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Comparative Genomic Analysis of Staphylococcal Cassette Chromosome *mec* Type V *Staphylococcus aureus* Strains and Estimation of the Emergence of SCC*mec* V Clinical Isolates in Korea

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Background: Staphylococcal cassette chromosome *mec* type V (SCC*mec* V) methicillin-resistant *Staphylococcus aureus* (MRSA) has been recovered from patients and livestock. Using comparative genomic analyses, we evaluated the phylogenetic emergence of SCC*mec* V after transmission from overseas donor strains to Korean recipient strains.

Methods: Sixty-three complete MRSA SCC*mec* V genomes (including six Korean clinical isolates) were used to construct a phylogenetic tree. Single-nucleotide polymorphisms were identified using Snippy, and a maximum-likelihood-based phylogenetic tree was constructed using RAxML. The possible emergence of the most common ancestor was estimated using BactDating. To estimate *mecA* horizontal gene transfer (HGT) events, Rangerdtl was applied to 818 SCC*mec* V strains using publicly available whole-genome data.

Results: The phylogenetic tree showed five major clades. German strains formed a major clade; their possible origin was traced to the 1980s. The emergence of Korean SCC*mec* V clinical isolates was traced to 2000–2010. *mecA* HGT events in *Staphylococcus* spp. were identified in seven strains. P7 (Hong Kong outbreak strain) served as the donor strain for two Korean sequence type (ST) 59 strains, whereas the other five recipient strains emerged from different SCC*mec* V donors.

Conclusions: Most Korean SCC*mec* V strains may have emerged during 2000–2010. A unique MRSA SCC*mec* V strain, ST72 (a Korean common type of community-associated MRSA), was also identified. The genomic dynamics of this clone with a zoonotic background should be monitored to accurately understand MRSA evolution.

Key Words: Clades, Comparative genomic analysis, Donor–recipient, Horizontal gene transfer, Korea, Methicillin, One Health, Phylogeny, SCC*mec* V, *Staphylococcus*, Whole genome sequence

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most prevalent nosocomial pathogens in Korea, where it was first discovered in 1969 [1]. MRSA accounted for 25% of *S. aureus* isolates obtained from Korean tertiary care hospitals in 1980, 54% in 1990, and 70% in 2000 [2, 3]. Korean invasive or non-invasive community-acquired MRSA strains, with or without genes encoding Panton–Valentine leucocidin (PVL)—a toxin and key virulence factor of community-acquired strains—emerged in the 2000s [4]. Methicillin resistance is associated with the clinical outcomes of *S. aureus* infections [5], and the early treatment is important for a good prognosis of *S. aureus* bloodstream infections. The European Committee on Antimicrobial Susceptibility Testing recommends rapid antimicrobial susceptibility testing of *S. aureus* from positive blood culture bottles [6].

Methicillin resistance is conferred by *mecA*, which encodes penicillin-binding protein 2a. *mecA* is carried on the staphylococcal cassette chromosome *mec* (SCC*mec*), which is a mobile genetic element containing cassette chromosome recombinase genes (*ccrA* and *ccrB*) [7]. Although the transfer of SCC*mec* is a rare event in *S. aureus*, methicillin-susceptible *S. aureus* (MSSA) strains become MRSA after acquiring *mecA* embedded in SCC*mec*; all MRSA clones appear to harbor specific SCC*mec* types [8, 9].

SCC*mec* typing is based on the amplification of nine different genes (eight loci [A–H] of *mec* element sequences in addition to the *mecA* gene as an internal positive control), and subtypes are determined based on the presence or absence of various genes [10, 11]. The major SCC*mec* types in clinical isolates are types II, IV, and III. In contrast, MRSA SCC*mec* type V (SCC*mec* V), which has a novel *ccrC* gene [12], is infrequent among clinical isolates and has been isolated from humans and animals in Europe, Korea, Japan, Taiwan, and China. In Korea, SCC*mec* V strains have recently been isolated from patient serum samples and are considered an emerging pathogen [13]. Accordingly, microbiological laboratories in hospitals are monitoring the emergence of novel staphylococcal strains with antimicrobial resistance (AMR) in clinical settings.

Colonization or infection with livestock-associated MRSA has been reported in several domesticated livestock animals [14]. Carrier animals not only serve as reservoirs for opportunistic infections but can also transmit livestock-associated MRSA to other animal species or humans [15]. In Korea, livestock-associated SCC*mec* V strains have recently been isolated from pigs and their environments, posing serious public health concerns [16]. Based on the "One Health" concept, which is a comprehensive health control strategy for humans, animals, and the environment [17], circulating SCC*mec* V strains must be carefully monitored to maintain overall health.

Recently, we analyzed six SCC*mec* V isolates from a Korean University-affiliated hospital (Kangdong Sacred Heart Hospital, Hallym University College of Medicine) using whole-genome sequencing (WGS) to characterize the emerging SCC*mec* V strains. SCC*mec* can share genetic content via horizontal gene transfer (HGT) from MRSA or other methicillin-resistant staphylococci to MSSA or other methicillin-susceptible staphylococci among different hosts. SCC*mec* mobility relies on the function of *ccr*, encoding a protein that catalyzes host chromosome excision and insertion, which is a crucial step in HGT. However, few studies have evaluated the emergence or prevalence of SCC*mec* V in Korea.

We evaluated the possible phylogenetic emergence of SCC*mec* V via *mecA* HGT/direct transmission from overseas donor strains to Korean recipient strains through comparative genomic analysis of SCC*mec* V strains. To the best of our knowledge, this is the first study to estimate the emergence of SCC*mec* V in Korea and the donor–recipient relationship among overseas and Korean SCC*mec* V strains.

MATERIALS AND METHODS

SCC*mec* V MRSA strains

We screened the SCC*mec* types of MRSA strains deposited at Kangdong Sacred Heart Hospital (Seoul, Korea) from 2017 to 2020. We analyzed six SCC*mec* V strains isolated from patients with bacteremia or wound infections (Table 1). Ethical approval was not required as all data used in this study (host, strain, isolation date, isolation source, geographic location, and disease) related to SCC*mec* V strains were obtained from the publicly available National Center for Biotechnology Information (NCBI) database.

The presence of a gene encoding methicillin resistance was confirmed via PCR screening for *mecA* [10]. A single locus of repeat region X of the *spa* gene was sequenced and analyzed using Ridom StaphType v.3 (http://spaserver.ridom.de/) [18], and *spa* types were assigned using BioNumerics (v.7.5; Applied Math, Sint-Martens-Latem, Belgium). SCC*mec* was typed based on the *mec* and *ccr* gene complexes [19]. Genes encoding the PVL toxin were detected as described previously [4].

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Table 1. Strain information

Strain	Isolation date	Host	Isolation source	MLST	*spa* type	SCC *mec*	PVL	Antibiotic resistance genes	Antibiotic resistance phenotypes	Virulence genes
HL20709	August 23, 2017	*Homo sapiens*	Blood	ST59	t437	V	PVL$^+$	*mecA, tet(38), mepA, glpT_A100V, murA_E291D_T396N, erm(B), aph(3')-IIIa, ant(6)-Ia, catA*	Phenicol, β-lactam, fosfomycin, macrolide, aminoglycoside, tetracycline, efflux	*selX, sek, seq, seb, lukF-PV, lukS-PV, scn, hld, hlgA, hlgC, hlgB, sey, aur, icaC*
HL23187	May 8, 2019	*Homo sapiens*	Blood	ST59	t437	V	PVL$^+$	*mecA, tet(38), mepA, glpT_A100V, murA_E291D_T396N, blaZ, blaR1, blaI*	β-Lactam, tetracycline, fosfomycin, efflux	*selX, sek, seq, seb, lukF-PV, lukS-PV, scn, hld, hlgA, hlgC, hlgB, sey, aur, icaC*
HL25274	June 10, 2020	*Homo sapiens*	Blood	ST45	t1081	V	PVL$^-$	*gyrA_S84L, mecA, tet(K), tet(38), mepA, glpT_A100V, murA_E291D_T396N, parC_S80F, blaI, blaR1, blaPC1, aac(6')-Ie/aph(2'')-Ia, erm(C)*	Quinolone, β-lactam, fosfomycin, macrolide, aminoglycoside, tetracycline, efflux	*hld, sak, scn, seo, sem, sei, seu, sen, sel26, hlgA, hlgC, hlgB, ser, sej, set, ses, aur, icaC, cna*
HL24830	March 6, 2020	*Homo sapiens*	Blood	ST72	t3092	V	PVL$^-$	*gyrA_S84L, mecA, aac(6')-Ie/aph(2'')-Ia, tet(38), mepA, parC_S80F, blaI, blaR1, blaZ, abc-f, fosB, dfrG, aac(6')-Ie/aph(2'')-Ia, mupA*	Quinolone, β-lactam, fosfomycin, trimethoprim, mupirocin, macrolide, aminoglycoside, tetracycline, efflux	*splE, splB, splA, lukD, lukE, sen, seu, sei, sem, seo, scn, sak, sel, sec3, tst, hld, hlgC, hlgB, aur, icaC*
HL25870	September 9, 2020	*Homo sapiens*	Blood	ST1232	t034	V	PVL$^+$	*mecA, tet(K), tet(38), mepA, glpT_A100V_F3I, erm(A), ant(9)-Ia, murA_D278E_E291D, blaZ, blaI, blaR1*	β-Lactam, fosfomycin, macrolide, aminoglycoside, tetracycline, efflux	*lukF-PV, lukS-PV, sel26, scn, sak, hlgA, hlgC, hlgB, aur, icaC, cna*
HL2018_N011	January 10, 2018	*Homo sapiens*	Pus	ST59	t437	V	PVL$^+$	*mecA, tet(38), mepA, glpT_A100V, murA_E291D_T396N, erm(B), aph(3')-IIIa, ant(6)-Ia, catA, blaZ, blaR1, blaI*	Tetracycline, phenicol, macrolide, efflux, fosfomycin, aminoglycoside, β-lactam	*selX, sek, seq, seb, lukF-PV, lukS-PV, scn, hld, hlgA, hlgC, hlgB, sey, aur, icaC*

Abbreviations: MLST, multilocus sequence type; PVL, Panton–Valentine leucocidin.

DNA extraction and WGS

Six SCC*mec* V strains were inoculated onto 5% sheep blood agar plates and incubated at 35°C in the presence of 5% CO_2 for 24 hrs. Single colonies were inoculated into broth cultures. Genomic DNA was extracted using the Blood and Cell Culture DNA Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For MiSeq sequencing (Illumina, San Diego, CA, USA), genomic libraries were prepared using the Nextera DNA Flex Library Prep Kit and sequenced on the Illumina MiSeq platform, as previously described [20]. Raw reads were processed using fastp v.0.20.0, with the default settings. For MinION sequencing (Oxford Nanopore Technologies, Oxford, UK), genomic libraries were constructed using a ligation sequencing kit (SQK-LSK109) according to the manufacturer's instructions, and MinION flow cells (FILO-MIN106D) were sequenced using MinION v.19.12.5.

Genome assembly and annotation

For the MiSeq reads, adapter sequences were removed, and quality-filtering was performed using Trimmomatic v.0.39 [21]. For the MinION reads, base calling and demultiplexing were conducted using the Guppy GPU basecaller v.6.0.6, and sequencing artifacts were removed using Porechop v.0.2.4. Genome sequences were constructed from the MiSeq and MinION data using SPAdes v.3.14.0. Hybrid assembly was performed using Unicycler v.0.4.8, as previously described [20]. Genome assembly completeness was assessed using BUSCO v.5.3.2 with the lineage dataset bacillales_odb10. The complete genome sequences were annotated using the Prokaryotic Genome Annotation Pipeline v.4.3.

Comparative genome hybridization (CGH)

A genome map of the constructed MRSA genome was generated using CGView v.2.0.3. The CGView Comparison Tool (CCT) was used for visual comparison of the CGH comprising six WGS datasets, as described previously [22, 23]. All five other WGS datasets were aligned with the complete genome of strain HL24830 as a reference sequence. Clusters of orthologous gene (COG) functional categories were assigned based on the NCBI COG database (https://www.ncbi.nlm.nih.gov/research/cog) and are displayed using different colors.

Comparative genomic analysis

Before conducting comparative genomic analysis, we retrieved all publicly available genome sequences of the genera *Staphylococcus* and *Mammalicoccus* assembled at the complete, chromosome, scaffold, and contig levels from the NCBI database. The sequences were reassigned for taxonomic classification based on the Genome Taxonomy Database (GTDB) using the GTDB-Tk toolkit v.2.0.0 before performing downstream analysis. SCC*mec* typing, multilocus sequence typing (MLST), and *spa* typing were performed using SCC*mec*Finder (https://bitbucket.org/genomicepidemiology/scc*mec*finder.git), MLST (https://bitbucket.org/genomicepidemiology/mlst/src/master/), and spaTyper (https://bitbucket.org/genomicepidemiology/spatyper/src/main/), respectively. AMR and virulence-associated genes were identified via homology-based screening using AMRfinderPlus v.3.10.30 and the Virulence Factor Database (http://www.mgc.ac.cn/VFs/) [24], respectively. Genome sequences were annotated using Prokka v.1.14.6 to confirm whether the detected genes were functional. Using PorthoMCL, gene clusters were defined in the 513 MRSA genomes, and the clusters specific to each group were further analyzed.

Dated phylogenetic tree construction

Sixty-three complete circular genome sequences of SCC*mec* V MRSA with available information (host, strain, isolation date, isolation source, geographic location, and disease) were used to construct a phylogenetic tree. Using Snippy v.4.6.0, reference-based mapping was performed and single-nucleotide polymorphisms were identified. Recombination regions detected using Gubbins v.3.2.1 and the GTRGAMMA model were excluded. Based on recombination-free alignments, a maximum likelihood-based phylogenetic tree was constructed using RAxML v.8.2.11, with 1,000 bootstrap inferences. To estimate the emergence of the most common ancestors of the corresponding clades, Bayesian analysis was performed using BactDating v.1.1.

Computational estimation of *mecA* HGT

WGS datasets on 818 methicillin-resistant staphylococcal strains (including MRSA and other staphylococci) were retrieved from NCBI, and the strains were typed as SCC*mec* V using SCC*mec*Finder. Multiple sequence alignments of the *mecA* genes from these strains and the core sequences of the corresponding genomes were generated using Clustal Omega v.1.2.4. Based on the multiple alignments, a maximum likelihood-based phylogenetic tree was constructed using RAxML with 1,000 bootstrap inferences and the GTRGAMMA model. *Macrococcus caseolyticus* FDAARGOS_868 was used to root the tree, and the sequence was removed using Newick Utility v.1.6. We used Ranger-dtl 2.0 to estimate *mecA* HGT events among SCC*mec* V strains, using a support value cutoff of 0.9 to exclude ambiguous HGT events. The tree reconciliation method, which detects HGT events only when the overall genome-wide similarity of two strains is low compared to that of the target gene regions, thus excluding the direct transmission of MRSA strains with similar clonal backgrounds, was applied.

RESULTS

Molecular epidemiological characterization of six SCC*mec* V strains

Among the six SCC*mec* V strains, ST59 (N=3) was isolated in 2017, 2018, and 2019 and harbored the PVL toxin gene with *spa* type t437 (Table 1). The remaining three PVL-negative ST72-t3092, PVL-negative ST45-t1081, and PVL-positive ST1232-t034 strains were identified in 2020 (Table 1).

WGS datasets of the six SCC*mec* V strains

Table 2 presents the MinION and MiSeq sequencing metrics of the two assemblies from both WGS datasets. Table 3 summarizes the metrics of the six circularly assembled and annotated SCC*mec* V genomes. The complete genomes ranged from 2.809 to 2.926 Mbp, with a GC content of 32.5% in all datasets. Five genomes harbored one or two plasmids, and the six WGS datasets contained 2,626–2,790 protein-coding genes and 66–83 non-coding genes.

CGH findings of the six SCC*mec* V genomes

Fig. 1 shows the circular map of the six complete SCC*mec* V genomes (including the complete genome of HL24830 as a reference) generated using CGH. The CCT map consists of several circles showing the reference genome features and the results of Basic Local Alignment Search Tool comparisons between the

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Table 2. Sequencing metrics for the two assemblies from two whole-genome sequencing datasets

Strain	MinION sequencing*					MiSeq sequencing†		
	Read length N50 (bp)	Mean read quality	N of reads	Total bases (bp)	Coverage (X)	N of reads	Total bases (bp)	Coverage (X)
HL20709	13,266	11.3	112,634	1,220,990,317	426.9	1,947,068	288,474,163	100.9
HL23187	16,867	11.2	42,440	557,671,576	196.4	1,837,608	272,537,071	96.0
HL25274	3,247	12.6	329,151	762,618,177	271.4	3,027,274	445,604,778	158.6
HL24830	7,148	8.7	132,592	528,929,395	181.5	1,881,702	278,662,641	95.6
HL25870	7,928	8.7	335,981	1,402,136,279	479.1	1,720,578	255,003,766	87.1
HL2018_N011	8,616	8.7	81,207	325,914,573	116.0	1,613,702	238,922,352	85.0

*Oxford Nanopore Technologies, Oxford, UK.
†Illumina, San Diego, CA, USA.

Table 3. Summarized metrics for the six circularly assembled and annotated genomes

Metric	HL20709	HL23187	HL25274	HL24830	HL25870	HL2018_N011
Completeness, %*	450 (100%)	450 (100%)	450 (100%)	450 (100%)	450 (100%)	450 (100%)
N of chromosomes	1	1	1	1	1	1
N of plasmids	0	1	2	1	1	1
Total length, bp	2,860,303	2,860,005	2,913,602	2,926,847	2,809,575	2,881,027
GC, %	32.5	32.5	32.5	32.5	32.5	32.5
N of protein-coding genes	2,706	2,695	2,714	2,790	2,626	2,705
N of non-coding genes	66	82	83	82	82	81

*Genome assembly completeness was assessed using BUSCO v.5.3.2 with the lineage dataset bacillales_odb10.

reference and comparative sequences. The GC content and GC skew+/− were also evaluated. Several portions of the HL24830 genome sequence differed from the other five genomes, suggesting a unique trait of the former (Korean community-associated MRSA representative clone with ST72-t3092) compared with the other SCC*mec* V genomes.

Dated phylogenetic tree findings with complete SCC*mec* V genomes available in NCBI

Fig. 2 shows the dated phylogenetic tree of the six Korean MRSA SCC*mec* V strains and reported MRSA SCC*mec* V strains with complete genomes (N=57) obtained from NCBI. The dated tree showed five major clades. Strain NGA71 (isolated from human urine at the University of Benin Teaching Hospital, Nigeria) was an outlier in the tree. The German strains formed a major clade (clade 1), with a possible origin traced to the 1980s. Korean HL25870, HL25274, HL2018_N011-HL23187-HL20709, and HL24830 belonged to clades 1, 2, 3, and 4, respectively, suggesting diverse clades. A distinct clade comprised HL24830, which emerged in 2010 and was situated close to ER03364.3 (human outbreak strain isolated from blood at Mount Sinai Hospital, New York, NY, USA). Based on the dated findings, all six Korean SCC*mec* V strains may have emerged during 2000–2010.

Evaluation of *mecA* HGT among staphylococci harboring SCC*mec* V

Possible *mecA* HGT events in the SCC*mec* V recipient strains isolated in Korea are shown in Table 4. Six *mecA* HGT events with a support value of 1 were identified. We found an association between the Korean HL2018_N011 and HL20709 recipient strains and the P7 donor strain (a 2017 human outbreak strain from Hong Kong) and between the Korean HL25870 recipient strain and 11A731 donor strain (a 2011 Chinese food-origin strain). We also observed associations between the PCFA-221 recipient strain (a 2017 Korean pig isolate) and ISU 924 donor strain (a 2010 USA pig isolate) and between the BDH17 recipient strain (a 2017 Korean human isolate) and Sau55 donor strain (a 2006 human blood-origin strain from Taiwan).

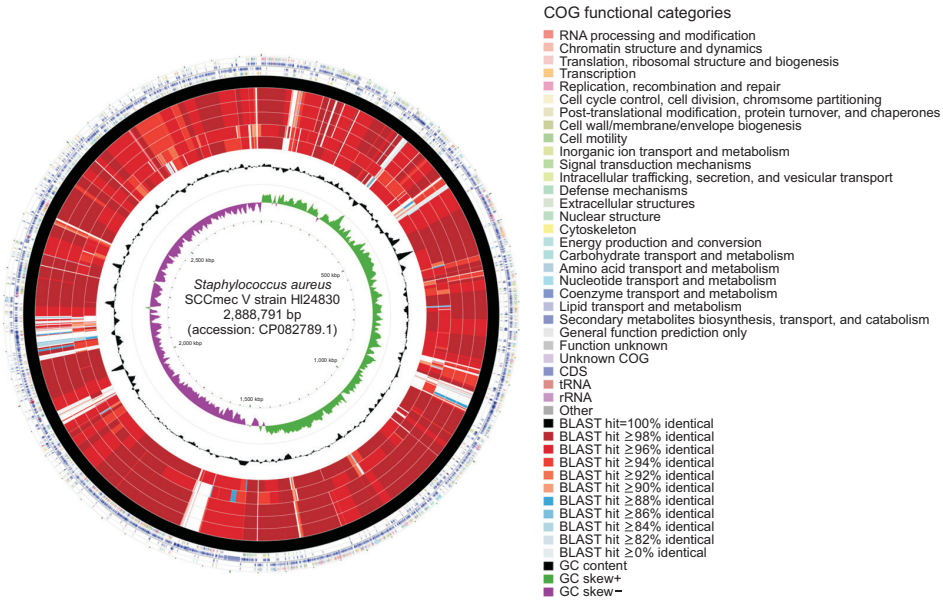

Fig. 1. Circular map of the six Korean complete MRSA SCC*mec* V strain genomes (including the HL24830 reference strain). A genome map of the constructed MRSA genome was generated using CGView v.2.0.3. The CGView Comparison Tool was used to construct the circular plot of *Staphylococcus aureus* DNA sequences. COG features for protein-coding genes and locations of protein-coding, transfer RNA, and ribosomal RNA genes are presented on the four outermost concentric rings. Similar regions of MRSA SCC*mec* V strains are presented on the next six concentric rings. Based on the percent identity determined using the Basic Local Alignment Search Tool between the similar regions, the inside rings of the MRSA genomes appear black (100% identical) to blue (≥88% identical). The black peaks depict GC content, and the innermost green and purple peaks describe positive and negative GC skews, respectively.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; COG, clusters of orthologous gene; SCC*mec* V, staphylococcal cassette chromosome *mec* type V.

DISCUSSION

MRSA strains with SCC*mec* V have been isolated from livestock and, less frequently, from humans. However, many people have companion animals in their homes, and medical hospitals and nursing homes have introduced animal-assisted therapy as a mental health service for patients and elderly residents [25]. Animals and humans are in constant close contact with the environment. Among bacterial pathogens with the potential to be transmitted between animals and humans, strains with AMR pose a serious threat to public health and must be closely monitored.

Staphylococcus pseudintermedius typically infects dogs. In 2008, *mecA*-positive *S. pseudintermedius* strains were recovered at two veterinary hospitals in Korea [26]. *S. pseudintermedius* with SCC*mec* V were the most prevalent isolates from veterinary staff (nose and hands), hospitalized animals (anus, skin, feet, ears, and wounds), and medical equipment (floors and phones). Pulsed-field gel electrophoresis analysis revealed genetic similarities among isolates from veterinary staff (hands), hospitalized animals (skin, feet, ears, and wounds), and medical equipment (floors) [26]. A recent study reported genetic relatedness in SCC*mec* between methicillin-resistant *Staphylococcus* isolates from companion dogs with pyoderma and their owners [27]. Among 31 dog–owner pairs, one pair with three isolates carrying SCC*mec* V, i.e., 18D20-1 (*S. pseudintermedius*, dog), 18D20-2 (*S. schleiferi*, dog), and 18H20-F2 (*S. epidermidis*, dog owner), was detected [27]. These observations suggest the clonal spread of SCC*mec* V strains between animals and humans.

Human invasive MRSA isolates harboring SCC*mec* V (N=3) were recovered at the Kangdong Sacred Heart Hospital between 2020 and 2021 [13]. A nosocomial outbreak of the community-associated MRSA P7 strain carrying SCC*mec* V–ST59 was reported in Hong Kong and originated from a clinical specimen from a neonatal intensive care unit [28]. We speculated that *mecA* was passed via HGT and/or direct transmission from the P7 donor strain (Hong Kong) to the HL2018_N011 and HL 20709 recipient strains (Korea) with ST59. The same ST59-t437 type was recovered in Hong Kong and Korea in 2018 [28], further supporting the notion of direct transmission occurring between these strains. However, HGT may have also played a role,

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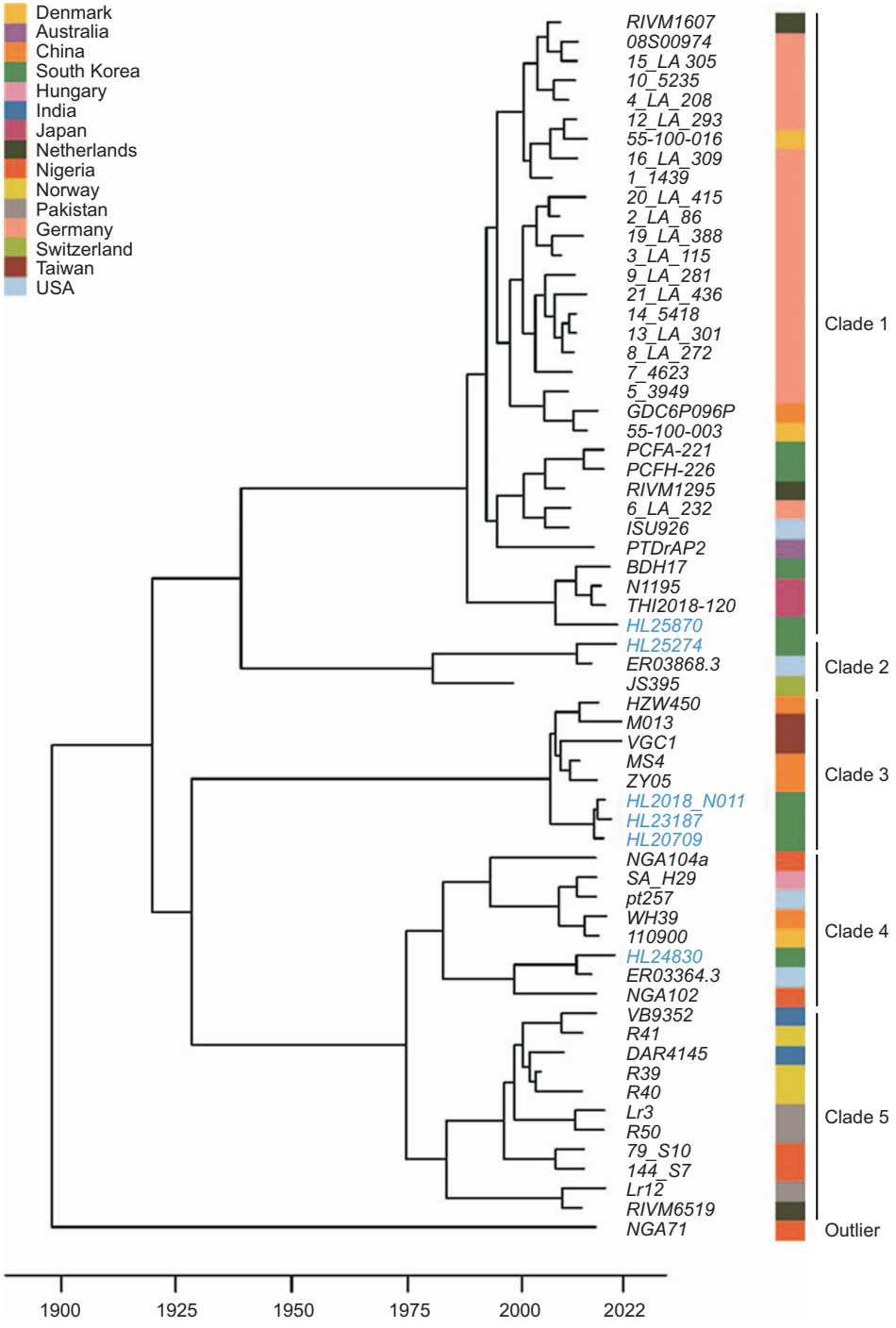

Fig. 2. Dated phylogenetic tree, including the six Korean MRSA SCC*mec* V strains and other MRSA SCC*mec* V strains with complete genomes (N = 57) available from the National Center for Biotechnology Information database. Strain HL24830 appears around 2010 and is situated near strain ER03364.3 from the USA. The six Korean SCC*mec* V stains may have emerged during 2000–2010.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; SCC*mec* V, staphylococcal cassette chromosome *mec* type V.

as the computational procedure excluded the relatedness of similar clones, and we manually checked and denied the similarities of the complete genome sequences and P7 contigs. We observed *mecA* HGT from the 11A731 donor strain (China) to the HL25870 recipient strain (Korea) with ST1232. Chlebowicz, *et al*. [29] reported that SCC*mec* V from the MRSA strain UMCG-M4 was packaged into its bacteriophage capsids. Although transduction of complete SCC*mec* V was not observed, purified staphylococcal phage particles encapsulated large portions (including *mecA*) of SCC*mec* V. Therefore, it is necessary to evaluate the SCC*mec* V transduction/encapsulation capabilities of phages from the Korean recipient strains in the future.

Table 4. Computational analysis of possible *mecA* HGT in staphylococcal SCC*mec* type V strains isolated in Korea

Donor strain	Host	Collection year	Oversea country	Recipient strain	Host (specimen)	Collection year	Location	Support value
S. aureus ISU 924	Pig	2010	The USA	*S. aureus* PCFA-221	Pig (nasal cavity)	2017	Chungcheongnam-do	1
S. aureus Sau55, 5_3949, VET0107R, Sau88, RR17, W, VET0421R, CFSA16SA028, CFSA15SA094, 08-01728, VET0241R, and VET0176R	Human	2006	Taiwan, Germany, and the Netherlands	*S. aureus* BDH17	Human	2019	Unknown location	1
S. aureus P7*	Human (pus)	2017	Hong Kong	*S. aureus* HL2018_N011	Human (pus)	2018	Seoul	1
S. aureus P7*	Human (pus)	2017	Hong Kong	*S. aureus* HL20709	Human (blood)	2017	Seoul	1
S. aureus VET0077R	Human	2008	Unknown	*S. schleiferi* OT1-1	Dog (ear)	2017	Seongnam-si	1
S. aureus 11A731	Food	2011	China	*S. aureus* HL25870	Human (blood)	2020	Seoul	1
S. epidermidis Se_BPH0736 and HD43-1	Human (nasal cavity and prosthetic joint)	2011 and 2018	Australia and Germany	*S. epidermidis* CDC121	Human (skin)	2017	Seoul	0.92

Recipient strains isolated in Korea are presented, and the methicillin-resistant *Staphylococcus aureus* strains analyzed in this study are shown in bold.
*Donor strain (*S. aureus* P7) for possible mecA direct transmission.
Abbreviation: HGT, horizonal gene transfer.

The current study had two limitations. First, although *mecA* HGT events were estimated, possible HGT events associated with another SCC*mec* V component gene, *ccrC*, should be evaluated. Second, it remains unclear how SCC*mec* V of overseas donor strains from China, the USA, and Taiwan were transmitted to the Korean recipient strains. Possible routes include imported foods and immigrant animals or humans. Further studies monitoring the dynamics of SCC*mec* V clones in similar populations are required.

In conclusion, we estimated the possible clades and donor–recipient relationships among MRSA SCC*mec* V strains. We identified a unique Korean community-acquired MRSA SCC*mec* V strain harboring PVL-negative ST72-t3092, HL24830, and found that this strain differed from the other five Korean SCC*mec* V strains. The ST72 clone is prevalent among community-acquired MRSA isolates in Korea [30]. Continuous nationwide surveillance is required to monitor the emergence and spread of SCC*mec* V isolates in other Korean hospitals. The same clones with ST72-t3092-SCC*mec* V were recently isolated from a raw buffalo milk tank in Italy [30], suggesting that the risk of human MRSA colonization or infection may be associated with the handling of raw milk or consumption of contaminated dairy products.

SCC*mec* V of MRSA is an emerging clone in Korea and East Asian and European countries in which the V clone has been associated with human-to-animal or animal-to-human transmission. We expect that the investigation of this clone can enhance our understanding of the importance of "One Health." The six Korean MRSA SCC*mec* V strains isolated from the blood or pus of patients may have emerged during 2000–2010. The clinical significance and implications of this clone among humans and pets/livestock/wild animals warrant further research to ensure that appropriate community health measures are taken. The silent AMR bacterial pandemic is an issue that requires prompt, optimized, and coordinated countermeasures.

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AUTHOR CONTRIBUTIONS

Kim J-S and Takahashi T conceptualized the study. Kim H and Kim J-S were involved in the investigation. Kim H, Kim H-S, Kim HS, Song W, and Kim J-S were involved in the formal analysis. Kim H-S, Kim HS, Song W, and Kim J-S provided resources. Takahashi T and Kim J-S drafted the manuscript. Kim J-S, Kim H, and Takahashi T reviewed and edited the manuscript.

CONFLICTS OF INTEREST

None declared.

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