Molecular characterization and antibiotic resistance of *Staphylococcus aureus* isolated from clinical specimens in an urban university hospital in Bangkok, Thailand

RESEARCH ARTICLE

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ABSTRACT

Little is known about the properties of the current strains of *Staphylococcus aureus* associated with human infections in Thailand. This study examined the rate of resistance to various antimicrobial agents, prevalence of virulence genes, and biofilm formation ability of 60 clinical *S. aureus* isolates from a single Thai hospital. Moreover, the *Staphylococcus* protein A gene (*spa*) type was determined among methicillin-resistant *S. aureus* (MRSA) isolates. Most methicillin-susceptible *S. aureus* isolates were susceptible to antimicrobials, whereas all MRSA isolates were resistant to erythromycin and clindamycin. The major virulence genes among the isolates were *hla* (100%), *sec* (26.7%), and *hlb* (20%). Meanwhile, 46.7% and 1.7% of the strains exhibited low-grade and high-grade biofilm formation, respectively. Our findings revealed the presence of *spa* types among MRSA isolates were: t032 (37.5%, 6/16), t088 (25%, 4/16), t001 (12.5%, 2/16), t008 (6.25%, 1/16), t034 (6.25%, 1/16), t439 (6.25%, 1/16), and t1928 (6.25%, 1/16). These findings will be useful for future research on anti-virulence therapies and the epidemiology of the strains circulating in our hospital.

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INTRODUCTION

Staphylococcus aureus is gram-positive coccus that normally colonizes the skin and nasal microbiota of healthy individuals [1–3]. However, if this bacterium enters the internal tissues or bloodstream, it can cause a variety of potentially serious infections in both community-acquired and hospital acquired settings [2]. *S. aureus* is a leading cause of bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, septic arthritis, device-related infections, pulmonary infections, gastroenteritis, urinary tract infections, and toxic shock syndrome (TSS) [2, 4].

S. aureus infections involve the production of several virulence factors associated with clinical symptoms such as hemolysins, leukotoxins, superantigens, and exfoliative toxins (ETs) [5–7]. Hemolysin lyses red blood cells by creating pores and disrupting the cell membranes or dissolving cell wall components [7, 8]. Panton–Valentine leukocidin (PVL, one type of leukotoxin) causes the destruction of white blood cells such as neutrophils and monocytes, and it is associated with tissue necrosis and the acceleration of apoptosis [7, 9]. The major types of pyrogenic exotoxins, called superantigens of *S. aureus*, are staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1). SEs are recognized as major causes of food poisoning [7]. Based on nucleotide and amino acid sequences, the 24 currently SEs and staphylococcal enterotoxin-like proteins (SE *ls*) have been discovered [7, 10], and the classical SEs are categorized into five major types: SEA, SEB, SEC, SED, and SEE [11]. TSST-1, another major superantigen, stimulates the release of pro-inflammatory cytokines from host T-cells and macrophages and then causes TSS. A cluster of manifestations including high fever, hypotension, rash, and hypovolemic shock rapidly progresses to severe or multisystem organ failure [7, 12, 13]. Staphylococcal ETs, specifically ETA and ETB, are etiologic agents that are linked to staphylococcal scalded skin syndrome (SSSS) [7, 14]. Moreover, *S. aureus* can produce a multilayer biofilm embedded in a glycocalyx or slime layer with heterogeneous protein expression throughout the structure [15, 16]. Biofilm plays an important role in the pathogenesis of diseases by facilitating bacterial colonization, infection, and antibiotic resistance [16–18]. Generally, *S. aureus* infection is multifactorial because of the combined action of several virulence determinants [19]. Remarkably, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) have emerged after the use of antibiotics in clinical practice [3, 20–22].

Molecular typing of MRSA isolates is critical for the rapid identification of prevalent strains, and this technique will help control and prevent MRSA spread in healthcare settings. *Staphylococcus* protein A gene (*spa*) typing, which relies on single-locus DNA sequencing of the repeat region of *spa*, exhibits excellent discriminatory ability for MRSA. The *spa* type distribution of *S. aureus* isolates varies in different regions globally. The most common *spa* types in Europe are t032, t008, and t002, whereas t037 is the most common MRSA strain found in clinical settings in most Asian countries [23, 24]. Although the distribution of *spa* types in Asia has been thoroughly studied, detailed reports on the distribution of the *spa* types of MRSA strains from clinical isolates in Thailand are lacking. Therefore, this study aimed to detect the occurrence of virulence factors and biofilm formation and presence of certain drug resistance genes produced by *S. aureus* strains isolated from clinical samples in an urban university hospital in Bangkok, Thailand. Furthermore, we genotyped *spa* types to gain insights into the epidemiology of MRSA isolates.

MATERIALS AND METHODS

Ethics approval

The Ethical Review Board of the Faculty of Medicine, Vajira Hospital, Navamindradhiraj University (Bangkok, Thailand) approved the study (COA194/2565).

Bacterial isolates and identification

Sixty non-repetitive clinical isolates of *S. aureus* from blood, tissue, sterile body fluid, and pus were collected by the Microbiology Laboratory, Central Laboratory and Blood Bank, Faculty of Medicine, Vajira Hospital between October 2021 and July 2022. The bacteria were first identified to the species level using a MALDI BioTyper (Bruker, Daltonik GmbH, Bremen, Germany) and stored in Soyabean Casein Digest Medium (TSB; HIMEDIA®, Mumbai, India) at 4 °C at the organism bank in the Department of Clinical Pathology, Faculty of Medicine, Vajira Hospital until use.

Antimicrobial susceptibility testing

In vitro antibacterial susceptibility testing was performed by the disk diffusion method according to the protocol described by the Clinical and Laboratory Standards Institute (CLSI) guidelines document M100-S31 [25]. The antibiotic disk contained cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), and trimethoprim–sulfamethoxazole (1.25/23.75 µg). The disk diffusion method was performed on Mueller–Hinton Agar (MHA: HIMEDIA®, Mumbai, India). The size of each inhibition zone size was interpreted according to the CLSI guideline as susceptible (S), intermediate (I), or resistance (R) [25]. Isolates with cefoxitin zones ≥22 mm in size were considered methicillin-susceptible *S. aureus* (MSSA) isolates, and those with zones ≤21 mm in size were deemed MRSA isolates [25]. Macrolide–lincosamide–streptogramin B (MLS$_B$) resistance was detected by placing 15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart on a MHA plate. Inducible clindamycin resistance (ICR) isolate was indicated by flattening of the clindamycin zone of inhibition adjacent to the erythromycin disk (termed a D-zone) [25].

Genomic DNA extraction; strain molecular identification; and *mec*A, *van*A, and *van*B detection

The studied strains were grown on brain heart infusion agar (BHI; HIMEDIA®, Mumbai, India) 24 h, and then the DNA of each strain was extracted following the protocol of the AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA). The extracted DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Isolates were identified to the species level using PCR with a specific primer pair targeting *fem*A (species-specific, Table A1) [26]. Then, PCR amplification using a *mec*A-specific primer pair (Table A1) was performed to identify MRSA isolates. The PCR reaction mixture (final volume,

25 μL) contained 0.4 μM of each primer, exTEN 2 × PCR Master Mix with loading dye (Axil Scientific Pte Ltd, Singapore), nuclease-free water, and genomic DNA. PCR was performed using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the following program: initial denaturation at 95 °C for 5 min; 35 cycles of amplification at 95 °C for 1 min, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 5 min. Moreover, the studied strains were subjected to PCR using *van*A- and *van*B-specific primer pairs to identify VRSA isolates (Table A1) [27–30]. The PCR program for the *van*A-specific primers was as follows: initial denaturation at 98 °C for 2 min; 35 cycles of amplification at 98 °C for 10 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min; and a final extension at 72 °C for 5 min. The PCR reaction mixture for *van*A and *van*B amplification was prepared as described for *fem*A and *mec*A amplification. Ten microliters of PCR products were electrophoresed on a 1.5% agarose gel in 1 × TAE buffer containing FloroSafe DNA stain (Axil Scientific Pte Ltd, Singapore) and photographed using a Gel Doc XR+ system (Bio-Rad, Hercules, CA, USA).

Virulence-associated gene detection

The extracted DNA was subjected to PCR to amplify 11 virulence genes encoding hemolysins [alpha-hemolysin (*hla*) and beta-hemolysin (*hlb*)], leukocidin; PVL (*pvl*), super-antigens (*sea, seb, sec, sed, see,* and *tsst*-1), and ETs (*eta* and *etb*) using specific primers (Table A1) [31, 32]. Each PCR mixture and PCR program followed those described for *fem*A amplification excluding changes in the specific annealing temperature of each gene (Table A1). PCR was performed using the same machine, and PCR amplicons were detected as previously described.

spa typing

MRSA strains were selected for further determination of *spa* types. *spa* typing was performed via PCR amplification using the standard primer pair spa-1113F and spa-1514R (Table A1 and available at http://www.spaserver.ridom.de) [33]. 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 1 min, 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 on a 1.5% agarose gel as previously described, purified, and bidirectionally sequenced using gene-specific forward and reverse primers at 1st BASE DNA Sequencing (Apical Scientific Sdn Bhd, Malaysia) by BNK Bioscience Limited (Nonthaburi, Thailand). The sequence files were analyzed using BioEdit software (https://www.mbio.ncsu.edu/bioedit/bioedit.html). The *spa* types were identified and grouped using the based upon repeat pattern algorithm with Ridom SeqSphere+ software [34].

Biofilm-producing ability on 96-well plates

Each strain was grown on BHI agar at 37 °C for 24 h. After that, bacterial cells were transferred to TSB and incubated at 37 °C for 24 h. Cell suspensions of each strain were prepared at a density of 1×10^8 CFU mL^{-1} in TSB, and then 200 μL of each adjusted cell suspension were inoculated into the wells (10 wells per isolate) of a flat-bottom 96-well plate (Costar®, Corning Incorporated, Corning, NY, USA). The plates were incubated without shaking at 37 °C for 24 h. Then, the supernatant and planktonic cells were removed, and the wells were washed three times with PBS. Biofilm was fixed with 200 μL of methanol per well for 15 min and stained with 200 μL of 1% crystal violet per well (YD Diagnostic, Republic of Korea) for 5 min. The crystal violet solution was then removed, and the wells were washed with sterile water. To destain the biofilms, 200 μL of 95% ethanol were added, and the plate was incubated for 45 min. One hundred fifty microliters of the solution were transferred to another microplate, and the absorbance was read at 570 nm using a Sunrise absorbance microplate reader (Tecan Austria GmbH, Grödig, Austria). The production of biofilm was quantified using crystal violet and categorized according to the absorbance of as high-grade biofilm formation ($OD_{570} \geq 1$), low-grade biofilm formation ($0.1 \leq OD_{570} < 1$), or no biofilm formation ($OD_{570} < 0.1$) for each isolate (35). The experiment was performed in duplicate, and biofilm measurements are expressed as the mean ± SD of 20 readings (wells) per isolate.

RESULTS

Strains and identification

In total, 60 isolates of *S. aureus* were collected between October 2021 and July 2022 (Table A2). *S. aureus* was recovered from different specimen types, including pus specimens (25, 41.6%), blood (25, 41.6%), and tissue (5, 8.3%), as well as ascetic fluid, synovial fluid, ear swab, peritoneal dialysis, and umbilical swab (1 each, 1.7%). The isolates were first identified to the species level using a MALDI BioTyper and then confirmed by *fem*A amplification.

Antimicrobial susceptibility testing and drug resistant gene detection

The antimicrobial susceptibility of the 60 *S. aureus* isolates was tested. Sixteen (26.7%) isolates were identified as MRSA as determined by resistance to cefoxitin and the presence of *mec*A, whereas the remaining 44 (73.3%) isolates were identified as MSSA. None of *S. aureus* isolates carried *van*A or *van*B. The *mec*A-positive *S. aureus* (MRSA) isolates exhibited different rates of resistance (25–100%) to five antimicrobials. The MRSA isolates were resistant to β-lactams, erythromycin, and clindamycin. One MRSA isolate (VJR-STPARS040) exhibited an MLS$_b$ resistance mechanism as confirmed by ICR phenomenon detection. However, the isolates were more sensitive to gentamicin (75%) and trimethoprim–sulfamethoxazole (100%), and no isolate carried both *van*A and *van*B. The *mec*A-negative *S. aureus* (MSSA) isolates were highly susceptible to all tested antibiotics (Tables 1 and A2).

Table 1. Antimicrobial susceptibility of the studied strains

Antimicrobial Agent	MRSA		MSSA	
	Susceptible No. (%)	Resistant No. (%)	Susceptible No. (%)	Resistant No. (%)
Cefoxitin	0 (0)	16 (100)	44 (100)	0 (0)
Erythromycin	0 (0)	16 (100)	43 (97.7%)	1 (2.3%)
Clindamycin	0 (0)	16 (100)	44 (100)	0 (0)
Gentamicin	12 (75)	4 (25)	43 (97.7%)	1 (2.3%)
Trimethoprim-sulfamethoxazole	16 (100)	0 (0)	44 (100)	0 (0)

Virulence gene detection

The frequencies of virulence gene detection among the 60 *S. aureus* isolates are presented in Tables 2, A3, and A4. All *S. aureus* isolates carried *hla*, whereas *sec*, *hlb*, *sed*, *pvl*, *seb*, *tsst*-1, and *sea* were detected in 26.7%, 20%, 11.7%, 10%, 10%, 3.3%, and 1.7% of these isolates, respectively. No isolates carried *see*, *eta*, or *etb*. Other than *hla* (100%), *hlb* (31.25%) was the most common virulence gene detected in MRSA isolates, followed by *pvl* (18.75%), *sed* (18.75%), and *sec* (12.5%). Aside from *hla* (100%), the most common virulence gene carried by MSSA isolates was *sec* (31.8%), followed by *hlb* (15.9%), *seb* (13.7%), *sed* (9.1%), *pvl* (6.8%), *tsst*-1 (4.5%), and *sea* (2.3%, Table 2). Thirty-two (53.3%) *S. aureus* isolates harbored multiple virulence genes. Of those 32 isolates, 19, 8, and 5 isolates carried 2, 3, and 4 virulence genes, respectively (Fig. 1, Tables A3 and A4).

spa typing

spa PCR products ranged in size from 250 to 600 bp. The 16 MSRA isolates were categorized into seven *spa* types. The most prevalent *spa* type was t032 (6, 37.5%), followed by t088 (4, 25%), t001 (2, 12.5%), t008 (1, 6.25%), t034 (1, 6.25%), t439 (1, 6.25%), and t1928 (1, 6.25%). t032 was the most frequently detected *spa* type among isolates from pus (50%) and blood (33%), whereas t088 was the only *spa* type detected in isolates from blood (100%; Fig. 2, Tables A2 and A5).

Biofilm formation under static conditions

The biofilm formation capability of the 60 *S. aureus* isolates was determined by measuring the absorbance after crystal violet staining of biofilm formed in flat-bottom microtiter plates. Biofilm formation capacity was classified according to the scheme of Kouidhi et al. [35]. According to the analysis of biofilm production, 51.7% of the isolates produced no biofilm, 46.67% exhibited low-grade biofilm production, and 1.7% exhibited high-grade biofilm production. One isolate with strong biofilm production was MRSA (Fig. 1, Table 3).

DISCUSSION

S. aureus is a major cause of numerous infections in both communities and healthcare facilities, and this bacterium is increasingly exhibiting resistance to multiple antimicrobial agents [36]. In this study, 60 clinical isolates of *S. aureus* were collected at an urban university hospital between 2021 and 2022 and then analyzed for antimicrobial susceptibility, the presence of virulence genes, and biofilm production. The prevalence of *S. aureus* was highest in blood and pus cultures. All *S. aureus* isolates were tested for antimicrobial resistance, and their rates of resistance to cefoxitin, erythromycin, clindamycin, and gentamicin were 26.7%, 28.3%, 26.7%, and 8.3%, respectively. All isolates were susceptible to trimethoprim–sulfamethoxazole. The prevalence of MRSA was 26.7% at both the phenotype (resistance to cefoxitin disks) and genotype levels (*mec*A gene-positive). The prevalence of MRSA has varied among different studies in Thailand, ranging from <20% to >40% [37, 38]. The majority of MSSA isolates were susceptible to antimicrobial drugs. No *S. aureus* isolates harbors *van*A or *van*B genes. An MRSA isolate (VJR-STPARS040) exhibited an MLS$_b$ resistance mechanism via an ICR phenomenon. The majority of

Table 2. Frequencies of virulence genes among MRSA and MSSA

Virulence gene		MRSA (N = 16)	MSSA (N = 44)	No. of isolates (N = 60)
Alpha-hemolysin	*hla*	16 (100%)	44 (100%)	60 (100%)
Beta-hemolysin	*hlb*	5 (31.25%)	7 (15.9%)	12 (20%)
Toxic shock syndrome toxin	*tsst*-1	0 (0%)	2 (4.5%)	2 (3.3%)
Panton-Valentine Leukocidin	*pvl*	3 (18.75%)	3 (6.8%)	6 (10%)
Enterotoxin A	*sea*	0 (0%)	1 (2.3%)	1 (1.7%)
Enterotoxin B	*seb*	0 (0%)	6 (13.7%)	6 (10%)
Enterotoxin C	*sec*	2 (12.5%)	14 (31.8%)	16 (26.7%)
Enterotoxin D	*sed*	3 (18.75%)	4 (9.1%)	7 (11.7%)
Enterotoxin E	*see*	0 (0%)	0 (0%)	0 (0%)
Exfoliative toxin A	*eta*	0 (0%)	0 (0%)	0 (0%)
Exfoliative toxin B	*etb*	0 (0%)	0 (0%)	0 (0%)

Fig. 1. The distribution pattern of virulence genes among 60 *S. aureus* isolates obtained in this study. Virulence genes encoding exotoxins and biofilm formation were screened (Gray squares = presence; Black squares = absence; Circle in gray squares = low-grade biofilm formation; Asterisk in gray squares = high-grade biofilm formation; * = MRSA)
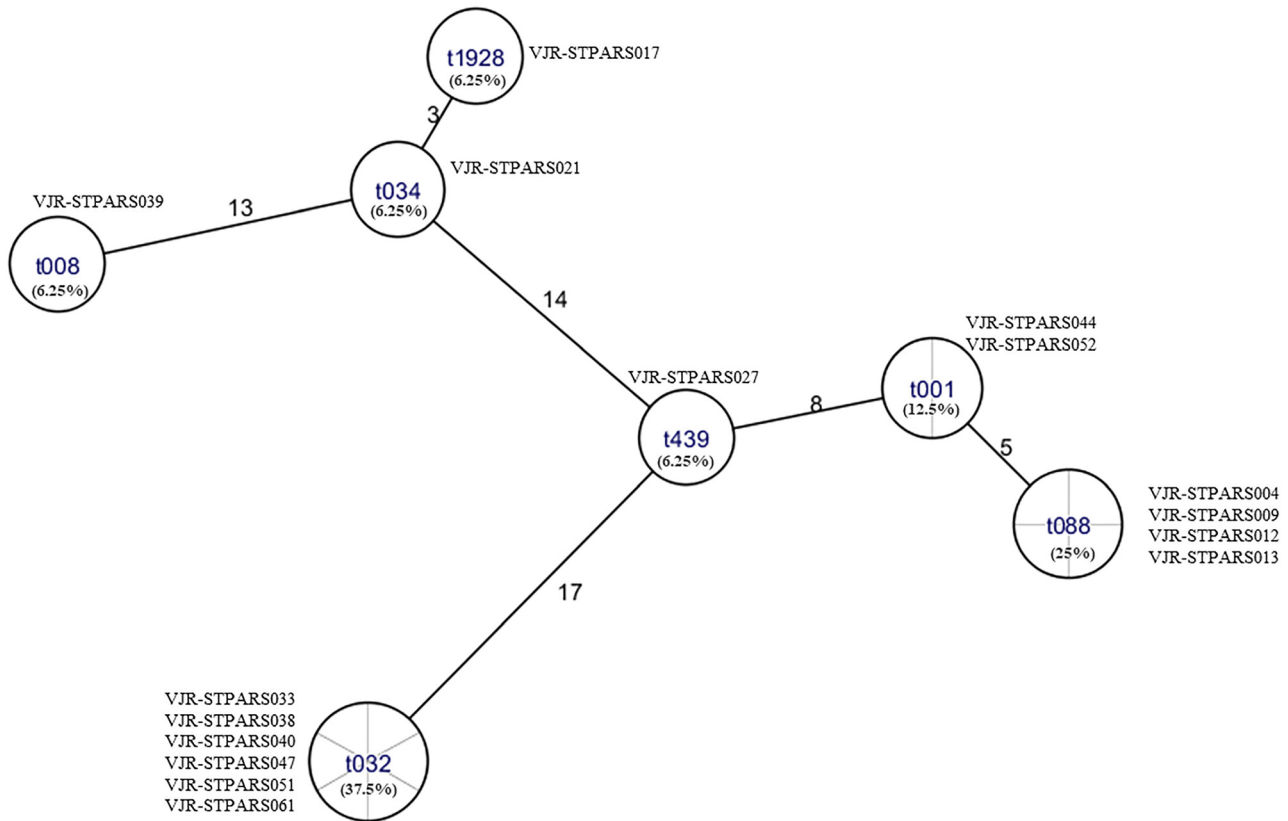
Fig. 2. Minimum spanning tree (Ridom SeqSphere+ software [34]) based on seven different *spa* types from all MRSA isolates (*n* = 16) presenting the distribution of the *spa* types among MRSA isolates. Each *spa* type is represented by a single node. The diameter of the node is proportional to the number of the isolates belonging to the *spa* type. The separated sections in each node represent the number of isolates. The distance between nodes represents the genetic diversity of the isolates

Table 3. Biofilm formation of the studied strains

Rate of biofilm formation	MRSA (*N* = 16)	MSSA (*N* = 44)	No. of isolates
High biofilm formation	1 (6.25%)	0 (0%)	1 (1.7%)
Low grade biofilm formation	9 (56.25%)	19 (43.2%)	28 (46.7%)
Non-biofilm formation	6 (37.5%)	25 (56.8%)	31 (51.7%)

MRSA strains in our study were resistant to erythromycin and clindamycin; moreover, 56.2% of the studied MRSA isolates were derived from bloodstream infections. Previous studies confirmed that MRSA isolates were frequently resistant to erythromycin and clindamycin; therefore, both drugs are ineffective for the treatment of MRSA infection in our hospital [37, 39, 40].

There are various trends in the *spa* type distribution of *S. aureus* isolates in various global geographic regions. We detected seven different *spa* types among 16 *spa*-typeable MRSA isolates. The three most common *spa* types among MRSA isolates in our study were t032, t088, and t001. t032 is the most prevalent *spa* type causing invasive MRSA infection in Europe and the dominant clone of hospital acquired-MRSA strains in the United Kingdom [41]. This *spa* type is uncommon outside European countries; however, a recent

study identified t032 as the dominant clone causing infection in Malaysia and Thailand [24, 42]. Taken together, our findings indicate that t032 has become the predominant variant in European and Asian countries, including Malaysia and Thailand. Regarding the other *spa* types in this study, t088 and t001 are the most frequent *spa* types found in Europe [43–45], but they have also been reported in Thailand [42]. Hence, the observation of t032, t088, and t001 among MRSA isolates in our study could reflect the ongoing expansion of these clones Asia.

S. aureus produces α- and β-hemolysins, which are encoded by *hla* and *hlb*, respectively. Hemolysin damages a large variety of host cells such as epithelial cells, endothelial cells, erythrocytes, monocytes, and keratinocytes, and it causes cell membrane damage and apoptosis [7, 19]. Our results illustrated that the frequencies of *hla* and *hlb* among our isolates were 100% and 20%, respectively, which were similar to the results of a previous study [46].

PVL is a leukotoxin that belongs to the bi-component Luk toxin family. PVL is most commonly associated with skin and soft tissue disease. PVL-positive strain infections appear to be more likely to require surgical intervention [7]. In our study, the distribution rate of *pvl* among *S. aureus* isolates was 10%. Of the *pvl*-positive isolates, 66.7% were recovered from pus samples, and 33.3% were isolated from

blood. Moreover, *pvl*-positive strains were identified as MRSA and MSSA at equal rates. These findings are consistent with previous reports that MRSA and MSSA isolates from blood cultures and skin lesions are both *pvl*-positive [47, 48]. PVL-positive *S. aureus* strains have been linked to skin infections, and some of these strains are MRSA, which has limited treatment options. TSST-1 produced by *S. aureus* has been associated with several acute diseases including TSS. The prevalence of *tsst*-1 in this study was low (3.3%) compared to that in previous reports, in which the prevalence usually exceeded 10%. These variations could be the consequence of the different sample groups.

SEs disrupt intestinal activity and induce staphylococcal food poisoning, which is characterized by nausea, vomiting, abdominal pain, and diarrhea without evidence of toxic effects such as fever or hypotension. Based on antigenic heterogeneity, more than 20 SEs (SEA—SEIV) have been discovered [7]. We only focused on classical SEs, i.e., SEA, SEB, SEC, SED, and SEE. Overall, SE genes were detected in 50% of *S. aureus* isolates in this study. SEC (*sec*) was the most predominant toxin, being present in 26.7% of isolates, followed by SED (*sed*), SEB (*seb*), and SEA (*sea*), which were detected in 11.7%, 10%, and 1.7% of isolates, respectively. Meanwhile, SEE (*see*) was not detected in any isolates. These findings are inconsistent with a previous report in which *sea* was the most predominant toxin (32.6%), followed by *seb* (4.3%), whereas *sec*, *sed*, and *see* were not found [49]. The differences in SE gene patterns of *S. aureus* could be associated with variable distributions in different regions.

ETs are specific serine proteases that recognize and hydrolyze desmosome proteins in the skin, thereby playing a role in SSSS [7]. The absence of *eta* and *etb* in this study is in line with other studies suggesting that the frequency of ET genes varies among *S. aureus* strains and that it could be close to zero [46, 50].

Biofilm formation is a key virulence factor of staphylococci and essentially the extracellular polymeric substance that provides unique niches to bacterial cells. Biofilm infections are clinically significant because bacteria in biofilms are resistant to antimicrobial agents [51]. The prevalence of biofilm formation among *S. aureus* isolates in the present study was 48.3%. According to prior studies, the prevalence of biofilm formation varies from <50% to >70% [52–55]. The differences in the prevalence of biofilm formation depends on many factors such as the environment, availability of nutrients, geographical origin, type of specimen, surface adhesion characteristics, and genetic makeup of the organism [56]. These factors could have influenced the data and contributed to the prevalence recorded in this study.

CONCLUSION

The present study investigated the antibiotic susceptibility and virulence patterns of *S. aureus* isolated from clinical samples between October 2021 and July 2022. The prevalence of MRSA in our hospital was 26.7%. The MRSA strains were mainly resistant to erythromycin and clindamycin. The current study also demonstrated the presence of *spa* types among MRSA isolates in Thailand and documented the spread of t032, t088, t001, t008, t034, t439, and t1928 in Thailand. Alpha-hemolysin was the main virulence factor in our isolates. Alpha-hemolysin and enterotoxin C co-existed in 13.3% of isolates, in line with the shared virulence pattern in this study. Forty-eight percent of isolates produced biofilm. Our findings will be useful for future studies on antivirulence therapies and the epidemiology of the strains circulating in our hospital.

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REFERENCES

1. Hussain K, Bandyopadhyay A, Roberts N, Mughal N, Moore LSP, Fuller LC. Panton-Valentine Leukocidin producing *Staphylococcus aureus*: a clinical review. Clin Exp Dermatol 2022; 47(12): 2150–8.

2. Taylor TA, Unakal CG. Staphylococcus aureus. Treasure Island (FL): StatPearls; 2022.

3. Hommes JW, Surewaard BGJ. Intracellular Habitation of *Staphylococcus aureus*: molecular mechanisms and prospects for antimicrobial therapy. Biomedicines 2022; 10(8).

4. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015; 28(3): 603–61.

5. Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl Environ Microbiol 2011; 77(22): 8097–105.

6. Otto M. *Staphylococcus aureus* toxins. Curr Opin Microbiol 2014; 17: 32–7.

7. Ahmad-Mansour N, Loubet P, Pouget C, Dunyach-Remy C, Sotto A, Lavigne JP, et al. *Staphylococcus aureus* Toxins: an update on their pathogenic properties and potential treatments. Toxins (Basel) 2021; 13(10).

8. Vandenesch F, Lina G, Henry T. *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: a redundant arsenal of membrane-damaging virulence factors? Front Cell Infect Microbiol 2012; 2: 12.

9. Bhatta DR, Cavaco LM, Nath G, Kumar K, Gaur A, Gokhale S, et al. Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant *Staphylococcus aureus* (MRSA) in Western Nepal: a matter of concern for community infections (a hospital based prospective study). BMC Infect Dis 2016; 16: 199.

10. Fisher EL, Otto M, Cheung GYC. Basis of Virulence in enterotoxin-mediated Staphylococcal food poisoning. Front Microbiol 2018; 9: 436.

11. Hait JM, Tallent SM, Bennett RW. Screening, detection, and serotyping methods for toxin genes and enterotoxins in *Staphylococcus* strains. J AOAC Int 2014; 97(4): 1078–83.

12. Stach CS, Herrera A, Schlievert PM. Staphylococcal superantigens interact with multiple host receptors to cause serious diseases. Immunol Res 2014; 59(1–3): 177–81.

13. Vergis N, Gorard DA. Toxic shock syndrome responsive to steroids. J Med Case Rep 2007; 1: 5.

14. Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 1999; 12(2): 224–42.

15. Silva-de-Jesus AC, Ferrari RG, Panzenhagen P, Conte-Junior CA. *Staphylococcus aureus* biofilm: the role in disseminating antimicrobial resistance over the meat chain. Microbiology (Reading) 2022; 168(10).

16. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. Virulence 2011; 2(5): 445–59.

17. Vestby LK, Gronseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics (Basel) 2020; 9(2).

18. Fitzpatrick F, Humphreys H, O'Gara JP. The genetics of staphylococcal biofilm formation–will a greater understanding of pathogenesis lead to better management of device-related infection? Clin Microbiol Infect 2005; 11(12): 967–73.

19. Bien J, Sokolova O, Bozko P. Characterization of virulence factors of *Staphylococcus aureus*: novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. J Pathog 2011; 2011: 601905.

20. da Silva RS, Duarte FC, Danelli T, Olak APS, Magalhaes GLG, Pelisson M, et al. High prevalence of Panton-Valentine Leukocidin-encoding genes in methicillin-resistant *Staphylococcus aureus* isolated from inpatients with invasive infections at a university hospital in southern Brazil. Infect Disord Drug Targets 2023; 23(2): e230822207951.

21. Rammelkamp Chm, T. Resistance of *Staphylococcus aureus* to the action of penicillin. Proc Soc Exp Biol Med 1942; 51(3): 386–9.

22. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W. Molecular Mechanisms of drug resistance in *Staphylococcus aureus*. Int J Mol Sci 2022; 23(15).

23. Asadollahi P, Farahani NN, Mirzaii M, Khoramrooz SS, van Belkum A, Asadollahi K, et al. Distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and -susceptible *Staphylococcus aureus* around the world: a review. Front Microbiol 2018; 9: 163.

24. Jones SU, Chua KH, Chew CH, Yeo CC, Abdullah FH, Othman N, et al. *spa* diversity of methicillin-resistant and -susceptible *Staphylococcus aureus* in clinical strains from Malaysia: a high prevalence of invasive European spa-type t032. PeerJ 2021; 9: e11195.

25. Weinstein MP, Lewis II JS, Bobenchik AM, Campeau S, Cullen SK, Galas MF, et al. Performance standards for antimicrobial susceptibility testing, M100, 31st ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2021.

26. Al-Talib H, Yean CY, Al-Khateeb A, Hassan H, Singh KK, Al-Jashamy K, et al. A pentaplex PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* and Panton-Valentine Leucocidin. BMC Microbiol 2009; 9: 113.

27. Handwerger S, Perlman DC, Altarac D, McAuliffe V. Concomitant high-level vancomycin and penicillin resistance in clinical isolates of enterococci. Clin Infect Dis 1992; 14(3): 655–61.

28. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995; 33(5): 1434.

29. Saha B, Singh AK, Ghosh A, Bal M. Identification and characterization of a vancomycin-resistant Staphylococcus aureus isolated from Kolkata (South Asia). J Med Microbiol 2008; 57(Pt 1): 72–9.

30. Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 1993; 37(11): 2311–7.

31. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. Infect Immun 2002; 70(2): 631–41.

32. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of methicillin resistant and sensitive, vancomycin intermediate *Staphylococcus aureus* strains isolated from different iranian hospitals. ISRN Microbiol 2012; 2012: 215275.

33. Larsen AR, Stegger M, Sorum M. *spa* typing directly from a *mec*A, *spa* and *pvl* multiplex PCR assay-a cost-effective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. Clin Microbiol Infect 2008; 14(6): 611–4.

34. Junemann S, Sedlazeck FJ, Prior K, Albersmeier A, John U, Kalinowski J, et al. Updating benchtop sequencing performance comparison. Nat Biotechnol 2013; 31(4): 294–6.

35. Kouidhi B, Zmantar T, Hentati H, Bakhrouf A. Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in *Staphylococcus aureus* associated to dental caries. Microb Pathog 2010; 49(1–2): 14–22.

36. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. Clin Microbiol Infect 2014; 20(7): 605–23.

37. Phokhaphan P, Tingpej P, Apisarnthanarak A, Kondo S. Prevalence and antibiotic susceptibility of methicillin resistant *Staphylococcus aureus*, collected at Thammasat University Hospital, Thailand, August 2012–July 2015. Southeast Asian J Trop Med Public Health 2017; 48(2): 351–9.

38. Waitayangkoon P, Thongkam A, Benjamungkalarak T, Rachayon M, Thongthaisin A, Chatsuwan T, et al. Hospital epidemiology and antimicrobial susceptibility of isolated methicillin-resistant *Staphylococcus aureus*: a one-year retrospective study at a tertiary care center in Thailand. Pathog Glob Health 2020; 114(4): 212–7.

39. Pomorska K, Jakubu V, Malisova L, Fridrichova M, Musilek M, Zemlickova H. Antibiotic resistance, *spa* typing and clonal analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from blood of patients hospitalized in the Czech Republic. Antibiotics (Basel) 2021; 10(4).

40. Rongsanam P, Yano T, Yokart W, Yamsakul P, Sutammeng S, Udpaun R, et al. Acquisition risk factors of the SCC*mec* IX-methicillin-resistant *Staphylococcus aureus* in swine production personnel in Chiang Mai and Lamphun Provinces, Thailand. Antibiotics (Basel) 2020; 9(10).

41. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, et al. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. Plos Med 2010; 7(1): e1000215.

42. Tabuchi F, Lulitanond A, Lulitanond V, Thunyaharn S, Kaito C. Epidemiological study on the relationship between toxin production and psm-mec mutations in MRSA isolates in Thailand. Microbiol Immunol 2020; 64(3): 219–25.

43. Ilczyszyn WM, Sabat AJ, Akkerboom V, Szkarlat A, Klepacka J, Sowa-Sierant I, et al. Clonal structure and characterization of *Staphylococcus aureus* strains from invasive infections in paediatric patients from south Poland: association between age, *spa* types, clonal complexes, and genetic markers. PLoS One 2016; 11(3): e0151937.

44. Tenover FC, Tickler IA, Goering RV, Kreiswirth BN, Mediavilla JR, Persing DH, et al. Characterization of nasal and blood culture isolates of methicillin-resistant *Staphylococcus aureus* from patients in United States hospitals. Antimicrob Agents Chemother 2012; 56(3): 1324–30.

45. Ruppitsch W, Indra A, Stoger A, Mayer B, Stadlbauer S, Wewalka G, et al. Classifying spa types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2006; 44(7): 2442–8.

46. Budzynska A, Skowron K, Kaczmarek A, Wietlicka-Piszcz M, Gospodarek-Komkowska E. Virulence factor genes and antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from blood and chronic wounds. Toxins (Basel) 2021; 13(7).

47. Ellis MW, Griffith ME, Jorgensen JH, Hospenthal DR, Mende K, Patterson JE. Presence and molecular epidemiology of virulence factors in methicillin-resistant *Staphylococcus aureus* strains colonizing and infecting soldiers. J Clin Microbiol 2009; 47(4): 940–5.

48. Nickerson EK, Wuthiekanun V, Wongsuvan G, Limmathurosakul D, Srisamang P, Mahavanakul W, et al. Factors predicting and reducing mortality in patients with invasive *Staphylococcus aureus* disease in a developing country. PLoS One 2009; 4(8): e6512.

49. Ahmed A. Baz EKB, Abdul-Raouf U, Abdelkhalek A. Prevalence of enterotoxin genes (SEA to SEE) and antibacterial resistant pattern of *Staphylococcus aureus* isolated from clinical specimens in Assiut city of Egypt. Egypt J Med Hum Genet 2021; 22: 84.

50. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Von Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J Clin Microbiol 2003; 41(4): 1434–9.

51. Hassan A UJ, Kaleem F, Omair M, Khalid A, Iqbal M. Detection and antibiotic susceptibility pattern of biofilm producing gram positive and gram negative bacteria isolated from a tertiary care hospital of Pakistan. Malays J Microbiol 2011; 7(1): 57–60.

52. Cha JO, Yoo JI, Yoo JS, Chung HS, Park SH, Kim HS, et al. Investigation of biofilm formation and its association with the molecular and clinical characteristics of methicillin-resistant *Staphylococcus aureus*. Osong Public Health Res Perspect 2013; 4(5): 225–32.

53. Samie A SN. Biofilm production and antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from HIV and AIDS patients in the Limpopo Province, South Africa. Afr J Biotechnol 2011; 10: 14625–36.

54. Khan F SI, Rizvi M, Mansoor T, Sharma SC. Detection of biofilm formation in *Staphylococcus aureus*. Does it have a role in treatment of MRSA infections. Trends Med Res 2011; 6(2): 116–23.

55. Neopane P, Nepal HP, Shrestha R, Uehara O, Abiko Y. In vitro biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. Int J Gen Med 2018; 11: 25–32.

56. Kokare CR CS, Khopade AN, Mahadik KR. Biofilm: importance and applications. Indian J Biotechnol 2009; 8: 159–68.

Appendix

Table A1. Primer used in this study

Target	Gene	Primer name	Sequence	PCR product size (bp)	Annealing	Reference
Species specific to *S. aureus*	*fem*A	*fem*A-F	CGATCCATATTTACCATATCA	450	55	[26]
		fem A-R	ATCACGCTCTTCGTTTAGTT			
MRSA	*mec*A	*mec*A-F	ACGAGTAGATGCTCAATATAA	293	55	[26]
		*mec*A-R	CTTAGTTCTTTAGCGATTGC			
S. aureus protein A	*spa*	*spa*-1113F	TAAAGACGATCCTTCGGTGAGC	varies	60	[33]
		spa-1514R	CAGCAGTAGTGCCGTTTGCTT			
VRSA	*van*A	*van*A-F	GGGAAAACGACAATTGC	857	50	[28]
		*van*A-R	TCACCCCTTTAACGCTAATA			[29]
	*van*B	*van*B-F	GTGACAAACCGGAGGCGAGGA	433	60	[27]
		*van*B-R	CCGCCATCCTCCTGCAAAAAA			
Alpha-hemolysin	*hla*	HLA-1	CTGATTACTATCCAAGAAATTCGATTG	209	57	[31]
		HLA-2	CTTTCCAGCCTACTTTTTTTATCAGT			
Beta-hemolysin	*hlb*	HLB-1	GTGCACTTACTGACAATAGTGC	309	57	[31]
		HLB-2-2	GTTGATGAGTAGCTACCTTCAGT			
Panton-Valentine Leukocidin	*pvl*	PVL-F	GGAAACATTTATTCTGGCTATAC	502	55	[32]
		PVL-R	CTGGATTGAAGTTACCTCTGG			
Toxic shock syndrome toxin	*Tsst*-1	TSST-1-F	TTATCGTAAGCCCTTTGTTG	398	55	[32]
		TSST-1-R	TAAAGGTAGTTCTATTGGAGTAGG			
SEA	*sea*	SEA-1	GAAAAAAGTCTGAATTGCAGGGAACA	560	55	[31]
		SEA-2	CAAATAAATCGTAATTAACCGAAGGTTC			
SEB	*seb*	SEB-1	ATTCTATTAAGGACACTAAGTTAGGGA	404	55	[31]
		SEB-2	ATCCCGTTTCATAAGGCGAGT			
SEC	*sec*	mpSEC-1	GTAAAGTTACAGGTGGCAAAACTTG	297	55	[31]
		mpSEC-2	CATATCATACCAAAAAGTATTGCCGT			
SED	*sed*	SED-1	GAATTAAGTAGTACCGCGCTAAATAATATG	492	55	[31]
		SED-2	GCTGTATTTTTCCTCCGAGAGT			
SEE	*see*	SEE-1	CAAAGAAATGCTTTAAGCAATCTTAGGC	482	55	[31]
		SEE-2	CACCTTACCGCCAAAGCTG			
ETA	*eta*	mpETA-1	ACTGTAGGAGCTAGTGCATTTGT	190	57	[31]
		mpETA-3	TGGATACTTTTGTCTATCTTTTTCATCAAC			
ETB	*etb*	mpETB-1	CAGATAAAGAGCTTTATACACACATTAC	612	57	[31]
		mpETB-2	AGTGAACTTATCTTTCTATTGAAAAACACTA			

Table A2. Strain information and antibiotic susceptibility result

Code	Specimen	*fem*A	*mec*A	*van*A	*van*B	Fox	DA	E	CN	SXT
VJR-STPARS001	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS002	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS003	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS004	Blood	+	+	−	−	R	R	R	S	S
VJR-STPARS005	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS006	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS007	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS008	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS009	Blood	+	+	−	−	R	R	R	S	S
VJR-STPARS011	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS012	Blood	+	+	−	−	R	R	R	S	S

(continued)

Table A2. Continued

Code	Specimen	*fem*A	*mec*A	*van*A	*van*B	Fox	DA	E	CN	SXT
VJR-STPARS013	Blood	+	+	−	−	R	R	R	S	S
VJR-STPARS014	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS016	Ear swab	+	−	−	−	S	S	S	S	S
VJR-STPARS017	Pus	+	+	−	−	R	R	R	S	S
VJR-STPARS018	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS019	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS020	Tissue	+	−	−	−	S	S	S	S	S
VJR-STPARS021	Pus	+	+	−	−	R	R	R	S	S
VJR-STPARS023	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS024	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS025	Tissue	+	−	−	−	S	S	S	S	S
VJR-STPARS026	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS027	Pus	+	+	−	−	R	R	R	R	S
VJR-STPARS028	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS029	Ascitic	+	−	−	−	S	S	S	S	S
VJR-STPARS030	PUS	+	−	−	−	S	S	S	S	S
VJR-STPARS031	Joint	+	−	−	−	S	S	S	S	S
VJR-STPARS032	Tissue	+	−	−	−	S	S	S	S	S
VJR-STPARS033	Peritoneal dialysis	+	+	−	−	R	R	R	S	S
VJR-STPARS034	Tissue	+	−	−	−	S	S	S	S	S
VJR-STPARS035	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS036	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS037	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS038	Pus	+	+	−	−	R	R	R	S	S
VJR-STPARS039	Blood	+	+	−	−	R	R	R	S	S
VJR-STPARS040	Blood	+	+	−	−	R	R	R	S	S
VJR-STPARS041	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS042	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS043	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS044	Blood	+	+	−	−	R	R	R	R	S
VJR-STPARS045	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS046	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS047	Blood	+	+	−	−	R	R	R	R	S
VJR-STPARS048	Blood	+	−	−	−	S	S	S	S	S
VJR-STPARS049	Umbilical swab	+	−	−	−	S	S	S	S	S
VJR-STPARS050	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS051	Pus	+	+	−	−	R	R	R	S	S
VJR-STPARS052	Blood	+	+	−	−	R	R	R	R	S
VJR-STPARS053	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS054	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS055	Tissue	+	−	−	−	S	S	S	S	S
VJR-STPARS056	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS057	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS058	Pus	+	−	−	−	S	S	R	R	S
VJR-STPARS059	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS060	Pus	+	−	−	−	S	S	S	S	S
VJR-STPARS061	pus	+	+	−	−	R	R	R	S	S
VJR-STPARS062	pus	+	−	−	−	S	S	S	S	S
VJR-STPARS063	Blood	+	−	−	−	S	S	S	S	S

S = susceptible, R = resistant, + = the presence, − = the absence, Fox = cefoxitin, DA = clindamycin, E = erythromycin, CN = gentamicin, and SXT = trimethoprim-sulfamethoxazole

Table A3. Distribution of virulence genes patterns among MRSA and MSSA

Profile No.	Virulence profile	MRSA (N =16)	MSSA (N = 44)	No. of isolates
1	*hla*	8 (50%)	20 (45.5%)	28 (46.7%)
2	*hla-sec*	0 (0%)	8 (18.2%)	8 (13.3%)
3	*hla-hlb*	2 (12.5%)	3 (6.8%)	5 (8.3%)
4	*pvl-hla*	2 (12.5%)	1 (2.3%)	3 (5%)
5	*hla-seb*	0 (0%)	2 (4.5%)	2 (3.3%)
6	*hla-sed*	0 (0%)	1 (2.3%)	1 (1.7%)
7	*hla-hlb-sec*	1 (6.25%)	1 (2.3%)	2 (3.3%)
8	*hla-hlb-sed*	2 (12.5%)	1 (2.3%)	3 (5%)
9	*tsst-1-hla-sec*	0 (0%)	2 (4.5%)	2 (3.3%)
10	*pvl-hla-sec*	0 (0%)	1 (2.3%)	1 (1.7%)
11	*hla-seb-sec-sed*	0 (0%)	2 (4.5%)	2 (3.3%)
12	*pvl-hla-sec-sed*	1 (6.25%)	0 (0%)	1 (1.7%)
13	*hla-hlb-sea-seb*	0 (0%)	1 (2.3%)	1 (1.7%)
14	*pvl-hla-hlb-seb*	0 (0%)	1 (2.3%)	1 (1.7%)

Table A4. Distribution of virulence genes patterns among the clinical samples

Profile No.	Virulence profile	Pus (n = 25)	Blood (n = 25)	Tissue (n = 5)	Ascetic (n = 1)	Synovial fluid (n = 1)	Swab (n = 2)	Peritoneal dialysis (n = 1)	No. of isolates (n = 60)
1	*hla*	13	11	3				1	28 (46.7%)
2	*hla-sec*		5		1	1	1		8 (13.3%)
3	*hla-hlb*	2	2	1					5 (8.3%)
4	*pvl-hla*	2	1						3 (5%)
5	*hla-seb*	2							2 (3.3%)
6	*hla-sed*				1				1 (1.7%)
7	*hla-hlb-sec*		2						2 (3.3%)
8	*hla-hlb-sed*		2				1		3 (5%)
9	*tsst-1-hla-sec*	2							2 (3.3%)
10	*pvl-hla-sec*	1							1 (1.7%)
11	*hla-seb-sec-sed*	2							2 (3.3%)
12	*pvl-hla-sec-sed*	1							1 (1.7%)
13	*hla-hlb-sea-seb*		1						1 (1.7%)
14	*pvl-hla-hlb-seb*		1						1 (1.7%)

Table A5. spa types of the 16 isolated MRSA

spa Type	Repeat Succession No.	Isolates
t088	26-23-17-34-17-20-17-12-12-17-16	VJR-STPARS 004, 009, 012, 013
t1928	08-02-25-02-25-34-24-25	VJR-STPARS 017
t034	08-16-02-25-02-25-34-24-25	VJR-STPARS 021
t439	26-17-20-17-17-16	VJR-STPARS 027
t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	VJR-STPARS 033, 038, 040, 047, 051, 061
t008	11-19-12-21-17-34-24-34-22-25	VJR-STPARS 039
t001	26-30-17-34-17-20-17-12-17-16	VJR-STPARS 044, 052