Phenotypic and genotypic characterization of *Listeria monocytogenes* in clinical ruminant cases in Korea

Jongho Kim[a,b], Jong Wan Kim[a], Ha-Young Kim[a#]

[a]Animal and Plant Quarantine Agency, Gyeongbuk, Republic of Korea

[b]Jeonbuk National University, Iksan, Republic of Korea

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#Address correspondence to Ha-Young Kim, kimhy@korea.kr or jnm4jesus@naver.com

ABSTRACT

Listeria monocytogenes is a foodborne human and veterinary pathogen. This study aimed to determine the phenotypic and genotypic characteristics of *L. monocytogenes* isolates from clinical cases of Korean ruminants. We collected 24 *L. monocytogenes* isolates from clinical cases with caprine neurological symptoms and bovine abortion. The most prevalent serotypes were 4b (IV_b), 1/2a (II_a; II_c), and 1/2b (II_b). All isolates, including two found in humans, formed three genetically diverse pulsed-field gel electrophoresis clusters according to serotype, lineage, and sequence type. The most prevalent sequence type was ST1, followed by ST365 and ST91. *L. monocytogenes* isolates from ruminant listeriosis were resistant to oxacillin and ceftriaxone. These clinical ruminant isolates showed diverse lineage, serotype (serogroup), and sequence type characteristics. Considering that the atypical sequence types exhibited clinical manifestations and histopathological lesions, further study is needed to elucidate the pathogenicity of genetically diverse ruminant *L. monocytogenes* isolates. Furthermore, continuous monitoring of antimicrobial resistance is required to prevent the emergence of *L. monocytogenes* strains resistant to common antimicrobials.

Keywords: ruminant listeriosis, serotype, PFGE, MLST, antimicrobial resistance

INTRODUCTION

Listeriosis caused by *Listeria monocytogenes* causes high morbidity and mortality primarily in ruminant farms. Listeriosis in ruminants manifests in two major forms: septicemia and (rhomb)encephalitis; the septicemic form of listeriosis can result in fetal infection and subsequent abortion (1). A Danish study showed that the diagnostic rate of *L. monocytogenes* in bovine abortion was 1.2% (2). In small ruminant surveillance studies conducted in Slovenia and Switzerland, the incidence rates of listeria rhombencephalitis and central nervous system disease in bovine adults were 5.3 and 26.3 cases per 100,000, respectively (3). In humans, *L. monocytogenes* is a serious foodborne pathogen causing various clinical conditions, including septicemia, abortion, gastroenteritis, and encephalitis (4).

Thirteen *L. monocytogenes* serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) have been identified, with lineages I (serotypes 1/2b, 4b, and 3b) and II (serotypes 1/2a, 1/2c, and 3c) more frequently isolated from ruminant listeriosis cases than lineages III or VI (1). Lineage I is most frequently isolated from human and ruminant encephalitis (1,5). Among the 13 serotypes, 1/2a and 3a have been associated with non-encephalitic infections (4). Using a multiplex PCR method to overcome the major drawbacks of conventional serotyping, these serotypes were reclassified into five serogroups: II_a (1/2a and 3a), II_b (1/2b, 3b, and 7), II_c (1/2c and 3c), IV_a (4a and 4c), and IV_b (4ab, 4b, 4d, and 4e) (6). Among them, PCR serogroup II_a is the most prevalent in both fetal infection and neurologic cases in ruminant listeriosis (1). Furthermore, *L. monocytogenes* serotypes 4b, 1/2a, and 1/2b were primarily responsible for the human cases of listeriosis in the EU in 2013 and USA between 2011 and 2016 (6,7). However, serotyping has a limited discriminatory ability when comparing bacterial genotypes (8).

Therefore, molecular typing is mostly conducted using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to investigate the zoonotic sources and genetic diversity of *L. monocytogenes* isolates (1,4).

Several virulence genes responsible for bacterial pathogenesis in host cells have been identified in *L. monocytogenes* isolates (9). Among them, *inlA*, *inlC*, and *inlJ*, which encode internalin-like proteins, are considered to be important for the initial stage of infection; *llsX* (listeriolysin S expression), *plcA* (phosphatidylinositol-phospholipase C), *plcB* (phosphatidylcholine-phospholipase C), *hly* (listeriolysin O), *lmo2672* (transcriptional regulator), and *prfA* (transcriptional regulator) are crucial for the development of human listeriosis (9,10).

Listeriosis is susceptible to most antimicrobials used to treat gram-positive bacteria (11,12). A combination of β-lactam antimicrobials, such as penicillin or ampicillin, and aminoglycosides, such as gentamicin, has been used as the first choice for human listeriosis. Trimethoprim-sulfamethoxazole, tetracycline, and erythromycin have been used as alternative therapies for listeriosis (9,11,13). However, several *L. monocytogenes* isolates are resistant to these antimicrobials (9,10,13,14). In Korea, *L. monocytogenes* isolates from swine and bovine carcasses have showed resistance to the recommended antimicrobials, such as penicillin (93.3%), ampicillin (26.7%), and tetracycline (20.0%) (15). There are currently no studies on the antimicrobial resistance of *L. monocytogenes* isolates from clinical ruminant cases.

The aim of the present study was to determine the phenotypic and genotypic characteristics of *L. monocytogenes* isolates from clinical cases of Korean ruminants. Furthermore, we monitored antimicrobial resistance and determined empirical antimicrobials of *L. monocytogenes* isolates from clinical cases of ruminants.

MATERIALS AND METHODS

Isolates and DNA extraction

For differential diagnosis, 360 stillborn bovine fetuses (2015–2019) and 99 goats (2013–2019) presenting with listeriosis-related symptoms, including neurological signs, abortion, and sudden death, were requested from farm owners. All isolates were subjected to biochemical identification using the VITEK® II system (BioMérieux, Marcy l'Etoile, France) and VITEK® MS system (matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MALDI-TOF MS, BioMérieux). The isolates were confirmed by the amplification of *hly* (16) and used to inoculate brain-heart infusion broth (Becton Dickinson, Sparks, MD, USA). These solutions were then aerobically cultured for 18 h at 37 °C. Genomic DNA was extracted using the Maxwell® RSC instrument (Promega, Madison, WI, USA) with the Maxwell® RSC Blood DNA kit (Promega) according to the manufacturer's instructions. In addition, two human *L. monocytogenes* isolates (NCCP 14714, NCCP 15743) were obtained from the National Culture Collection for Pathogens (NCCP) of the Korea Disease Control and Prevention Agency (KDCA).

PCR serogrouping and conventional serotyping

Multiplex PCR was used to determine *L. monocytogenes* molecular serogroups as previously described (1). For agglutination analysis, a commercially available serotyping kit (Denka Seiken Co., Tokyo, Japan) was used to identify O and H antigens according to the manufacturer's instructions.

Detection of virulence genes

92 The 11 virulence genes *inlA*, *inlC*, *inlJ*, *lmo2672*, *llsX*, *prfA*, *plcA*, *hlyA*, *mplA*, *actA*, and
93 *plcB* were identified from *L. monocytogenes* isolates using PCR, as described previously (9).

94 **PFGE**

95 PFGE of the *L. monocytogenes* isolates was performed using a previously described
96 protocol, with some modifications (15). Briefly, bacterial DNA was digested using the restriction
97 enzyme *AscI* (Thermo Scientific, Waltham, MA, USA) for at least 5 h at 37°C. Size separation of
98 restricted DNA fragments was performed using a CHEF MAPPER XA system (Bio-Rad,
99 Richmond, CA, USA) with 1% SeaKem gold agarose gel for 21 h. *XbaI*-digested *Salmonella*
100 *enterica* serotype Braenderup H9812 DNA was used as a standard to compare each band of the
101 *L. monocytogenes* isolates. The banding patterns were then interpreted and compared using
102 GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). A combined
103 dendrogram was analyzed using the unweighted pair group method with arithmetic mean with
104 Dice correlation coefficient and 1% tolerance.

105 **MLST**

106 *Listeria monocytogenes* isolates were subtyped using the MLST scheme, as described in the
107 Institut Pasteur MLST database (http://bigsdb.pasteur.fr/listeria). Sequence data for seven
108 housekeeping genes (*abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh*, and *lhkA*) were analyzed using an ABI
109 Prizm 3730XL analyzer (Applied Biosystems, Foster City, CA, USA) at Macrogen (Macrogen
110 Inc., Seoul, Korea). Allelic profiles and phylogenetic lineage corresponding sequence types
111 (STs) with clonal complexes (CC) were determined according to the criteria in the MLST
112 Pasteur database. MEGA 7.0 (Pennsylvania State University, State College, Pennsylvania, USA)
113 was used to construct a neighbor-joining phylogenetic tree from the concatenated alignment of

allele sequences, with Kimura's two-parameter model and 1,000 replicates (17). Concatenated sequences obtained from clinical ruminant isolates in this study and human *L. monocytogenes* strains from the NCCP of KDCA were phylogenetically analyzed. ST72 from lineage III was chosen as the root. For comparison with common clinical ruminant *L. monocytogenes*, ruminant listeriosis-associated STs from Europe and common human listeriosis-associated STs, such as ST2, ST6, and ST37, were used for phylogenetic analysis.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of the *L. monocytogenes* isolates was performed using a Sensititre GPN3F plate (Trek Diagnostic Systems, Cleveland, USA), which contained 18 antimicrobials, following the manufacturer's instructions. The antimicrobials and their ranges in the GPN3F plate are shown in Table S1. The antimicrobial resistance of the isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute, adopting the criteria for *L. monocytogenes* and other gram-positive bacteria, except for ampicillin, erythromycin, and penicillin, for which the European Committee on Antimicrobial Susceptibility Testing breakpoints were used (18,19).

Statistical analysis

Statistical testing was performed using GraphPad Prism (v5.01; GraphPad Software, San Diego, CA, USA). A 2 × 2 contingency table using Fisher's exact test was used to assess associations among serotypes and virulence genes. Statistical significance was set at $P < 0.05$.

RESULTS

Clinical features of caprine and bovine listeriosis

The prevalence rates of listeriosis in bovine and caprine samples were 2.5% (9/360) and 16.2% (16/99), respectively. Among them, 24 *L. monocytogenes* isolates were examined in this study, including 8 bovine fetal abortion cases and 16 caprine listeriosis. According to the clinical features of the ruminant listeriosis cases observed (Table 1), most cases (66.7%, 16/24) occurred in the spring between March and May, and all bovine abortions caused by *L. monocytogenes* occurred during late-term pregnancy (8–9 months). *L. monocytogenes* was the only pathogen in all caprine listeriosis cases that presented neurological symptoms, including circling. Furthermore, all caprine cases showed suppurative meningoencephalitis with microabscesses in the brainstem. In bovine abortion cases, *L. monocytogenes* was also the only pathogen identified, with the exception of two cases that displayed co-infection with *Leptospira* species and *Neospora caninum*, respectively. Among the eight bovine abortion cases, suppurative placentitis was confirmed in two cases, and histopathological lesions were not identified in other cases.

PCR serogroups and serotypes of *L. monocytogenes* isolates

Among the 24 *L. monocytogenes* isolates, PCR serogroup IV_b (n = 11, 45.8%) was the most prevalent, followed by II_a (n = 9, 37.5%), II_b (n = 2, 8.3%), and II_c (n = 2, 8.3%), with most belonging to 1/2a (n = 11, 45.8%) and 4b (n = 11, 45.8%) serotypes. The remaining two isolates were 1/2b (Table 1). Interestingly, serotypes 4b and 1/2b corresponded to serogroups IV_b and II_b, respectively. However, serotype 1/2a included the two serogroups, II_a and II_c.

Virulence genes expressed in *L. monocytogenes* isolates

All isolates expressed the virulence genes *inlA*, *inlC*, *inlJ*, *lmo2672*, *plcA*, *actA*, *plcB*, *mpl*, *hly*, and *iap* (Table 1). In contrast, *llsX*-encoding listeriolysin was expressed in 13 of the

evaluated isolates (54.2%); however, *llsX* was not observed in isolates with the serotype 1/2a and was significantly correlated with serotype 4b ($P < 0.001$).

PFGE analysis of *L. monocytogenes* isolates

Among the 24 *L. monocytogenes* isolates, 14 PFGE banding patterns were obtained, and the isolates were grouped into three clusters according to lineage, serotype, and ST, regardless of geographical and host source (Fig. 1). Cluster I had a similarity level of 76.6%, which included six *L. monocytogenes* isolates (lineage I, serotype 4b, and ST1). Cluster III (n = 4) included two isolates (lineage I, serotype 1/2b, and ST224) with 92.9% similarity and two other isolates (lineage I, serotype 4b, and ST1) with 96.6% similarity. All the *L. monocytogenes* isolates in Cluster II belonged to lineage II and serotype 1/2a. Cluster II (similarity level of 56.0%) contained three ST91 and two ST9 isolates.

MLST of *L. monocytogenes* isolates

Among the 24 ruminant *L. monocytogenes* isolates, the most common ST (CC) was ST1 (CC1; 37.5%, n = 9/24), followed by ST365 (CC14; 16.7%, n = 4/24) and ST91 (CC14; 12.5%, n = 3/24) (Fig. 2). The most prevalent lineage in ruminant isolates was lineage I (66.7%, n = 13), which included ST1, ST4, ST219, and ST224. The remaining STs included ST365, ST91, ST9, ST8, and ST18 and belonged to lineage II (33.3%, n = 11) (Fig. 2). The distribution of STs and genetic relatedness of *L. monocytogenes* isolates, including a clinically common bovine ST in France and Slovenia (ST37) and human isolates from common STs (ST2 and ST6), are illustrated in the phylogenetic tree (Fig. 3). STs from lineage I were more closely genetically related than those in lineage II (Fig. 3).

177 **Antimicrobial resistance in ruminant *L. monocytogenes* isolates**

178 The antimicrobial resistance patterns of the *L. monocytogenes* isolates are shown in Table 2.
179 All isolates tested were susceptible to 11 antimicrobials; however, many isolates were resistant to
180 oxacillin (70.8%) and ceftriaxone (62.5%). Furthermore, several isolates showed intermediate
181 resistance to clindamycin (58.3%), ceftriaxone (29.2%), ciprofloxacin (29.1%), and linezolid
182 (12.5%). We did not identify isolates exhibiting multiresistance to the antimicrobials tested.

183 **DISCUSSION**

184 *L. monocytogenes* is a major foodborne pathogen associated with high morbidity and
185 mortality in infected animals. Although there have been several studies on *L. monocytogenes*
186 isolates from foods and environments, characterization studies of ruminant *L. monocytogenes*
187 isolates are limited in Korea (15,20). Moreover, there is little information available on the
188 prevalence, serotypes, antimicrobial resistance, and molecular characteristics of *L.*
189 *monocytogenes* isolates from ruminant clinical cases worldwide. In our study, the diagnostic rate
190 of bovine fetal abortion was slightly higher than that in a Danish study (2). The high incidence
191 rate in goat encephalitis was hypothesized to be from the sample collection of listeriosis cases
192 that presented neurological symptoms. In a previous study, PCR serogroup II_a (serotypes 1/2a
193 and 3a) was the dominant serogroup from ruminant listeriosis cases in the USA (1). Conversely,
194 PCR serogroup IV_b and serotype 4b, which are responsible for human listeriosis, were also the
195 most prevalent type identified from ruminant clinical listeriosis cases analyzed in this study
196 (Table S2). The identified serotypes were limited to serotypes 1/2a, 1/2b, and 4b, which are the
197 predominant serotypes that cause human listeria infection (21,22).

198 All the *L. monocytogenes* isolates examined in this study expressed 10 virulence genes,
199 indicating that these isolates may be potentially pathogenic to humans. Indeed, *llsX* is important
200 for the pathogenesis of human listeriosis and was expressed in the isolates that belonged to
201 serotype 4b ($P < 0.001$) and 1/2b (10). Therefore, the serotype 4b strains from ruminant
202 listeriosis could present a potential risk factor for human listeriosis.

203 Our PFGE results revealed that *L. monocytogenes* isolates from ruminant listeriosis cases
204 were correlated with serotype and ST rather than host source or geographical origin. Based on
205 these results, three clusters that demonstrated 55% similarity showed high genetic variation.
206 Interestingly, two isolates (serotype 1/2b/ST224) from caprine listeriosis had relatively high
207 similarity (85.2%) with the human blood strain NCCP 14714, which belongs to the same
208 serotype (PCR serogroup), virulence profile, and ST reported in two caprine isolates. These
209 caprine and human listeriosis isolates were not related to the outbreak year or geographical
210 origin. Further epidemiological investigations and more powerful molecular characterization
211 methods, such as whole-genome sequencing, are required to elucidate the possibility of zoonotic
212 listeriosis and genetic relatedness of these cases.

213 We found that *L. monocytogenes* lineage I (n = 13, 54.2%) was more frequently detected
214 than lineage II (n = 11, 45.8%), which was consistent with previous MLST studies of ruminant
215 listeriosis in Europe (3,5). In contrast, a USA survey showed that lineage II was the most
216 prevalent in ruminant listeriosis, and the number of STs was more diverse than that observed in
217 this study (1). Moreover, ST1, ST4, and ST412 accounted for 51% of the cases, with ST1
218 (33.3%) being the most prevalent type in ruminant listeriosis cases in Europe (5). In another
219 ruminant clinical case from France and Slovenia, the three most prevalent clones were CC1

(39.1%), CC4-CC217 (12.9%), and CC37 (6.0%) (3). In the USA, ST7 (15.2%) was the most frequently isolated from ruminant listeriosis, followed by ST91 (10.9%) (1). When comparing these and our results, we estimated that ST1 and ST91 were common in ruminant listeriosis despite their regional differences. Interestingly, ST365, which was the second most frequently isolated ST in this study, is mainly isolated from vegetation and rarely from ruminant listeriosis, according to the Institut Pasteur MLST database (http://bigsdb.pasteur.fr/listeria). In the phylogenetic tree, ST365 was clustered with ST37, the third most prevalent ruminant listeriosis ST in France and Slovenia cases (Fig 3). Considering the clinical manifestations and genetic relatedness with ST37, these results suggest that ST365 is a significant factor in ruminant listeriosis that should be considered in future investigations.

The lineage I clones CC1, CC4, CC2, and CC6 were described as clinically associated and hypervirulent in humans (3). However, the possibility of zoonotic infection should be noted because ST1 and ST4 strains comprised 41.7% of the cases we characterized. Moreover, according to the Pasteur MLST *L. monocytogenes* database, other STs, including ST8, ST9, ST18, ST91, ST224, ST219, and ST365 that have mainly been isolated from food and the environment have also been isolated from human infections worldwide (http://bigsdb.pasteur.fr/listeria/). Therefore, these STs may pose a risk to public health.

Although antimicrobial resistance in *L. monocytogenes* isolates has not yet been unnoticed, it is important to evaluate the effectiveness of antimicrobials and monitor the emergence of resistant strains. Fortunately, we found no multi-drug resistant (MDR) isolates, and all *L. monocytogenes* isolates we analyzed were susceptible to 11 different antimicrobials. The frequency of oxacillin (70.8%) and ceftriaxone (62.5%) resistance was relatively high, which

might have resulted from the intrinsic resistance to these antimicrobials, consistent with previous studies (6,18,23). In contrast, 20.0% of the *L. monocytogenes* isolates in a Korean slaughterhouse study were MDR (15). In other Korean studies using slaughterhouse, food, and environmental samples, the resistance rates against therapeutic antimicrobials like penicillin, ampicillin, and tetracycline were 53.3–100%, 26.6–97.0%, and 13.3–20.0%, respectively (15,24,25).

Several studies have reported *L. monocytogenes* isolates from foods and environments with resistance to therapeutic antimicrobials (9,13,26–28). Although comparisons are arduous due to differences in the antimicrobial agents and breakpoints used in these studies, *L. monocytogenes* isolates from ruminant listeriosis have relatively lower resistance rates than those reported for food and environmental origins, except for intrinsic antimicrobial resistance. These results might be related to sample source differences and antimicrobial resistance transfer from cross-contamination through the food chain (13,29). The antimicrobial resistance of clindamycin classified into the class of lincosamides, generally used in the treatment of diseases of human and veterinary medicine showed relatively higher intermediate rates (58.3%) than those reported in Poland (34.6%), Italy (29%), and the USA (27%) (12,18,23,26). Furthermore, the resistance of ciprofloxacin-resistant isolates classified into critically important antimicrobial class in WHO also showed slightly higher intermediate rates (29.1%) than those reported in Poland (20.4%) and Italy (24%) (23,26). Considering the acquisition of additional gene mutations, intermediate MIC values in clindamycin and ciprofloxacin widely used in hospitals to treat Gram-positive infections could indicate a possible future shift to resistance phenotypes (26). To our knowledge, there are limited reports of antimicrobial resistance in ruminant clinical listeriosis. Therefore, continuous surveillance of antimicrobial-resistant *L. monocytogenes* is necessary to improve animal and human health. For the treatment of listeriosis, beta-lactams (penicillin and

ampicillin), macrolides (erythromycin), aminoglycoside (gentamicin and streptomycin), tetracycline, and trimethoprim/sulfamethoxazole are more effective than ceftriaxone, oxacillin, and clindamycin. Therefore, these agents are recommended as empirical antimicrobials for the treatment of clinical listeriosis.

The significant relevance of antimicrobial resistance patterns among the animal sources, serotypes, PFGE clusters, and STs was not observed in this study, potentially due to the diverse phenotypic and genetic origins of the isolates tested. To elucidate the relevant characteristics that contribute to antimicrobial resistance in *L. monocytogenes*, further studies with larger numbers of isolates from diverse locations and sources are required.

In conclusion, this report is the first comprehensive characterization of *L. monocytogenes* in clinical ruminant listeriosis cases in Korea. Our results indicated that the ruminant *L. monocytogenes* isolates belonged to serotypes 4b, 1/2a, and 1/2b. Based on molecular characterization, clinical ruminant *L. monocytogenes* isolates were genetically diverse and associated with isolates from humans, food, and the environment. According to the clinical signs and histopathological lesions of ruminant *L. monocytogenes* isolates, ruminant listeriosis pathogens, such as ST365, ST9, ST101, ST18, ST224, and ST219, cannot be ignored. Although the ruminant *L. monocytogenes* isolates were susceptible to the primary antimicrobial used for treating human listeriosis, continuous monitoring of the antimicrobial resistance in *L. monocytogenes* isolates is needed. Further research is required to predict the virulence phenotypes and improve the genetic characterization of clinical ruminant *L. monocytogenes* isolates.

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*Authors' contributions***:**

Jongho Kim and Jong Wan Kim: conceptualization, methodology, writing of the original draft, review and editing of the final manuscript.

Ha-Young Kim: conceptualization, supervision, review and editing of the manuscript.

Competing interests:

The authors declare that they have no competing interests.

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Figure legends

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400

401 Table 1. Clinical features and characterization of ruminant (n = 24) and human (n=2) *Listeria monocytogenes* isolates in Korea

Isolates	Source (isolated organs)	Region	Clinical	Year (month)	Age or pregnancy period	Serogroup	Serotype	Lineage	*IlsX*[1]	Sequence types	Clonal complex
LM4	Caprine (Brain)	Gyeongbuk	Circling	2013 (April)	2 years	II_a	1/2a	II	-	ST18	CC18
LM7	Caprine (Brain)	Gyeongbuk	Circling	2013 (May)	1 year	II_a	1/2a	II	-	ST365	CC14
LM9	Caprine (Brain)	Gyeongbuk	Circling	2013 (May)	1 year	II_a	1/2a	II	-	ST365	CC14
LM11	Bovine (Placenta, lung, gastric juice)	Jeonbuk	Abortion	2015 (March)	8 months	IV_b	4b	I	+	ST1	CC1
LM15	Bovine (Lung, gastric juice)	Jeonbuk	Abortion	2015 (March)	9 months	IV_b	4b	I	+	ST1	CC1
LM17	Caprine (Brain)	Jeonnam	Circling	2015 (June)	< 1 year	IV_b	4b	I	+	ST1	CC1
LM18	Caprine (Brain)	Gyeongnam	Circling	2016 (March)	3 years	II_b	1/2b	I	+	ST224	CC224
LM19	Caprine (Brain)	Gyeongnam	Circling	2016 (April)	2 years	II_b	1/2b	I	+	ST224	CC224
LM20	Caprine (Brain)	Gyeongbuk	Circling	2016 (June)	< 1 year	IV_b	4b	I	+	ST219	CC475
LM21	Caprine (Brain)	Jeonbuk	Circling	2016 (June)	1 year	II_c	1/2a	II	-	ST9	CC9
LM22	Bovine (Placenta)	Chungnam	Abortion	2017 (January)	8 months	IV_b	4b	I	+	ST1	CC1
LM25	Caprine (Brain)	Chungnam	Circling	2017 (January)	2 years	IV_b	4b	I	+	ST1	CC1
LM26	Caprine (Brain)	Chungnam	Circling	2017 (April)	NT*	II_a	1/2a	II	-	ST365	CC14
LM27	Caprine (Brain)	Chungnam	Circling	2017 (April)	NT	II_a	1/2a	II	-	ST365	CC14
LM29	Caprine (Brain)	Chungnam	Circling	2017 (May)	< 1 year	IV_b	4b	I	+	ST1	CC1
LM30	Caprine (Brain)	Gyeonggi	Circling	2017 (June)	3 years	II_c	1/2a	II	-	ST9	CC9
LM32	Bovine (Lung, gastric juice)	Gyeongbuk	Abortion	2018 (March)	8 months	II_a	1/2a	II	-	ST91	CC14
LM34	Bovine (Lung, gastric juice, muscle)	Gangwon	Abortion	2018 (March)	9 months	II_a	1/2a	II	-	ST91	CC14
LM36	Caprine (Brain)	Chungnam	Circling	2018 (March)	NT	II_a	1/2a	II	-	ST91	CC14
LM39	Bovine (Placenta)	Chungnam	Abortion	2018 (April)	8 months	IV_b	4b	I	+	ST1	CC1
LM40	Bovine (Brain)	Chungnam	Abortion	2018 (June)	8 months	IV_b	4b	I	+	ST1	CC1
LM41	Bovine (Muscle)	Jeonnam	Abortion	2019 (January)	9 months	II_a	1/2a	II	-	ST8	CC8
LM42	Caprine (Brain)	Jeonnam	Circling	2019 (March)	1 year	IV_b	4b	I	+	ST4	CC4
LM43	Caprine (Brain)	Gangwon	Circling	2019 (May)	< 1 year	IV_b	4b	I	+	ST1	CC1
NCCP14714[2]	Human (Blood)	Jeonbuk	listeriosis	2009 (May)	NT	II_b	1/2b	I	+	ST224	CC224
NCCP15743[2]	Human (Blood)	Gyeonggi	listeriosis	2012 (February)	NT	II_a	1/2a	II	-	ST101	CC101

402 * ND: Not determined.

403 [1] All isolates harbored the following 10 virulence genes: *inlA, inlC, inlJ, lmo2672, plcA, actA, plcB, mpl, hly,* and *iap*.

404 [2] human isolates of *L. monocytogenes* were obtained from the Korea Disease Control and Prevention Agency (KDCA).

Table 2. Antimicrobial susceptibility and cumulative percentage of *L. monocytogenes* (n = 24) inhibited by 18 antimicrobials

Antimicrobial	Cumulative percentage of strains inhibited at antimicrobial concentration (μg/mL) of:													MIC_{50} (μg/mL) [a]	MIC_{90} (μg/mL) [a]	S (%) [b]	I (%) [b]	R (%) [b]	MIC Breakpoint (μg/mL) [c]
	<0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>1000						
Oxacillin					4.2	29.2	66.7	100						4	8	**29.2**	ND [d]	**70.8**	≥4
Ceftriaxone								8.3	37.5	54.2	100			32	64	**8.3**	**29.2**	**62.5**	≥32
Ampicillin		12.5	66.7	100										0.25	0.5	100	0	0	>1
Ciprofloxacin				4.2	70.9	100								1	2	**70.9**	**29.1**	0	≥4
Clindamycin			4.2	41.7	87.5	100								1	2	**41.7**	**58.3**	0	≥4
Erythromycin			100											≤0.25	≤0.25	100	0	0	>1
Gatifloxacin					100									≤1	≤1	100	0	0	≥8
Gentamicin						100								≤2	≤2	100	0	0	≥16
Levofloxacin				4.2	75	100								1	2	100	0	0	≥8
Linezolid					25	87.5	100							2	4	**87.5**	**12.5**	0	≥8
Penicillin		8.3	66.6	100										0.25	0.5	100	ND	0	>1
Quinupristin/dalfopristin			4.2	54.2	100									0.5	1	100	0	0	≥4
Rifampin				100										≤0.5	≤0.5	100	0	0	≥4
Streptomycin													100	≤1,000	≤1,000	ND	0	0	≥1,024
Tetracycline						100								≤2	≤2	100	0	0	≥16
Trimethoprim/Sulfamethoxazole				100										≤0.5	≤0.5	100	0	0	≥4/76
Vancomycin					100									≤1	≤1	100	0	0	≥32
Daptomycin						4.2	58.4	100						4	8	ND	ND	ND	-

The gray zone represents the tested concentration range for each antimicrobial from the GPN3F plate.

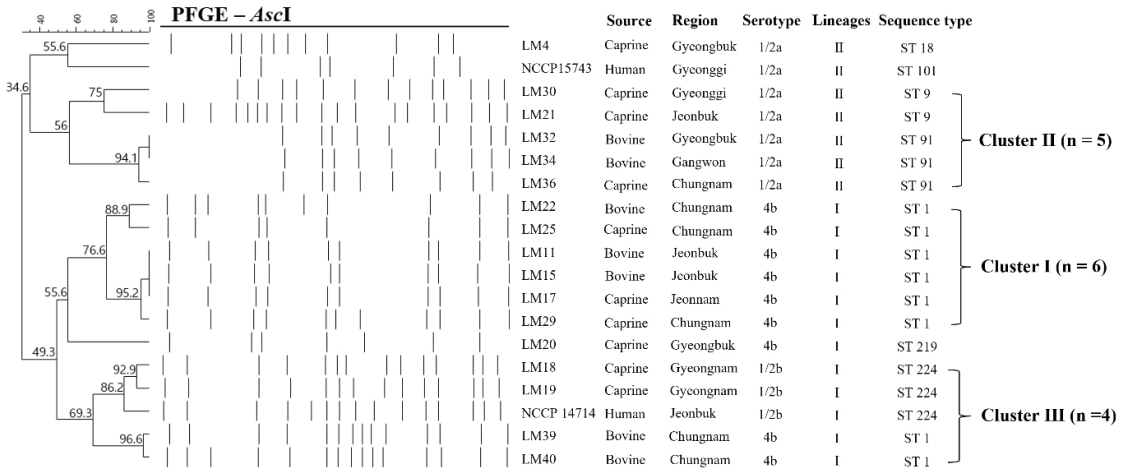
Susceptibility and resistance are indicated as vertical double (sensitive) and single (resistant) lines according to the guidelines for each reference.

[a] MIC_{50} and MIC_{90} are concentrations at which the growth of isolates was inhibited by 50% and 90%, respectively.

[b] S, susceptible; I, intermediate; R, resistant.

[c] MIC breakpoints are those that were recommended by a previous study (*18*); Ampicillin, Erythromycin, and Penicillin interpretations are based on (*19*).

[d] ND, not determined



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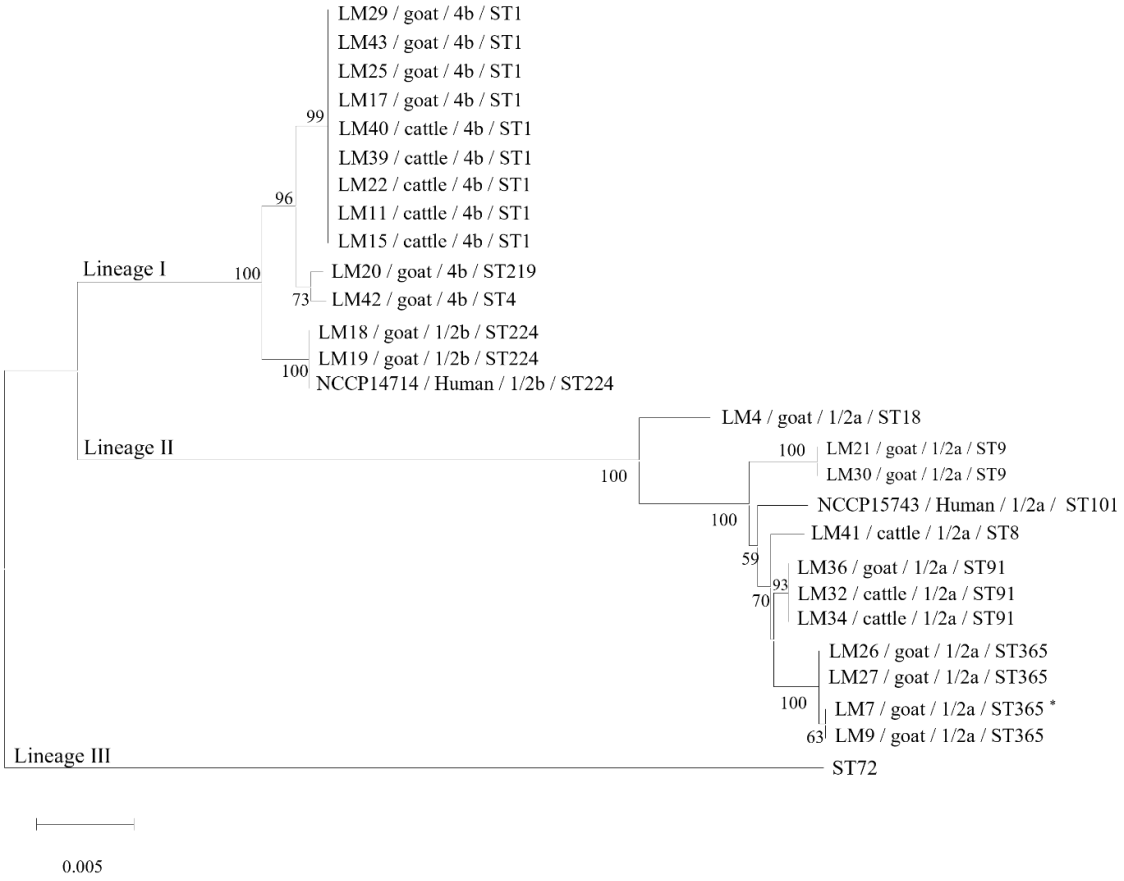

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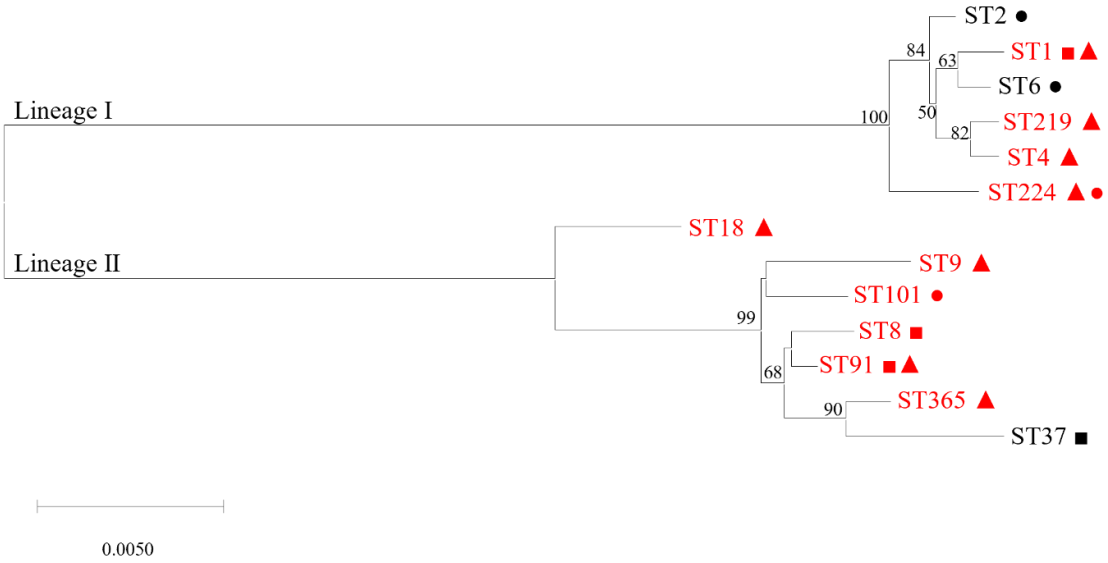

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