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Antibiotic Resistance and Genetic Diversity of *Listeria monocytogenes* Isolated from Chicken Carcasses in Korea

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Abstract *Listeria monocytogenes* is a well-known high-risk foodborne pathogen that grows at refrigeration temperature and is responsible for outbreaks of listeriosis. We report here the incidence of *L. monocytogenes* in fresh chicken carcasses and present genetic diversity of *L. monocytogenes* isolates. In this study, 25 g of chicken carcasses from markets in Korea were examined according to the FDA method, and presumptive isolates were confirmed by multiplex PCR assay. *L. monocytogenes* isolates were analyzed by Pulsed-Field Gel Electrophoresis using restriction enzymes, ApaI and AscI, to obtain strain-specific DNA fragments profiles. Antimicrobial resistance of *L. monocytogenes* strains against generally used antibiotics (Penicillin G, Kanamycin, Tetracycline, Vancomycin, Cephalothin, Rifampicin, Erythromycin, Ampicillin, Gentamicin, Streptomycin, and Chloramphenicol) were analyzed by NCCLS protocols to examine the presence of antimicrobial resistance in natural *L. monocytogenes*. Of a total 274 chicken samples, 81 samples (29.6%) were positive for *L. monocytogenes*. *Listeria innocua* (50.1%), *Listeria welshimeri* (6.9%), and *Listeria grayi* (11.3%) were also detected. PFGE analysis, using restriction enzymes ApaI and AscI, showed 27 pulsotypes of *L. monocytogenes*. Antimicrobial resistance analysis confirmed the existence of antimicrobial resistance for penicillin G and tetracycline in isolated *L. monocytogenes* strains.

Key words: *Listeria monocytogenes*, PFGE, chicken, subtyping

Listeria monocytogenes is a foodborne pathogenic bacteria that is responsible for foodborne listeriosis outbreaks. Symptoms of listeriosis include meningitis, central nervous system infection, and stillbirth and mortality was reported to be 30–40%. In the U.S.A., *L. monocytogenes* infections have showed a high hospitalization rate (90% of all foodborne pathogens) and 30% of all reported deaths associated with foodborne illness [15].

L. monocytogenes is detected in a range of foods such as meat, seafoods, and dairy products. In this range of food products, chickens have frequently been reported to harbor many pathogenic bacteria, and the incidence of *L. monocytogenes* in chickens is reported as 23–60% [32].

Detection methods for *L. monocytogenes* consist of selective enrichment, plating onto selective differential agar media, and a range of biochemical and physiological confirmation tests [2, 51, 29]. However, it is well known that only a few serotypes or strains of *L. monocytogenes* [47] are responsible for listeriosis. Therefore, to ensure for food safety, it is necessary to examine food products to have information on the presence as well as the characterization of target microorganisms.

However, in Korea, only a few reports are available on the presence of *L. monocytogenes* in foods and the characterization of isolated strains [6, 19, 18]. Thus, we report herein the incidence, genetic diversity, and serotype of *L. monocytogenes* in raw chicken carcasses that were purchased at the Seoul city area in Korea.

Materials and Methods

Isolation and Identification of *L. monocytogenes*

Raw chicken carcasses (n=274) were purchased from local markets in the Seoul city area in Korea from February to September 2004. All chicken carcasses were immediately

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transferred to the laboratory in an ice cooler after purchase and kept at 4°C prior to assays.

All samples were analyzed for the presence of *L. monocytogenes* and other *Listeria* spp. using the procedures recommended by the Food and Drug Administration [29].

Confirmation of *L. monocytogenes* by Multiplex Polymerase Chain Reaction (PCR) Assay

The isolated colonies were analyzed by a PCR method that was modified from that of Bansal [8], for confirmation. The colonies were streaked on plates of TSAYE, and one healthy colony was inoculated into 1 ml of Tryptone Soya Broth (Oxoid) with 6% Yeast Extract (TSBYE). It was incubated overnight at 37°C, and cells were centrifuged at 13,000 ×*g* for 10 min. The pellet was suspended in 1 ml of sterile distilled water and centrifuged again at 13,000 ×*g* for 10 min. To extract DNA, the pellet was resuspended in 100 µl of sterile distilled water and boiled for 20 min. The cellular debris was pelleted by centrifugation at 13,000 ×*g* for 10 min, and the supernatant containing nucleic acids was taken for PCR assay.

Two primers (LIS-F and LIS-R), based on 16S ribosomal RNA, were used for the generic detection of *Listeria* spp. Two other primers (LM-F and LM-R), based on the sequence of the listeriolysin O gene, were used to specifically detect *L. monocytogenes*. The sequences and sources of these primers are indicated in Table 1. The primers were purchased from Corebio System Co. Ltd. (Seoul, Korea) and were stored at −20°C before assays.

PCR assays were performed using a thin-wall 8-tube strip (200 µl) (Bioplastics, Netherlands) in a Mastercycler Gradient (Eppendorf). The total reaction volume was 25 µl, containing 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 200 mmol/l each of deoxynucleoside triphosphates, 1 mmol/l each of primers, and 2.5 U of Taq DNA polymerase. Ingredients were purchased from Takara Korea Biomedical Inc. and were mixed according to the manufacturer's instructions. The cycling parameters were initial denaturation at 95°C for 1 min followed by 30 cycles, each consisting of 30 s at 94°C, 20 s at 51°C, and 30 s at 72°C. The reaction mixture was incubated for 10 min at 72°C for final extension and held at 4°C until electrophoresis.

Electrophoresis was performed on 1.2% agarose gels (Bio Basic Inc.). Gels were initially run for 50 min at 110 V in TBE buffer using Mupid-21 (Cosmo Bio Co., Ltd., Korea). The gels were stained with ethidium bromide (0.5 µg/ml) for 30 min and destained for 20 min in clean distilled water. The result of gel electrophoresis was visualized by UV transillumination and photography. The molecular weight marker was a Ready-Load™ 1-kb plus DNA ladder (Invitrogen, Korea).

Table 1. Primers used for the PCR detection of *Listeria* spp.

Name	Sequence	Reference
LM-F	5'CAAACGTTAACAACGCAGTA3'	[40]
LM-R	5'TCCAGAGTGATCGATGTTAA3'	[40]
LIS-F	5'CAGCMGCCGCGGTAATWC3'	[34]
LIS-R	5'CTCCATAAAGGTGACCCT3'	[49]

Identification of Other *Listeria* spp.

Other *Listeria* spp. isolates confirmed by PCR assay were identified by CAMP test and carbohydrate utilization tests according to the FDA manual [29].

Subtyping of *Listeria* Isolates by Pulsed-Field Gel Electrophoresis (PFGE)

Listeria cultures were subcultured on TSA plates at 37°C for 24 h. A well-developed colony was transferred into 1 ml of TSBYE in an Eppendorf tube for incubation at 30°C for 14–16 h to obtain exponential phase cells. Cells were centrifuged at 1,500 ×*g* for 10 min using a HM-150IV centrifuge (Hanil Science Industrial Co., Ltd., Korea). The pellet was suspended in 1 ml of cold TE buffer (10 mM Tris-HCl, pH 7.4, and 1 mM EDTA, pH 8.0) and centrifuged again at the same speed for 10 min. The pellet was resuspended in 500 µl of 1% low gelling temperature agarose (Sigma) in TE buffer. Approximately 100 ml of this mixture was dispensed into each well of the moulds (BioRad) to obtain agarose plugs and left at room temperature (20–25°C) for 20 min.

Agarose plugs with embedded cells were incubated overnight at 37°C in a lysis buffer containing 5 mg/ml of lysozyme (Sigma) and 20% sucrose in 0.1 M phosphate buffer (pH 8.0) in sterile distilled water. They were washed twice for 30 min in 1 ml of TE buffer and suspended again in a lysis solution containing 0.25 M EDTA, 0.5% n-lauroylsarcosine (Sigma), and 1 mg/ml of proteinase K (Sigma), and the mixture was incubated for 24–48 h at 50°C. They were cooled to 4°C and washed twice for 30 min with 1 ml of TE buffer. They were then washed with 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) in TE buffer for at least 2 h to inactivate proteinase K. The plugs were rinsed again in TE buffer for 1 h before enzyme treatments.

Restriction enzymes ApaI and AscI were used. For ApaI, the plugs were digested overnight at 37°C with 100 U of ApaI restriction enzyme (Promega) in 100 µl of the enzyme buffer A [60 mM Tris-HCl (pH 7.5), 60 mM NaCl, 60 mM $MgCl_2$, and 10 mM dithiothreitol (DTT)] provided by the manufacturer. For AscI (New England Biolabs), the plugs were digested overnight at 37°C with 5 U of AscI in 100 µl of NE Buffer 4 (pH 7.9, 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, and 1 mM dithiothreitol).

One-hundred ml of 1% agarose gel (Pulsed-Field Certified Agarose, BioRad) was prepared in TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH

8.4). Each plug was cut by a sterile surgical blade to fit the size of well in the gel. One percent of low melting agarose gel (Sigma) in TE was used to seal the wells after inserting the plugs.

Electrophoresis was run using the CHEF-DRIII pulsed-field electrophoresis system (BioRad). Running conditions were 5 and 40 s for the initial and final pulse times, respectively, for 18 h at 200 V (6 V/cm, 120°). The buffer was kept at 12°C during electrophoresis. A pulse marker (50–1,000 kb, Sigma D2416) was used as a molecular weight standard. The gels were stained for 2 h in 1 μg/ml ethidium bromide (Sigma) solution and destained for 3 h in distilled water. The gels were photographed under an UV transilluminator (Sigma) using a gel documentation system (Korea Bio-tech Inc.).

The data obtained from PFGE were analyzed using BioNumerics (version 4.01, Applied Maths, Kortrijk, Belgium). The images of gels were normalized by alignment with appropriate size standard lanes. Dendrograms of the fingerprintings were obtained by UPGMA (unweighted pair group method with averages) using the Dice coefficient with 1% tolerance.

Serotyping

The serotypes of identified *L. monocytogenes* pulsotypes were determined by a commercial kit (Denka Seiken, Japan) according to the manufacturer's instruction.

Antibiotic Susceptibility Test

Isolated *L. monocytogenes* strains were subcultured on a plate of TSAYE at 37°C for 24 h prior to assays. Three to five well-isolated colonies from each plate were mixed and transferred into a tube of TSBYE (10 ml), and they were incubated for 24 h at 37°C. The concentration of culture was adjusted to an optical density of 0.5 McFarland standards (approximately $1-2\times10^8$ cfu/ml) with sterile TSBYE. The standardized cell suspension was inoculated onto the entire surface of a dried Mueller-Hinton Agar (MHA, Oxoid) plate containing 5% Horse Blood (Oxoid) using a cotton swab. The plates were dried at room temperature (20–25°C) for 10 min. Then, antibiotic disks (Oxoid) were placed onto the surface of each plate using a disk dispenser (Oxoid). Antibiotic disks used were Penicillin G (10 μg, P10), Kanamycin (30 μg, K30), Tetracycline (30 μg, TE30), Vancomycin (30 μg, VA30), Cephalothin (30 μg, KF30), Rifampicin (5 μg, RD5), Erythromycin (15 μg, E15), Ampicillin (10 μg, AMP10), Gentamicin (10 μg, CN10), Streptomycin (10 μg, S10), and Chloramphenicol (30 μg, C30).

After incubation for 24–48 h at 37°C, the diameter of growth inhibition zones surrounding each antibiotic disk was measured and the mean diameter of inhibition zones for *Listeria* was recorded. Results were interpreted as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) guidelines informational supplement to "susceptible," "intermediate," or "resistant" [1]. Since the specification for *Listeria* spp. is not provided in the current edition of the NCCLS guideline informational supplement, the data obtained for most antibiotics, except rifampicin, were interpreted according to the specifications for *Staphylococcus aureus*. The data for rifampicin were interpreted according to the specifications for *Enterococcus* spp.

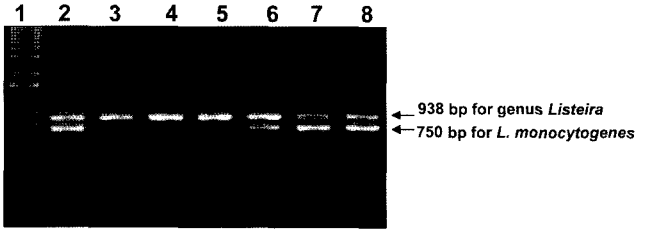

Fig. 1. PCR assay of presumptive isolates from raw chickens. Lane 1, 1-kb DNA plus ladder; lane 2, positive control (*L. monocytogenes* ATCC 19113); lanes 3 to 8, isolates from raw chickens. Lanes 6, 7, and 8 are *L. monocytogenes*; Lanes 3, 4, and 5 are other *Listeria* spp.

RESULTS

Incidence of *L. monocytogenes* and other *Listeria* spp. in Raw Chicken Carcasses

Samples were purchased from local traditional markets. To minimize the effect of temperature, purchased samples were kept in an ice cooler during transport and stored at 4°C prior to assays. The number of purchased chicken carcasses in a tradional market was about three to five, but only one sample was purchased from each shop. A total of 274 raw chickens were assayed for the presence of *L. monocytogenes* and other *Listeria* spp. Approximately five suspicious colonies with typical blackish or gray colonies with a black sunken center were isolated from each selective differential plate and confirmed by multiplex PCR.

Table 2. Incidence of *Listeria. monocytogenes* and other spp. in raw chicken carcasses in Korea.

Species	Number of positive samples (%)[a]	Number of positive isolates (%)[b]
L. monocytogenes	81 (29.56%)	150 (21.12%)
L. innocua	139 (50.07%)	479 (67.46%)
L. welshimeri	19 (6.93%)	37 (5.21%)
L. grayi	31 (11.31%)	44 (6.20%)
Total	274	710

[a]N=274 raw chicken samples.
[b]The number of isolates of *Listeria* spp. other than *L. monocytogenes* was 560.

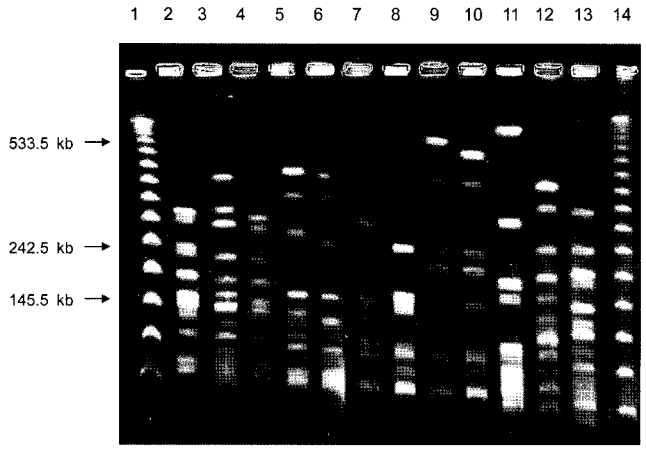

Fig. 2. DNA fragments profile of different pulsotypes of isolated *Listeria monocytogenes* using ApaI.
Lanes: 1 and 14, pulse markers (50–1,000 kb, Sigma D2416); 2–13, pulsotypes of *L. monocytogenes* isolates.

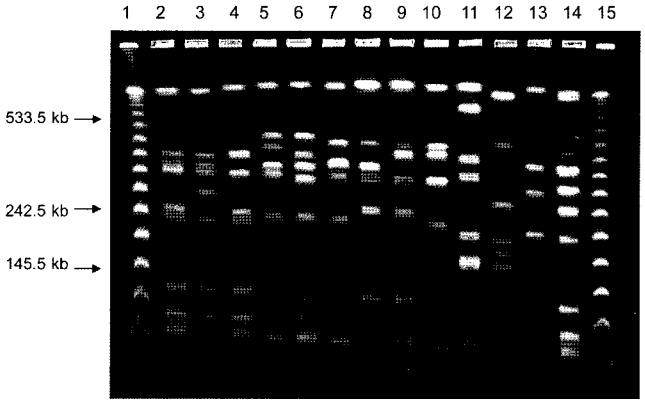

Fig. 3. DNA fragments profile of different pulsotypes of isolated *Listeria monocytogenes* using AscI.
Lanes: 1 and 15, pulse markers (50–1,000 kb, Sigma D2416); 2–14, pulsotypes of *L. monocytogenes* isolates.

Figure 1 shows the amplication products of the multiplex PCR assay. All of the isolates in the genus *Listeria* gave a common band of 938 bp, and strains of *L. monocytogenes* gave an additional band of 750 bp. It was possible to distinguish *L. monocytogenes* and other *Listeria* spp. from suspicious isolates. Therefore, the PCR assay could confirm suspicious *Listeria* colonies much faster than biochemical confirmation tests. Incidence data were analyzed according to the results of PCR confirmation.

Table 2 shows the incidence of *L. monocytogenes* and other *Listeria* spp. In total, 150 *L. monocytogenes* isolates and 560 other *Listeria* spp. isolates were found. Results showed that half of the examined samples (n=139) were contaminated with *L. innocua*, and that *L. monocytogenes* was associated with 81 samples (29.6%). *L. welshimeri* (19 samples) and *L. grayi* (31 samples) were also detected. *L. innocua* was the most predominant spp. in chickens.

Subtyping of *L. monocytogenes* Isolates by PFGE

Four *Listeria* spp. of genus *Listeria* were detected in chicken samples. However, only *L. monocytogenes* isolates were assayed for subtyping studies, since only *L. monocytogenes* is harmful to humans. Several sample profiles of DNA

Table 3. Diversity of pulsotypes of *L. monocytogenes* in chickens by PFGE using restriction enzymes ApaI and AscI.

Restriction enzyme	Pulsotype	Number of isolates	Number of related samples	Restriction enzyme	Pulsotype	Number of isolates	Number of related samples
ApaI	P01	13	4	AscI	S01	27	14
	P02	2	1		S02	2	1
	P03	4	1		S03	5	2
	P04	38	20		S04	40	21
	P05	43	18		S05	40	17
	P06	4	3		S06	7	5
	P07	2	2		S07	1	1
	P08	2	2		S08	5	3
	P09	10	4		S09	2	2
	P10	1	1		S10	1	1
	P11	2	2		S11	4	2
	P12	3	3		S12	6	2
	P13	2	2		S13	3	2
	P14	1	1		S14	2	1
	P15	1	1		S15	1	1
	P16	1	1		S16	1	1
	P17	1	1		S17	1	1
	P18	19	10		S18	2	1
	P19[a]	1	1				

[a]The band profile could not be obtained by ApaI. Analyses were performed four times independently.
N=150 examined isolates.

fragments obtained by restriction enzymes ApaI and AscI, are given in Figs. 2 and 3. The number of DNA fragments detected was about 10 and the sizes of major bands were less than 500 kb. It was possible to distinguish isolates from each other by comparison of band profiles.

The results of PFGE are shown in Tables 3 and 4. Table 3 shows that PFGE by ApaI or AscI produced 20 and 19 macrorestriction patterns (MRP), respectively. In the case of ApaI, three major MRPs (P04, P05, and P19) were obtained, and their total percentage was 66.7%. The number of samples associated with each major MRP was at least 10. In the case of AscI, the result was similar to that of ApaI, and three major MRPs (S01, S04, and S05, 71.4%) were also obtained. The number of samples with each major MRP ranged from 14 to 21. When MRPs from ApaI and AscI were combined, they presented 27 different pulsotypes (Table 4). The pulsotypes were named as SNU01, SNU02 and up to SNU27. Pulsotypes SNU07, SNU08, and SNU27 were detected in at least 10 samples. However, 18 out of 27 pulsotypes were detected only in one sample.

Table 4. Pulsotypes of *L. monocytogenes* classified by restriction patterns using ApaI and AscI.

Pulsotype	Restriction pattern by ApaI/AscI	No. of isolates	No. of related samples
SNU01	P1/S11	3	1
SNU02	P1/S1	7	3
SNU03	P2/S18	2	1
SNU04	P1/S2	2	1
SNU05	P1/S3	1	1
SNU06	P3/S3	4	1
SNU07	P4/S4	37	19
SNU08	P5/S5	40	18
SNU09	P5/S4	3	2
SNU10	P6/S7	1	1
SNU11	P6/S6	3	2
SNU12	P7/S9	2	2
SNU13	P8/S6	2	2
SNU14	P9/S8	5	3
SNU15	P10/S10	1	1
SNU16	P19[a]/S1	1	1
SNU17	P9/S12	5	1
SNU18	P11/S13	2	1
SNU19	P12/S6	2	1
SNU20	P12/S10	1	1
SNU21	P13/S14	2	1
SNU22	P4/S15	1	1
SNU23	P14/S13	1	1
SNU24	P15/S16	1	1
SNU25	P16/S17	1	1
SNU26	P17/S12	1	1
SNU27	P18/S1	19	10

[a]The band profile could not be obtained by ApaI.
[b]Could not be typed.

Interestingly, the isolates of MRP P1 by ApaI were divided into four different pulsotypes, and other MRPs by ApaI (P4, P5, P6, P9, and P12) into two strains.

Figure 4 shows a dendrogram of the isolates, based on MRPs produced by ApaI and AscI. The isolates formed six clusters at a similarity level of 60%. In several cases of pulsotypes, they were closely related with more than 90% similarity. Furthermore, the pulsotypes associated with MRP P1 by ApaI (*L. monocytogenes* SNU01, 02, 04, and 05) were closely related with each other, although they were divided into four pulsotypes.

Therefore, the overall results of subtyping of *L. monocytogenes* isolates suggested the possibility that several typical pulsotypes of *L. monocytogenes* exist in chicken carcasses in the Korean market.

Serotype

An isolate from isolates of the same pulsotype was selected for serotyping, and results are shown in Table 5. Serotypes 1/2a, 1/2b, 1/2c, 3b, and 4b were detected. However, the *L. monocytogenes* isolate of pulsotype SNU25 could not be typed by serotyping. About half of the isolates were found to have serotype 1/2a and these isolates had 13 different pulsotypes. In addition, 1/2b was another major serotype and 10 pulsotypes were associated with it. Other serotypes were only found in one sample. Overall, the percentage of 1/2 serotypes was over 95%.

Antimicrobial Resistance of Isolated *L. monocytogenes* Strains

Twenty-seven pulsotypes of *L. monocytogenes* were examined for resistance and sensitivity to several antibiotics, by testing at least twice to minimize experimental errors. One to seven isolates of the same pulsotype were also examined to find out the difference of antibiotic resistance between the isolates of the same pulsotype. Antibiotic resistance profiles of isolates with the same pulsotype were identical in all of the examined cases. All *L. monocytogenes* strains tested were sensitive to kanamycin, vancomycin, cephalothin, rifampicin, erythromycin, ampicillin, gentamicin, streptomycin, and chloramphenicol (Table 6). Seventeen strains were resistant to penicillin G and seven strains to tetracycline. Five strains were resistant to both antibiotics.

Discussion

In this study, analysis of raw chicken carcasses purchased from Korean markets revealed that chickens were heavily contaminated with *L. monocytogenes* and other *Listeria* spp. The incidence of *L. monocytogenes* was about 30%. Other reports [6, 19] on the incidence of *L. monocytogenes* in Korea was 30–33%, in agreeement with the results of this study. However, comparison of our present result with

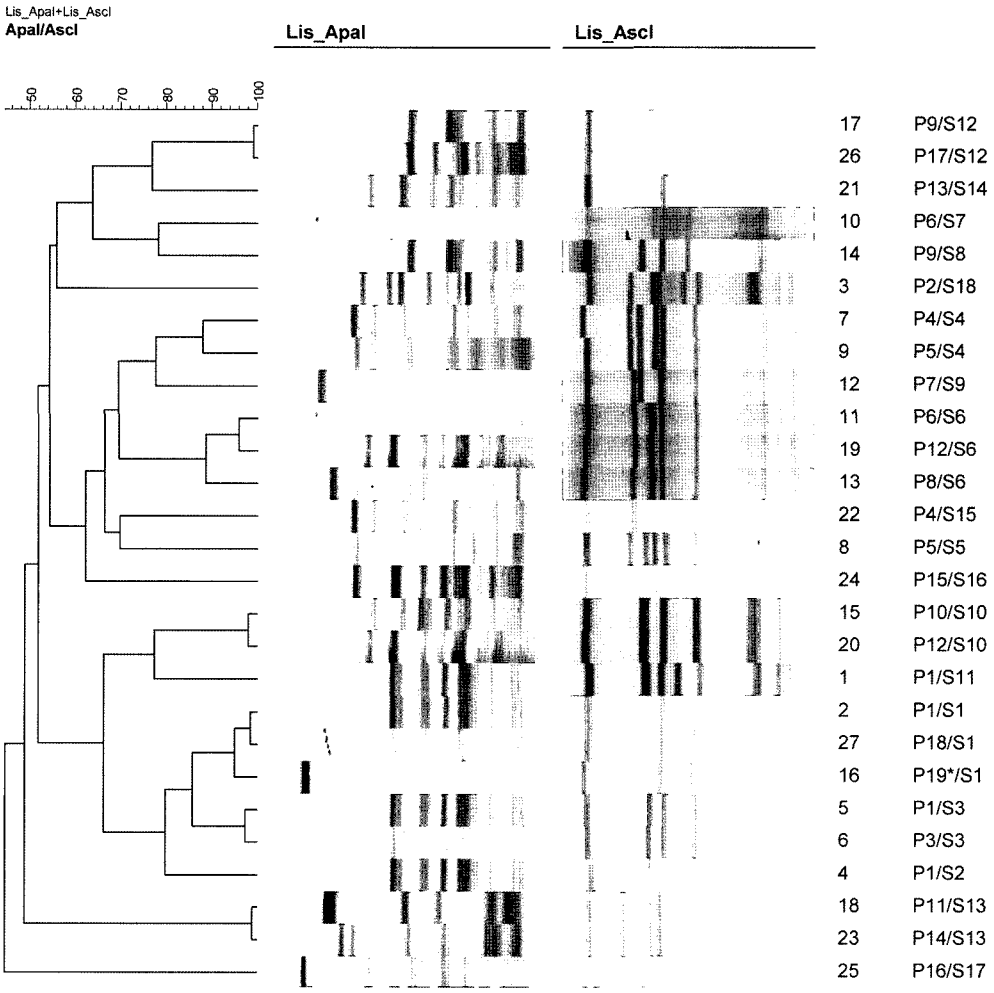

Fig. 4. Dendrogram displaying the genetic diversity of *Listeria monocytogenes* strains by ApaI and AscI.

those from other countries revealed differences: Reported incidences of several countries were 38% in the U.S.A. [7], 94% in Denmark [48], 48% in New Zealand [38], 69% in the U.K. [43], and 76.3% in Spain[53]. Therefore, the incidence of *L. monocytogenes* in chicken carcasses in Korea is comparable with or lower than those reported for European countries. Possible reasons for the inconsistency might be different sample sizes, season of sampling, and detection methods.

Interestingly, *L. innocua* was found in 50% of the chicken samples examined, suggesting that *L. innocua* is the most predominant species in chicken. It is consistent with the result of Choi *et al.* [19], who reported a 71.7% incidence. Although not harmful to humans, the existence

Table 5. Serotypes of isolated *L. monocytogenes*.

Serotype	No. of isolates (%)	No. of related samples (%)	Pulsotype
1/2a	68 (45.3)	36 (45.6)	SNU08, SNU09, SNU10, SNU11, SNU12, SNU13, SNU14, SNU17, SNU18, SNU21, SNU22, SNU23, SNU26
1/2b	41 (27.3)	21 (26.6)	SNU01, SNU02, SNU04, SNU05, SNU06, SNU15, SNU19, SNU20, SNU24, SNU27
1/2c	37 (24.6)	19 (24.1)	SNU07
3b	1 (0.6)	1 (1.3)	SNU16
4b	2 (1.3)	1 (1.3)	SNU03
UT[a]	1 (0.6)	1 (1.3)	SNU25

[a]Untypeable.

Table 6. Number of antimicrobial resistant pulsotypes of of *L. monocytogenes* strains.

Antibiotic disk	P10	K30	TE30	VA30	KF30	RD5	E15	AMP10	CN10	S10	C30
	17	0	7	0	0	0	0	0	0	0	0

*Abbreviation for antibiotic disks: Penicillin G (P10); Kanamycin (K30); Tetracycline (TE30); Vancomycin (VA30); Cephalothin (KF30); Rifampicin (RD5); Erythromycin (E15); Ampicillin (AMP10); Gentamicin (CN10); Streptomycin (S10); Chloramphenicol (C30).

of *L. innocua* means a high potential risk of contamination of *L. monocytogenes*. It is well known that *L. innocua* is more tolerant to some selective agents used in *L. monocytogenes* enrichment media and grows faster [42, 9, 21]. Therefore, it may block the growth of *L. monocytogenes* in selective media and compromise the detection of *L. monocytogenes* in the same sample. Therefore, even if *L. monocytogenes* is not detected and other spp. of *Listeria* are detected, the possibility of contamination by *L. monocytogenes* could be high.

PFGE analysis by enzyme ApaI presented 19 macrorestriction profiles, as shown in Table 3. However, one isolate (SNU19, serotype 1/2b) could not produce distinguishable DNA fragments profiles by PFGE using ApaI, although the experiment was repeated at least four times in this study. Regarding the use of ApaI enzyme, Carriere *et al.* [14] reported that the DNA of many isolates of *Listeria* serovar 1/2c was not digested by ApaI and NotI. Similarly, Brosch *et al.* [13] noted that the DNA of isolates of *Listeria* serovar 4c was not digested by ApaI. Therefore, the use of another enzyme or a combination of two different enzymes has been considered. Several kinds of restriction enzymes have been reported for PFGE analysis of *Listeria* spp. The most popular enzyme for *Listeria* is ApaI, as reported elsewhere [24, 28], and other enzymes include AscI [30] and SmaI [41]. Generally, AscI and ApaI are considered to be the most useful enzymes for DNA profiling of *Listeria* spp. [30, 13, 31]. Therefore, a combination of two enzymes, such as ApaI and AscI, was tested in this study. Combined use of these restriction enzymes could produce discernable DNA fragments profiles for all of isolates. It was clearly shown in Table 4 that at least two enzymes were required to obtain more pulsotypes, because isolates with the same pulsotype by ApaI could be divided into several pulsotypes by the enzyme AscI. A combination of two different enzymes, such as ApaI+SmaI [12, 11, 36, 37, 22, 46, 20], ApaI+AscI [13, 26, 5], AscI+SmaI [4], and ApaI+NotI [12, 14], has also been reported.

Serotyping is a classical tool for epidemiological and sporadic case studies of *L. monocytogenes*, and different serotypes of a certain organism are predominant in different continents or countries. Serotype 1 has been known to be predominant in Europe. In contrast, serotype 4b is more common in the U.S.A. [27]. Most human infections are reported to be associated with serogroup 4 strains [23] or 1/2a, 1/2b, and 4b [27]. Table 5 shows that the predominant serotypes in this study were 1/2a, 1/2b, and 1/2c, and the percentage of these three serotypes was more than 95%, in agreement with a report in Korea [6], showing that 90% of *L. monocytogenes* isolates were serotype 1 and only 4.1% were type 4. Serotype 1/2a was classified into 13 different pulsotypes, and 1/2b into 10 pulsotypes. We detected one strain that we could not type. It is reported that isolates of food and environmental origin cannot often be typed by standard typing antisera [27]. Therefore, serotyping appeared to be less discriminatory than other subtyping methods such as PFGE.

The increased resistance of foodborne pathogenic bacteria to antibiotics has been reported, and more attention is paid by public health authorities and consumers [35, 10]. The resistance is attributed to the heavy use of antibiotics in hospitals for treatment of patients and the use of antibiotics as growth promoters in the production of animal foods [16, 50, 25, 33]. *Listeria* spp. are generally considered to be still susceptible to most antibiotics [16, 55], and it has even been reported that the susceptibility patterns of *L. monocytogenes* from humans have not been altered in the 25 years between 1958 and 1985 [44]. However, a *Listeria* strain with antibiotic resistance was firstly reported in 1988 [38], and since then, several antibiotic resistant *Listeria* strains have been reported. Walsh *et al.* [54] reported that 10.9% of *L. monocytogenes* isolates were resistant to one or more antibiotics, and resistance to tetracycline (6.7%) and penicillin (3.7%) was the most frequently observed. These findings are consistent with the results of this study, suggesting that dissemination of *L. monocytogenes* with resistance to tetracycline and penicillin has already occurred in Korean food ecosystems. Others reported the resistance to ampicillin, streptomycin, tetracycline, penicillin G, phosphomycin, lincomycin, and chloramphenicol [39, 45, 17, 52, 3]. The profile of resistance has been found to vary by the origin of food, the method of analysis, and geographic region. In this study, the isolate of each pulsotype was investigated for antibiotic resistance, and the results in Table 6 showed that several isolates were resistant to penicillin G (17 strains) and tetracycline (7 strains), but susceptible to the other antibiotics tested.

Possible mechanisms of emergence of antibiotic resistance in *Listeria* spp. have been suggested to involve self-transferable and mobilizable plasmids and conjugative transposons [16]. Sources of resistance genes for *L. monocytogenes* are considered as enterococci, streptocci, and other microorganisms, due to a flux of genes among Gram-positive and Gram-negative bacteria through conjugative mobilization [55].

In conclusion, although human listeriosis by chickens is not remarkable in Korea, the incidence and genetic diversity of *L. monocytogenes* are comparable to that of other countries with a higher incidence of listeriosis. The reason for the low incidence of listeriosis might be due to different consumption patterns of chicken products from western countries. However, the consumption pattern of chicken meat is becoming more westernized in Korea, consuming more ready-to-eat products. Therefore, the handling and processing procedures of chicken products should be appropriately improved to minimize the contamination of food pathogens and to enhance public health concerns imposed by *L. monocytogenes*.

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