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ARTICLE

Occurrence and Characteristics of Methicillin-Resistant and -Susceptible *Staphylococcus aureus* Isolated from the Beef Production Chain in Korea

Haeng Ho Lee, Gi Yong Lee, Hong Sik Eom, and Soo-Jin Yang*

School of Bioresources and Bioscience, Chung-Ang University, Anseong 17547, Korea

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***Corresponding author** : Soo-Jin Yang
School of Bioresources and Bioscience, Chung-Ang University, Anseong 17547, Korea
Tel: +82-31-670-3256
Fax: +82-31-675-1381
E-mail: soojin@cau.ac.kr

***ORCID**
Haeng Ho Lee
https://orcid.org/0000-0002-7360-365X
Gi Yong Lee
https://orcid.org/0000-0001-5308-0065
Hong Sik Eom
https://orcid.org/0000-0002-1840-3399
Soo-Jin Yang
https://orcid.org/0000-0003-3253-8190

Abstract The emergence and persistence of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in livestock animals have been reported as a potential risk factor for transmission to humans. In this study, we investigated the nationwide prevalence and characteristics of MRSA and MSSA in the Korean beef production system, including retail markets, slaughterhouses, and cattle farms. From a total of 1,285 samples, only 5 MRSA strains were isolated: from a farmer (1 ST72 MRSA), a carcass sample from a slaughterhouse (1 ST72 MRSA), and beef cattle (3 ST5 MRSA). In addition, 11 MSSA strains were isolated from beef cattle (n=3), humans (1 farmer, 1 slaughterhouse worker, and 4 retail market workers), and carcass samples (n=1) and slaughterhouse environment (n=1). Although the prevalence of MRSA and MSSA in beef cattle was much lower than that reported in pigs, 5/5 MRSA and 2/11 MSSA strains displayed multiple drug resistance (MDR) phenotypes. Unlike the swine-associated MRSA, no correlation was found between tetracycline/zinc resistance and MDR phenotype. However, MRSA strains had an identical set of staphylococcal enterotoxins and exhibited enhanced levels of resistance to antimicrobial peptides (PMAP-36 and LL-37) compared to the MSSA strains. In conclusion, continued and systemic surveillance of livestock, meat products, and humans in close contact with livestock/meat products is necessary to prevent the transmission of MRSA and MSSA to humans.

Keywords methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA), beef cattle, antimicrobial resistance

Introduction

Staphylococcus aureus is a primary cause of human infections and one of the most important foodborne pathogens (Hennekinne et al., 2012; Turner et al., 2019). *S. aureus* has been implicated in a number of infectious diseases such as minor skin and soft tissue infections, toxic shock syndrome, and septicemia (Turner et al., 2019). Coupled with the virulence, *S. aureus* can also develop antimicrobial resistance to methicillin (methicillin-resistant *S. aureus*; MRSA), vancomycin (vancomycin-

intermediate or -resistant *S. aureus*; VISA/VRSA), and daptomycin (Turner et al., 2019). In addition to the infections in humans, a recent increase in the occurrence of livestock-associated (LA)-MRSA has been observed in various livestock animals, including the prevalence of sequence type (ST) 398 LA-MRSA in pigs worldwide (Price et al., 2012). The LA-MRSA strains tend to develop multidrug resistance (MDR) to several classes of antimicrobials, particularly tetracycline (Larsen et al., 2016; Price et al., 2012). Although there has been less focus on methicillin-susceptible *S. aureus* (MSSA) than MRSA, an increase in MSSA infections has been reported both in humans and animals (Carfora et al., 2016). Because MRSA and MSSA in livestock can be transmitted to farm workers and foods of animal origin and thus to CA settings, several studies were performed to monitor the prevalence of MRSA and MSSA in chicken, pork, and beef meat samples (Moon et al., 2015; Osman et al., 2016; Pauly et al., 2019).

Although the prevalence of MRSA and MSSA in swine farms and pork has been studied extensively (Moon et al., 2015), information is lacking on the occurrence and genetic profiles of MRSA and MSSA in the beef production system (retail markets, slaughterhouses, and beef cattle farms). Previous studies on bovine-associated MRSA or MSSA have mostly focused on bovine mastitis in the dairy industry (Song et al., 2016; Vanderhaeghen et al., 2010). Song et al. (2016) characterized profiles of antimicrobial resistance and staphylococcal enterotoxin (SE) genes in MRSA strains isolated from mastitic milk samples in Korea. Although several previous studies also identified MRSA in major livestock animals and meat samples in Korea (Lim et al., 2010; Moon et al., 2015), to the best of our knowledge, this is the first study in Korea that performed a nationwide screening of both MRSA and MSSA in the beef production system including beef cattle, beef meat, farmers or workers, and facilities.

In this study, we investigated the prevalence and genotypes of MRSA and MSSA throughout the beef production system in Korea, encompassing beef cattle farms, retail markets, and slaughterhouses. In addition, we examined genotypic and phenotypic factors associated with antimicrobial resistance and analyzed virulence characteristics of the MRSA and MSSA isolates.

Materials and Methods

Isolation of *S. aureus* and culture conditions

A total of 1,148 swabs and 137 meat samples were obtained from the following: 20 beef cattle farms, 7 slaughterhouses, and 20 retail markets from eight different provinces of Korea during 2018. Samples from beef cattle farm were obtained from healthy cattle (n=169), the cattle farm environment (n=60), and farmers (n=21); retail market samples included fresh beef meat (n=137), the environments within retail facility (n=13), and retail market workers (n=7); slaughterhouse samples were collected from cattle carcasses (n=264), the facility environments (n=21), and workers (n=9). All samples were transported to the laboratory within 24 h of sampling for isolation of *S. aureus*.

The swab samples were inoculated into 5 mL of fresh tryptic soy broth (TSB; Difco Laboratories, Detroit, MI, USA) containing 10% NaCl (NaCl-TSB), and then incubated at 37°C for 16–18 h. Beef meat samples (25 g) were homogenized in 10% NaCl-TSB (225 mL) and enriched at 37°C for 24 h, and then 10 µL samples were streaked onto Baired-Parker agar (BPA; Difco Laboratories) and cultured overnight at 37°C. Three presumptive *S. aureus* colonies per plate were selected and subcultured for identification. *S. aureus* isolates were identified using 16S rRNA sequencing and the Vitek 2 system (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility assays

Susceptibilities to antimicrobial agents were determined according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2019). The antimicrobial agents tested were ampicillin (10), cefoxitin (30), chloramphenicol (30), ciprofloxacin (5), clindamycin (2), dalfopristin-quinupristin (15), erythromycin (15), gentamicin (30), mupirocin (200), rifampin (5), sulfamethoxazole-trimethoprim (23.73–1.25), and tetracycline (30). All antibiotic disks were obtained from BD BBL™, except mupirocin, which was purchased from Oxoid (UK). Standard E-test (bioMérieux) assays were performed to determine minimum inhibitory concentrations (MICs) to vancomycin, oxacillin, tetracycline, and teicoplanin on Meuller-Hinton agar (MHA) plates.

Determination of MICs to zinc chloride and detection of czrC

The MICs to zinc chloride on all *S. aureus* isolates were determined by the standard agar dilution assay (ranging from 0.25 to 32 mM) in MHA II as reported before (Aarestrup and Hasman, 2004). Break point for zinc resistance was set at MIC of >2 mM according to the previous report (Aarestrup and Hasman, 2004). All susceptibility tests were repeated at least three times on separate days. The presence of the *czrC* (zinc resistance gene) was detected by PCR amplification as described before (Cavaco et al., 2010).

Molecular characterization of MRSA and MSSA strains

Multilocus sequence typing (MLST) was performed on the 5 MRSA and 11 MSSA isolates as described before (Enright et al., 2000). Briefly, seven target loci (*arcC*, *gmk*, *tpi*, *aroE*, *glpF*, *pta*, and *yqiL*) were PCR-amplified for subsequent sequencing analyses to determine the STs based on the MLST database (http://pubmlst.org/saureus/).

Types of SCC*mec* were determined on the 5 MRSA strains by multiplex PCR analysis as reported previously (Kondo et al., 2007). Multiplex PCR assays were performed to detect *mec* regulatory elements and cassette chromosome recombinase genes (*ccrA1*, *ccrA2*, *ccrA3*, *ccrB1*, *ccrB2*, *ccrB3*, *ccrB4*, and *ccrC*). The SCC*mec* types were determined based on the types of *ccr* and *mec* gene complexes.

The *agr* (I–IV) and *spa* types were determined for the MRSA and MSSA isolates, respectively, by PCR-based methods as described before (Gilot et al., 2002). For *spa* typing, the amplified genes were sequenced to determine the number of tandem repeats, and the *spa* type was assigned to each *S. aureus* strain according to the SpaServer database (http://spa.ridom.de/) (Harmsen et al., 2003). All MRSA and MSSA isolates were PCR-screened for the presence of *tetK*, *tetL*, *tetM*, *tetO*, and *tetS* genes, which confer resistance to tetracycline (Ng et al., 2001).

Detection of staphylococcal enterotoxin (SE) genes and PVL genes

Multiplex-PCR analyses were performed on all MRSA and MSSA isolates to examine the presence of 19 SE genes and the *tst1* gene (toxic shock syndrome toxin-1 gene) (Park et al., 2011). Briefly, four sets of multiplex PCRs were performed for the detection of the SE genes (PCR 1: *sea*, *seb*, *sec*, *sed*, and *see*; PCR 2: *seg*, *seh*, *sei*, *sej*, and *sep*; PCR 3: *sek*, *sem*, *seo*, and *tst1*; and PCR 4: *sel*, *sen*, *seq*, *ser*, and *seu*). Genomic DNA samples from the reference *S. aureus* strains were used for positive control PCR reactions (FRI913 strain for *sea*, *sec*, *see*, *sek*, *seq*, and *tst1* ; FRI472 strain for *sed*, *seg*, *sej*, *sel*, *sem*, *sen*, *seo*, *ser*, and *seu*; MW2 strain for *seh*; COL strain for *seb*; and N135 strain for *sei*, and *sep*). Detection of Panton-Valentine leukocidin (PVL) genes, *lukF* and *lukS*, were performed by using a PCR-based method as reported previously (Lina et al., 1999).

Biofilm assays under static conditions

Static biofilm formation assays were performed on all the MRSA and MSSA isolates as described previously (Pompilio et al., 2015). Briefly, staphylococci from overnight cultures were adjusted to OD_{600} of 0.1, diluted ~1:100 with fresh brain heart infusion (BHI) broth containing 30 mM glucose, and then 200 μL of the *S. aureus* culture was aliquoted to 96-well culture plates (SPL Life Sciences, Pochun, Korea). After 48 h incubation at 37°C, the culture supernatant was discarded, and then the plates were washed four times with phosphate-buffered saline. Next, the plates were air dried, and cells attached to the surface were stained with 5% safranin for 5 min. After the safranin staining, 30% acetic acid (Difco laboratories) was added to each well and absorbance was determined at OD_{492nm}. A minimum of four independent experiments were performed on separate days.

In vitro antimicrobial peptide susceptibility assays

Cationic antimicrobial peptides (CAPs) represent key elements in the host innate immune response. Porcine cathelicidin (PMAP-36) (Scheenstra et al., 2019) and human cathelicidin (LL-37) (Ouhara et al., 2008; Scheenstra et al., 2019) were synthesized with a purity of ≥95% (GL Biochen, Shanghai, China). *In vitro* survival assays against PMAP-36 and LL-37 were performed as described before using a 2 h microdilution method (Xiong et al., 2005). CAP susceptibility assays were performed with PMAP-36 (0.1 μg/mL) and LL-37 (3 μg/mL) using an initial *S. aureus* inoculum of ~5×10^3 CFUs in RPMI-1640 containing 5% LB broth. The concentrations of PMAP-36 and LL-37 were selected after extensive preliminary assays that showed sub-lethality with <50% reductions in CFUs over the 2 h time in both MSSA and MRSA strain groups. The data were analyzed and expressed as the % of relative mean survival±SDs of CAP-exposed versus CAP-unexposed controls. Three independent CAP susceptibility assays were performed for each MRSA and MSSA strain.

Statistical analysis

The statistical significance was analyzed by Mann-Whitney *U* test (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was determined at p-value <0.05.

Results

Occurrence of MRSA and MSSA in the beef production chain

Sixteen strains of MRSA (n=5, 0.39%) and MSSA (n=11, 0.86%) were cultured from 1,285 samples obtained from beef cattle farms, retail markets, and slaughterhouses over the twelve months of the sampling period (Table 1). The overall prevalence of MRSA was 0.5% in cattle farms and 0.3% in slaughterhouses. No MRSA isolates were recovered from beef meat, workers, or facility environment samples in retail markets. As shown in Table 1, four of the MRSA strains were recovered from cattle farms and one MRSA strain was cultured from a carcass sample in a slaughterhouse. Three of the five MRSA strains from beef cattle were isolated from the Gangwon province, and the two other MRSA strains were cultured from a farm worker and a carcass sample in Gyeongsang province (Table 1).

The overall prevalence of MSSA strain was 0.5% (n=4), 2.3% (n=4), and 0.9% (n=3) in cattle farms, retail markets, and slaughterhouses, respectively. Three of the MSSA strains were isolated from beef cattle, six MSSA strains were isolated from facility workers (one farm worker, one slaughterhouse worker, and four retail market workers), and two MSSA strains were isolated; one from a carcass and another from a slaughterhouse environment (Table 2). The geographic distribution of the 11

Table 1. Prevalence of MRSA and MSSA isolates in beef cattle farms, slaughterhouses and retail markets in Korea

	Positivity of *S. aureus* (No. of *S. aureus* positive/ no. of samples, %)					
	Provinces					
	Gyeonggi	Gangwon	Chungcheong	Jeolla	Gyeongsang	Total
MRSA	0/290	3/151 (2.0)	0/215	0/403	2/226 (0.9)	5/1,285 (0.4)
Cattle farms	0/190	3/86 (3.5)	0/143	0/220	1/156 (0.6)	4/795 (0.5)
Slaughterhouses	0/63	0/43	0/43	0/126	1/43 (2.3)	1/318 (0.3)
Retail markets	0/37	0/22	0/29	0/57	0/27	0/172
MSSA	4/290 (1.4)	0/151	1/215 (0.5)	4/403 (1.0)	2/226 (0.9)	11/1,285 (0.9)
Cattle farms	1/190 (0.5)	0/86	0/143	1/220 (0.5)	2/156 (1.3)	4/795 (0.5)
Slaughterhouses	1/63 (1.7)	0/43	1/43 (2.3)	1/126 (0.8)	0/43	3/318 (0.9)
Retail markets	2/37 (5.4)	0/22	0/29	2/57 (3.5)	0/27	4/172 (2.3)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

Table 2. Genotypes, antimicrobial resistance profiles, tetracycline resistance, and zinc chloride resistance in MSSA and MRSA isolates

Strains	MLST	Origin	Sample sites	*spa*	SCC*mec*	*agr*	Resistant phenotype	*tet* genes	TET MICs (μg/mL)	*crzC*	Zinc MICs (μg/mL)
MRSA											
BGFA-222E	ST5	Cattle-skin	F-1[1)]	t002	II	II	AMP, CEF, CIP, CLI, ERY	-	1	+	4
BGFA-262E	ST5	Cattle-skin	F-1	t002	II	II	AMP, CEF, CIP, CLI, ERY	-	1	–	4
BGFA-292E	ST5	Cattle-skin	F-1	t002	II	II	AMP, CEF, CIP, CLI, ERY	-	1	–	4
BKFH-321E	ST72	Farm worker-nasal	F-2	t664	IV	I	AMP, CEF, TET	*tet*(M)	16	+	12
BKSM-133E	ST72	Slaughterhouse-carcass	S-1[2)]	t664	IV	I	AMP, CEF, MUP	*tet*(M)	0.125	+	4
MSSA											
BJFA-222	NT	Cattle-skin	F-3	t008	-	III		-	0.125	+	4
BSFA-4104	ST1	Cattle-fecal	F-4	t127	-	III	AMP, CIP, GEN	-	0.125	+	6
BKFA-211	ST2416	Cattle-nasal	F-5	t008	-	I	AMP	-	0.125	–	4
BKFH-451	NT	Farm worker-nasal	F-6	t017	-	III	AMP, ERY	-	0.125	–	4
BJSM-273	ST1	Slaughterhouse-carcass	S-2	t127	-	III	AMP	-	0.125	+	6
BSSH-171	ST2199	Slaughterhouseworker-nasal	S-3	t571	-	I	AMP, CHL, CIP, CLI, GEN, SYN, TET	*tet*(M)	32	+	6
BCSE-104	ST188	Slaughterhouse-environ.	S-4	t189	-	I		-	0.125	–	4
BJMH-426	ST72	Retail market worker-hands	R-1[3)]	t126	-	I		-	0.125	+	4
BJMH-111	ST7	Retail market worker-nasal	R-1	t304	-	I	AMP	-	0.125	+	4
BSMH-611	ST1	Retail market worker-nasal	R-2	t18104	-	III	ERY	-	0.25	+	4
BSMH-616	ST188	Retail market worker-hands	R-2	t189	-	I	AMP	-	0.25	+	4

[1)] F, cattle farms; [2)] S, slaughterhouses; [3)] R, retail markets.
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MLST, Multilocus sequence typing; MIC, minimum inhibitory concentrations; ST, sequence type; NT, non-typeable; AMP, ampicillin; CEF, cefoxitin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; MUP, mupirocin; GEN, gentamicin; CHL, chloramphenicol; SYN, quinupristin-dalfopristin.

MSSA strains were as follows: Gyeonggi (n=4, 36.4%), Chungcheong (n=1, 9%), Jeolla (n=4, 36.4%), and Gyeongsang (n=2, 18.2%) provinces (Table 1).

Genetic profiles of the MRSA and MSSA isolates

Seven different STs were assigned to the 16 *S. aureus* strains through MLST-analyses, with two non-typeable MSSA strains: BJFA-222 and BKFH-451 (Table 2). The three MRSA strains isolated from cattle were ST5 with SCC*mec* type II (ST5-SCC*mec* II), and two other MRSA strains each isolated from a farm-worker and a carcass sample were ST72-SCC*mec* type IV. The 3 ST5-SCC*mec* II MRSA strains were isolated from a single cattle farm and had identical *spa* type of t002 and *agr* type II. The two ST72-SCCmec IV MRSA strains had *spa* types of t664 and *agr* type of I.

As presented in Table 2, MSSA strains exhibited 6 different ST types: ST1 (n=3, 27.3%), ST188 (n=2, 18.2%), ST7 (n=1, 9.1%), ST72 (n=1, 9.1%), ST2199 (n=1, 9.1%), and ST2416 (n=1, 9.1%), and two non-typeable strains. Similar to the diverse MLST types, the 11 MSSA strains had 8 different *spa* types and two different *agr* types. Combined analyses of MLST, *spa*, and *agr* types in the MSSA strains revealed that the 11 MSSA strains were each genetically distinct, except the two ST1 MSSA strains, BSFA-4104 and BJSM-273. Although one ST72 MSSA strain (BJMH-426) and two ST72 MRSA strains (BKFH-321E and BKSM-133E) had identical MLST and *agr* types, differences in *spa* types were observed between the MSSA and MRSA strains (t664 *vs.* t126).

Antimicrobial resistance of the MRSA and MSSA strains

All the 16 *S. aureus* strains were susceptible to rifampin, sulfamethoxazole-trimethoprim, teicoplanin, and vancomycin. In contrast, the 5 MRSA strains and 7 of the 11 MSSA strains showed resistance to ampicillin (Table 2). Of note, the 5 MRSA strains displayed a multidrug resistance (MDR) phenotype by showing resistance to more than three antimicrobial agents tested. The ST72 MRSA-SCC*mec* IV strain, BKSM-133E, showed resistance to mupirocin, which is not used in animals. Similar to the MRSA strains, two of the MSSA strains, BSFA-4104 and BSSH-171, displayed an MDR phenotype. The BSSH-171 MSSA strain (ST2199) showed resistance to 7 different antimicrobial agents. In contrast to the two ST72 MRSA strains, the ST72 MSSA strain (BJMH-426) was susceptibility to all the antimicrobial agents tested.

PCR analyses of five tetracycline resistance genes (*tetK*, *tetL*, *tetM*, *tetO*, and *tetS*) revealed that the two ST72 MRSA strains and the ST2199 MSSA strain harbored *tetM* gene. Interestingly, although two of the three *tetM*-positive *S. aureus* strains (BKFH-321E and BSSH-171) exhibited a high level of tetracycline MICs (16 and 32 μg/mL, respectively), the BKSM-133E strain (ST72 MRSA strain) showed tetracycline MIC of 0.125 μg/mL (Table 1).

Zinc chloride MICs and detection of *czrC* gene in MRSA and MSSA strains

Resistance to zinc chloride has been proposed to be associated with specific genotypes of LA-MRSA isolates, such as CC398 MRSA with SCC*mec* V (Graveland et al., 2011). Although 3/5 MRSA (60%) and 8/11 MSSA (72.7%) strains were positive for *czrC*, all 16 *S. aureus* strains exhibited resistance to zinc chloride (MICs >2 mM) (Table 2). Of note, only *czrC*-positive strains displayed a high level of resistance (MICs ≥6 mM) to zinc chloride.

Profiles of staphylococcal enterotoxin (SE) genes in MRSA and MSSA strains

Although the five MRSA strains differed in MLST, SCC*mec*, *agr*, and *spa* types (ST5-SCC*mec* II-*agr* II-*spa* t002 vs. ST72-SCC*mec* IV-*agr* I-*spa* t664), all MRSA strains carried an identical set of SE genes (Table 3). The six SE genes (*seg*, *sei*,

Table 3. Detection of staphylococcal enterotoxin genes (SEs) in MRSA and MSSA isolates

Strain	*sea*	*seb*	*sec*	*sed*	*see*	*tst1*	*seg*	*seh*	*sei*	*sej*	*sek*	*sel*	*sem*	*sen*	*seo*	*sep*	*seq*	*ser*	*seu*
MRSA																			
BGFA-222E							+		+				+	+	+				+
BGFA-262E							+		+				+	+	+				+
BGFA-292E							+		+				+	+	+				+
BKFH-321E							+		+				+	+	+				+
BKSM-133E							+		+				+	+	+				+
MSSA																			
BJFA-222															+				
BSFA-4104								+			+					+			
BKFA-211	+										+								
BKFH-451							+						+	+					+
BJSM-273								+			+					+			
BSSH-171	+										+				+				
BCSE-104																			
BJMH-426							+		+				+	+	+				+
BJMH-111																			
BSMH-611								+			+								
BSMH-616	+																		

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

sem, *sen*, *seo*, and *seu*) detected in the five MRSA strains were within enterotoxin gene cluster (*egc*) 2 locus (Table 4). In contrast to the identical SE gene profile seen in MRSA strains, the eleven MSSA strains had ten different SE gene profiles. Interestingly, the ST72 MSSA strain (BJMH-426) had a set of SE genes that was identical to the two ST72 MRSA strains, suggesting a correlation between MLST type and SE gene profile.

Biofilm formation

The formation of biofilm usually results in decreased susceptibility to antimicrobial drugs and host immune defense (i.e. antimicrobial peptide). Hence, the biofilm formation assays have been performed to assess virulence characteristics of the MRSA and MSSA strains in human and animal hosts (Abdelhady et al., 2014; Osman et al., 2016). As shown in Fig. 1A, ability to form biofilm under static condition was not different between MRSA and MSSA strains. However, as shown in Fig. 1B, in a comparison of *S. aureus* strains as groups of bovine-, human-, and non-bovine/non-human-associated strains regardless of methicillin-resistance, *S. aureus* strains from human hosts exhibited a significantly higher level of biofilm formation than the other two groups of *S. aureus* strains ($p<0.01$).

Susceptibilities to PMAP-36 and LL-37

In vitro CAP susceptibility assays of all 16 strains revealed that MRSA strains tended to have significantly higher levels of resistance to PMAP-36 ($p<0.05$) and LL-37 ($p<0.01$) than MSSA strains. When the *S. aureus* isolates were compared as

Table 4. Profiles of staphylococcal enterotoxin genes (SEs) and *egc* locus in MRSA and MSSA isolates

Strain	Classical SEs[1)]	*egc1*[2)]	*egc2*[3)]	*egc3*[4)]	*tst1*	Other *se* and *sel* genes
MRSA						
BGFA-222E	-	-	+	-	-	-
BGFA-262E	-	-	+	-	-	-
BGFA-292E	-	-	+	-	-	-
BKFH-321E	-	-	+	-	-	-
BKSM-133E	-	-	+	-	-	-
MSSA						
BJFA-222	-	-	-	-	-	*seo*
BSFA-4104	-	-	-	-	-	*seh*, *sek*, *sep*
BKFA-211	*sea*	-	-	-	-	*sek*
BKFH-451	-	-	-	-	-	*seg*, *sem*, *sen*, *seu*
BJSM-273	-	-	-	-	-	*seh*, *sek*, *sep*
BSSH-171	*sea*	-	-	-	-	*sek*, *seo*
BCSE-104	-	-	-	-	-	-
BJMH-426	-	-	+	-	-	-
BJMH-111	-	-	-	-	-	-
BSMH-611	-	-	-	-	-	*seh*, *sek*
BSMH-616	*sea*	-	-	-	-	-

[1)] Screened for *sea*, *seb*, *sec*, *sed*, and *see* gene.
[2)] Screened for the *egc* locus genes; *seg*, *sei*, *sem*, *sen*, and *seo* (Collery et al., 2009).
[3)] Screened for the *egc* locus genes; *seg*, *sei*, *sem*, *sen*, *seo*, and *seu* (Collery et al., 2009).
[4)] Screened for the *egc* locus genes; seg_v sei_v, sem_v, sen_v, seo_v and seu_v (Collery et al., 2009).
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

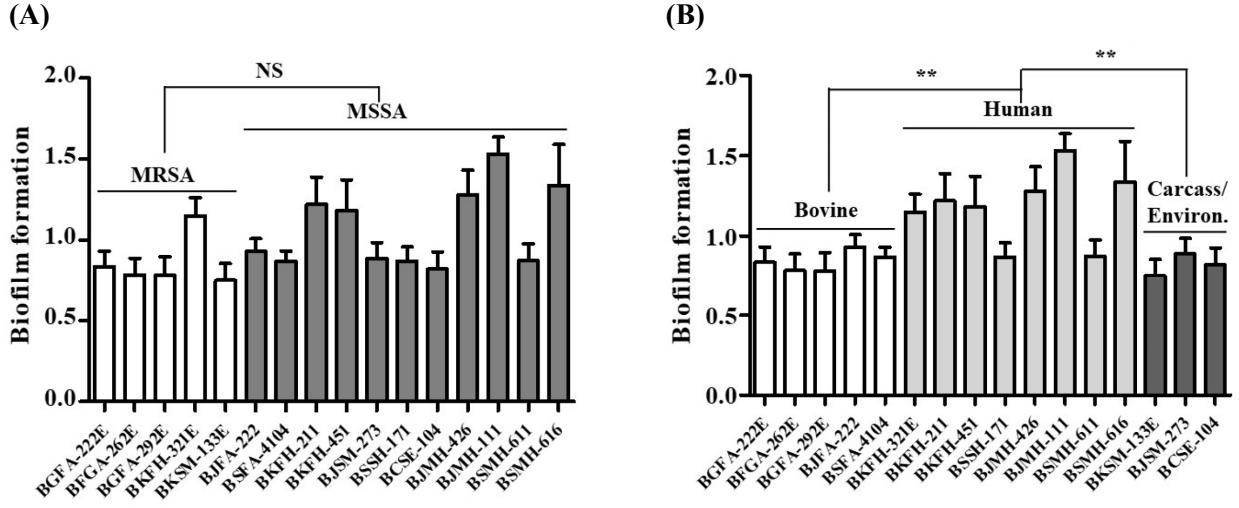

Fig. 1. Biofilm formation of MRSA and MSSA strains isolated from the beef production chain. Biofilm formation under static culture condition was compared between *Staphylococcus aureus* groups of MRSA and MSSA (A) or bovine-, human-, and non-bovine/non-human-associated strains (B). The data were normalized to the *S. aureus* Newman strain. ** $p<0.01$. NS, not significant; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

groups of bovine-, human-, and non-bovine/non-human-associated strains, no significant differences in susceptibilities were observed among the three strain groups (data not shown).

Discussion

Food-producing animals are well-known reservoirs for MRSA and MSSA, and an increasing number of cases have been reported in which humans are infected with *S. aureus* via foods of animal origin (Lim et al., 2010; Osman et al., 2016; Pauly et al., 2019). Recently, swine-associated MRSA has been reported worldwide; particularly, the ST398 MRSA in European countries (Graveland et al., 2011; Price et al., 2012; Vanderhaeghen et al., 2010). Although the presence and antimicrobial resistance of MRSA and MSSA have been investigated extensively in dairy cattle farms and beef meat samples (Lim et al., 2010; Osman et al., 2016; Song et al., 2016; Vanderhaeghen et al., 2010), few studies have been conducted on the occurrence and molecular composition of the MRSA and MSSA in beef cattle farms.

In this study, we investigated the occurrence and characteristics of MRSA and MSSA in the beef production chain, covering beef cattle farms, slaughterhouses, and meat samples in retail markets. Because MRSA and MSSA can be transmitted between livestock animals or beef meat and farmers or workers through frequent contact, samples were collected systematically from beef cattle, beef meat, humans, and facility environment. Overall, the prevalence of MRSA was 0.5% and 0.3% in cattle farms and slaughterhouses, respectively (Table 1). Several previous studies in Korea reported detection rates of 1.3%–13.9% for MRSA in bovine mastitic milk samples (Moon et al., 2007; Nam et al., 2011; Song et al., 2016). However, no previous studies have been conducted on the national prevalence of MRSA in beef cattle farms in Korea. The prevalence of bovine-originated MRSA in this investigation was lower than the prevalence rates reported in pigs from previous studies (2%–8.6%) in Korea (Lim et al., 2010; Moon et al., 2015). Weese et al. (2012) also reported the inability to isolate MRSA in a large group of beef cattle (491 nasal swabs and 488 fecal samples) in Canada. However, the prevalence of MRSA in beef cattle farms in Belgium was 10.16% (Nemeghaire et al., 2014), suggesting geographical variation. The overall prevalence of MSSA in cattle farm in this study was 0.5%, indicating that the occurrence of *S. aureus* in beef cattle farm is very low, regardless of methicillin resistance. Interestingly, as shown in Table 1, the isolation rates of MSSA in samples from slaughterhouses and retail markets (0.9% and 2.3%, respectively) were slightly higher than those of MRSA (0.3% and 0%, respectively). In a previous study, Kim et al. (2015) also reported isolation rates of MSSA (~6%) and MRSA (0.2%) in domestic beef meat samples. However, none of the MRSA and MSSA strains was isolated from beef meat samples in this study (Table 2).

The spread of MRSA in livestock and foods of animal origin has been linked to a high prevalence of CC398 LA-MRSA strains in the pig population and pork production system worldwide (Graveland et al., 2011; Moon et al., 2015; Price et al., 2012). Similarly, the most frequent swine-associated MRSA strain in Korea has been CC398 with t571 or t034 *spa* types (Moon et al., 2015). In contrast to the pork production chain, no CC398 genotype was observed among the MRSA and MSSA strains isolated from the beef production chain in this study (Table 2). Interestingly, the three MRSA strains from beef cattle were ST5 and two MRSA strains, one each from a farm-worker and a slaughterhouse carcass sample, were ST72, which are the most significant HA-MRSA and CA-MRSA strains in Korea (Kang et al., 2019). The three ST5 MRSA strains were isolated from different bovine hosts on a single farm and had identical genotypes (*spa* type of t002, SCC*mec* II, and *agr* type II) and antimicrobial resistance phenotypes, indicating colonization of beef cattle with a single clone of an ST5 MRSA strain. The two ST72 MRSA strains (BKFH-321E and BKSM-133E) each displayed resistance to three antimicrobial agents, while

the ST5 MRSA strains exhibited an MDR phenotype to five antimicrobial agents (Table 2). A higher prevalence of MDR phenotype in ST5 MRSA vs. ST72 MRSA has previously been reported in Korea (Kang et al., 2019; Park et al., 2015). These data suggest that the two ST72 MRSA strains originated from human hosts rather than beef cattle. Unlike the MRSA strains, which displayed two major genotypes (ST5-SCC*mec* II-*spa* t002-*agr* II and ST72-SCC*mec* IV-*spa* t664-*agr* I), MSSA strains displayed diverse MLST, *spa*, or *agr* types (Table 2). Of note, the three MSSA strains from bovine hosts differed in their ST, *spa*, or *agr* types from the MSSA strains from human hosts, suggesting that transmission of MSSA from beef cattle to retail meat is still rare in Korea.

The high prevalence of CC398 LA-MRSA strains in swine farms seems to be associated with resistance to antimicrobial agents, particularly tetracycline compounds (Larsen et al., 2016; Moon et al., 2015; Price et al., 2012). In addition, zinc resistance conferred by the *czrC* has been proposed to be involved in the persistence of CC398 LA-MRSA strains in pigs (Aarestrup and Hasman, 2004; Cavaco et al., 2010; Price et al., 2012). As shown in Table 2, 3/5 MRSA strains (60%) and 8/11 MSSA strains (72.7%) harbored *czrC*, and a high level of zinc resistance was observed only in *czrC*-positive strains. However, due to the limited number of strains used in this study, a correlation between zinc resistance and other genotypic or phenotypic factors (e.g. MLST, SCC*mec* type, *agr* type, or origin of the strains) could not be determined. Although two ST72 MRSA strains and one ST2199 MSSA strain were positive for *tetM*, the BKSM-133E (ST72 MRSA) strain showed susceptibility to tetracycline (MIC 0.125 μg/mL) (Table 2).

Virulence factors may have played a crucial role in the prevalence and evolution of epidemic strains of MRSA and MSSA (Park et al., 2015). A variety of virulence-related factors have been identified in *S. aureus* strains, such as SE genes and toxic shock syndrome toxin-1 (TSST-1) (Hennekinne et al., 2012; Park et al., 2011; Park et al., 2015). These toxins are known to be pyrogenic and are often associated with human food poisoning and toxic shock syndrome (Park et al., 2011; Weese et al., 2012). As presented in Tables 3 and 4, all five MRSA strains possessed an identical set of *seg*, *sei*, *sem*, *sen*, *seo*, and *seu* genes that belongs to the *egc*2 locus (Collery et al., 2009). Similar to the ST5 and ST72 MRSA strains, the ST72 MSSA strain, BJMH-426, also carried the SE genes of *egc*2, suggesting a genetic correlation between ST72/ST5 and SE gene profiles.

The ability of *S. aureus* to adhere to extracellular adhesion molecules on host cells is an important virulence determinant for colonization in human and animal hosts (Osman et al., 2016). As shown in Fig. 1A, no significant difference was observed in biofilm formation between MRSA and MSSA strain groups ($p=0.102$). However, *S. aureus* strains isolated from human hosts tended to have a significantly enhanced level of biofilm formation versus strains from bovine hosts (Fig. 1B). These data, in combination with the genetic profiles of MRSA and MSSA strains, suggest that *S. aureus* strains need to orchestrate virulence factors such as biofilm formation to colonize new hosts and for successful transmission between human and animal hosts.

In addition to biofilm formation, *S. aureus* must overcome the bactericidal action of host antimicrobial peptides (Kang et al., 2019). Cathelicidins, small amphipathic peptides with a net positive charge, are abundant in host cells such as neutrophils and epithelial cells of the skin and upper respiratory tract (Kang et al., 2019). The persistence and successful colonization of the skin and upper respiratory tract would require *S. aureus* to resist the bactericidal action of cathelicidins. As presented in Fig. 2A and B, MRSA strains exhibited significantly higher levels of resistance to PMAP-36 ($p<0.05$) and LL-37 ($p<0.01$) than the MSSA strains. Although a difference in resistance to host antimicrobial peptides was observed in a small number of MRSA and MSSA strains, we speculate that the MRSA strains with MDR phenotype in combination with cathelicidin resistance can have an enhanced ability to survive on human and animal hosts under antimicrobial exposure.

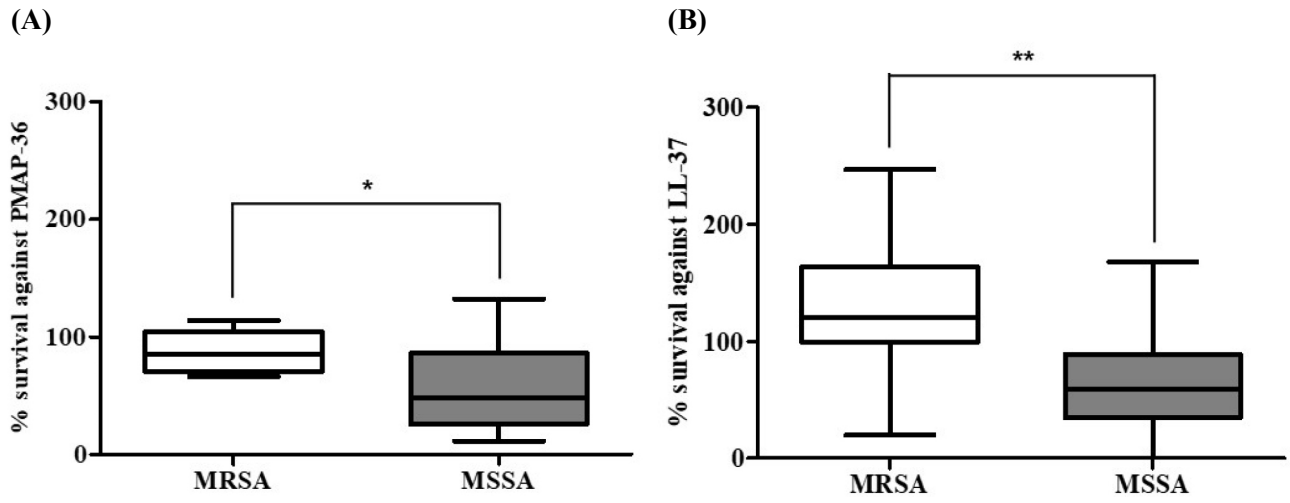

Fig. 2. Box and Whisker plots of *in vitro* susceptibilities to PMAP-36 (A) and LL-37 (B). The median % survival under PMAP-36 treatment (0.1 μg/mL) for MRSA and MSSA strains were 85% and 48%, respectively. The median % survival under LL-37 treatment (3 μg/mL) for MRSA and MSSA strains were 120% and 59%, respectively. * $p<0.05$, ** $p<0.01$. MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

We recognize that the present study has several limitations. Most data were resulted from a limited number of MRSA and MSSA isolates due to a very low prevalence of *S. aureus* in the samples collected in 2018. Information on the usage of antimicrobial agents and the concentration of zinc chloride in the animal feed in cattle farms were not available in this study. However, the current study is the first to systematically sample beef cattle, humans, and carcass/beef meat samples in different sectors of the beef production chain in Korea.

In conclusion, our data provide important information on the occurrence of MRSA and MSSA in the beef production chain in Korea. The prevalence of bovine-associated MRSA and MSSA was much lower than that of swine-associated MRSA and MSSA in Korea and other Asian and European countries. Unlike CC398 LA-MRSA in pigs, resistance to tetracycline and zinc chloride did not correlate with enhanced levels of MDR phenotype nor the prevalence of specific MLST types in bovine-associated MRSA and MSSA strains. In addition, increased levels of resistance to cathelicidins (PMAP-36 and LL-37) were observed in MRSA strains compared to MSSA strains. Although prevalence of MRSA is low in beef cattle in Korea, increased resistance to antimicrobial drugs and antimicrobial peptides may play an important role in the transmission of MRSA to humans via direct and/or indirect contact. Since livestock animals can serve as a reservoir for MRSA and MSSA, extensive surveillance for antimicrobial resistant pathogens and antimicrobial usage in the beef production chain is necessary to prevent potential transmission of the pathogens to humans.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Yang SJ. Data curation: Yang SJ, Lee HH. Formal analysis: Yang SJ, Lee HH, Lee GY, Eom HS. Methodology: Yang SJ, Lee HH. Writing - original draft: Yang SJ, Lee HH. Writing - review & editing: Lee HH, Lee GY, Eom HS, Yang SJ.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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