Short communication: First detection of Pantone-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* ST30 in raw milk taken from dairy cows with mastitis in South Korea

Abraham Fikru Mechesso, Su-Jeong Kim, Ho-Sung Park, Ji-Hyun Choi, Hyun-Ju Song, Mi Hyun Kim, Suk-kyung Lim, Soon-Seek Yoon, and Dong-Chan Moon*
Bacterial Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, Republic of Korea

ABSTRACT

We identified 199 *Staphylococcus aureus* isolates from quarter milk samples of 1,289 dairy cattle between 2014 and 2018. About 66% of the isolates were resistant to at least 1 antimicrobial agent; the highest rate of resistance was to penicillin, followed by resistance to ampicillin, erythromycin, and sulfadimethoxine. We obtained 30 methicillin-resistant *S. aureus* (MRSA) strains from 6 farms in 3 provinces. The MRSA strains exhibited a significantly higher resistance rate to most of the tested antimicrobials than the oxacillin-susceptible strains. The MRSA strains represented 5 genotypes: ST72-t324-SCCmec IV (n = 14), ST30-t1752-SCCmec IV (n = 8), ST188-t189-SCCmec NT (n = 6), ST188-t2284-SCCmec NT (n = 1), and NT-NT-SCCmec IV (n = 1). One of the ST188 MRSA strains represented a novel *staphylococcal protein A* (*spa*) type (t2284). In addition, 7 of the 8 ST30 MRSA strains were Pantone-Valentine leukocidin (PVL)-positive and carried various staphylococcal enterotoxin encoding genes. This is the first report of PVL-positive ST30 MRSA-t1752-SCCmec IV from bovine mastitis in Korea. All of ST72-t324-SCCmec IV MRSA strains carried staphylococcal enterotoxin and leukotoxin encoding genes. They were also sensitive to most of the tested non- β -lactam antimicrobials. In contrast, ST188-t189 MRSA strains were resistant to multiple antimicrobials and predominantly carried the leukotoxin encoding gene. Taken together, these findings may indicate that dairy cows could be a major source for spreading MRSA strains, and contaminated milk could be a vehicle for transmission. Suitable hygienic measures should be established in dairy farms and processing plants to limit the likelihood of introducing MRSA into the food chain.

Key words: antimicrobial, bovine, milk, Pantone-Valentine leukocidin toxin, *Staphylococcus aureus*

Short Communication

Staphylococcus aureus continues to be one of the leading pathogens causing mastitis in dairy cows in Korea (Nam et al., 2011) and other countries (Kateete et al., 2013; Wang et al., 2018). It causes considerable financial losses for the global dairy industry because of reduced milk production and quality, as well as increased treatment costs for infected animals. Raw milk contaminated with staphylococcal toxins may also cause further problems along the food chain, giving rise to mild to severe life-threatening infections in humans (Wang et al., 2018).

Staphylococcus aureus is characterized by its rapid ability to develop resistance to antimicrobials. Virulent strains of methicillin-resistant *S. aureus* (MRSA) are increasingly being recovered from food and companion animals, livestock farms, hospitals, nursing homes, and the community (Nam et al., 2011; Pantosti, 2012). Pantone-Valentine leukocidin (PVL) is an *S. aureus*-specific leukocytolytic toxin that interferes with immune defenses by disrupting phagocytic cell membranes and contributing to the severity of staphylococcal infections. The presence of members of this toxin family in dairy ruminants has been associated with bovine mastitis (Lo and Wang, 2011). In humans, however, it is related to severe cutaneous infections and occasionally necrotizing pneumonia that could lead to death (Nimmo, 2012).

Pantone-Valentine leukocidin has been considered the main virulence determinant or a simple marker of community-associated MRSA. Community spread of PVL-positive *S. aureus* strains (ST8, ST30, and ST80) has been frequently shown in samples recovered from patients with skin, soft tissue, and bone infections in Korea (Lee et al., 2018; Park et al., 2019). Recently, Song et al. (2019) reported invasive sporadic cases of *S. aureus* infections that were predominantly associated with PVL-harboring ST8 and ST30 MRSA. Many authors reported the prevalence and molecular characteristics of MRSA isolated from mastitic milk in Korea before 2013 (Nam et al., 2011; Song et al., 2016). In the

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*Corresponding author: ansehdcks@korea.kr

current study, we aimed to provide further information on the resistance profiles of *S. aureus* and molecular characteristics of MRSA isolated from bovine mastitis cases from 2014 to 2018.

We collected a total of 3,422 milk samples from 1,289 dairy cattle on 69 farms from 2014 to 2018 (Supplemental Table S1; <https://doi.org/10.3168/jds.2020-19004>). Approximately 50 mL of milk was collected from individual quarters of lactating cows as part of routine testing to determine the cause of mastitis. Samples were then aseptically divided into 2 sterile tubes (each containing 25 mL of milk) for SCC and bacteriological examination. The SCC of milk samples were determined using a Fossmatic System 4000 (Foss Electric, Hillerød, Denmark). Samples with SCC $\geq 200,000$ cells/mL were subjected to bacteriological examination. Primary culture of the milk samples, identification of *S. aureus* using MALDI-TOF MS (bioMérieux, Marcy L'Etoile, France), and PCR confirmation of presumptive *S. aureus* and MRSA colonies were performed as described by Mason et al. (2001).

Antimicrobial susceptibility was determined with the broth microdilution method according to CLSI (2018) guidelines, using antibiotic-containing plates (CMV1A-MAF; TREK Diagnostics Systems, Cleveland, OH). Ten antimicrobials were tested: ampicillin (0.12–8 $\mu\text{g/mL}$), ceftiofur (0.5–4 $\mu\text{g/mL}$), cephalothin (2–16 $\mu\text{g/mL}$), erythromycin (0.25–4 $\mu\text{g/mL}$), oxacillin + 2% NaCl (2–4 $\mu\text{g/mL}$), penicillin (0.12–8 $\mu\text{g/mL}$), penicillin/novobiocin (1/2–8/16 $\mu\text{g/mL}$), pirlimycin (0.5–4 $\mu\text{g/mL}$), sulfadimethoxine (32–256 $\mu\text{g/mL}$), and tetracycline (1–8 $\mu\text{g/mL}$). The concentration range of oxacillin in the plates did not include the breakpoint (0.5 $\mu\text{g/mL}$), so the agar dilution assay was performed at the oxacillin (Sigma-Aldrich, St. Louis, MO) test range of 0.0064–16 $\mu\text{g/mL}$. Antimicrobial resistance breakpoints were determined based on CLSI (2018) guidelines and Saini et al. (2012). *Staphylococcus aureus* ATCC 25923 was used as a quality-control strain. Isolates demonstrating resistance to at least 3 antimicrobial subclasses were considered multidrug-resistant.

Multilocus sequence typing was performed according to Enright et al. (2000). Specific primers (Genotech, Daejeon, Korea) were used to amplify and sequence the following 7 housekeeping genes: *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*. Chromosomal DNA was digested with *SmaI* (TaKaRa, Shiga, Japan) and pulsed-field gel electrophoresis (PFGE; Bio-Rad, Hercules, CA) was conducted to determine the genetic relatedness of the isolates, according to McDougal et al. (2003). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was conducted using multiplex PCR as described by Boyle-Vavra and Daum (2007). *S. aureus* protein A (*spa*) typing was performed using the method de-

scribed by Enright et al. (2000), and the *spa* types were assigned using the Ridom Staph Type server (Ridom GmbH, Würzburg, Germany; www.spaserver.ridom.de). As well, a PCR assay was used to determine the presence of staphylococcal toxin genes encoding PVL, leukotoxins (*LukED*), exfoliatins (*eta* and *etb*), toxic shock syndrome toxin 1 (*tsst1*), and staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *seli*, *selj*, *sek*, *sell*, *sem*, *sen*, *seo*, *sep*, *seq*, and *ser*; Yamada et al., 2005).

Among 3,422 milk samples collected in this study, 1,395 (40.8%) samples had high SCC ($\geq 200,000$ cells/mL; Supplemental Table S1; <https://doi.org/10.3168/jds.2020-19004>). Of the samples with high SCC, we recovered 199 *S. aureus* isolates (14.3%), a lower rate than found in a previous report from Korea (25%; Song et al., 2016). The isolates exhibited resistance rates to most of the tested antimicrobials that were comparable with the findings of Moon et al. (2007) and Nam et al. (2011). However, the sulfadimethoxine (19.6%), oxacillin (15.1%), and pirlimycin (12.6%) resistance rates were high compared with the above studies (Table 1). Although the erythromycin resistance rate decreased over time ($P < 0.05$), the overall resistance rate (17.6%) was higher than the studies above. The penicillin resistance rate (54.8%) was consistent with previous reports from Japan (Hata et al., 2010) and Turkey (Güler et al., 2005). However, it was higher than other reports from Japan (27%; Yoshimura et al., 2002), Kenya (24%; Shitandi and Sternesjö, 2004), the United States (13%; Anderson et al., 2006), and Sweden (7%; Bengtsson et al., 2009). The ampicillin resistance rate remained high throughout the study period, a finding consistent with those of Erskine et al. (2002) in the United States and Güler et al. (2005) in Turkey. We also identified a relatively small number of isolates that were resistant to ceftiofur, cephalothin, penicillin/novobiocin, and tetracycline. Although the rate of resistance to multiple antimicrobials decreased over time ($P < 0.05$), the overall proportion of multidrug-resistant isolates in this study (16.1%) was higher than our previous report (Nam et al., 2011). Resistance to ampicillin, erythromycin, penicillin, and pirlimycin was the major multidrug resistance pattern (Supplemental Table S2; <https://doi.org/10.3168/jds.2020-19004>). The high resistance rate of *S. aureus* isolates to these antibiotics was not difficult to explain, because these antimicrobials are commonly used in the Korean dairy industry to treat bovine mastitis (APQA, 2018).

We identified a total of 30 (15.1%) MRSA strains from 6 farms in 3 provinces, 13 of which (43.3%) were recovered in 2015 (Tables 1 and 2). The percentage of MRSA strains found in this study was in close agreement with Song et al. (2016) in Korea and Türkyilmaz

Table 1. Antimicrobial resistance profiles of *Staphylococcus aureus* isolates from mastitic milk in Korea between 2014 and 2018^{1,2}

Subclass and antimicrobial	Breakpoint (µg/mL)	2014 (n = 44)				2015 (n = 79)				2016 (n = 57)				2017 (n = 19)				Total (n = 199)				P-value
		MIC ₅₀ (mg/mL)	MIC ₉₀ (mg/mL)	R, n (%)		MIC ₅₀ (mg/mL)	MIC ₉₀ (mg/mL)	R, n (%)		MIC ₅₀ (mg/mL)	MIC ₉₀ (mg/mL)	R, n (%)		MIC ₅₀ (mg/mL)	MIC ₉₀ (mg/mL)	R, n (%)		MIC ₅₀ (mg/mL)	MIC ₉₀ (mg/mL)	R, n (%)		
Penicillins																						
Ampicillin	≥0.5	1	4	63.6 (28)		0.25	8	48.1 (38)		0.12	8	42.1 (24)		1	8	68.4 (13)		0.5	8	51.8 (103)		0.9130
Oxacillin + 2% NaCl	≥8	2	4	13.6 (6)		2	4	16.5 (13)		2	4	12.3 (7)		2	4	21.1 (4)		2	4	15.1 (30)		0.3943
Penicillin	≥32	1	8	70.5 (31)		0.25	8	50.6 (40)		0.12	8	43.9 (25)		2	8	68.4 (13)		0.25	8	54.8 (109)		0.8722
Cephalexin I																						
Cephalexin	≥4	2	2	0 (0)		2	4	1.3 (1)		2	16	7.0 (4)		2	4	0 (0)		2	4	2.5 (5)		0.7797
Cephalexin III																						
Ceftiofur	≥8	1	8	11.4 (5)		1	8	16.5 (13)		1	1	0 (0)		1	8	21.1 (4)		1	8	11.1 (22)		0.8208
Macrolides																						
Erythromycin	≥0.25	0.25	8	29.5 (13)		0.5	8	20.3 (16)		0.25	8	10.5 (6)		0.25	0.5	0 (0)		0.25	8	17.6 (35)		0.0004*
Penicillins/other class																						
combinations																						
Penicillin/novobiocin	≥4	1	1	0 (0)		1	1	0 (0)		1	8	14.0 (8)		1	1	0 (0)		1	1	4.0 (8)		0.7418
Lincosamides																						
Pirlimycin	≥4	0.5	4	13.6 (6)		0.5	8	15.2 (12)		0.5	4	12.3 (7)		0.5	0.5	0 (0)		0.5	4	12.6 (25)		0.1885
Folate pathway inhibitors																						
Sulfadimethoxine	≥512	64	256	0 (0)		256	512	15.2 (12)		256	512	45.6 (26)		64	256	5.3 (1)		256	512	19.6 (39)		0.7067
Tetracyclines																						
Tetracycline	≥16	1	1	0 (0)		1	1	1.3 (1)		1	4	8.8 (5)		1	1	0 (0)		1	1	3.0 (6)		0.7710
MDR				22.7				17.7				14				0				16.1		0.0486*

¹MDR = multidrug-resistant (resistant to at least 3 antimicrobial subclasses); MIC₅₀ = concentration at which 50% of the isolates were inhibited; MIC₉₀ = concentration at which 90% of the isolates were inhibited; R = resistance rate.

²*Staphylococcus aureus* was not identified from samples collected in 2018.

*P < 0.05; significant change in antibiotic resistance trend.

et al. (2010) in Turkey. However, it was higher than previous reports in Korea (Moon et al., 2007; Nam et al., 2011) and other countries (Bengtsson et al., 2009; Vanderhaeghen et al., 2010). Difference in sampling and detection methodology should be considered when comparing and contrasting our results with others. In the above studies, *S. aureus* isolates were identified either from milk samples with high SCC (Moon et al., 2007; Bengtsson et al., 2009; Nam et al., 2011; Song et al., 2016) or from animals with clinical mastitis (Türkyılmaz et al., 2010; Vanderhaeghen et al., 2010). As well, we observed differences in the types of growth media and PCR conditions used to identify *S. aureus* and MRSA strains. Further, MRSA strains were identified either after an initial screening on a medium containing various β -lactam antibiotics (Moon et al., 2007; Bengtsson et al., 2009; Türkyılmaz et al., 2010; Nam et al., 2011) or through direct detection of the *mecA* gene (Vanderhaeghen et al., 2010; Song et al., 2016).

Regular surveillance of MRSA susceptibility patterns is essential to provide clinicians with the most up-to-date information about changes in resistance patterns. In agreement with Song et al. (2016), we found that 16 MRSA strains (53.3%) were resistant to multiple antimicrobials (Figure 1). Except for pirlimycin and sulfadimethoxine, we observed a significantly high ($P < 0.001$, Student's *t*-test) antimicrobial resistance rate in MRSA strains compared with the oxacillin-susceptible strains. The resistance of MRSA to multiple antimicrobials in this study was not surprising, because MRSA strains carry *SCCmec* elements and additional resistance determinants (Zhang et al., 2005).

Continuous monitoring and characterization of MRSA strains in raw milk are vital to determine the evolution and dissemination of specific clones and establish an effective control program. We identified 3 major MRSA genotypes from 5 different farms. Of the 30 MRSA strains originating from 3 farms, 14 belonged to ST72-t324-*SCCmec* IV without the PVL-encoding gene, which has been reported to be the most common clonal type circulating in the community in Korea (Park et al., 2016; Bae et al., 2019). It has also been identified from the raw milk of dairy cows with mastitis, from cattle and pig carcasses, and from slaughterhouse workers (Nam et al., 2011; Moon et al., 2015).

All PVL-negative MRSA strains from farm H belonged to ST188-t189-NT. The ST188-t189 MRSA strain has been frequently identified from pigs, farmers, and hospital patients in Korea (Ko et al., 2008; Bae et al., 2019; Moon et al., 2019), but this is the first report of this strain from bovine milk in Korea. Similar to the findings of Wang et al. (2014), we found that ST188-t189 MRSA was resistant to multiple antimicrobials.

As well, the only ST188 MRSA strain from farm B in Gyeongbuk province presented a novel spa type (t2284).

In this study, except for a single untypable isolate, all of the isolates from farm F in Gyeonggi were identified as ST30-t1752-*SCCmec* IV. The ST30 MRSA-*SCCmec* IV strain has been spreading between the community and hospitals in various Asian countries including Korea (Ko et al., 2008; Song et al., 2011; Park et al., 2019). Consistent with the findings of Williamson et al. (2013) in New Zealand and Wang et al. (2017) in Taiwan, ST30 MRSA-*SCCmec* IV MRSA strains were spa type t1752. We also detected PVL-encoding genes in 7 of the 8 ST30 MRSA strains. The proportion of PVL-positive *S. aureus* isolates in the current study was higher than in reports from Italy (61%; Zecconi et al., 2006), China (42%; Wang et al., 2015), and India (42%; Mitra et al., 2013). Although PVL-positive ST30 has been commonly reported in patients in Korea (Lee et al., 2018; Park et al., 2019), this study is the first to report PVL-positive ST30 MRSA strains from non-human sources in Korea. The increased rate of PVL-positive MRSA in raw milk could be an additional risk factor for public health, because it is likely to be related to recurrent or chronic skin and soft tissue infections, as well as rapidly fatal pneumonia in humans (Cavalcante et al., 2015).

Analysis using PFGE revealed 6 distinct PFGE types, with identical PFGE types in each farm (Supplemental Figure S1; <https://doi.org/10.3168/jds.2020-19004>). The results suggest that transmission of MRSA could occur between cattle or between the milker's hand and cattle during milking. The ST72-t324-*SCCmec* IV MRSA strains from farms A and C in Gyeonggi presented an identical PFGE pattern (P2). These results might suggest the presence of an epidemic clone in dairy farms, or clonal spread between farms A and C.

It has been reported that MRSA strains differed in their carriage of enterotoxin and leucotoxin encoding genes. Consistent with previous reports (Song et al., 2016; Kim et al., 2020), the majority (92.9%) of ST72 MRSA strains carried identical staphylococcal enterotoxin and leukotoxin encoding genes: *seg*, *sei*, *sem*, *sen*, *seo*, and *LukED*; only *LukED* was predominant in the ST188 MRSA strains. We also found that the majority of ST30-IV MRSA isolates carried *sei* (100%) and *LukED* (87.5%) genes. However, *seg* was detected in a single isolate. This was slightly different from the findings of previous researchers (Aschbacher et al., 2012; Goudarzi et al., 2018), who reported that all PVL-positive ST30-IV carried *seg* and *sei* genes. Strains of MRSA harboring the classical staphylococcal enterotoxins and leukotoxins can spread to farm personnel as well into the food chain. Recent studies (Macori et al., 2017; Adame-Gomez et al., 2018; Papadopoulos et

Table 2. Characteristics of methicillin-resistant *Staphylococcus aureus* isolates from mastitic milk in Korea between 2014 and 2018^{1,2}

Strain ID	Cattle ID	Farm ID	Province	Year of isolation	MIC (µg/mL)			Non-β-lactam antimicrobial resistance	MLST	spa type	SCCmec type	Pulsotype	Virulence genes
					Penicillin	Cefoxitin	Clotrimazole						
14-A-89	7	A	Gyeonggi	2014	2	>4	—	—	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
14-A-90	12	A	Gyeonggi	2014	8	>4	—	Erythromycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
14-A-92	12	A	Gyeonggi	2014	8	>4	—	Erythromycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
14-A-93	12	A	Gyeonggi	2014	4	>4	—	Erythromycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
14-A-94	212	A	Gyeonggi	2014	4	>4	—	Erythromycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
14-A-97	180	A	Gyeonggi	2014	2	>4	—	Erythromycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
15-C-107	1	C	Gyeonggi	2015	>8	>4	—	Erythromycin, sulfadimethoxine	ST72	t324	IV	P2	<i>LukED</i>
15-C-111	19	C	Gyeonggi	2015	>8	>4	—	Sulfadimethoxine	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
15-C-112	19	C	Gyeonggi	2015	>8	>4	—	—	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
15-C-113	20	C	Gyeonggi	2015	>8	>4	—	Erythromycin, pirlimycin	ST72	t324	IV	P2	<i>LukED seg sei sem sen seo</i>
15-F-125	3	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei seo</i>
15-F-127	9	F	Gyeonggi	2015	8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei sem sen seo</i>
15-F-128	22	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei sem sen seo</i>
15-F-129	23	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>pml seg sei sem sen seo</i>
15-F-130	24	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml seg sei sem sen seo</i>
15-F-131	26	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei seo</i>
15-F-132	28	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei sem sen seo</i>
15-F-134	40	F	Gyeonggi	2015	>8	>4	—	—	NT	NT	IV	P6	<i>LukED sei sem seo</i>
15-F-135	42	F	Gyeonggi	2015	>8	>4	—	—	ST30	t1752	IV	P5	<i>LukED pml sei seo</i>
16-B-195	40	B	Gyeongbuk	2016	4	>4	—	Sulfadimethoxine, tetracycline	ST188	t2284	NT	P3	<i>LukED sen</i>
16-H-214	180	H	Gyeonggi	2016	>8	>4	—	Erythromycin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline	ST188	t189	NT	P4	<i>LukED seo</i>
16-H-215	16	H	Gyeonggi	2016	>8	4	—	Penicillin/novobiocin, pirlimycin	ST188	t189	NT	P4	<i>LukED sem seo</i>
16-H-216	19	H	Gyeonggi	2016	>8	>4	—	Erythromycin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline	ST188	t189	NT	P4	<i>LukED seo</i>
16-H-217	20	H	Gyeonggi	2016	>8	>4	—	pirlimycin, sulfadimethoxine, tetracycline	ST188	t189	NT	P4	<i>LukED</i>
16-H-219	36	H	Gyeonggi	2016	0.5	4	—	Erythromycin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline	ST188	t189	NT	P4	<i>LukED</i>
16-H-220	38	H	Gyeonggi	2016	>8	>4	—	pirlimycin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline	ST188	t189	NT	P4	<i>LukED sen</i>
17-AH-255	11	AH	Chungbuk	2017	>8	>4	—	—	ST72	t324	IV	P1	<i>LukED seg sei sem sen seo</i>
17-AH-256	12	AH	Chungbuk	2017	>8	>4	—	—	ST72	t324	IV	P1	<i>LukED seg sei sem sen seo</i>
17-AH-257	13	AH	Chungbuk	2017	>8	>4	—	—	ST72	t324	IV	P1	<i>LukED seg sei sem sen seo</i>
17-AH-258	14	AH	Chungbuk	2017	>8	>4	—	—	ST72	t324	IV	P1	<i>LukED seg sei sem sen seo</i>

¹MLST = multi-locus sequence types; NT = untypable; SCCmec = staphylococcal cassette chromosome mec; spa = *Staphylococcus aureus* protein; ST = sequence type.

²*Staphylococcus aureus* was not identified from samples collected in 2018.

Figure 1. Antimicrobial resistance profiles of methicillin-resistant and oxacillin-susceptible *Staphylococcus aureus* isolated from raw milk taken from dairy cows with mastitis in Korea between 2014 and 2018. * $P < 0.001$; significant compared with the resistance rate in MSSA. MDR = multidrug-resistant; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA, oxacillin-susceptible *Staphylococcus aureus*.

al., 2019) revealed that MRSA isolated from the dairy chain, dairy products, and dairy farm personnel were enterotoxigenic and capable of causing food-related illnesses (Jones et al., 2002; Kerouanton et al., 2007).

Our results indicate a rise in the prevalence of MRSA in bovine milk in Korea. We identified new genotypes related to community-acquired MRSA strains (PVL-positive ST30-t 1752-*SCCmec* type IV MRSA and PVL-negative ST188-t189 MRSA) from raw milk taken from dairy cows with mastitis in Korea. Dairy farms could be major sources for spreading toxigenic and virulent MRSA strains, and contaminated milk could be a vehicle for transmission through the food chain. Inadequate hygiene (farm, dairy cattle, and farm personnel), as well as insufficient cleaning and disinfection of milking equipment, are among the most common contributing factors for the persistence and spread of infectious agents that cause mastitis. Therefore, this study supports the use of suitable hygienic measures to limit the likelihood of introducing MRSA into the food chain. In addition to the inspection of animals, regular inspection of personnel working on dairy farms should be considered. As well, further studies are needed to ex-

plore the epidemiological relationship between human and animal MRSA isolates.

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ORCIDS

Ho-Sung Park <https://orcid.org/0000-0003-1719-6233>
 Suk-kyung Lim <https://orcid.org/0000-0002-7549-9411>
 Soon-Seek Yoon <https://orcid.org/0000-0003-0908-8785>
 Dong-Chan Moon <https://orcid.org/0000-0003-1244-201X>