions were heated at 121 °C for 30 min, they were centrifuged at 3000 rpm. The precipitant was stored in 1 mL of 0.2% (w/v) sodium chloride for agglutination tests. The manufacturer's serotyping protocol was followed and results were analyzed according to Denka Seiken Co. Ltd. (12).

2.5. Antibiotic susceptibility

The susceptibility of *L. monocytogenes* isolates to 10 antimicrobial agents (ampicillin Oxoid-CT 0003B-AMP 10 µg, gentamicin Oxoid-CT 0024B-CN 10 µg, erythromycin Oxoid-CT 0020B-E 15 µg, tetracycline Oxoid-CT 0054B-TE 30 µg, chloramphenicol Oxoid-CT 0013B-C 30 µg, cefalotin Oxoid-CT 0010B-KF 30 µg, streptomycin Oxoid-CT 0047B-S 10 µg, vancomycin Oxoid-CT 0058B-VA 30 µg, penicillin Oxoid-CT 0043B-P 10 IU, and sulfamethoxazole/trimethoprim Oxoid-CT 0052B-SXT 25 µg) was determined by the disk diffusion method (13). The diameter of the inhibition zone was measured and the results were evaluated according to the Clinical and Laboratory Standards Institute's (14) guidelines for gram-positive bacteria.

2.6. Genotyping of isolates

The CDC's PulseNet (15) was used to genotype the isolates. For this purpose, agarose plugs containing bacterial DNA were prepared. The plugs were digested with restriction enzymes (10 U/µL AscI-Fermentas, ER 1891 and 10 U/µL ApaI-Fermentas, ER 1411) for 5 h. Ultrapure DNA grade agarose 1% (w/v) (Thermo Fisher Scientific, 16500100) was prepared for electrophoresis.

The electrophoresis conditions were selected according to PulseNet on CHEF Mapper for *L. monocytogenes* strains. The gel was stained with ethidium bromide (1 µg/mL) for 20–30 min in a covered container. PFGE patterns were visualized in a Bio-Rad VersaDoc 4000MP gel displaying system (Bio-Rad, Hercules, CA, USA). The band intervals were selected with BIO 1D++ gel analysis software (Vilber, Eberhardzell, Germany) by using band images belonging to isolates and dendrogram band profiles were formed by using the unweighted pair group method with arithmetic mean. In dendrogram analysis, the homology coefficient was optimized at 0.7% and determined for both of the enzymes.

2.7. Statistical analysis

The data obtained in the study were analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using the chi-square test.

3. Results

3.1. Cultural isolation and identification

Seventeen out of 192 samples (8.85%) were determined to be contaminated with *Listeria* spp. The rate of samples contaminated with *Listeria* spp. was 16.66% (8/48), 8.33% (4/48), and 10.41% (5/48) in processing plants A, B, and C, respectively. In plant D, no contamination was detected. The samples contaminated with *Listeria* spp. did not present statistical significance ($P > 0.05$) in terms of distribution by plant. Distribution of the isolates according to plants is shown in Table 1.

In order to determine *Listeria* species in the 17 contaminated samples, 5 isolates were examined from each sample. Fifty-two of 85 isolates (61.18%) were identified as *L. ivanovii*, 20 (23.53%) as *L. welshimeri*, 5 (5.58%) as *L. grayi*, 5 (5.58%) as *L. seeligeri*, and 3 of them (3.53%) as *L. monocytogenes* (Figure 1). *L. monocytogenes* isolates were obtained from raw milk, wall/ground, and press cases.

3.2. Molecular identification

All of the 3 *L. monocytogenes* isolates identified by culture methods were determined to have the *hly* gene and to form band profiles at 732 bp (Figure 2). The remaining *Listeria* isolates tested negative for the *hly* gene according to PCR (Figure 2).

3.3. Serotyping

The *L. monocytogenes* isolates belonged to serotype 4b (Table 2) according to serotyping with antisera used against somatic (O) and flagellar (H) antigen.

3.4. Antibiotic susceptibility

The antibiotic susceptibility profiles of *L. monocytogenes* isolates are presented in Table 3.

3.5. Genotyping

As a result of restriction with the AscI enzyme, 7–9 fragments and 3 pulsotypes were observed in PFGE dendrogram profiles (Figure 3). The homologies of the isolates showed an indistinguishable band pattern.

As a result of restriction with the ApaI enzyme, 14–15 fragments and 4 pulsotypes were observed in PFGE dendrogram profiles (Figure 4). Homologies of wall/ground with raw milk, press cases with raw milk, and wall/ground with press cases were 94%, 80%, and 80%, respectively. The isolate obtained from the raw milk and the ATCC (13932) strain displayed indistinguishable band patterns. The homology of isolates from the wall/ground and press cases to the ATCC (13932) strain was determined as approximately 94% and approximately 80%, respectively. The isolates exhibited high homology to ATCC (13932) and lower homology to EGD SLCC (5835) obtained by both enzymes.

4. Discussion

High contamination levels of *L. monocytogenes* in raw milk in cheese production lines have been observed by many researchers (16,17). In this study, the isolation of *L. monocytogenes* from 3 different points of the

Table 1. Distribution of *Listeria* spp. isolates according to processing plants.

	Plant A	Plant B	Plant C	Total
Listeria monocytogenes	2	-	1	3
Listeria ivanovii	23	10	19	52
Listeria seeligeri	5	-	-	5
Listeria welshimeri	10	5	5	20
Listeria grayi	-	5	-	5
Total	40	20	25	85

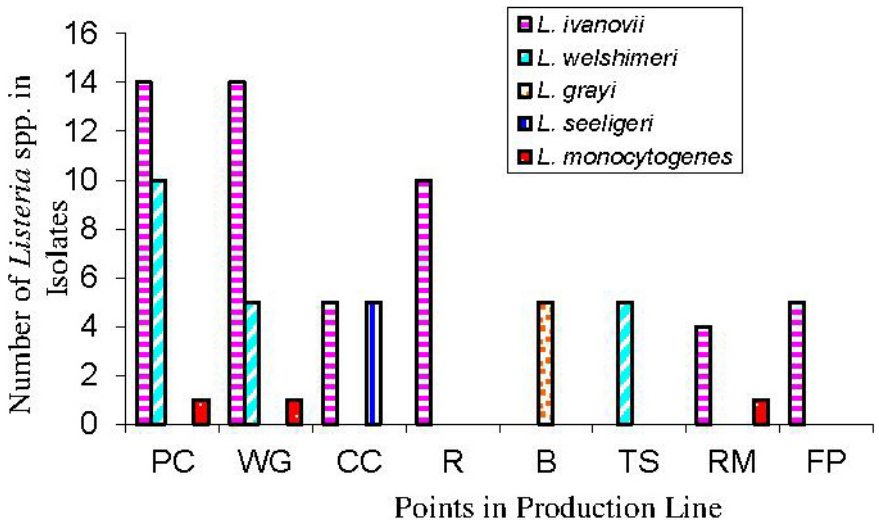

Figure 1. Distribution of the *Listeria* spp. isolates in sampling points. PC: Press cases. WG: Wall/ground. CC: Cheese cloth. R: Rennet. B: Brine. TS: Tank surface. RM: Raw milk. LP: Final product.

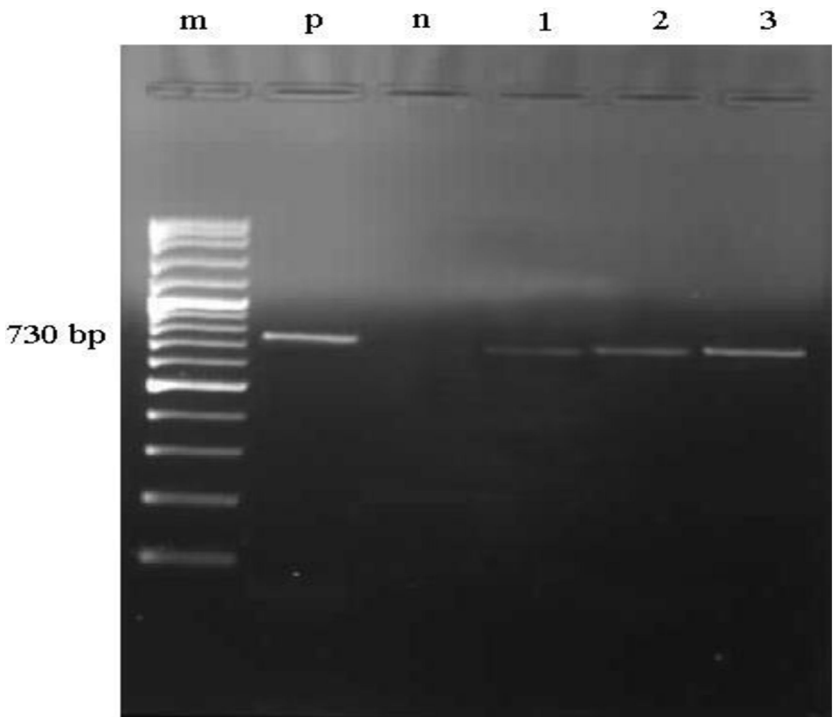

Figure 2. UV transilluminator image of amplified DNA samples including the specific *hly* gene. m: 100-bp DNA marker. p: Positive control (*L. monocytogenes* ATCC 13932). n: Negative control. 1: Raw milk. 2: Wall/ground. 3: Press cases.

production process, and especially from raw milk, illustrates the contamination risk during pickled white cheese production. Contamination of raw milk may occur on a dairy farm. In a dairy farm, *L. monocytogenes* contaminations could be sourced from poor quality of silage, inadequate cleanliness of animals and their beddings, and poor hygienic conditions in the milking process. Although pasteurization is applied during cheese production, cross-contamination is a major problem. Contamination may occur at different points (4,5), from

Table 2. Serotyping results of *Listeria monocytogenes* isolates. [-] : Agglutination negative; [+] : agglutination positive.

Serotype	*Listeria* O antigen								*Listeria* H antigen			
	I/II	I	IV	V/VI	VI	VII	VIII	IX	A	AB	C	D
Raw milk isolate (4b)	[-]	[-]	[-]	[+]	[-]	[-]	[-]	[-]	[+]	[+]	[+]	[-]
Wall/ground isolate (4b)	[-]	[-]	[-]	[+]	[-]	[-]	[-]	[-]	[+]	[+]	[+]	[-]
Press case isolate (4b)	[-]	[-]	[-]	[+]	[-]	[-]	[-]	[-]	[+]	[+]	[+]	[-]

Table 3. The diameter of the inhibition zone of isolates. S: Susceptible, R: resistant, I: intermediate. 1: Wall/ground. 2: press case. 3: raw milk.

Antibiotic	The diameter of the inhibition zone (mm)			Isolates		
	Resistant (R)	Intermediate (I)	Susceptible (S)	1	2	3
Ampicillin (10 µg)	≤19	-	≥20	S	S	S
Gentamicin (10 µg)	≤12	13–14	≥15	S	S	S
Erythromycin (15 µg)	≤13	14–22	≥23	S	R	R
Tetracycline (30 µg)	≤14	15–18	≥19	S	S	S
Chloramphenicol (30 µg)	≤12	13–17	≥18	S	I	S
Cefalotin (30 µg)	≤14	15–17	≥18	S	S	S
Streptomycin (10 µg)	≤11	12–14	≥15	S	I	I
Vancomycin (30 µg)	≤9	10–11	≥12	S	S	S
Penicillin (10 U)	≤19	-	≥20	S	S	S
Sulfamethoxazole/trimethoprim (25 µg)	≤10	11–15	≥16	S	S	S

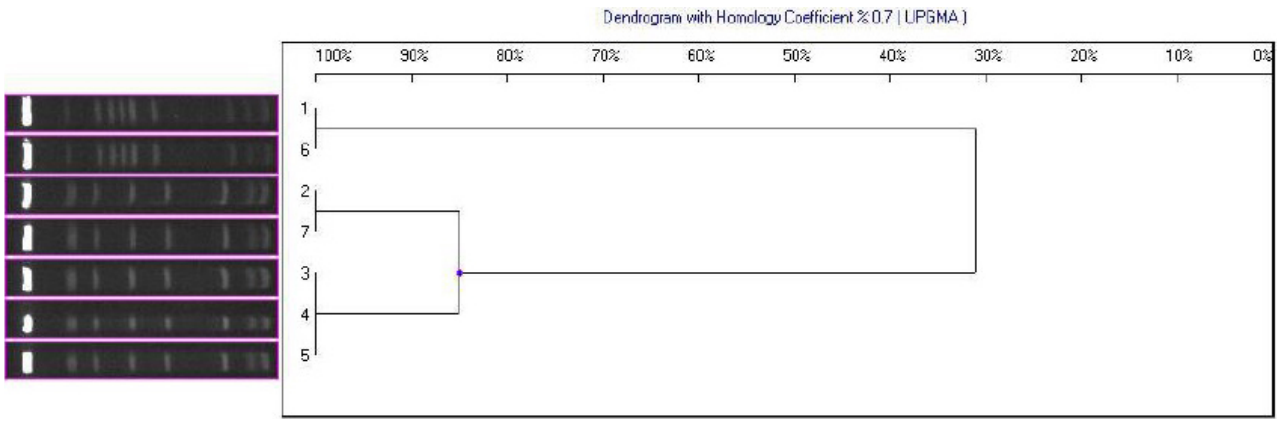

Figure 3. PFGE dendrogram profiles with the application of the AscI enzyme. 1: *L. monocytogenes* EGD SLCC (5835). 2: *L. monocytogenes* ATCC (13932). 3: Wall/ground. 4: Press cases. 5: Raw milk. 6: *L. monocytogenes* EGD SLCC (5835). 7: *L. monocytogenes* ATCC 13932.

the contaminated raw milk, to the plant environment and the equipment, to the heating process. The ubiquity of bacteria (2) and biofilm formation on many various surfaces (e.g., stainless steel, glass, wood, plastic, carton) are considered major factors in the increasing incidence of cross-contamination (18,19).

The three isolates of *L. monocytogenes* identified through the cultural method were determined to have the *hly* gene (732 bp). Furthermore, the isolates belonging to other *Listeria* species were found to be negative in terms of the *hly* gene according to PCR. When our findings were evaluated, the PCR method was effective in confirming the cultural results. Likewise, Salmanzadeh Ahrabi et al. (20) and Aznar and Alancon (21) also claimed that PCR was a fast, reliable, and precise method for confirming *L. monocytogenes* in different food products.

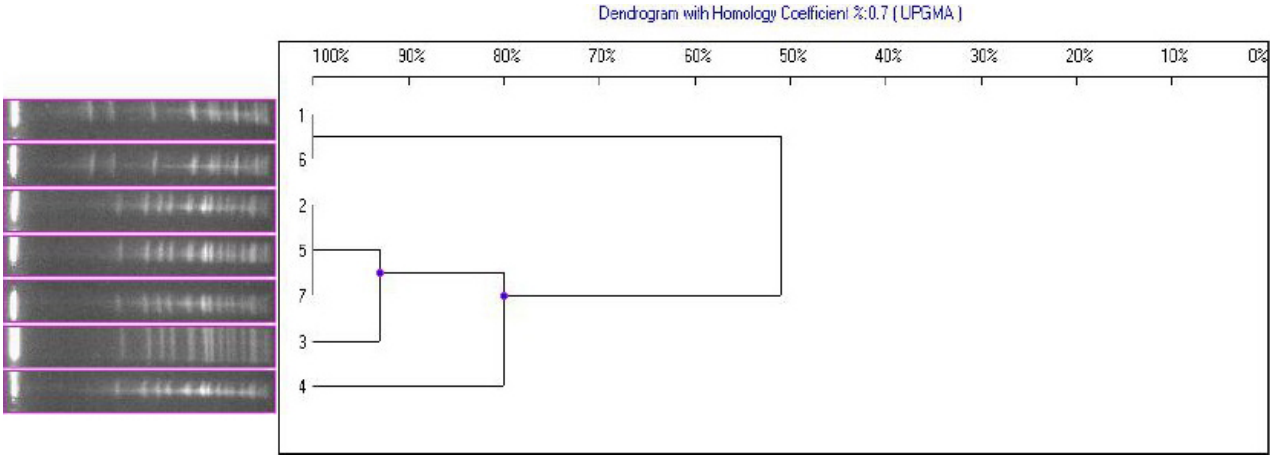

Figure 4. PFGE dendrogram profiles with the application of the ApaI enzyme. 1: *L. monocytogenes* EGD (SLCC) 5835. 2: *L. monocytogenes* ATCC 13932. 3: Wall/ground. 4: Press cases. 5: Raw milk. 6: *L. monocytogenes* EGD (SLCC) 5835. 7: *L. monocytogenes* ATCC 13932.

L. monocytogenes isolates belonged to serotype 4b. Dominant serotypes causing human listeriosis belonged to the 4b, 1/2a and 1/2b serotypes (1). Serotype 4b was determined to be dominant in Europe and the most virulent serotype in listeriosis epidemics (1,22).

When considering using erythromycin (23) in the treatment of human listeriosis, resistance to erythromycin of isolates from press cases and raw milk samples were evaluated to be important. Although *L. monocytogenes* is known to be susceptible to effective antibiotics used against gram-positive bacteria, antibiotic resistance was first determined in these bacteria and then multiresistant isolates were detected from foods and sporadic cases in 1988 (24). Poros-Gluchowska and Markiewicz (25) stated that mutations in chromosomal genes or gene transfer via transposons and plasmids of other gram-positive bacteria could result in antimicrobial resistance in the pathogen.

As a result of digestions made with ApaI and AscI enzymes, *L. monocytogenes* isolates from different points were determined to have close relations among themselves and with the ATCC (13932) strain, and low similarity to the EGD SLCC (5835) strain. The homology differences between the isolates and the reference strains can be explained by their serotype distributions. The isolates and reference strains belonged to the 4b serotype and lineage I, whereas the EGD SLCC (5835) strain belongs to the 1/2a serotype and lineage II (1,26).

The lower homology of the wall/ground and the press case isolates to the ATCC (13932) strain compared with the raw milk isolates can be explained by changes in their genetic structures, especially in biofilm layers on food processing surfaces, to adapt to environmental stress factors (27,28).

Digestion with AscI resulted in 7–9 fragments and 3 pulsotypes, while digestion with ApaI resulted in 14–15 fragments and 4 pulsotypes. Additionally, in the results obtained with ApaI enzyme, the raw milk isolate was divided from two other isolates as a pulsotype. In restriction with ApaI, DNA was cut more frequently and more pulsotypes and DNA fragments were formed; thus, it described the clonal relations with more sensitivity. Hamdi et al. (29) genotyped 11 *L. monocytogenes* from the raw milk and the milk tanks using ApaI and AscI with PFGE. Researchers stated that they obtained 11–19 and 5 pulsotypes with AscI and 9–10 fragments and 4 pulsotypes with ApaI. In the same way, Yde and Genicot (30) used ApaI and AscI enzymes to determine clonal relations among 48 *L. monocytogenes* obtained from listeriosis cases seen in humans in Belgium. In their study, restrictions performed with ApaI resulted in 8–21 fragments and 38 pulsotypes, and restrictions performed with AscI resulted in 6–12 fragments and 34 pulsotypes.

In conclusion, the isolates in serotype 4b that display antibiotic resistance to erythromycin indicate the public health risk of *L. monocytogenes* in pickled white cheese. Taking all precautions to avoid contamination in raw milk, environmental sources, and the production environment as well as meticulous routine control are essential. Carrying out more studies on other food products would increase epidemiological knowledge and improve the understanding of the genetic features of *L. monocytogenes* in Turkey.

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References

1. Bhunia AK. Foodborne Microbial Pathogens. 1st ed. New York, NY, USA: Springer Science Business Media; 2011.
2. Adams MR, Moss MO. Food Microbiology. 3rd ed. Cambridge, UK: RSC Publishing; 2008.
3. Turkish Statistical Institute (TÜİK). The Amount of Milk and Milk Products Production. Ankara, Turkey: TÜİK; 2014 (in Turkish).
4. El-Hofi M, El-Tanboly ES, Ismail A. Implementation of the hazard analysis critical control point (HACCP) system to UF white cheese production line. Acta Sci Pol Technol Aliment 2010; 9: 331-342.
5. Temelli S, Anar S, Sen C, Akyuva P. Determination of microbiological contamination sources during Turkish white cheese production. Food Control 2006; 17: 856-861.
6. Fugett EB, Schoonmaker-Bopp D, Dumas NB, Corby J, Wiedmann M. Pulsed-field gel electrophoresis (PFGE) analysis of temporally matched *Listeria monocytogenes* isolates from human clinical cases, foods, ruminant farms, and urban and natural environments reveals source-associated as well as widely distributed PFGE types. J Clin Microbiol 2007; 45: 865-873.
7. Jamali H, Thong KL. Genotypic characterization and antimicrobial resistance of *Listeria monocytogenes* from ready-to-eat foods. Food Control 2014; 44: 1-6.
8. Moorman M, Pruett P, Weidman M. Value and methods for molecular subtyping of bacteria. In: Kornacki JL, editor. Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment. 1st ed. New York, NY, USA: Springer Science Business Media; 2010. pp. 157-175.
9. Graves LM, Swaminathan B, Hunter SB. Subtyping *Listeria monocytogenes*. In: Ryser ET, Marth EH, editors. *Listeria*, Listeriosis, and Food Safety. 3rd ed. Boca Raton, FL, USA: CRC Press; 2007. pp. 283-305.
10. FDA. Detection and enumeration of *Listeria monocytogenes* in foods. In: Bacteriological Analytical Manual Online. Silver Spring, MD, USA: Food and Drug Administration; 2003.
11. Gouws PA, Liedemann I. Evaluation of diagnostic PCR for the detection of *Listeria monocytogenes* in food products. Food Technol Biotechnol 2005; 43: 201-205.
12. Denka Seiken. *Listeria* Antisera "Seiken." Tokyo, Japan: Denka Seiken; 2000.
13. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45: 493-496.
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 9th ed. CLSI Document M2-A9. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2006.
15. CDC PulseNet. One-Day (24–28 h) Standardized Laboratory Protocol for Molecular Subtyping of *Listeria monocytogenes* by Pulsed Field Gel Electrophoresis (PFGE). Atlanta, GA, USA: Centers for Disease Control and Prevention; 2009.
16. Kabuki DY, Kuaye AY, Wiedmann M, Boor KJ. Molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants. J Dairy Sci 2004; 87: 2803-2812.
17. Brito JRF, Santos EMP, Arcuri EF, Lange CC, Brito MAVP, Souza GN, Cerqueira MMPO, Beltran JMS, Call JE, Liu Y et al. Retail survey of Brazilian milk and minas frescal cheese and a contaminated dairy plant to establish prevalence, relatedness, and sources of *Listeria monocytogenes* isolates. Appl Environ Microbiol 2008; 74: 4954-4961.
18. Montanez-Izquierdo VY, Salas-Vazquez DI, Rodriguez-Jerez JJ. Use of epifluorescence microscopy to assess the effectiveness of phage P100 in controlling *Listeria monocytogenes* biofilms on stainless steel surfaces. Food Control 2011; 1: 1-8.
19. Tenenhaus-Aziza F, Daudin JJ, Maffre A, Sanaa M. Risk-based approach for microbiological food safety management in the dairy industry: the case of *Listeria monocytogenes* in soft cheese made from pasteurized milk. Risk Anal 2014; 34: 56-74.
20. Salmanzadeh Ahrabi S, Erguven S, Gunalp A. Incidence of *Listeria* spp in raw milk samples and rapid identification of *Listeria monocytogenes* by the polymerase chain reaction. Microbiol Bult 1997; 31: 263-268.
21. Aznar R, Alarcon B. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. J Appl Microbiol 2003; 95: 958-966.
22. Lunden J, Tolvanen R, Korkeala H. Human listeriosis outbreaks linked to dairy products in Europe. J Dairy Sci 2004; 87: 6-11.
23. Wagner M, McLauchlin J. Biology. In: Liu D, editor. Handbook of *Listeria monocytogenes*. 1st ed. Boca Raton, FL, USA: CRC Press; 2008. pp. 3-26.
24. Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianieri A. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. Int J Food Microbiol 2009; 128: 497-500.
25. Poros-Gluchowska J, Markiewicz Z. Antimicrobial resistance of *Listeria monocytogenes*. Acta Microbiol Pol 2003; 52: 113-129.
26. Rocourt J, Buchrieser C. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy and identification. In: Ryser ET, Marth EH, editors. *Listeria*, Listeriosis, and Food Safety. 3rd ed. Boca Raton, FL, USA: CRC Press; 2007. pp. 1-20.
27. Fuente-Nunez C, Reffuveille F, Fernandez L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol 2013; 16: 580-589.
28. Begley M, Hill C. Stress adaptation in foodborne pathogens. Annu Rev Food Sci Technol 2015; 6: 191-210.
29. Hamdi TM, Naim M, Martin P, Jacquet C. Identification and molecular characterization of *Listeria monocytogenes* isolated in raw milk in the region of Algiers (Algeria). Int J Food Microbiol 2007; 116: 190-193.
30. Yde M, Genicot A. Use of PFGE to characterize clonal relationships among Belgian clinical isolates of *Listeria monocytogenes*. J Med Microbiol 2004; 53: 399-402.