

Original article

Complete genome analysis of representative methicillin-resistant *Staphylococcus aureus* clinical strains prevalent in Korea during 2014-2017

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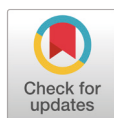
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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen responsible for various clinical infections. The investigation of representative MRSA genomes is important for understanding their molecular epidemiology and genetic evolution, as well as MRSA infections. We characterized the complete genome sequences of representative MRSA clinical strains prevalent in Korea between 2014 and 2017.

Methods: Ten representative clinical MRSA strains were selected based on the staphylococcal cassette chromosome *mec* (SCC*mec*) type. Complete genomes were generated via hybrid assembly using long- and short-read sequencing. Analyses of resistance and virulence genes, whole-genome alignment, phylogenetic tree construction, and comparative genome hybridization were performed.

Results: The average chromosomal lengths were 2.916 Mb in SCC*mec* II (n = 6), 2.920 Mb in SCC*mec* IV (n = 2), and 2.777 Mb in SCC*mec* IVA (n = 2). The number of genome coding sequences ranged from 2,713 to 3,026, with an average of 2,946 in SCC*mec* II, 3,001 in SCC*mec* IV, and 2,740 in SCC*mec* IVA. Only the SCC*mec* IV and *spa* t008 strains (n = 2) harbored the Pantón-Valentine leukocidin gene, which is rarely detected in Korea. The SCC*mec* IVA strains of ST72 showed a distinct genetic group compared with other representative SCC*mec* IV strains, as determined by single-nucleotide polymorphism analysis.

Conclusion: In the present study, the complete and gap-filled genome sequences of representative MRSA clones prevalent in Korea were derived and characterized by genome size, virulence, antimicrobial resistance genes, and their evolutionary relationships.

Information on these clinical MRSA strains would enhance our understanding of the pathogenicity and molecular epidemiology of Korean MRSA isolates.

Keywords: Antimicrobial resistance, Methicillin-resistant *Staphylococcus aureus*, Molecular epidemiology, Staphylococcal protein A, Whole-genome sequencing

Introduction

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare- and community-associated infections and causes significant morbidity and mortality in patients [1]. In Korea, *S. aureus* is the second most common pathogen causing bloodstream infections, with an incidence of 2.8 cases per 10,000 patient-days among inpatients; more than half of these cases (54.3%) were attributable to MRSA [2]. In the United States, approximately 323,700 MRSA infections and 10,600 associated deaths were reported among hospitalized patients in 2017 [3]. Over the past few decades, the epidemiology of MRSA infections has changed, resulting in severe clinical infections caused by both healthcare-associated MRSA clones and community-associated MRSA (CA-MRSA) clones [4,5]. Understanding the genomic characteristics of the prevalent MRSA strains according to their genetic subtypes is necessary to study MRSA infections and epidemiology.

Whole-genome sequencing (WGS) has revealed the comprehensive and high-resolution genomic characteristics of various bacterial pathogens. Conventional typing methods, such as pulsed-field gel electrophoresis and multilocus sequence typing (MLST), have been successfully used to characterize bacterial strains responsible for disease outbreaks, but offer limited discrimination between closely related strains. WGS has enabled the investigation of pathogens with much greater discriminatory power, resulting in a more comprehensive understanding of the transmission of isolates and their evolutionary relationships [6]. In addition, WGS provides a complete view of antimicrobial resistance genes, virulence factor genes, and other genomic characteristics related to pathogenicity [7,8].

The genome sequence of fragmented DNA contigs with gaps, usually obtained by short-read sequencing technology, has been used to investigate the antimicrobial resistance, epidemiological characteristics, and virulence factors of bacteria. In addition, short-read sequencing data can be further analyzed using reference-based sequence alignment, allowing genome-wide comparisons with other strains with high accuracy [6].

In contrast, the Oxford Nanopore Technologies DNA sequencing platform generates long reads that facilitate complete genome assembly. However, these long reads have relatively higher error rates. Hybrid assembly, which combines short- and long-read sequencing data, using software such as Unicycler [9], can produce complete circular bacterial genomes with high accuracy.

Complete genomes of pathogens provide advantages over fragmented bacterial genomes with gaps because they precisely identify all the genes (i.e., virulence genes and antimicrobial resistance genes) present,

and also provide structural information relating to genes, mobile genetic elements, and gene rearrangements. This comprehensive information provides novel structural insights into bacterial genetics [10-12]. In addition, complete genomic sequences enable accurate phylogenetic and evolutionary analyses, including the identification of the horizontal gene transfer of antimicrobial resistance elements [13,14]. Although several complete circular genome sequences of MRSA strains have been published [14-16], comprehensive genomic analyses of MRSA clinical isolates from Korea remain scarce, with only approximately five MRSA isolates to date. The paucity of genomic representation limits our understanding of the molecular characteristics of Korean MRSA strains.

Objectives

The present study was conducted to address this limitation by performing complete genome sequencing of MRSA clinical isolates from Korea. We selected ten representative MRSA strains from blood specimens collected at a university hospital according to the molecular genotypes of their staphylococcal cassette chromosome *mec* (*SCCmec*) and staphylococcal protein A (*spa*) type. To reveal the characteristics of these MRSA strains, complete circular genomes were derived using hybrid assembly. The genome sizes of representative MRSA clones in Korea and the characteristics of their virulence factors and antimicrobial resistance were investigated herein. Phylogenetic analyses were performed to compare the genomic characteristics of Korean MRSA strains with those of representative *S. aureus* strains from other countries.

Methods

MRSA clinical isolates and their phenotypic and molecular characterization

Ten MRSA clinical isolates obtained from the blood cultures of patients presenting at Kangdong Sacred Heart Hospital, Seoul, Korea, between 2014 and 2017, were selected according to their *SCCmec* and *spa* types (Table 1). We performed *SCCmec* typing on all MRSA blood isolates at our hospital during this period. Among these isolates, representative strains were selected to reflect the major genotypes prevalent in Korea: *SCCmec* II (n = 6) with *spa* t002, t2460, and t9353; *SCCmec* IV (n = 2) with *spa* t008; *SCCmec* IVA (n = 2) with *spa* t324. These combinations are commonly associated with the predominant hospital- and community-associated MRSA clones in Korea. The strains were also identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Microflex LT; Bruker Daltonics GmbH), and MRSA was confirmed by polymerase chain reaction amplification of *mecA* [15] and cefoxitin disk diffusion tests. The *SCCmec* [17] and *spa* types [18] were determined for each MRSA isolate.

Table 1. Summary metrics of the assembled (circular) and annotated genomes of methicillin-resistant *Staphylococcus aureus* strains

Strain	HL18888	HL20835	HL17064	HL18807	HL18883	HL21008	HL17078	HL18380	HL16278	HL18840
SCC _{mec} type	II	II	II	II	II	II	IV	IV	IVA	IVA
<i>spa</i> type	4002	4002	42460	42460	49353	49353	4008	4008	4324	4324
Isolation year	2016	2017	2015	2016	2016	2017	2015	2016	2014	2016
Completeness (%) ^a	100	100	100	100	100	100	100	100	100	100
Chromosomes	1	1	1	1	1	1	1	1	1	1
Total length (bp)	2,893,276	2,913,324	2,931,169	2,903,929	2,904,018	2,947,630	2,922,932	2,917,999	2,757,377	2,796,963
GC (%)	32.89	32.91	32.98	32.92	32.92	32.93	32.81	32.80	32.82	32.85
CDS	2,923	2,970	2,974	2,905	2,906	3,000	3,026	2,975	2,713	2,766
rRNAs	16	16	16	16	16	16	19	19	16	19
tRNAs	59	59	59	59	59	59	59	59	59	59
ncRNAs	4	4	4	4	4	4	4	4	4	4
GenBank No.	CP080548.1	CP080566.1	CP080560.1	CP080552.1	CP080550.1	CP080562.1	CP080556.1	CP080553.1	CP080564.1	CP080551.1
Plasmids	1	1	1	0	0	1	3	2	1	0
Plasmid length (bp)	24,653	24,653	25,109			25,109	42,253/19,845/3,125	23,058/6,118	3,332	
GC (%)	28.5	28.5	29			29	28.5/29/28.5	30.5/28.5	29.5	
GenBank No.	CP080549.1	CP080567.1	CP080561.1			CP080563.1	CP080557.1/ CP080558.1/ CP080559.1	CP080554.1/ CP080555.1	CP080565.1	
Genome assembly No.	ASM1955137v1	ASM1955141v1	ASM1955094v1	ASM1955131v1	ASM1955103v1	ASM1955109v1	ASM1955117v1	ASM1955123v1	ASM1955135v1	ASM1955087v1

The table was created with analysis of genome statistics of Fasta sequences

^aCompleteness of genome assembly was analyzed by BUSCO v5.3.2 with bacteria_odb10 lineage dataset

Abbreviations: SCC_{mec}: staphylococcal cassette chromosome mec; *spa*: staphylococcal protein A; bp: base pairs; GC: guanine and cytosine; CDS: coding sequences or protein-coding genes; BUSCO: benchmarking universal single-copy orthologs.

WGS of short-read and long-read sequencing

The MRSA strains were cultured on 5% sheep blood agar plates at 37°C under 5% CO₂ for 16–18 h. Genomic DNA was extracted using QIAamp® DNA Mini Kit (Qiagen), and the DNA concentration was measured with a Quantus™ Fluorometer (Quantus Inc.).

For short-read sequencing, genome libraries were prepared using Nextera DNA Flex Library Prep Kit (Illumina Inc.). The size and concentration of DNA libraries were measured using a TapeStation 4200 capillary electrophoresis platform (Agilent Inc.). Paired-end libraries were sequenced using the Illumina MiSeq platform [15].

For long-read sequencing, genome libraries were prepared using a ligation sequencing kit (SQK-LSK109; Oxford Nanopore Technologies) and sequenced using a MinION flow cell (FLO-MIN106D; R9; Oxford Nanopore Technologies) controlled by the MinKNOW software (v.20.10.3).

Genomes were assembled with Unicycler (v.0.4.8) [9] using MiSeq paired-end sequences and MinION sequences. Genomes were annotated using Prokka (v1.14.6) [19]. Complete genome sequence data for MRSA strains have been deposited in the NCBI database with BioProject PRJNA693997 (Dataset 1).

Multiple genome alignment and phylogenetic analysis

Mauve analysis using MegAlign Pro (DNASTAR v.17.0.2.1) was performed to align the MRSA circular genome sequences and identify the structural characteristics of the genome.

With the genome of the study isolates and the previously reported representative strains, single-nucleotide polymorphism (SNP)-based genome alignment was performed using Snippy (v.4.6.0) and Gubbins (v.3.4) was used to exclude recombination. The N315 complete genome was selected as the reference genome. Based on recombinant-free alignments, phylogenetic analysis was performed using RAxML-NG (v.1.2.2) [20] for the maximum-likelihood tree. A phylogenetic tree was constructed using FigTree software (v.1.4.4). Core- and whole-genome MLST analyses were performed using ChewBBACA software (v.3.3.10). Newick-formatted tree files of MSTreeV2, a minimal spanning tree method, were generated using Grapetree (v.1.5.0), and trees were drawn using FigTree.

Comparative genome hybridization (CGH)

Maps of the constructed MRSA genomes were generated. A visual comparison of the ten MRSA WGS datasets was performed using the CGView Comparison Tool (CCT; v.2.0.3) [21]. 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. Roary (v.3.13.0) was used herein to determine the presence and absence of genes and the number of core genes [22].

Antimicrobial resistance and virulence gene detection

The MicroScan WalkAway 96 Plus system (Beckman Coulter) was used to determine the phenotypic antimicrobial susceptibility testing (AST) of each MRSA strain. Using the MRSA genome sequences,

the presence of antimicrobial resistance and virulence genes was identified using ResFinder (v.4.4.1) and VirulenceFinder (v.2.0.3) from the Center for Genomic Epidemiology server. Both ResFinder and VirulenceFinder were set to a default threshold ID of 90% and minimum length of 60%.

Results

WGS analysis of the MRSA strains

Each of the ten MRSA strains possessed a single circular chromosome, ranging from 2,757,377 to 2,947,630 base pairs (bp), with guanine and cytosine (GC) content between 32.80% and 32.98% (average 32.88%) (Table 1). The average chromosomal lengths were 2.916 Mb for SCC*mec* II (n = 6), 2.920 Mb for SCC*mec* IV (n = 2), and 2.777 Mb for SCC*mec* IVA (n = 2).

The SCC*mec* II and SCC*mec* IVA strains carried either no plasmids or a single plasmid, whereas the *spa* t008 SCC*mec* IV strains carried two or three plasmids (Table 1). Notably, two SCC*mec* II strains of *spa* t002 harbored plasmids of identical size (24,653 bp), whereas one t2460 strain and one t9353 strain also carried plasmids of the same size (25,109 bp), regardless of the isolation year.

The MRSA genome contains 2,713–3,026 protein-coding genes and 79–82 non-coding genes. The number of coding sequences (CDS) ranged from 2,713 to 3,026, with an average of 2,946 in SCC*mec* II, 3,001 in SCC*mec* IV, and 2,740 in SCC*mec* IVA. Roary pan-genome analysis identified 3,661 total genes, including 2,204 core genes (present in $\geq 99\%$ of strains), 0 soft-core genes (95%–98%), 1,151 shell genes (15%–94%), and 306 cloud genes (< 15%). Information pertaining to the gene content of the MRSA strains is listed in supplementary Table 1 in the online-only Data Supplement. The GC content of the genomes showed minimal variation among strains (range: 32.80–32.98, average: 32.88).

Ten MRSA strains represented five *spa* types (t002, t008, t324, t2460, and t9353), with two isolates per *spa* type. The *spa* strains t002, t2460, and t9353 were associated with SCC*mec* II (n = 6), t008 with SCC*mec* IV (n = 2), and t324 with SCC*mec* IVA (n = 2). All SCC*mec* II isolates belonged to MLST ST5, while two SCC*mec* IV strains belonged to ST8 and ST5863, and two SCC*mec* IVA strains belonged to ST72.

Comparative genome alignment of the MRSA complete genomes

Mauve analysis of the complete genomes revealed high levels of synteny between these representative MRSA genomes (Fig. 1), although structural variations, such as intraspecific recombination, were noted. Gene order in the proximal and distal regions was conserved; however, structural differences, such as recombination, inversion, and deletions, occurred mainly between ~0.9 Mb and ~2.2 Mb. A large genomic inversion (~0.8 Mb) was observed in strain HL17064.

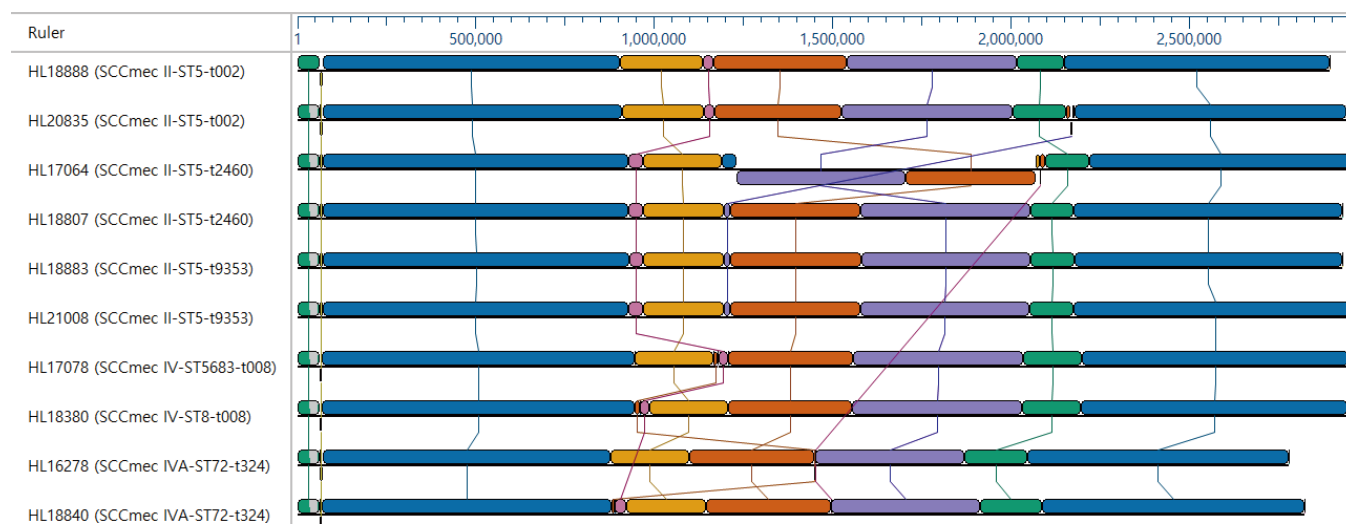


Fig. 1. Genome alignments of MRSA strains using multiple alignment of conserved genomic sequences (MAUVE). Pairwise comparisons of MRSA genome sequences revealed a large genomic inversion in strain HL17064.

MRSA: methicillin-resistant *Staphylococcus aureus*

Phylogenetic relationships among representative MRSA strains

Phylogenetic tree analysis based on SNPs of the genome showed that strains grouped by SCCmec type formed three distinct clades (Fig. 2). Korean MRSA strains of the same SCCmec type clustered closely. The SCCmec II strain, ST5 (CC5)-t002/t2460/t9353, is closely related to representative ST5 strains, such as Mu50, N315, and JH1. The SCCmec IV strain, ST8/ST5863 (CC8)-t008, clustered with the representative ST8 strains TW20, Newman, USA300_FPR3757, COL, and NCTC_8325. Interestingly, the Korean SCCmec IVA strain ST72 (CC8)-t324 formed a unique phylogenetic clade distinct from the CC8 representative strains.

For the Korean SCCmec IVA strain of ST72-t324, additional analyses with cgMLST and wgMLST, the allele-based genotyping methods using fewer loci (2,009 and 3,567 loci), also showed findings compatible with those of Snippy (112,574 loci), the whole-genome SNP-based analysis (Fig. 3).

CGH of the MRSA strains

Circular genome maps were generated using the largest genome (HL21008) as reference (Fig. 4). Genes in the SCCmec II strains showed $\geq 98\%$ identity, while the SCCmec IV strains displayed 94%–98% similarity in some genomic elements. A lack of genetic elements was observed in specific SCCmec and *spa* types. For instance, the SCCmec IV and IVA strains (inner four circles) lacked phenol-soluble modulins (*psm-mec*), *mecI*, and part of *mecR* in the SCCmec region (~37–39 kb region) of the proximal part of the MRSA genome (supplementary Fig. 1 in the online-only Data Supplement).

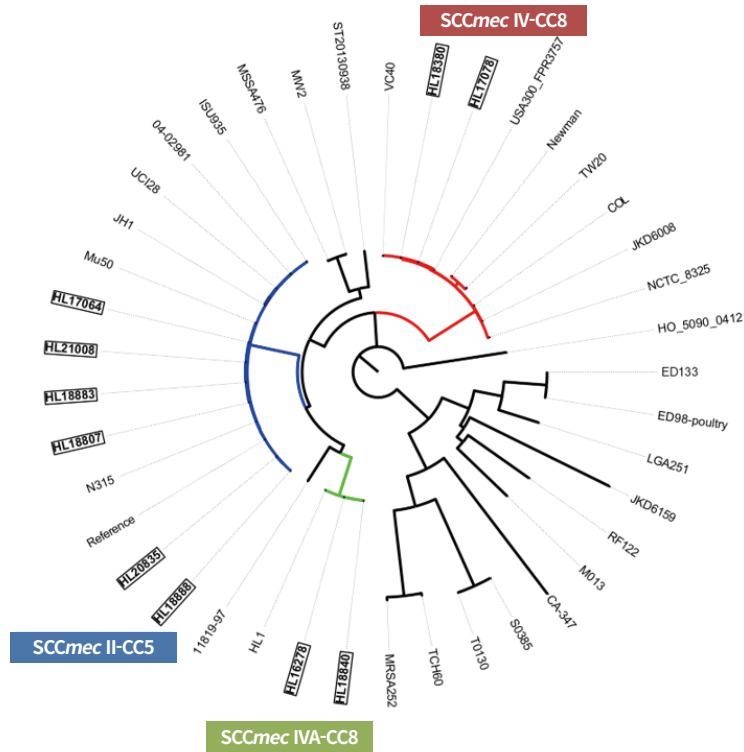


Fig. 2. SNP-based phylogenetic tree with Snippy, Gubbins, and RAXML-NG analysis of the representative *S. aureus* strains, illustrating relationships between major clones. The Korean clinical MRSA strains are indicated by bolded rectangles. SCCmec II strains from Korea clustered closely with other representative SCCmec II strains. In contrast, SCCmec IVA strains of CC8 (HL16278 and HL18840) formed a distinct clade, separate from other SCCmec IV reference strains of CC8. A total of 112,574 SNP loci were included in the analysis. SNP: single-nucleotide polymorphism; MRSA: methicillin-resistant *Staphylococcus aureus*; SCCmec: staphylococcal cassette chromosome mec

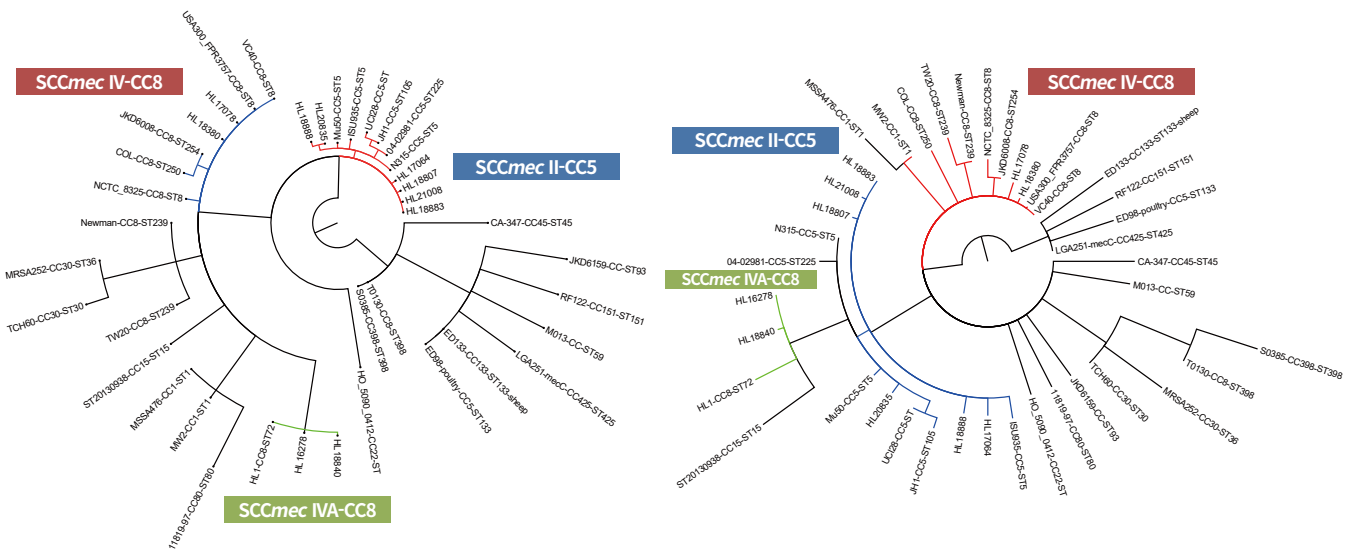


Fig. 3. Minimal spanning tree (MSTreeV2) based on cgMLST (left) and wgMLST (right) allelic profiles of the representative *S. aureus* strains, illustrating relationships between major clones. The Korean clinical MRSA strains are indicated by bolded rectangles. SCCmec II strains from Korea clustered closely with other representative SCCmec II strains. In contrast, SCCmec IVA strains of CC8 (HL16278 and HL18840) formed a distinct clade, separate from other SCCmec IV reference strains of CC8 in both allelic analysis of cgMLST and wgMLST, which is consistent with the findings of SNP-based phylogenetic tree analysis. A total of 2,009 cgMLST loci and 3,567 wgMLST loci were included in this analysis. MLST: multilocus sequence typing; SCCmec: staphylococcal cassette chromosome mec; SNP: single-nucleotide polymorphism

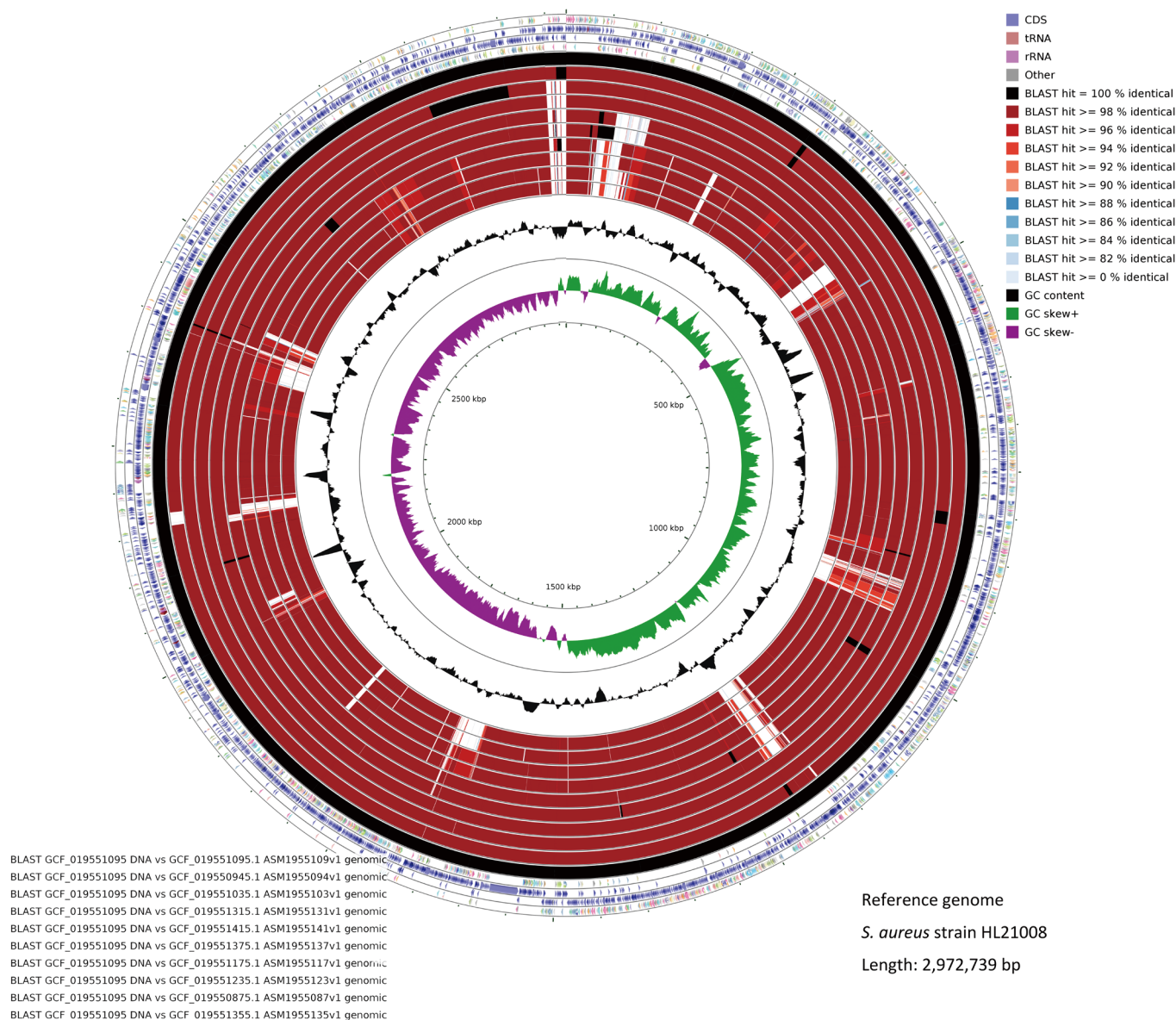


Fig. 4. Circular visualization of MRSA complete genomes with HL21008 as a reference strain. The inner genome rings showed 100% identity (black), $\geq 98\%$ (dark magenta) and $\geq 96\%$ (red) for these reference isolates, reflecting similarity percentages determined by BLAST hit. Black peaks indicate GC content, and the innermost green and purple peaks denote positive and negative GC skews, respectively. MRSA: methicillin-resistant *Staphylococcus aureus*

Antimicrobial resistance profiles of the MRSA strains

The *mecA* gene was detected in all the MRSA strains using ResFinder. The *blaZ* beta-lactamase gene was found in strains *spa* t002, t008, and t324, but was absent in the t2460 and t9353 strains of SCC_{mec} II (Table 2). All isolates were phenotypically resistant to beta-lactam antibiotics ampicillin, oxacillin, and penicillin.

There was full concordance between the phenotypic AST and genotypic AST for gentamicin (*aac(6')*-*aph(2'')*), ciprofloxacin (*griA* and *gyrA* mutations), clindamycin (*erm(A)*), erythromycin (*erm(A)* or *msr(A)*), fusidic acid (*fusC* or *fusA* (L461K)), and mupirocin (*ileS* (V588F) or *mupA*).

Table 2. Phenotypic and genotypic antimicrobial susceptibility test (AST) results of methicillin-resistant *Staphylococcus aureus* strains

Strain	HL18888	HL20835	HL17064	HL18807	HL18883	HL21008	HL17078	HL18380	HL16278	HL18840
SCC <i>mec</i> type	II	II	II	II	II	II	IV	IV	IVA	IVA
<i>spa</i> type	t002	t002	t2460	t2460	t9353	t9353	t008	t008	t324	t324
Isolation year	2016	2017	2015	2016	2016	2017	2015	2016	2014	2016
MLST ST	ST5	ST5	ST5	ST5	ST5	ST5	ST5863	ST8	ST72	ST72
MLST CC	CC5	CC5	CC5	CC5	CC5	CC5	CC8	CC8	CC8	CC8
Phenotypic resistance profile ^a	OX, PEN, AMP, AMC, AZI, CD [†] , CIP, ERY, FA [‡] , IMI, LVX, MXF	OX, PEN, AMP, AMC, AZI, CD, CIP, ERY, GM, IMI, LVX, MXF	OX, PEN, AMP, AMC, AZI, CD, CIP, ERY, FA, FOS, GM, IMI, LVX, MXF, TE	OX, PEN, AMP, AMC, AZI, CD, CIP, ERY, FA, FOS, IMI, LVX, MUP, MXF, TE	OX, PEN, AMP, AMC, AZI, CD, CIP, ERY, FA, FOS, IMI, LVX, MXF, TE	OX, PEN, AMP, AMC, AZI, CD, CIP, ERY, FA, FOS, GM, IMI, LVX, MXF	OX, PEN, AMP, AMC, AZI, CIP, ERY, GM, IMI, LVX, MUP, MXF	OX, PEN, AMP, AMC, AZI, CIP, ERY, IMI, LVX, RIF	OX, PEN, AMP, AMC, IMI	OX, PEN, AMP, AMC, IMI
Genotypic antimicrobial resistance ^b	<i>mecA</i> , <i>blaZ</i> , <i>aadD</i> , <i>erm(A)</i> , <i>tetM</i> , <i>fusC</i>	<i>mecA</i> , <i>blaZ</i> , <i>aac(6)-aph(2)</i> , <i>erm(A)</i> , <i>tetM</i>	<i>mecA</i> , <i>aac(6)-aph(2)</i> , <i>erm(A)</i> , <i>tetM</i>	<i>mecA</i> , <i>erm(A)</i> , <i>tetM</i>	<i>mecA</i> , <i>erm(A)</i> , <i>tetM</i>	<i>mecA</i> , <i>aac(6)-aph(2)</i> , <i>erm(A)</i> , <i>tetM</i>	<i>mecA</i> , <i>blaZ</i> , <i>aac(6)-aph(2)</i> , <i>mst(A)</i> , <i>mph(C)</i> , <i>mupA</i>	<i>mecA</i> , <i>blaZ</i> , <i>aph(3)-III</i> , <i>aadD</i>	<i>mecA</i> , <i>blaZ</i> , <i>aadD</i>	<i>mecA</i> , <i>blaZ</i>
Mutations in AMR genes ^d	<i>grlA</i> (S80F, E84K) + <i>gyrA</i> (S84L, S85P)	<i>grlA</i> (S80F) + <i>gyrA</i> (S84L)	<i>grlA</i> (S80F) + <i>gyrA</i> (S84L), <i>fusA</i> (L461K)	<i>grlA</i> (S80F) + <i>gyrA</i> (S84L), <i>fusA</i> (L461K)	<i>grlA</i> (S80F) + <i>gyrA</i> (S84L), <i>fusA</i> (L461K)	<i>grlA</i> (S80F) + <i>gyrA</i> (S84L), <i>fusA</i> (L461K)	<i>grlA</i> (S80Y) + <i>gyrA</i> (S84L)	<i>grlA</i> (S80Y) + <i>gyrA</i> (S84L)		

^aPhenotypic AST was performed using MicroScan WalkAway 96 Plus system, as part of the routine clinical practice. ^bAntimicrobial resistance genes were identified using ResFinder 4.4.1 ($\geq 90\%$ sequence similarity with $\geq 60\%$ minimum identity length); ^cHL18888 strain showed intermediate resistance to CD encoded by *erm(A)* and FA encoded by *fusC* gene; ^d() within parentheses detected mutations are indicated.

Abbreviations: SCC*mec*, staphylococcal cassette chromosome *mec*; *spa*, staphylococcal protein A; MLST, multilocus sequence type; ST, sequence type; CC, clonal complex; AMR, antimicrobial resistance; AMP, ampicillin; AMC, amoxicillin/clavulanate (β -lactam/ β -lactamase inhibitor combination); AZI, azithromycin; CD, clindamycin; CIP, ciprofloxacin; ERY, erythromycin; FA, fusidic acid; FOS, fosfomycin; GM, gentamicin; IMI, imipenem (β -lactam antibiotic of subgroup carbapenem); LVX, levofloxacin; MUP, mupirocin; MXF, moxifloxacin; OX, oxacillin; PEN, penicillin; RIF, rifampin; TE, tetracycline.

No antimicrobial resistance genes were detected for sulfamethoxazole-trimethoprim, fosfomycin, vancomycin, teicoplanin, quinupristin-dalfopristin, linezolid, or chloramphenicol, and all isolates were susceptible to these antimicrobial agents (supplementary Table 2 in the online-only Data Supplement).

Virulence gene profiles of the MRSA strains

A total of 26 virulence genes were identified by the VirulenceFinder (Table 3). All isolates carried aureolysin (*aur*), gamma-hemolysin chain II precursor (*hlgA*), gamma-hemolysin B precursor (*hlgB*), gamma-hemolysin component C (*hlgC*), leukocidin D component (*lukD*), leukocidin E component (*lukE*), serine protease *splA* (*splA*), or serine protease *splB* (*splB*) genes. Among the SCC*mec* II strains, toxic shock syndrome toxin-1 (*tst*) was detected in all but one strain (HL17064; *spa* t2460). No *tst* genes were found in the SCC*mec* IV and IVA strains.

The *lukF-PV* and *lukS-PV* genes, encoding Pantone-Valentine leukocidin (PVL), and the *ACME* gene, encoding the arginine catabolic mobile element (ACME), were observed only in the two SCC*mec* type IV *spa* t008 strains (HL17078 and HL18380). The SCC*mec* IV strains also showed unique virulence profiles, including enterotoxin K (*sek*), enterotoxin Q (*seq*), and serine protease *splE* (*splE*), in contrast to the SCC*mec* II and IVA strains.

Table 3. Virulence gene profiles of methicillin-resistant *Staphylococcus aureus* strains

Strain	HL18888	HL20835	HL17064	HL18807	HL18883	HL21008	HL17078	HL18380	HL16278	HL18840
SCC <i>mec</i> type	II	II	II	II	II	II	IV	IV	IVA	IVA
<i>spa</i> type	t002	t002	t2460	t2460	t9353	t9353	t008	t008	t324	t324
Isolation year	2016	2017	2015	2016	2016	2017	2015	2016	2014	2016
MLST ST	ST5	ST5	ST5	ST5	ST5	ST5	ST5863	ST8	ST72	ST72
MLST CC	CC5	CC5	CC5	CC5	CC5	CC5	CC8	CC8	CC8	CC8
Arginine catabolic mobile element	-	-	-	-	-	-	ACME	ACME	-	-
Aureolysin	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>	<i>aur</i>
Gamma-hemolysin chain II precursor	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>	<i>hlgA</i>
Gamma-hemolysin component B precursor	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>	<i>hlgB</i>
Gamma-hemolysin component C	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>	<i>hlgC</i>
Leukocidin D component	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>	<i>lukD</i>
Leukocidin E component	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>	<i>lukE</i>
Leukocidin F component	-	-	-	-	-	-	<i>lukF-PV</i>	<i>lukF-PV</i>	-	-
Leukocidin S component	-	-	-	-	-	-	<i>lukS-PV</i>	<i>lukS-PV</i>	-	-
Staphylokinase	<i>sak</i>	<i>sak</i>	-	-	-	-	<i>sak</i>	<i>sak</i>	<i>sak</i>	<i>sak</i>
Staphylococcal complement inhibitor	<i>scn</i>	<i>scn</i>	-	-	-	-	<i>scn</i>	<i>scn</i>	<i>scn</i>	<i>scn</i>
Enterotoxin C	-	<i>sec</i>	-	<i>sec</i>	<i>sec</i>	<i>sec</i>	-	-	-	-
Enterotoxin K	-	-	-	-	-	-	<i>sek</i>	<i>sek</i>	-	-
Enterotoxin Q	-	-	-	-	-	-	<i>seq</i>	<i>seq</i>	-	-
Enterotoxin G	<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>	<i>seg</i>	-	-	<i>seg</i>	<i>seg</i>
Enterotoxin I	<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>	<i>sei</i>	-	-	<i>sei</i>	<i>sei</i>
Enterotoxin L	-	<i>sel</i>	-	<i>sel</i>	<i>sel</i>	<i>sel</i>	-	-	-	-
Enterotoxin M	<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>	<i>sem</i>	-	-	<i>sem</i>	<i>sem</i>
Enterotoxin N	<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>	<i>sen</i>	-	-	<i>sen</i>	<i>sen</i>
Enterotoxin O	<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>	<i>seo</i>	-	-	<i>seo</i>	<i>seo</i>
Enterotoxin P	<i>sep</i>	<i>sep</i>	-	-	-	-	-	-	-	-
Enterotoxin U	<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>	<i>seu</i>	-	-	<i>seu</i>	<i>seu</i>
Serine protease <i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>	<i>splA</i>
Serine protease <i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>	<i>splB</i>
Serine protease <i>splE</i>	-	-	-	-	-	-	<i>splE</i>	<i>splE</i>	-	-
Toxic shock syndrome toxin-1	<i>tst</i>	<i>tst</i>	-	<i>tst</i>	<i>tst</i>	<i>tst</i>	-	-	-	-

Abbreviations: SCC*mec*, staphylococcal cassette chromosome *mec*; *spa*, staphylococcal protein A; MLST, multilocus sequence type; ST, sequence type; CC, clonal complex.

Discussion

Key results

In the present study, the genomes of 10 clinical MRSA isolates were sequenced using short-read-based MiSeq and long-read-based MinION platforms, and hybrid assembly was performed to assemble the complete MRSA genome sequences and understand the genome structures of representative Korean MRSA strains. Furthermore, WGS analysis of these MRSA strains provided a comprehensive overview of their genetic characteristics (such as genome size, CDS number, and GC content), highlighted differences in virulence factors and antimicrobial resistance genes, and identified molecular epidemiological markers corresponding to the SCC*mec* and/or *spa* types.

Interpretation/comparison with previous studies

Investigations of complete bacterial genome sequences have been conducted to understand the full complement of genetic elements and the genome structure of major pathogens, such as *S. aureus*, *E. coli*, and *Helicobacter pylori* [10,12,23]. The availability of whole-genome sequences not only provides information on genetic variations and pathogenicity factors but is also valuable for the investigation of metabolic networks, drug development, and evolution [24-26]. In addition, the complete genome sequences of pathogens are the basis for mutant analysis [26,27], metabolic system analysis [24], and precise comparative genomic analysis [15].

We obtained the complete genomes of 2,038 *S. aureus* strains isolated worldwide from the NCBI genome database. Our search of this database, using the filters of “Korea” or “Seoul” (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=1280&assembly_level=3:3. Accessed 2025-05-08), revealed only five human MRSA isolates, two methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates, and four *Bos taurus* (cattle) MSSA isolates from Korea, excluding the MRSA genome sequences submitted by our group. Four of the five human MRSA strains were isolated in 2011 and 2013.

The genome sizes of the MRSA strains obtained herein ranged from 2.7 to 2.9 Mb, with an average GC content of 32.88% (Table 1), which is consistent with previous published data. Genome size variation probably reflects differences in the presence or absence of mobile genetic elements, virulence factors, and other accessory genetic elements [8,15,23]. The average MRSA chromosomal lengths observed herein were 2.916 Mb in SCCmec II, 2.920 Mb in SCCmec IV, and 2.777 Mb in SCCmec IVA, respectively. SCCmec IVA-ST72-*spa* t324, a prevalent community-associated MRSA lineage, tends to have a smaller genome than healthcare-associated (i.e., SCCmec II) and community-associated strains (i.e., SCCmec IV). This was because of the smaller size of the SCC regions and the absence of several regions throughout the genome, as indicated by the circular map generated following CGH analysis (Fig. 4).

Genome analysis of global ST72 isolates showed that the ST72 MRSA and MSSA lineages have evolved differently in Asia [8]. Phylogenetic analysis with SNPs and recombinant-free alignment showed that MLST ST72 (CC8) MRSA, a Korean CA-MRSA lineage, was placed in a different clade from other representative CC8 strains (Fig. 2). HL16278, HL18840, and HL1 of SCCmec IVA-ST72 (CC8) were genetically distinct from other SCCmec IV strains of CC8 (i.e., HL17078, HL18380, USA300_FPR3757, and TW20) despite sharing SCCmec elements and four alleles of the MLST genes ST72 (1-4-1-8-4-4-3) and ST8 (3-3-1-1-4-4-3). These ST72 strains of SCCmec IVA were close to strain11819-97 (SCCmec IVA-ST80); however, the MLST profiles of ST72 (1-4-1-8-4-4-3) and ST80 (1-3-1-14-11-51-10) shared only two alleles. The differences in the genetic background of MLST and SNPs analyzed by WGS suggested that ST72 (CC8) evolved from a genetically distinct lineage from the CC8 strains of other CA-MRSA representative strains, such as the USA300 clone, irrespective of their similarity to the ST and CC types.

Complete genome sequencing, SCCmec typing, and MLST analysis suggest that this successful CA-MRSA lineage may have evolved independently from other strains with similar SCCmec types, highlighting the importance of genome studies for epidemiological surveillance. Further studies on bacterial genomes are needed to understand the epidemiology and molecular characteristics of the successful CA-MRSA ST72

lineage in Korea and other neighboring countries.

Plasmid analysis revealed that most MRSA strains possessed a single or no plasmid, whereas SCCmec IV strains ($n = 2$) harbored two or three plasmids. Small plasmids (< 10 kb) were found only in the SCCmec IV and IVA strains ($n = 3$; 3,125 bp, 6,118 bp, and 3,332 bp), whereas the SCCmec II strains all had a plasmid > 25 kb in size. The mechanism of replication of plasmids differs based on their size: plasmids smaller than 10 kb generally use the rolling circle mechanism, whereas plasmids larger than 14.5 kb replicate using the theta-mode [28]. The biological significance of plasmid size variations among strains remains to be elucidated.

Whole-genome variability using comparative genome analysis was determined by Roary analysis of core and accessory genes. The entire gene pool comprised of 3,661 genes, of which 2,204 were core genes and 1,457 were accessory genes. Large core gene numbers may reflect a high degree of conservation of genes required for the maintenance of essential metabolic mechanisms, virulence, and antimicrobial resistance. In the present study, the presence or absence of genes was used to characterize the MRSA strains (supplementary Table 1 in the online-only Data Supplement); however, further studies with more MRSA strains are needed to understand the general gene pools and genetic plasticity of MRSA in Korea.

For phylogenetic analysis of various representative MRSA strains, we used the Snippy, Gubbins, and RAxML-NG tools. Whole-genome SNP-based and phylogenetic analyses revealed that the MRSA strains clustered into three distinct clades according to their SCCmec types, and into very close clades according to their *spa* types (Fig. 2). Interestingly, the Korean SCCmec IV and SCCmec IVA strains with the same clonal complex clustered into distinct clades, indicating genomic divergence (Fig. 4 and supplementary Fig. 1 in the online-only Data Supplement).

Single-nucleotide polymorphism-based analysis using Snippy provides the highest resolution for phylogenetic reconstruction by identifying single-nucleotide differences in the core genome relative to the reference genome. By contrast, cgMLST and wgMLST offer allele-based typing approaches that are portable and scalable for large-scale surveillance. While cgMLST focuses on conserved core loci, wgMLST includes both core and accessory genes, providing broader genome coverage but potentially more missing data. We analyzed these methods to provide complementary insights into the genetic relatedness and evolutionary relationships of *S. aureus* strains.

Studies have shown that a high level of synteny (gene order) is retained among different staphylococcal strains, but intraspecific homologous recombination occasionally occurs [13,29]. In the present study, complete whole genomes allowed Mauve analysis to indicate a relatively well-conserved synteny among most MRSA strains (Fig. 1), although evidence of intraspecific recombination and the presence or absence of accessory genes were observed, particularly between the 0.9 Mb and 2.2 Mb regions of MRSA genomes. The findings obtained herein indicate that frequent structural variations occur primarily in specific genomic regions, as revealed by analysis of complete MRSA genomes. Interestingly, a large genomic inversion was observed in the HL17064 strain. Although substantial information on virulence, epidemiological markers, and resistance genes can be obtained using short-read sequencing[1], complete genome assembly is required to fill the gap in contigs for the identification of precise structural variations and synteny.

Comparative genome hybridization analysis showed genetic homology among the MRSA strains (Fig. 4)

and the absence of some genetic elements (i.e., lack of *psm-mec*, *mecI*, and part of *mecR*) near the *SCCmec* regions, especially in the *SCCmec* IV and IVA strains (supplementary Fig. 1 in the online-only Data Supplement), is a well-known characteristic of *SCCmec* type IV and IVA strains [30]. We also observed the absence of the *cst* operon (*cstB* and *cstR*), an essential element for sulfide detoxification [31,32] and an erythromycin-resistance gene (*ermA*) adjacent to the *SCCmec* region (data not shown). The absence of specific genes near the *SCCmec* regions in the *SCCmec* IV and IVA strains suggests evolutionary events that could affect their pathogenicity, adaptation to hospital environments, and antimicrobial-resistance phenotypes [30].

The phenotypic and genotypic AST results of the studied isolates showed 100% concordance with the antimicrobials tested (Table 2).

The MRSA strains were analyzed for the presence of genes encoding 26 different virulence factors using VirulenceFinder (Table 3). All MRSA strains possessed *aur*, *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *splA*, and *splB*, regardless of the *SCCmec* or *spa* type, suggesting a core set of virulence factors among the MRSA clinical isolates. The gamma-hemolysin (*hlgAB*, *hlgCB*) locus is present in ~99% of sequenced *S. aureus* genomes, whereas the leukocidin (*lukED*) locus is present in ~70% of *S. aureus* isolates and is conserved in a lineage-specific manner [33]. Both *SCCmec* IV *spa* t008 strains harbored PVL- and ACME-encoding genes. Such strains are infrequently isolated in Korea [34,35] and exhibit virulence gene profiles that are different from those of the *SCCmec* II and IVA strains, indicating distinct pathogenic and genomic characteristics. The *SCCmec* IV strains, in contrast to *SCCmec* IVA, showed distinct genomic characteristics, suggesting different origins from other countries. The toxic shock syndrome toxin-1 gene (*tst*) was associated with five of the six *SCCmec* II strains, but not with the *SCCmec* IV or IVA strains, suggesting differences in the pathogenic potential of the MRSA strains according to their *SCCmec* types [36].

Complete genome sequences could provide insight into the regulation of antimicrobial resistance and virulence mechanisms, and identify networks of genes/operons that are coordinately expressed [26], especially for experiments with specific reference or clinical isolates. In addition, reference genomes are necessary for RNA sequencing to obtain a global view of gene expression [37,38]. Such transcriptomic analyses can be useful for investigating bacterial responses to different environmental stresses, such as antimicrobial agents, and for developing novel methods to inhibit MRSA infections [38]. We believe that the complete genomes of Korean MRSA clinical strains would be useful for the investigation of MRSA antimicrobial resistance, gene function, and gene regulation in RNA-seq studies of these clinical isolates.

Limitations

Although the aim of the present study was to determine the complete genomes of the prevalent Korean MRSA strains, only a limited number of isolates from a single hospital were analyzed. Follow-up studies on a broader and more diverse collection of MRSA strains are required to fully explore the genomic diversity of clinical MRSA isolates.

Conclusions

Complete circular bacterial genome sequences have advantages over fragmented genomes with gaps, as they provide precise genetic information, intact genomic structures, and reference templates for gene-expression studies, such as RNA-seq. These complete genomes of representative clinical MRSA strains provide comprehensive and detailed genomic knowledge and are essential for accurate epidemiological and evolutionary studies on *S. aureus*.

Supplementary Materials

The following supplementary materials are available on the journal's website:

- **Supplementary Table 1.** The list of genes in the MRSA study isolates. The number 1 means presence and number 0 means absence of the gene
- **Supplementary Table 2.** Antimicrobial susceptibility of MRSA strains in this study
- **Supplementary Figure 1.** The *mecA* and adjacent regions of the MRSA strains in the present study. SCC*mec* type IV strains (inner four circles) showed the absence of phenol-soluble modulins (*psm-mec*), *mecI*, and part of *mecR* in the SCC*mec* region, and some genes, such as *cstB* and *cstR*, for sulfide detoxification were absent when compared with the corresponding genes in the SCC*mec* type II strain HL21008. MRSA: methicillin-resistant *Staphylococcus aureus*; SCC*mec*: staphylococcal cassette chromosome *mec*.

Ethics statement

The present study was approved by the Institutional Review Board of Kangdong Sacred Heart Hospital (NON2023-001).

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Data availability

Dataset 1. Complete genome sequence data for MRSA strains have been deposited in the NCBI database with BioProject PRJNA693997

Additional datasets generated during the current study are available from the corresponding author upon request.

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