Original Article

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Predominance of the ST22-MRSA-IV-t32 Clone and Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Hospital in Thailand

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Abstract:

Objective: This study aimed to determine the molecular characteristics of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains in a tertiary hospital in Bangkok, Thailand.

Material and Methods: Twenty-one MRSA isolates were collected from clinical specimens; between December 2022 and May 2023. These isolates were characterized by determining their antimicrobial susceptibility profiles, Staphylococcal Cassette Chromosome *mec* and staphylococcal protein A types, multilocus sequence types, biofilm-forming abilities and whole-genome sequences from six representative strains.

Results: ST22-MRSA-IV-t032 was the most common clone (57.1%), followed by ST22-MRSA-IV-t628, ST22-MRSA-IV-t1467, ST5-MRSA-II-t21248, ST3976-MRSA-IV-t32, ST764-MRSA-II-t045, ST8502-MRSA-V-t21247; ST1-MRSA-IV-t1784, ST188-MRSA-IV-t189 and ST8-MRSA-IV-t008 (4.8% each). Most (66.6%) MRSA strains produced a strong biofilm: 28.6% produced a moderate biofilm and 14.8% produced a weak biofilm. When the strains were divided into

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two groups (ST22 and non-ST22), ST22 strains had a greater ability to produce biofilm than non-ST22 strains. All six genomes carried the exoenzyme aureolysin gene, hemolysin-associated *hlg*A, *hlg*B, and *hlg*C genes, and host-immune system modulator genes; including a staphylokinase and staphylococcal complement inhibitor.

Conclusion: This study revealed that ST22-MRSA-IV-t32 was the dominant clone related to the ST22 EMRSA-15 epidemic in Europe. These results emphasize the necessity of continuous surveillance for effective management and control measures to understand microbial infections and their patterns of antibiotic resistance, as well as to identify the emergence of new clones.

Keywords: antibiotic resistance, genome, MLST, MRSA, SCC*mec* typing

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a known superbug that causes serious infections[1]. An important risk factor for hospital infections is the widespread use of central venous catheters, heart valves, artificial lenses, and prosthetic joints. Colonization with MRSA not only increases the risk of MRSA infection but is also linked to invasive MRSA infections[2]. MRSA develops from Methicillin-susceptible *Staphylococcus aureus*, via staphylococcal cassette chromosome *mec (*SCC*mec)* gene transfer, which contains the *mec*A or *mec*C gene. Then, MRSA confers resistance to most β-lactam antibiotics[1]. Additionally, MRSA is often resistant to other antibiotic classes. *S. aureus*; including MRSA, and has demonstrated a concerning ability to develop resistance to many common antibiotics. Although certain antibiotics, like vancomycin, carbapenems, and select beta-lactam, remain crucial treatment options, their long-term effectiveness is threatened by the continued spread of antibiotic resistance. This, in turn, impacts the current and future treatment options for this pathogen[1]. *S. aureus* infections involve the production of major virulence factors; such as Panton-Valentine leukocidin *(PVL)* and toxic shock syndrome toxin 1 (*TSST*-1), that manifest as clinical symptoms. *PVL* destroys white blood cells (such as neutrophils and monocytes) and accelerates apoptosis as well as tissue necrosis. *TSST*-1, a superantigen, induces host T-cells and macrophages to release proinflammatory cytokines, resulting in toxic shock syndrome. This manifests as high fever, hypotension, rash, hypovolemic shock, and end-organ damage[1,2]. Therefore, this study aimed to determine the occurrence of these two major virulence factors among the studied strains.

The genotype of *S. aureus* influences the severity, complications, and mortality in patients. Presently, various molecular subtyping approaches; including SCC*mec*, staphylococcal protein A *(spa),* and multilocus sequence typing (MLST), have been developed to characterize and elucidate the molecular evolution and relationships of *S. aureus*[3]. In Thailand, sequence type 239 (ST239)-MRSA-SCC*mec* type III[4], ST5-MRSA-SCC*mec* type II[4], ST764-MRSA-SCC*mec* type II[5] and ST9-MRSA-SCC*mec* type IX[6] have been isolated from clinical samples. In our previous study *spa* typing was used to identify the prevalence of major clones among 16 MRSA strains, with *spa* type numbers t032, t088, t001, t008, t034, t439, and t1928 being detected[7]. However, using molecular techniques to determine the clonal relationships of MRSA in our hospital has not been conducted. To improve our understanding of the clonal lineage characteristics of MRSA isolated from our patients, we also aimed to determine the diversity, relationships and epidemiological characteristics of isolated MRSA strains during a 6-month period in a single university hospital. Different MRSA clinical isolates

were compared by SCC*mec* typing, MLST, *spa* typing, and antibiotic resistance profiling. Additionally, whole-genome sequencing (WGS) for six representative isolates having different ST types was performed.

Material and Methods

Ethics approval

This study was approved by the institutional ethical review board of the Faculty of Medicine, Vajira Hospital, Navamindradhiraj University (Bangkok, Thailand) (COA013/2566).

Bacterial isolates, identification, and antimicrobial susceptibility testing

Overall, 21 nonrepetitive isolates of MRSA from blood, sterile body fluid, tissue, and wounds were collected at the Central Laboratory and Blood Bank, Faculty of Medicine, Vajira Hospital, from December 2022 and May 2023. Bacteria were first identified to the species level using a MALDI BioTyper (Bruker, Daltonik GmbH, Bremen, Germany). The studied isolates were stored in Soyabean Casein Digest Medium (TSB; HIMEDIA®, Mumbai, India), at 4 °C at the Department of Clinical Pathology, Faculty of Medicine; Vajira Hospital until further use.

In vitro antimicrobial susceptibility testing was conducted using a BD Phoenix PMIC/ID-95 commercial kit (Becton Dickinson Diagnostic Systems, Sparks, Maryland, USA) on the BD Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems, Spark, Maryland, USA) according to the manufacturer's instructions. Determination of the minimum inhibitory concentration breakpoint was performed according to the Clinical and Laboratory Standards Institute guidelines. The test antimicrobials included: cefoxitin (FOX: 2–8 µg/mL), linezolid (LZD: 1–4 µg/mL), trimethoprim-sulfamethoxazole (SXT: 1/19 – 4/76 µg/mL), ciprofloxacin (CIP: 0.5–2 µg/mL), levofloxacin (LVX: 1–4 µg/mL), clindamycin (CC: 0.5–2 µg/mL), erythromycin (E: 0.25–4 µg/mL), gentamicin (GM: 2–8 µg/mL); vancomycin (VA: 1–16 µg/mL), teicoplanin (TEC: 1–16 µg/mL), nitrofurantoin (NIT: 16–64 µg/mL); minocycline (MIN: 1–8 µg/mL), tetracycline (TE: 0.5–8 µg/mL), doxycycline (DOX: 0.5–8 µg/mL) and daptomycin (DAP: 1–4 µg/mL). *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were the control strains.

Genomic deoxyribonucleic acid (DNA) extraction

The MRSA isolates were grown on brain heart infusion agar (BHI; HIMEDIA®, Mumbai, India) for 24 hr, and the DNA of each strain was extracted using the Presto™ Mini gDNA Bacteria Kit Quick Protocol (Geneaid Biotech Ltd., Shijr District, New Taipei City, Taiwan ROC). The extracted DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Polymerase chain reaction (PCR) of *fem*A, *mec*A, *pvl*, and *tsst*-1

To identify MRSA isolates, PCR amplification using *fem*A, *mec*A, *pvl*, and *tsst*-1-specific primer pairs was performed. The PCR reaction mixture contained 0.4 µM of each primer, 1X GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), nuclease-free water, and genomic DNA (final volume, 25 µL). PCR was performed using the following program: initial denaturation at 95 °C for 5 minutes (min); 35 cycles of amplification at 95 °C for 1 min; annealing at 55 °C for 45 seconds (s); extension at 72 °C for 45 s; and a final extension at 72 °C for 5 min on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). Overall, 5 µL of PCR products were electrophoresed on a 1.5% agarose gel in 1X TAE buffer containing FluoroSafe DNA stain (Axil Scientific Pte Ltd), and photographed using a Gel Doc XR+ system (Bio-Rad).

MLST

All isolates were PCR and sequenced using primers specific for the MLST scheme of *S. aureus* using the PubMLST database (https://pubmlst.org/)[8,9]. The MLST scheme amplifies sequences at the following genetic loci: carbamate kinase *(arc*C*),* shikimate dehydrogenase *(aro*E*),* glycerol kinase *(glp),* guanylate kinase *(gmk),* phosphate acetyltransferase *(pta),* triosephosphate isomerase *(tpi)* and acetyl coenzyme A acetyltransferase *(yqi*L*)*[9]. The PCR reaction mixture was generated as previously described. PCR was performed and PCR products were electrophoresed as described above. Then, all PCR products were purified, and bidirectionally sequenced using specific forward and reverse primers of each gene at 1st BASE DNA Sequencing (Apical Scientific Sdn Bhd, Malaysia). The sequence files were analyzed using BioEdit software (https://www. mbio.ncsu. edu/bioedit/bioedit.html) and compared with sequences in the PubMLST database using the: "Typing" platform (https://pubmlst.org/bigsdb?db=pubmlst_saureus_seqdef). Allelic profiles (allele type number of seven loci) defined the corresponding sequence types (STs). The primers used for the amplification of the MLST genes are listed in Supplementary Table 1. The relationship between the *S. aureus* STs in this study and the globally reported strains was analyzed using the eBURST (goeBURST) algorithm[10].

SCC*mec* typing

Two multiplex PCR, adapted from the protocol of Yamaguchi et al., were used to determine SCC*mec* type-specific primers: as listed in Supplementary Table 1[11,12]. Multiplex PCR 1 (M1) was used to determine the ccr gene complex type, while Multiplex PCR 2 (M2) was used to determine the *mec* gene complex class. Each PCR reaction mixture (50 µL final volume) was generated as previously described. PCR was performed under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 60 s, 57 °C (M1) or 60 °C (M2) for 45 s, and 72 °C for 60 s; and a final extension of 5 min at 72 °C. All PCR products were electrophoresed on a 1.5% agarose gel. *S. aureus* NCTC10442 SCC*mec* type I (carrying type 1 of *ccr* gene complex type (*ccr*A1-*ccr*B1) and class B of *mec* gene complex class (*mec*A-IS1272); *S. aureus* N315 SCC*mec* type II (carrying type 2 of *ccr* gene complex type [*ccr*A2-*ccr*B2] and class A of *mec* gene complex class [*mec*A-*mec*I]); *S. aureus* 85/2082 SCC*mec* type III (carrying type 3 of *ccr* gene complex type (*ccr*A3-*ccr*B3) and class A of *mec* gene complex class). *S. aureus* 81/108 SCC*mec* type IVc (carrying type 2 of *ccr* gene complex type (*ccr*A2-*ccr*B2) and class B of *mec* gene complex class) were used as positive controls.

Spa typing

Spa typing was conducted by PCR amplification, with the *spa*-1113F and *spa*-1514R primes (available at http://www.spaserver.ridom.de)[13]. The PCR reaction mixture was generated as previously described, and PCR was performed under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 60 s, 60 °C for 45 s, and 72 °C for 45 s; and a final extension of 5 min at 72 °C. All PCR products were electrophoresed, sequenced, and primarily analyzed as described above. The *spa* types were identified and grouped with *spa*Typer 1.0 (https://cge.food.dtu.dk/services/spaTyper/)[14].

Biofilm formation test

Overall, 21 MRSA strains were cultured overnight at 37 °C, and 0.5 McFarland bacterial suspensions were prepared with Tryptic Soy Broth (TSB). In total, 200 µL of the prepared bacterial suspension was added to each well of a 96-well microplate, with 10 parallel wells prepared for each strain. TSB without bacterial suspension was used as the blank control. The 96-well microplate was incubated at

37 °C for 24 hr. The supernatant was discarded, and the 96-well plate was washed twice with phosphate-buffered saline. The biofilm that adhered to the microwells was fixed with 200 µL of methanol for 15 min and then stained with 200 µL of 1% crystal violet stain for 5 min at room temperature. The crystal violet was then removed, and the wells were slowly washed twice with distilled water. Each well was decolorized by adding 200 µL of 95% ethanol for 45 min. Then 150 L of the solution was transferred to another microplate to measure the absorbance at 570 nm using a spectrophotometer. The value obtained was compared with the cut-off optical density (OD) value. The cut-off OD (ODnc) was defined as the standard deviation above the mean OD of the negative control. Strains were interpreted as follows: nonbiofilm producers (OD ≤ODnc), weak biofilm producers (ODnc <OD ≤2 × ODnc), moderate biofilm producers (2 × ODnc <OD ≤4 × ODnc) and strong biofilm producers (4 × ODnc <OD).

Whole genome sequencing and analysis

WGS of six representative isolates having different ST types was performed using a short-read, paired-end sequencing strategy on the Illumina platform model Nextseq550 at CELEMICS (Celemics, Inc., Seoul, Korea), by U2Bio (Thailand) Co., Ltd. (Bangkok, Thailand). The obtained sequencing reads were trimmed and filtered using AfterQC software (version 0.9.6). De novo Genome assembly was performed by Unicycler (version 0.5.0), and the quality of the assembled genomes was evaluated using quality assessment tool for genome assemblies (version 5.0.2). The estimates of genome completeness and contamination were performed by CheckM (version 1.1). The assembled genomes were annotated using Prokka (version 1.14.6). The genome-based taxonomy was identified by the Type (Strain) Genome Server (TYGS) (https://tygs.dsmz.de/). The assembled contigs were analyzed using ResFinder 4.3.3 via http://genepi.food.dtu.dk/resfinder to identify the AMR genes with: i) chromosomal point mutations, ii) acquired AMR genes, and iii) disinfectant (90% of the threshold for %ID and 60% of the select minimum length)[15]. Virulence genes were identified by VirulenceFinder 2.0 via https://cge.food.dtu.dk/services/VirulenceFinder/, with 90% of the minimum percentage of nucleotides that are identical between the best matching virulence gene in the database in addition to the corresponding sequence in the genome and 60% of the select minimum length (the number of nucleotides a sequence must overlap a virulence gene to count as a hit for that gene). MLST 2.0 (https://cge.food.dtu.dk/services/MLST/) was used to perform MLST of *S. aureus* assemblies[14,16-22]. Clusters of orthologous groups (COGs) were assigned to essential genes using Eggnog Mapper (v2) (http://eggnog-mapper.embl.de/).

Data availability

Sequence reads were deposited in the national center for biotechnology information as BioProject ID: PRJNA1173083 and BioSample accessions were: SAMN44287465, SAMN44287466, SAMN44287467, SAMN44287468, SAMN44287469, SAMN44287470 corresponding to VAJIRA-MRSA001, 011, 016, 020, 035 and 040, respectively.

Results

Strains, identification, and *mec*A detection

Overall, 21 isolates of *S. aureus* were collected between December 2022 and May 2023 (Table 1). *S. aureus* was recovered from different specimen types; including wound/pus specimens (eight isolates), blood (six isolates), tissue (four isolates), abdominal fluid, sputum and peritoneal dialysis (one isolate). The isolates were first identified as *S. aureus* using a MALDI BioTyper, and then confirmed by *fem*A gene amplification. For MRSA confirmation, the *mec*A gene was detected in all studied strains by conventional PCR.

Antimicrobial susceptibility testing

Based on the cefoxitin disk diffusion test, all 21 isolates were resistant to methicillin, whilst according to PCR, all isolates were positive for *mec*A. To assess the antibiotic resistance pattern MRSA isolates were subjected to broth microdilution testing (commercial kit), with the pattern determined using 15 antibiotics. Of the 21 MRSA isolates, 95.2% (n=20) were resistant to macrolide (E), lincosamides (CC), and fluoroquinolones (LVX or CIP) ;additionally 4.7% (n=1) were resistant to folate pathway inhibitors (SXT), lincosamides (CC), macrolide (E) and TE. Thus, all MRSA isolates were multidrug-resistant (MDR, defined as nonsusceptibility to at least one agent in three or more antimicrobial categories not including beta-lactams)[1,23]. According to the results (Table 2), the MRSA isolates were resistant to LVX (95.2%), CIP (85.7%), GM (9.5%, TE (4.7%) and SXT (4.7%), whereas they were susceptible to Vancomycin, Nitrofurantoin, Teicoplanin and Linezolid. Three isolates (14.2%) were positive for the D-test, indicating the presence of the inducible CC resistance ($iMLS_B$) phenotype. The proportion of strains with simultaneous resistance patterns was 71.4% (n=15/21) for five antibiotics (FOX, CC, E, LVX, CIP), 9.5% (n=2/21) for four antibiotics (FOX, CC, E, LVX), 4.7% (n=1/21) for seven antibiotics (FOX, CC, E, LVX, CIP, GN, MIN), 4.7% (n=1/21) for five antibiotics (FOX, CC, E, TE, SXT), and 4.7% (n=1/21) for seven antibiotics (FOX, CC, E, LVX, CIP, GN, MIN) (Table 1).

Presence of *pvl* and *tsst*-1

Among the 21 strains, *pvl* was detected in 2 isolates (VJR-MRSA040 and VJR-MRSA041). *Pvl* and *tsst*-1 were present in only VJR-MRSA041 (Table 1).

SCC*mec* typing, MLST, and *spa* typing

Based on the results of SCC*mec* typing, 3 types were identified within the 21 MRSA isolates; including types IV (85.7%, 18/21), II (9.5%, 2/21), and V (4.8%, 1/21).

The MLST data revealed that the 21 isolates of *S. aureus* strains included eight STs and four clonal complexes. The most frequent ST was ST22 (66.7%, 14/21), with the remaining STs (ST1, ST5, ST8, ST188, ST764, ST3976 and novel ST8502) in one isolate each. The prediction from goeBURST (Figure 1) showed that our MRSA isolates were clustered into four major groups consisting of: CC1, CC5, CC8, and CC22. The first three were more closely linked evolutionarily, whereas CC22 was considered an out-group. The predominant CC was CC22 (71.4%; ST22 and ST3976), followed by CC5 (14.3%; ST5, ST8502, and ST764), CC1 (9.5% ; ST1 and ST188) and CC8 (4.8%; ST8). Characterization of the novel ST8502 (VJR-MRSA020) contained the novel *yqi*L allele number 1116, which has a 1 nucleotide difference compared with a close match to *yqi*L allele number 453, at position 361T-361C. VJR-MRSA020 was assigned to ST8502 (*arc*C-*Aro*E-*glp*F-*gmk*-*pta*-*tpi*-*yqi*L: 1-4-1-4-12-1-1116) belonging to CC5, with ST5 as founder ST (Figure 1).

Six *spa* types were identified by *spa* typing (Table 1). Among these, t032 was the most prevalent (61.9%, 13/21), with the others found in only one isolate each (t008, t045, t189, t628, t1467, t1784, t21247 and t2148). In this study, VJR-MRSA001 and VJR-MRSA020 contained the novel *spa* types t21248 (26-23-17-34-23-17-34-17-20-17-12-12-17-17-16) and t21247 (14-17-34-17-20-17-12-16), respectively.

Based on the MLST-SCC*mec* typing-spa typing, the most prevalent MRSA type strain in our study was ST22-MRSA-IV-t032 (12 strains of ST22-MRSA-IV), followed by ST22-MRSA-IV-t628 (1/14 of ST22-MRSA-IV) and ST22-MRSA-IV-t1467 (1/14 of ST22-MRSA-IV). ST1-MRSA-IV-t1784 (VJR-MRSA027), ST5-MRSA-II-t21248 (VJR-MRSA001: novel *spa* type), ST8-MRSA-IV-t008 (VJR-MRSA040), ST188-MRSA-IV-t189, ST764-MRSA-II-t045 (VJR-MRSA016), ST3976-MRSA-IV-t032 (VJR-MRSA011) and ST8502-MRSA-V-t21247 (novel ST and novel *spa* type).

Biofilm formation

We also evaluated all 21 MRSA strains for their capacity to produce biofilms and compared ST22 strains, which were the most common in this study, with non-ST22 strains. Semi-quantitative biofilm formation tests were conducted. Most (66.6%, 14/21) of the strains produced a strong biofilm, while 28.6% (6/21) and 14.8% (1/21) produced moderate and weak biofilms, respectively. When the strains were divided into the ST22 and non-ST22 groups, ST22 strains exhibited a greater ability to produce biofilm than non-ST22 strains (Figure 2). Thus, there was a significant difference between ST22 and non-ST22 strains in the ability to produce biofilm.

Genome analysis

To understand the genetic background of different STs circulating in our hospital, WGS was performed on six different ST strains; including ST5-MRSA-II-t21248 (VJR-MRSA001), ST3976-MRSA-IV-t032 (VJR-MRSA011), ST764-MRSA-II-t045 (VJR-MRSA016), ST8502-MRSA-V-t21247 (VJR-MRSA020), ST22-MRSA-IV-t1467 (VJR-MRSA035), and ST8-MRSA-IV-t008 (VJR-MRSA040). The selected genomic DNA of *S. aureus* isolates was successfully sequenced on the illumina sequencing platform. The genome-base taxonomy identified by TYGS and the TYGS was *S. aureus* DSM 20231 for all six strains[24]. Among the *S. aureus* isolates, VJR-MRSA016 had the largest genome size (2,886,742 bp) however, MRSA 001 had the highest percentage of G+C contents. The number of protein-coding sequences in the studied strains varied from 2,557 (VJR-MRSA035) to 2,706 (VJR-MRSA016) (Table 3). A whole-genome comparisons between the six MRSA genomes and the reference genomes were generated against the ST22-SCC*mec* type IV *S. aureus* subsp. aureus HO 5096 0412 (HE681097.1) genome using Proksee (https://proksee.ca), by JSON with the CGViewBuilder script, based on BLAST sequence similarities using the BLAST+ 2.12.0 tool[25,26]. In the circular genome, each genome is indicated by a different color shade, and the darker areas show 100% sequence similarity with the reference genome, whereas the lighter areas show different degrees of sequence similarity, from 98.0% to 82.0% (Figure 3). The map revealed a high degree of conserved genomic regions between two MRSA genomes (ST22-MRSA-IV-t1467 (VJR-MRSA035) and ST3976-MRSA-IV-t032 (VJR-MRSA011)) and *S. aureus* subsp. aureus HO 5096 0412 genome (ST22-SCC*mec* type IV), respectively. The ST8502-MRSA-V-t21247 (VJR-MRSA020) genome had several gaps, with a white color than the other genomes, reflects regions present in the reference genome but absent in the draft genome. ST22 and ST3979 belong to the same clonal complex (CC22, which includes ST22); meanwhile, ST764 belongs to CC5 (which includes ST5 and ST8502). The comparison of the draft genome assemblies is summarized in Table 3 and Figure 3.

COG analysis

Assigned COGs provide insights into the dominant essential gene functions. Each COG comprises homologous sequences that can be used to deduce the functions of proteins. The COG database was divided into 25 parts according to function. The COG categories in the genome of MRSA were divided into five main domains: 1) Information storage and processing, 2) Cellular process and signaling, 3) Metabolism, 4) Poorly characterized, and 5) Ambiguously characterized. Overall, 2508, 2527, 2584, 2550, 2443, and 2544 genes of VJR-MRSA001, –011, –016, –020, –035, and –040, respectively, were annotated and classified into 19 functional groups. No genes were allocated to the chromatin structure and dynamics, extracellular structure, cytoskeleton, nuclear structure or mobility. The COG categories most highly enriched for essential genes were category S (Function unknown). Moderately enriched COG categories (150–200 genes) were Category E (Amino

acid transport and metabolism), Category J (Translation, ribosomal structure and biogenesis) and Category K (Transcription). Genes that were poorly characterized or have no COG annotation accounted for 166, 171, 179, 182, 159, and 172 genes of the essential gene set of VJR-MRSA001, –011, –016, –020, –035 and –040, respectively. Ambiguously characterized groups show the number of essential genes that have been assigned to more than one COG category (Figure 4 and Supplementary Table 2).

Virulence factor-encoding genes

The six MRSA genomes were analyzed, and the secondary metabolite biosynthetic gene clusters were identified using antibiotics & secondary metabolite analysis shell version 6.2; revealing aureusimine, kijanimicin, staphyloferrin A, staphyloferrin B and staphyline in all strains. The virulence genes were then analyzed using VirulenceFinder 2.0, which revealed different virulence factors (Figure 5 and Supplementary Table 3) classified as exoenzyme genes, toxin genes and host-immune system modulators. All six MRSA genomes contained identical virulence factors for exoenzyme genes, i.e., aur, for toxin genes, i.e., gamma-hemolysin chain II precursor *(hlg*A*)*, gamma-hemolysin component B precursor *(hlg*B*)* and gamma-hemolysin component C *(hlg*C*)*, for host-immune system modulators: i.e., sak and scn. In the exoenzyme genes profile, serine protease *spl*A and serine protease *splB* were found in VJR-MRSA001, –016, –020, and –040. Only VJR-MRSA040 contained serine protease *spl*E. Regarding the toxin gene profile, leukocidin D component *(luk*D*)* and leukocidin E component *(luk*E*)* were found in VJR-MRSA001, –016, –020, and –040. Only VJR-MRSA040 contained the PVL F *(luk*F–PV*)* and S *(luk*S–PV*)* components. The six MRSA enterotoxin genes are shown in Figure 5 and Supplementary Table 1; enterotoxin G *(seg)*, enterotoxin I *(sei)*, enterotoxin M *(sem)*, enterotoxin N *(sen)*, enterotoxin O *(seo)* and enterotoxin U *(seu)* were found in VJR-MRSA001, –011, –016, –020, and –035, enterotoxin B *(seb)* was found in VJR-MRSA016 and –020, enterotoxin D *(sed)*, enterotoxin J *(sej)*, and enterotoxin R *(ser)* were found only in VJR-MRSA020; enterotoxin P *(sep)* was found only in MRSA 001, and enterotoxin K and enterotoxin Q were found only in VJR-MRSA040. Regarding the last virulence profile, host-immune system modulators, only VJR-MRSA040 differed from the rest due to the presence of an arginine catabolic mobile element (ACME) in its genome.

AMR genes

Based on the phenotypic characteristics of the antibiotic resistance profile, the identified AMR genes were searched using ResFinder (Figure 5 and Supplementary Table 4). All six genomes carried the methicillin resistance gene *(mec*A*)* and macrolide-resistance determinant genes (*erm*A, *erm*C, *msr*A, or *mph*C). Additionally, the coexistence of both *mec*A and *bla*Z was observed in VJR-MRSA035 (ST22), VJR-MRSA011 (ST3976), VJR-MRSA020 (ST8502) and VJR-MRSA040 (ST8). Furthermore, mutations associated with quinolone resistance *(grl*A*, grl*B*,* and *gyr*A*)* in MRSA were found in all genomes; except VJR-MRSA020 (ST8502), which had no chromosomal mutations mediating AMR. VJR-MRSA001 (ST5) showed resistance to aminoglycoside (*ant* [9]-*Ia*, and *aad*D) and bleomycin *(ble*O*)*. VJR-MRSA016 (ST764) was resistant to aminoglycosides (ant [9]-*Ia* and *aac* [6']-*aph* [2'']), fosfomycin *(fos*D*)*, TE *(tet*M*)* as well as carried a disinfectant resistance gene *(gac*B*)*. VJR-MRSA020 (ST8502) carried genes associated with TE, chloramphenicol, and trimethoprim resistance. The genomic analysis results were in accordance with the *in vitro* antimicrobial susceptibility results, with most MRSA strains being MDR.

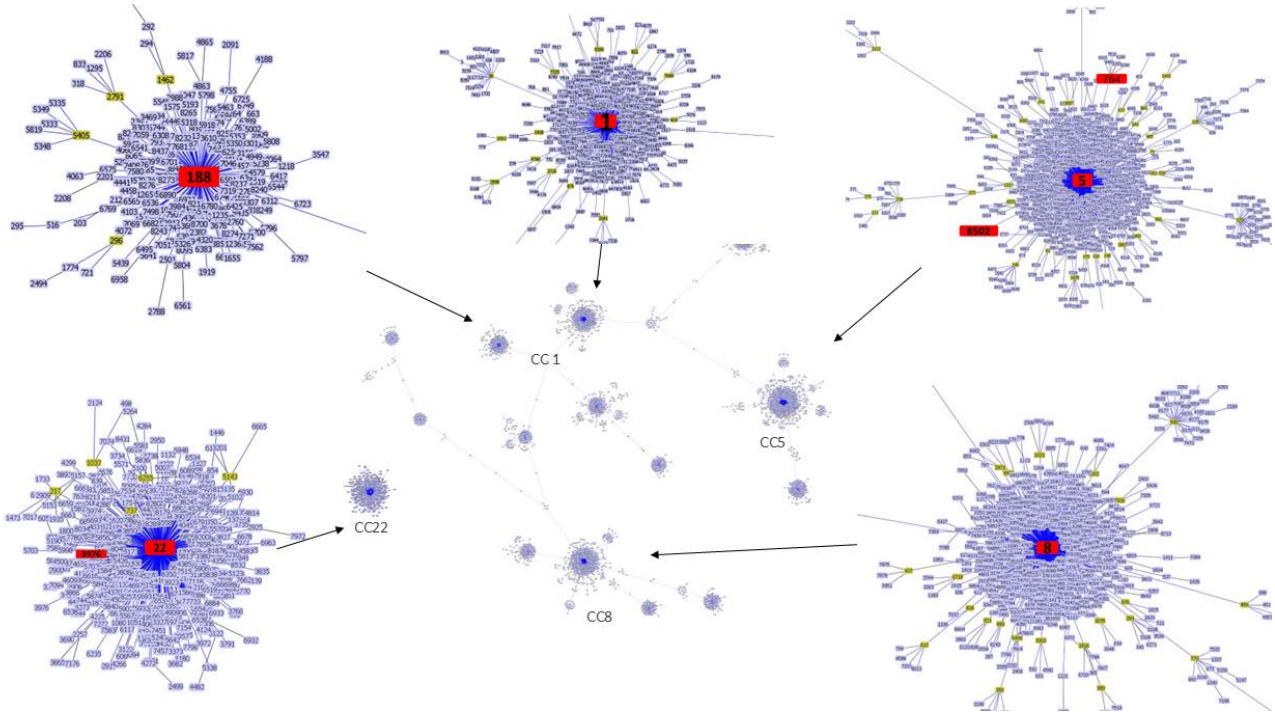
Table 1 Bacterial strains antimicrobial susceptibility (resistant phenotype), *tsst*-1, *pvl*, STs and *spa* types of the studied strains

Strain	Date of isolation	Specimens	Resistant phenotype / AMR genes (R =resistant, I=Intermediate)	*tsst*-1	*pvl*	ST	CC	*Spa*	SCC*mec*
VJR-MRSA001	12/12/2022	tissue	R: FOX, CC, E, LVX: I: CIP **grl*A *(S80F), *gyr*A *(S84L), ant(9)-Ia, aad*D, *mec*A, *erm*A, *ble*O	-	-	ST5	5	t21248	II
VJR-MRSA008	08/12/2022	wound	R: FOX, CC, E, LVX: I: CIP	-	-	ST22	22	t032	IV
VJR-MRSA010	21/12/2022	blood	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA011	12/12/2022	blood	R: FOX, CC, E, LVX, CIP **grl*A *(S80F), *grl*B *(D432N), gyr*A *(S84L, E88G), mec*A, *erm*C, *bla*Z	-	-	ST3976	22	t032	IV
VJR-MRSA013	06/12/2022	wound	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA016	17/02/2023	pus	R: FOX, CC, E, LVX, CIP, GN, MNO: I: DOX **grl*A *(S80Y, E84K), *gyr*A *(S84L, E88G), ant(9)-Ia, aac(6')-aph(2'')*, *mec*A, *erm*A, *fos*D, *tet*M, *gac*B	-	-	ST764	5	t045	II
VJR-MRSA017	18/02/2023	pus	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA019	16/02/2023	pus	R: FOX, CD, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA020	02/02/2023	pus	R: FOX, CC, E, TE, SXT *mec*A, *erm*C, *bla*Z, *tet*K, *cat(pC221), dfr*G	-	-	ST8502	5	t21247	V
VJR-MRSA021	01/02/2023	blood	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t628	IV
VJR-MRSA024	18/02/2023	tissue	R: FOX, CC, E, LVX, CIP, GN, MIN: I: DOX, NIT	-	-	ST22	22	t032	IV
VJR-MRSA026	03/01/2023	tissue	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA027	17/02/2023	sputum	R: FOX, CC, E, LVX, CIP	-	-	ST1	1	t1784	IV
VJR-MRSA029	26/04/2023	blood	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032t032	IV
VJR-MRSA034	04/03/2023	blood	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA035	26-03-2023	blood	R: FOX, CC, E, LVX, CIP **grl*A *(S80F), *gyr*A *(S84L), mec*A, *erm*C, *bla*Z	-	-	ST22	22	t1467	IV
VJR-MRSA038	05/04/2023	dialysis	R: FOX, CC, E, LVX, CIP, GN, MIN	-	-	ST188	1	t189	IV
VJR-MRSA039	21/03/2023	pus	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV
VJR-MRSA040	17/04/2023	pus	R: FOX, CC, E, LVX, CIP **grl*A *(S80Y), *gyr*A *(S84L), bla*Z, *mec*A, *msr*A, *mph*C, *erm*C	-	+	ST8	8	t008	IV
VJR-MRSA041	14/04/2023	tissue	R: FOX, CC, E, LVX, CIP	+	+	ST22	22	t032	IV
VJR-MRSA043	17/05/2023	abdomen fluid	R: FOX, CC, E, LVX, CIP	-	-	ST22	22	t032	IV

PVL=panton-valentine leukocidin, ST=sequence type, CC=clonal complex, *Spa*=staphylococcal protein A, SCC*mec*=staphylococcal cassette chromosome *mec* *Parentheses indicates *grl*A, *grl*B or *gyr*A amino acid point mutation related with fluoroquinolone antibiotic resistance

Table 2 Antibiotic resistance profiles of 21 Methicillin-resistant *Staphylococcus aureus* strains

Antimicrobial category	Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
B-lactam	Cefoxitin	21 (100)	-	-
Oxazolidinones	Linezolid	-	-	21 (100)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	1 (4.7)	-	20 (95.2)
Fluoroquinolones	Ciprofloxacin	18 (85.7)	2 (9.5)	1 (4.7)
Fluoroquinolones	Levofloxacin	20 (95.2)	-	1 (4.7)
Lincosamides	Clindamycin	21 (100)	-	-
Macrolides	Erythromycin	21 (100)	-	-
Aminoglycosides	Gentamicin	2 (9.5)	-	-
Glycopeptides	Vancomycin	-	-	21 (100)
Glycopeptides	Teicoplanin	-	-	21 (100)
Nitrofuran	Nitrofurantoin	-	1 (4.7)	20 (95.2)
Tetracyclines	Minocycline	1 (4.7)	-	20 (95.2)
Tetracyclines	Tetracycline	1 (4.7)	-	20 (95.2)
Tetracyclines	Doxycycline	-	2 (9.5)	19 (90.4)
Cyclic lipopeptides	Daptomycin	-	-	21 (100)



MRSA=methicillin-resistant *Staphylococcus aureus*, MLST=multilocus sequence typing

Figure 1 Minimum spanning trees of 21 clinical strains of MRSA were created by the goeBURST algorithm using Phyloviz software 2.0. Allelic profiles were downloaded from the MLST website. The founding genotypes of clonal complexes are the central sequence type, and the related STs are shown in blue. The sequence types reported in this study are indicated in red.

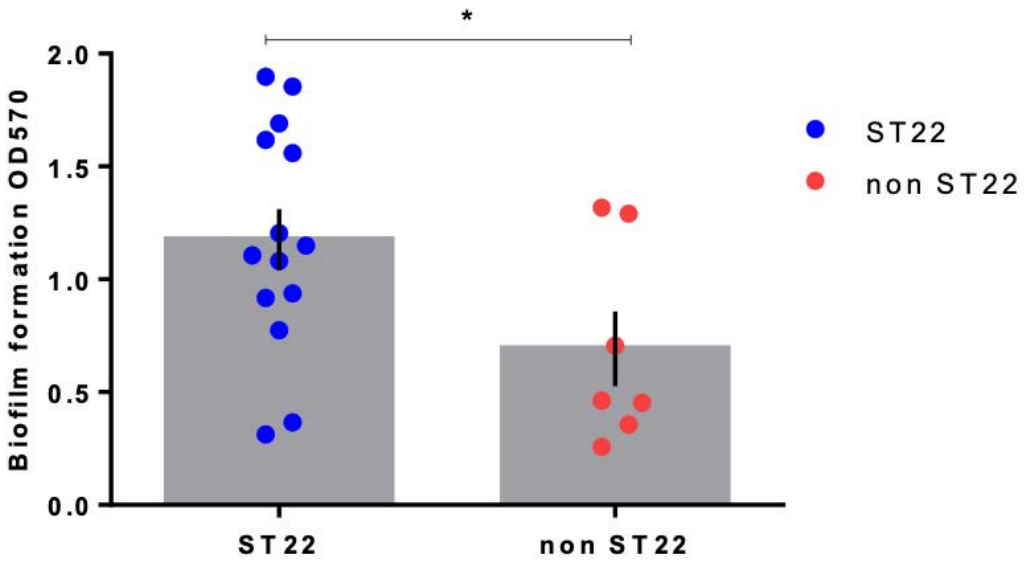


Figure 2 Semiquantitative biofilm analysis of ST22 and non-ST22 strains. Biofilm formation was evaluated using a semi-quantitative biofilm assay, by testing absorbance at 570 nm *in vitro*. **p-value*<0.05 (unpaired t-test, ST22 vs. non ST22). Data were analyzed using GraphPad Prism 6.0, and error bars in all graphs represent mean±SEM. P-values<0.05 were considered statistically significant.

Table 3 Detail of draft genome sequences

Strain	VJR-MRSA001 (ST5)	VJR-MRSA011 (ST397)	VJR-MRSA016 (ST764)	VJR-MRSA020 (ST8502)	VJR-MRSA035 (ST22)	VJR-MRSA040 (ST8)
Depth of coverage	264 x	252 x	267 x	242 x	257 x	245 x
Total reads (M)	2.781	2.892	2.920	2.798	2.813	2.721
Genome size (bp)	2,806,190	2,838,861	2,886,742	2,858,626	2,763,002	2,853,930
G+C contents (%)	32.76	32.71	23.72	32.66	32.70	32.61
Number of contigs	73	73	58	46	56	50
Largest contig (bp)	277,345	283,322	276,502	320,812	283,321	488,530
N50	152,258	76,875	121,983	171,407	108,311	145,042
Genes	2,656	2,699	2,757	2,732	2,616	2,708
Coding sequence	2,604	2,640	2,706	2678	2,557	2,647
rRNA genes	4	4	3	4	4	4
tRNA genes	47	54	47	47	54	55
tmRNA genes	1	1	1	1	1	1

bp=base pair, M=megabases, ST=sequence type

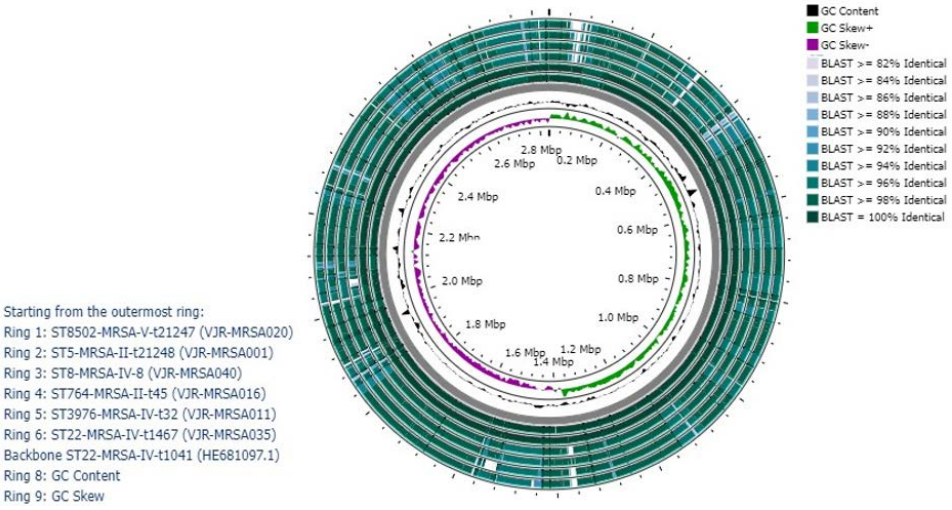


Figure 3 Circular genome comparison map showing homologous chromosome segments of six methicillin-resistant *Staphylococcus aureus* genomes, with the reference genome of *Staphylococcus aureus* subsp. aureus HO 50960412 (HE681097.1). The inner scales designate the coordinates in kbp. White spaces indicate regions with no identity to the reference genome.

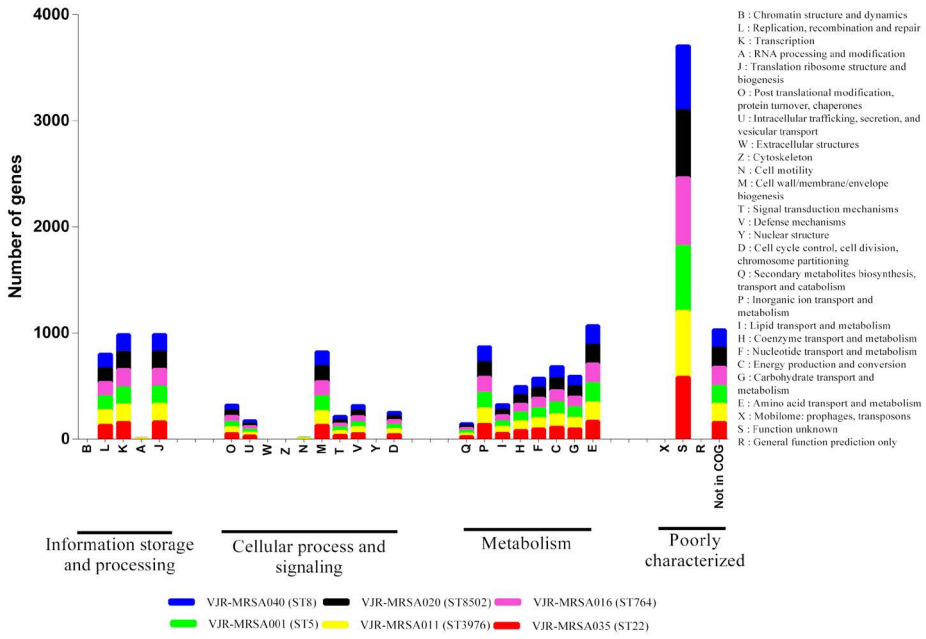


Figure 4 Comparisons of COG functional categories of six MRSA strains. The MRSA strain's blocks annotate with blue, black, pink, green, yellow, and red for VJR-MRSA040 (ST8), VJR-MRSA020 (ST8502), VJR-MRSA016 (ST764), VJR-MRSA001 (ST5), VJR-MRSA011 (ST3976) and VJR-MRSA035 (ST22), respectively. Abbreviations for COG categories are shown in the legend. No genes belonging to the COG category Chromatin structure and dynamics; RNA processing and modification; Extracellular structures; Cytoskeleton.

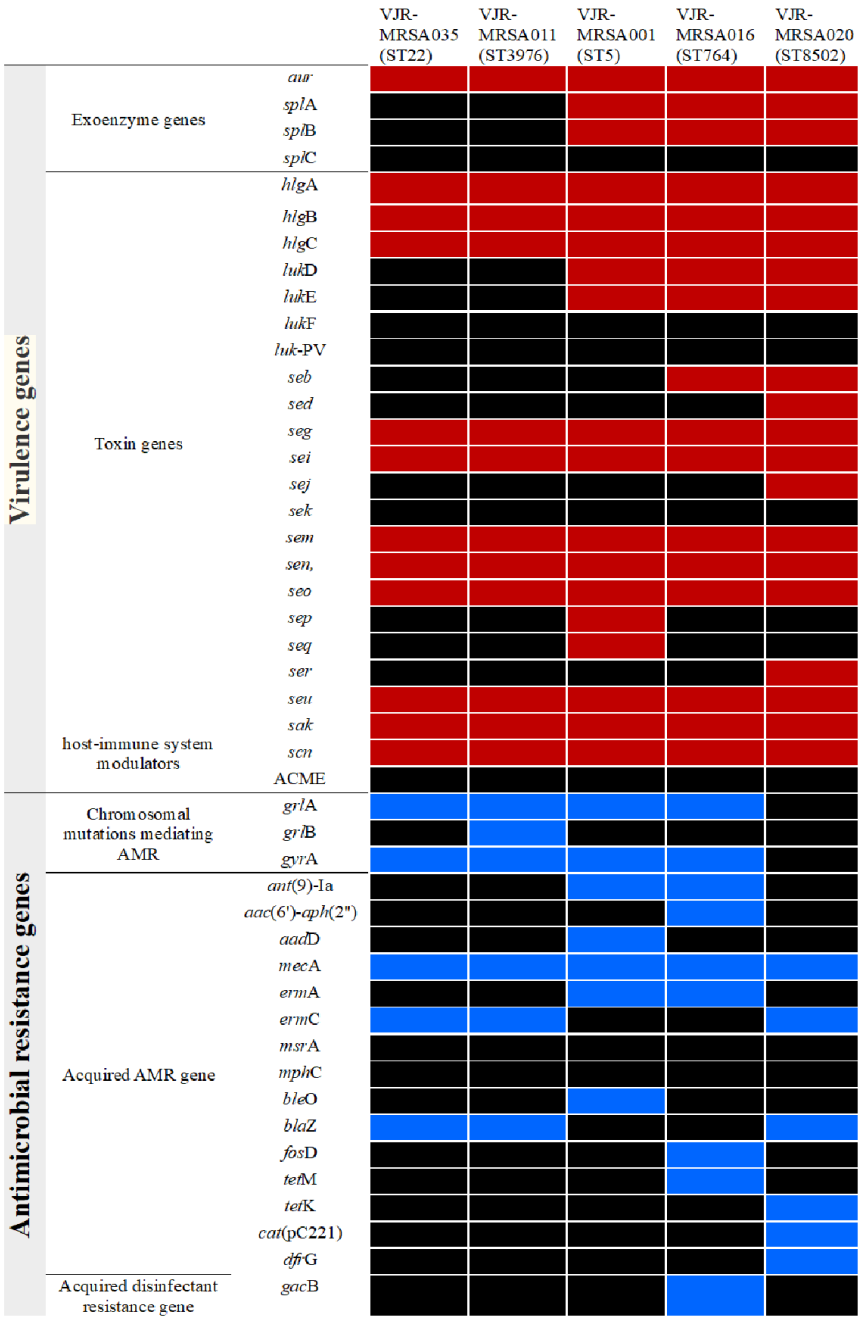


Figure 5 Heatmap of virulence genes and antimicrobial resistance gene profiles among six methicillin-resistant *Staphylococcus aureus* genomes in this study. Black blocks represent the absence of virulence or antimicrobial resistance genes. The red and blue blocks represent the presence of virulence genes and resistance to antibiotics, respectively.

Discussion

MRSA is an important pathogen that is associated with high rates of morbidity and mortality[27,28]. During the past few decades, MRSA strains have spread globally and are some of the most important nosocomial pathogens. In this study, 21 MRSA isolates obtained from our hospital over 6 months exhibited MDR and AMR resistance to several classes, including β-lactams, macrolides, lincosamides, and fluoroquinolones. Most strains exhibited high resistance to E and CC, while showing good antibacterial activity against VA and LZD. Additionally, three MDR-MRSA isolates in our study showed *in vitro* inducible CC resistance via the D-test. Currently, the prevalence of the $iMLS_B$ phenotype among MRSA strains has been documented worldwide, ranging from 0.6% to 76.4%[29]. Our results underscore the fact that selecting appropriate antimicrobial regimens for initial empiric therapy is becoming more challenging.

The epidemic ST239 MRSA strains are hospital-associated MRSA (HA-MRSA) clones prevalent in Asian countries[23]. From 1998 to 1999[30] and 2004 to 2006[23] in central Thailand, two surveillance studies identified the dominance of ST239-MRSA-III; however, the ST239-MRSA-III clone was not detected in this study. In contrast to previous studies, ST22-MRSA-IV was the most common strain in this study. ST22-MRSA-IV is known as the epidemic clone EMRSA-15. It is a hospital-associated pathogen[31] that is typically CIP and E resistant, which aligns with our results[32]. To the best of our knowledge, this is the first report on the emergence of ST22-MRSA-IV isolates in Thailand. *Spa* type classification further subdivided ST22-MRSA-IV into three subgroups: ST22-MRSA-IV-t032 (12/14), ST22-MRSA-IV-t628 (1/14) and ST22-MRSA-IV-t1467. According to the literature, these strains have not been isolated from clinical specimens in Thailand. This study provides informative molecular typing data among ST22-MRSA-IV isolates from a hospital in Thailand for epidemiological studies. Regarding other MRSA strains in this study, ST5-MRSA-II was previously reported in Thailand by Lulitanond et al. in 2010, at Srinagarind Hospital, Khon Kaen University, Thailand[4] and is one of the global HA-MRSA clones (New York/Japan clone)[27]. This study found that one strain carried this clone via VJR-MRSA001 (ST5-MRSA-II-t21248). Kondo et al. reported that the MRSA genotype ST764-SCC*mec* type II is the first HA occurrence in Southeast Asia[5]. This ST belongs to the global ST5 lineage and is found mainly in Japan. We also detected that one strain carried this clone via VJR-MRSA016 (ST764-MRSA-II-t045). Another clone in our study, namely ST1-MRSA-IV-t1784, is predominantly found in bloodstream infections in Japan[33]. We found one strain of the USA300 clone (ST8-MRSA-IV), which is a major clone of community-associated MRSA (CA-MRSA) in the USA. The prevalence of ST188-MRSA-IV-t189 is associated with livestock-associated MRSA[34]. ST3976-MRSA-IV was first reported in India in 2019, by Bakthavatchalam et al. (ST3976-MRSA-IVd-t030)[35]. Biofilm formation is a key virulence factor of Staphylococci and is the extracellular polymeric substance that is resistant to antimicrobial agents[7]. In this study, it was found that ST22 strains had a significantly greater ability to form biofilms than non-ST22 strains. This study described the emergence of a highly virulent biofilm. ST22 is an HA-MRSA lineage that first appeared in the United Kingdom as EMRSA-15. Currently, ST22 MRSA clones are spreading rapidly worldwide, and have even replaced other dominant clones in some regions[36].

When *S. aureus* infects a host, it produces several virulence factors; including secreted toxins (exotoxins)[37]. These promote manipulation of the host's immune responses while ensuring its survival. The six MRSA genomes revealed the presence of γ-Hemolysins *(Hlg*AB, *Hlg*CB*)*, which are exotoxins that can cause acute tissue

injury and inflammation. Additionally, they contribute to *S. aureus* disease in various animal models through its pore-forming function[37]. Moreover, *luk*E and *luk*D are found in VJR-MRSA001, –016, –020 and –040. *Luk*ED (broad leukocidal activity) mediates lysis of cells, because *Luk*ED targets G-protein-coupled receptors (CXCR1, CXCR2, CCR5 and DARC) on dendritic cells, macrophages monocytes, neutrophils, NK cells, T-cells and red blood cells[37]. *PVL* is a major toxin that could significantly enhance the pathogenicity of *S. aureus*, is linked with CA-MRSA, and has more virulence potential than HA-MRSA[29]. Notably, *luk*F–PV and *luk*S–PV were found in VJR-MRSA040 cells. The *luk*SF-PV *(PVL)* carrier strain is associated with skin and soft tissue infections, necrotizing pneumonia, diffuse cellulitis, and osteomyelitis[37]. Patients with *PVL*-positive MRSA infections had a higher mortality rate. Although the prevalence of *PVL*-producing *S. aureus* isolates differs across various regions, reports indicate that the increase in *PVL*-positive MRSA cases constitutes a significant public health challenge.

The major types of pyogenic exotoxins, called superantigens of *S. aureus*, are staphylococcal enterotoxins (SEs) and *TSST*-1. SEs are major causes of food poisoning. Based on amino acid and nucleotide sequences, 24 available SEs and SE-like proteins have been discovered, with the classical SEs categorized into five major types: SEA, SEB, SEC, SED, and SEE[7]. In this study, SEG, SEI, SEM, SEN, SEO, and SEU had the highest prevalence in the genome of the six studied strains. The emetic activity of these enterotoxins has been demonstrated in staphylococcal food poisoning outbreaks[38]. In our study, two strains harbored the classic SE genes: VJR-MRSA016 harbored SEB, and VJR-MRSA020 harbored SEB and SED. *TSST*-1 induces host T-cells and macrophages to release the proinflammatory cytokines, causing TSS that manifests as high fever, hypotension, rash, hypovolemic shock, and rapidly progresses to severe or multisystem organ failure[7]. Among the 21 MRSA in this study, *tsst*-1 was found in one strain (VJR-MRSA041) via conventional PCR. *Sak* and *scn*, which allow for adaptation to human hosts, are part of the immune evasion cluster present in the six MRSA genomes[39]. Moreover, ACME was found in VJR-MRSA040 ST8-IV. ACME plays an important role in the growth and survival of bacteria. ST8-MRSA-IVa, also known as the USA300 clone, has been isolated in several countries[40]; including from patients in our hospital. The identified AMR genes were in accordance with the antimicrobial susceptibility results. COG distribution analysis revealed that among core genes, most were genes involved in metabolism; especially in class E (amino acid transport and metabolism) and class P (inorganic ion transport and metabolism). However, regarding the function of information and processing, the major proportion was obtained from class J (transcription, ribosomal structure, and biogenesis) and class K (transcription).

Several limitations inherent to this study should be acknowledged. Firstly, the isolates were collected from a single hospital. Secondly, the study period was relatively short, spanning only six months. Lastly, the sample size of MRSA isolates was limited

Conclusion

In conclusion, this study has provided comprehensive knowledge on the correlation among AMR, SCC*mec,* ST, and *spa* types from a large-scale clinical study of *S. aureus* over a lengthy period. This is the first report of ST22 MRSA clones from clinical isolates predominantly in Thailand.

Authors contributions

Conceptualization and study design: SB and TW; Investigation: SB, AH, and TW; Methodology: SB, TA, PH, PP (Pholkla), VB, NS, TU, PP (Porramatikul), AH, TW; Interpretation and Analysis: SB, TA, PH, PP (Pholkla), VB, NS, TU, PP (Porramatikul), and TW; Data curation: SB and TW; Helped in coordinating the project: AH and JC;

Drafting the original manuscript: SB and TW; Review and editing manuscript: SB, TA, PH, PP (Pholkla), VB, NS, TU, PP (Porramatikul), AH, JC, and TW. All authors approved this manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1: Primers used for the amplification of the multilocus sequence typing genes and SCC*mec*

Target	Primer names	Primer sequences
Carbamate kinase *(arc*C*)*	*arc*C-Up	TTGATTCACCAGCGCGTATTGTC
	*arc*C-Dn	AGGTATCTGCTTCAATCAGCG
	*aro*E-Up	ATCGGAAATCCTATTTCACATTC
Shikimate dehydrogenase *(aro*E*)*	*aro*E-Dn	GGTGTTGTATTAATAACGATATC
Glycerol kinase *(glp*F*)*	*glp*F-Up	CTAGGAACTGCAATCTTAATCC
	*glp*F-Dn	TGGTAAAATCGCATGTCCAATTC
Guanylate kinase *(gmk)*	*gmk*-Up	ATCGTTTTATCGGGACCATC
	gmk-Dn	TCATTAACTACAACGTAATCGTA
	pta-Up	GTTAAAATCGTATTACCTGAAGG
Phosphate acetyltransferase *(pta)*	*pta*-Dn	GACCCTTTTGTTGAAAAGCTTAA
	tpi-Up	TCGTTCATTCTGAACGTCGTGAA
Triosephosphate isomerase *(tpi)*	*tpi*-Dn	TTTGCACCTTCTAACAATTGTAC
	*yqi*L-Up	CAGCATACAGGACACCTATTGGC
Acetyl coenzyme A acetyltransferase *(yqi*L*)*	*yqi*L-Dn	CGTTGAGGAATCGATACTGGAAC
Class A *mec (MecI-mecR1)*	mI6	CATAACTTCCCATTCTGCAGATG
	mA7	ATATACCAAACCCGACAACTACA
Class B *mec (IS1272)*	IS5	AACGCCACTCATAACATATGGAA
	mA6	TATACCAAACCCGACAAC
Class C *mec (IS431-mec*A*)*	IS2	TGAGGTTATTCAGATATTT CGATGT
	mA2	AACGTTGTAACCACCCCAAGA
ccr1 *(ccr*A1*)*	α 1	AACCTATATCATCAATCAGTACGT
	Bc	ATTGCCTTGATAATAGCCITCT
	B2	TGGACTTGGGGTTTTTGA
ccr2 *(ccr*A2*)*	α 2	TAAAGGCATCAATGCACAAACACT
	Bc	ATTGCCTTGATAATAGCCITCT
	B2	TGGACTTGGGGTTTTTGA
ccr3 *(ccr*A3*)*	α 3	AGCTCAAAAGCAAGCA ATAGAAT
	Bc	ATTGCCTTGATAATAGCCITCT
	B2	TGGACTTGGGGTTTTTGA
cr4 *(ccr*A4*)*	A4.2	GTATCAATGCACCAGAACTT
	B4.2	TTGCGACTCTCTTGGCGTTT
ccr5 *(ccr*A5*)*	r-F	CGTCTATTACAAGATGTTAAGGATAAT
	r-R	CCTTTATAGACTGGATTATTCAAAATAT

Supplementary Table 2: Comparison of COG among the genome of 6 MRSA

Category	Class	Functional description	Strains					
			1	11	16	20	35	40
Information storage and processing	B	Chromatin structure and dynamics	0	0	0	0	0	0
	L	Replication, recombination and repair	130	139	134	133	129	136
	K	Transcription	159	164	172	163	159	168
	A	RNA processing and modification	1	1	1	1	1	1
	J	Translation, ribosomal structure and biogenesis	164	163	165	164	164	166
Cellular process and signaling	O	Posttranslational modification, protein turnover, chaperones	53	53	53	53	53	56
	U	Intracellular trafficking, secretion, and vesicular transport	29	29	30	29	28	28
	W	Extracellular structures	0	0	0	0	0	0
	Z	Cytoskeleton	0	0	0	0	0	0
	N	Cell motility	1	2	1	2	1	1
	M	Cell wall/membrane/envelope biogenesis	141	129	141	142	129	141
	T	Signal transduction mechanisms	35	35	36	34	35	37
	V	Defense mechanisms	49	54	53	54	53	52
	Y	Nuclear structure	0	0	0	0	0	0
	D	Cell cycle control, cell division, chromosome partitioning	41	46	43	40	43	41
Metabolism	Q	Secondary metabolites biosynthesis, transport and catabolism	25	25	25	23	24	25
	P	Inorganic ion transport and metabolism	147	144	148	143	141	146
	I	Lipid transport and metabolism	53	55	54	54	55	55
	H	Coenzyme transport and metabolism	82	82	82	83	82	83
	F	Nucleotide transport and metabolism	95	96	96	95	96	95
	C	Energy production and conversion	112	117	113	113	113	114
	G	Carbohydrate transport and metabolism	100	98	98	100	97	99
	E	Amino acid transport and metabolism	183	172	183	180	169	183
Poorly characterized	X	Mobilome: prophages, transposons	0	0	0	0	0	0
	S	Function unknown	613	622	645	633	581	609
	R	General function prediction only	0	0	0	0	0	0
	Not in COG		166	171	179	182	159	172

Supplementary Table 2: (continued)

Category	Class	Functional description	Strains					
			1	11	16	20	35	40
Ambiguously characterized	BQ	–	1	1	1	1	1	1
	CE	–	1	1	1	1	1	1
	CH	–	5	5	5	5	5	5
	CO	–	5	5	5	5	5	5
	CP	–	5	5	5	5	5	5
	DZ	–	2	2	2	2	2	2
	EF	–	0	0	0	0	0	1
	EG	–	5	4	6	5	4	5
	EGP	–	23	26	24	24	26	25
	EH	–	7	7	7	7	7	7
	EI	–	2	2	2	2	2	2
	EJ	–	1	1	1	1	1	1
	EM	–	1	1	1	1	1	1
	EP	–	9	9	9	9	9	11
	ET	–	1	1	1	1	1	1
	FG	–	1	1	1	1	1	1
	FJ	–	1	1	1	1	1	1
	GH	–	1	1	1	1	1	1
	GK	–	2	2	2	2	2	2
	GKT	–	3	3	3	3	3	4
	GM	–	15	15	15	16	16	16
	HP	–	1	1	1	1	1	1
	HQ	–	1	1	1	1	1	1
	IM	–	1	1	1	1	1	1
	IQ	–	10	10	10	10	10	10
	IU	–	2	2	2	2	2	2
	JK	–	1	1	1	1	1	1
	JKL	–	2	2	2	2	2	2
	KLT	–	1	1	1	1	1	1
	KO	–	1	1	1	1	1	1
	KOT	–	1	1	1	1	1	1
	KT	–	4	3	4	3	3	3
	KTV	–	1	2	3	1	2	2
	LU	–	2	2	1	1	2	1
	MU	–	1	1	1	1	1	1
	NOT	–	1	1	1	1	1	1
	NOU	–	1	1	1	1	1	1
	NU	–	4	4	4	4	4	4
	OP	–	1	0	2	1	0	1
	OU	–	2	1	1	2	1	2
	UW	–	0	0	0	0	0	1

Supplementary Table 3: Virulence determinants found in the MRSA strains analysed in this study (6 isolates genome)

Strains	Exoenzyme genes	Toxin genes	Hostimm genes*
VJR-MRSA001 (ST5)	*aur, splA, splB*	*hlgA, hlgB, hlgC, lukD, lukE, seg, sei, sem, sen, seo, sep, seu*	*sak, scn*
VJR-MRSA011 (ST3976)	*aur*	*hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu*	*sak, scn*
VJR-MRSA016 (ST764)	*aur, splA, splB*	*hlgA, hlgB, hlgC, lukD, lukE, seb, seg, sei, sem, sen, seo, seu*	*sak, scn*
VJR-MRSA020 (ST8502)	*aur, splA, splB*	*hlgA, hlgB, hlgC, lukD, lukE, seb, sed, seg, sei, sej, sem, sen, seo, ser, seu*	*sak, scn*
VJR-MRSA035 (ST22)	*aur*	*hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu*	*sak, scn*
VJR-MRSA040 (ST8)	*aur, splA, splB, splE*	*hlgA, hlgB, hlgC, lukD, lukE, lukF-PV, lukS-PV, sek, seq*	*ACME, sak, scn*

*Host-immune system modulators

Supplementary Table 4: Antimicrobial resistance genes were present by ResFinder

Strains	Chromosomal mutations mediating antimicrobial resistance	Acquired antimicrobial resistance gene hits (%)	Acquired disinfectant resistance gene hits
VJR-MRSA001	*grlA (tcc>ttc; p.S80F) gyrA (tca>tta; p.S84L)*	*ant(9)-Ia (100, aadD (99.87), mecA (100), erm(A) (100) (100), bleO (100)*	-
VJR-MRSA011	*grlA (tcc>ttc; p.S80F) grlB (gat>aat;D432N) gyrA (tca>tta; p.S84L gaa>gga; p.E88G)*	*mecA (100), blaZ (100), erm(C) (100)*	-
VJR-MRSA016	*grlA (tcc>tac; p.S80Y gaa>aaa; p.E84K) gyrA (tca>tta; p.S84L gaa>gga; p.E88G)*	*ant(9)-Ia (100), mecA (99.95) aac(6')-aph(2'') (100), fosD (100), erm(A) (100), tetM (100)*	*qacB (99.94%)*
VJR-MRSA020	-	*blaZ (99.89), mecA (100), erm(C) (100), tet(K) (99.98) cat(pC221) (100), dfrG (100)*	-
VJR-MRSA035	*grlA (tcc>ttc; p.S80F) gyrA (tca>tta; p.S84L)*	*mecA (100), blaZ (100), erm(C) (100)*	-
VJR-MRSA040	*grlA (tcc>tac; p.S80Y) gyrA (tca>tta; p.S84L)*	*blaZ (100), mecA (100), msr(A) (99.38), mph(C) (100), erm(C) (99.86)*	-