의학박사 학위논문

지속성 황색포도알균 균혈증의 미생물학적, 유전학적, 임상적 특성 연구

A Study on

Persistent *Staphylococcus aureus* Bacteremia: Microbiological,

Genotypic, and Clinical Characteristics

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이 논문을 의학박사 학위 논문으로 제출함

2024 년　 9 월

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ABSTRACT

Background: *Staphylococcus aureus* bacteremia (SAB) may persist despite proper antibiotic treatment. Persistent SAB is associated with poor clinical outcomes and high mortality. Several studies have analyzed the risk factor, clinical outcome, and microbiological characteristics of persistent SAB, but are limited. Therefore, this study aims to extensively analyze the clinical, microbiological and genotypic characteristics of persistent SAB and the factors affecting the poor clinical outcome.

Methods: This prospective cohort study was conducted from August 2008 to February 2021 at the Asan Medical Center, a 2,700-bed tertiary referral center in South Korea. Among enrolled patients, The clinical characteristics, management, outcomes, microbiological, and genetic characteristics of patients with persistent SAB, including those with persistent methicillin-resistant *S. aureus* (MRSA) or methicillin-susceptible *S. aureus* (MSSA) bacteremia, were compared and analyzed. Persistent bacteremia was defined as a period of 3 days or more from the time appropriate antibiotics were administered until a negative follow-up blood culture was confirmed, or even if the period was less than 3 days, if a follow-up blood culture conducted within those 3 days was positive again. The comparator was defined as resolving bacteremia. Microbiologic data, including genotyping, sequence type (ST), *Staphylococcus* protein A (*spa*), staphylococcal cassette chromosome *mec* (SCC*mec*), and virulence genes, were analyzed. The clinical and microbiological characteristics and outcomes of major ST versus other STs in persistent SAB as well as persistent MRSA or MSSA bacteremia were also analyzed.

Results: Among 1877 patients with SAB, 826 had resolving SAB and 1,051 had persistent SAB. Among these, 953 had MRSA bacteremia (resolving MRSA bacteremia, n = 318; persistent MRSA bacteremia, n = 635), and 924 had MSSA bacteremia (resolving MSSA bacteremia, n = 508; persistent MSSA bacteremia, n = 416). The multivariate analysis revealed that vascular grafts (adjust OR, 1.493; 95% CI, 1.036-2.153; $P = 0.032$), metastatic infection (adjusted OR, 3.447; 95% CI, 2.604-4.562; $P < 0.001$) and methicillin resistance (adjusted OR, 2.582; 95% CI, 2.219-3.130; $P < 0.001$) were

significant independent risk factors for persistent SAB. For persistent MRSA bacteremia, solid organ transplantation (adjusted OR, 1.975; 95% CI, 1.088-3.583; P = 0.025), metastatic infection (adjusted OR, 3.479; 95% CI, 2.227-5.435; P < 0.001), and ST5 (adjusted OR, 1.457; 95% CI 1.103-1.925; P = 0.008) were significant independent risk factors. For persistent MSSA bacteremia, community-acquired acquisition (adjusted OR, 1.637; 95% CI 1.189-2.255; P = 0.003), vascular grafts (adjusted OR, 1.990; 95% CI, 1.190-3.328; P = 0.009), metastatic infection (adjusted OR, 3.301; 95% CI, 2.286-4.767; P < 0.001), and ST72 (adjusted OR, 1.587; 95% CI, 1.127-2.236; P = 0.008) were identified as significant risk factors. The most predominant clone in persistent MRSA bacteremia was ST5-*SCCmec* II-t2460 (211/635, 33.2%), and in persistent MSSA it was ST72-t126 (63/416, 15.1%). In persistent MRSA bacteremia, *agr* dysfunction (P = 0.025) and *agr* type II (P = 0.008) were significantly more prevalent. In virulence gene analysis, *sec* was frequent in persistent MRSA bacteremia (P = 0.033). When using bacteremia day 1 as the reference point, analysis of whether there is a significant increase in mortality with each additional day of bacteremia duration revealed significant mortality on day 3 for of SAB-related mortality (relative risk, 1.589; 95% CI, 1.060-2.355; P = 0.024) in overall SAB. The analysis revealed that ST5-*spa*-t9353 and ST72-*spa*-t126 in persistent SAB, as well as ST72-*spa*-t126 in persistent MSSA bacteremia, had statistically significantly higher 90-day and SAB-related mortality rates compared to resolving bacteremia.

Conclusions: As a result of this study based on a large cohort, it can be helpful to treat patients with persistent SAB and achieve better outcomes by understanding the clinical, microbiological and genetic characteristics of persistent SAB as well as the characteristics of dominant ST.

Keywords: *Staphylococcus aureus*, persistent bacteremia, sequence type, virulence genes

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INTRODUCTION

Staphylococcus aureus bacteremia (SAB) can persist even with the use of appropriate antibiotics and can progress to severe infections, accompanied by multiple complications or leading to high morbidity and mortality.[1,2] Among SAB, persistent SAB accounts 6-38%,[2-4] and in previous studies, the mortality rate of persistent SAB is 21~51%, which is higher than that of resolving SAB.[1,5]

However, in previous studies, the duration of persistent bacteremia was defined differently for each study, and accordingly, persistent bacteremia ranges from 2 days or more,[6] 3 days,[7,8] 4 days,[9-11] 5 days,[12] to 7 days[1,2,7,13] or even longer. In addition, the definition of the onset of bacteremic days varies from study to study, and in some studies, it is counted from the first positive blood culture, and in others, it is counted after the appropriate antibiotic is administered.[7,14,15] Because of these heterogeneous definitions, the analysis or outcome compared to the shorter duration group is bound to vary depending on the duration of the bacteremia, and the ambiguous results have been the limitations of the study so far.

The Infectious Diseases Society of America (IDSA) recommends that the median clearance of methicillin-resistant *S. aureus* (MRSA) bacteremia is 7-9 days based on two studies,[1,16] patients with persistent bacteremia over 7 days should be re-assessed to determine whether a change in therapy is indicated. However, despite the use of appropriate antimicrobial agents against SAB, it is a very complex question at which point to consider treatment failure when positive is consistently confirmed in the follow-up blood culture.

Recent studies have reported that the duration of persistent SAB should be shorter than 7 days. Some other cohorts documented that median duration of 2-3 days of SAB could be a cutoff of increased poor clinical outcome including mortality.[7,9] Minejima, E. *et al*. reported that 3 days of SAB was the most significant duration to differentiate survival versus death by ROC analysis,[15] and Kuehl, *R. et al.* reported that persistent SAB 2 days or more despite active antibiotics is the best cutoff to predict mortality.[14] However, even in these latest studies, there were limitations that there were not enough sample sizes, especially for intermediate or prolonged SAB group, and the microbiologic or genotypic

characteristics were not be thoroughly analyzed. And MRSA or methicillin-susceptible *S. aureus* (MSSA) bacteremia was analyzed as a subgroup.

As mentioned above, persistent SAB is related to higher mortality and poor clinical outcomes compared to resolving SAB. Most recent studies have shown higher mortality rates, but MRSA bacteremia has only a slightly higher adjusted mortality compared to MSSA bacteremia.[17] Recent high-quality studies in the field indicate that a modest increase in the odds ratio (OR) or relative risk (RR) of death, ranging from approximately 1.3 to 1.8.[17] Mortality in patients with SAB can be reduced through standardized clinical management practices, including mandatory infectious diseases consultation, routine echocardiography, follow-up blood cultures, and appropriate antibiotic therapy.[17-19] Despite these measures, approximately 25% of patients with SAB will die within three months of diagnosis. This is because the etiology of persistent SAB is considered to result from the complex interaction among the host factors, the pathogen, and the antibiotic treatment.[17] Furthermore, persistent SAB is recognized as the strongest predictor of complicated SAB.[13] One of the unique and disturbing features of SAB is the tendency of the organism to persist in the bloodstream despite the presence of microbiologically appropriate antibiotics. However the phenomenon of persistent bacteremia remains poorly understood, due to lack of studies.

As for host clinical risk factors, the presence of retained intravascular devices or foreign bodies, metastatic infection including endocarditis, bone and joint infection, underlying disease such as chronic renal failure, cirrhosis, and diabetes are associated with persistent SAB.[1,2,5,7,20] However, the majority of these studies do not distinguish MSSA bacteremia from MRSA bacteremia. Regarding host genetic factors, a study by Oestergaard et al. in 2016 found that first-degree relatives of patients hospitalized for SAB, particularly siblings, had a significantly increased risk of experiencing SAB themselves, suggesting heritable risk factors, although the specific genetic defect remains unidentified.[21] In relation to genome-wide association studies, genetic loci near the human leukocyte antigen class II histocompatibility antigen, differentiation region alpha chain (HLADRA) and leukocyte antigen - differentiation region beta-1 Chain (HLA-DRB1) genes in the HLA class II region were found to be associated with SAB in both European and African American populations through

admixture mapping. This groundbreaking discovery in *S. aureus* research underscores the role of HLA haplotypes in influencing susceptibility and severity of bacterial infections.[22-27] Studies on host genetic variation in persistent SAB are rare, but Mba Medie et al. discovered a protective association between a deoxyribonucleic acid (cytosine-5)-methyltransferase 3A (DNMT3A) gene variant and reduced risk of persistent MRSA bacteremia, linked to lower interleukin (IL)-10 levels and altered deoxyribonucleic acid (DNA) methylation patterns influencing immune response dynamics.[28]

And Chang et al. found distinct DNA methylation patterns in leukocytes of patients with persistent versus resolving MRSAB, implicating immune-regulatory transcription factors and histone modifiers in the pathogenesis of the infection.[29] As for biomarkers of persistent SAB, Guimaraes et al. identified eight proteins, including IL-17A, IL-10, and soluble E-selectin, strongly associated with persistent SAB,[12] while Cao et al. showed these biomarkers can predict microbiologic failure and mortality better than traditional clinical risk factors.[30] To survive and replicate in the bloodstream, *S. aureus* must evade host defenses and adhere to endothelial surfaces. This process involves coordinated expression of adhesins, exotoxins, and exoenzymes, while also resisting neutrophil phagocytosis and oxidative stress, as well as platelet-derived antimicrobial peptides.

Virulence factors, regulatory mechanisms such as accessory gene regulator,[1,31] and susceptibility to antimicrobial peptides contribute significantly to these complex interactions.[32] The *agr* system in *S. aureus*, a key regulator of virulence factor production, is often dysfunctional in persistent MRSA bloodstream infections, leading to increased bacterial survival and reduced antibiotic susceptibility.[13,33,34] Despite decades of research on *S. aureus* virulence factors, no single factor has been pinpointed as crucial for survival in bloodstream infections, indicating that combinations of factors likely vary by infection site. Studies comparing persistent and resolving MRSA bloodstream infections have found some differences, such as increased resistance to neutrophil antimicrobial peptides and greater binding to fibrinogen and fibronectin in persistent isolates.[35] However, other studies have not found consistent differences in virulence genes or binding capabilities, suggesting variability in findings may be due to epidemiological differences in isolates from different regions.[1,36] Genotypic analysis has helped differentiate persistent from resolving MRSAB isolates, but functional

differences often result from multiple interacting processes. Studies by Seidl et al. confirmed that persistent MRSAB isolates showed significantly less killing by neutrophil-derived antimicrobial peptide human neutrophil peptide-1 (AMP hNP-1) and platelet-derived thrombin-induced platelet microbicidal proteins (tPMPs).[35] Although no significant differences in overall biofilm biomass were noted, biofilms from persistent isolates had a lower carbohydrate content. These findings suggest that decreased killing by tPMPs is associated with increased virulence and persistence of MRSAB, particularly in endovascular infections.[35-37] Antibiotic tolerance in *S. aureus* allows bacterial survival in the presence of lethal antibiotic concentrations without altering the minimum inhibitory concentration (MIC), posing challenges for detection and clinical management. Mechanisms such as altered metabolic activity and immune evasion contribute to tolerance, complicating treatment outcomes, particularly in persistent bloodstream infections.[38] Studies suggest antibiotic tolerance may precede resistance development, highlighting its potential role in treatment failure and emphasizing the need for further research into its clinical implications and management strategies.[39]

Vancomycin remains a cornerstone treatment for MRSA, with rare instances of resistance (VRSA) and more common occurrence of VISA. Elevated vancomycin MIC (>1.5 μg/mL) in MRSA is associated with increased mortality, despite treatment adjustments. Heterogeneous VISA (hVISA) complicates treatment further, with uncertain impact on clinical outcomes, necessitating ongoing research into effective management strategies for persistent bacteremia in MRSA.

However, research on persistent SAB remains still limited, with most studies focusing on overall SAB or MRSA. For MSSA, the evidence is even more scarce and derived from studies that have important limitations. Consequently, the phenomenon of persistent bacteremia continues to be poorly understood.

Therefore, based on large prospective cohort, this study aims to investigate persistent MRSA and MSSA bacteremia, as well as overall SAB, compared with resolving bacteremia to analyze microbiological, genotypic and clinical characteristics by various period of bacteremia. In addition, by identifying factors related to poor clinical outcome, which can lead to find strategies that are helpful in the treatment of persistent SAB patients.

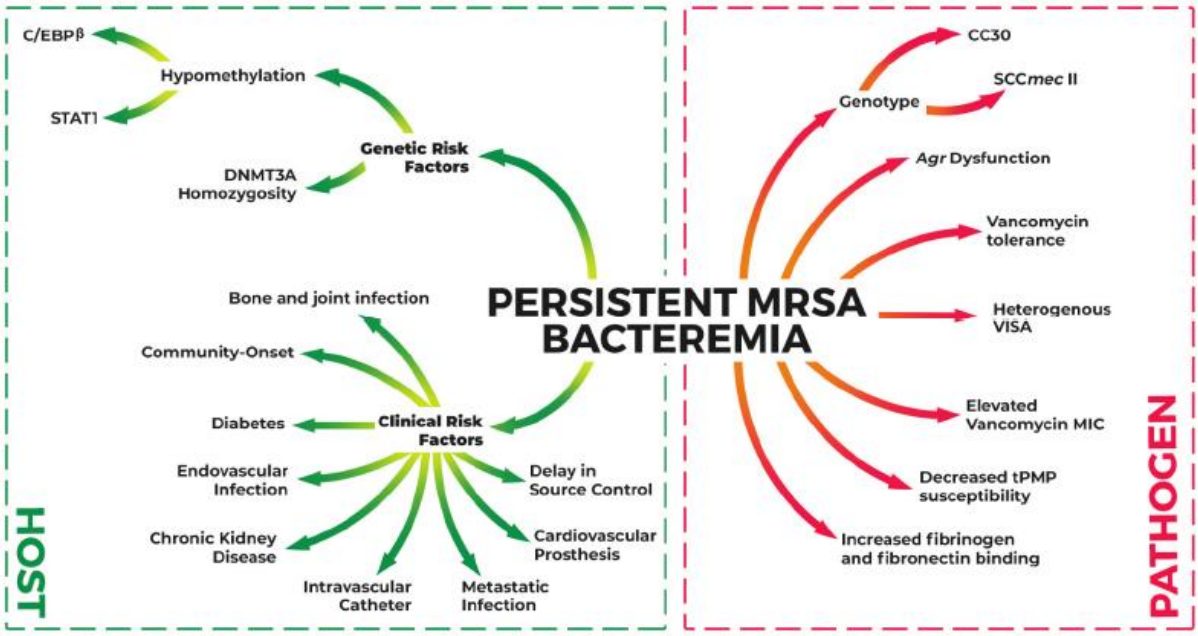


Figure 1. Summary of host and pathogen factors contributing to persistent SAB.[40]

MATERIALS AND METHODS

1. Study design and population

This study was conducted at Asan Medical Center, a 2700-bed tertiary care hospital in Seoul, South Korea. Adult patients (≥ 16 years of age) with SAB were prospectively enrolled and followed up in accordance with this study protocol over a period of 13 years (August 2008 and February 2021).

Patients were excluded from the analysis if: (1) they had polymicrobial bacteremia, (2) they died or had been discharged before obtaining positive blood culture results, (3) they had SAB within the previous three months, (4) the first follow-up blood culture was done after more than 7 days (ie, after day 8), (5) or if active antibiotic therapy was started more than 3 days after the first positive blood culture.

Persistent bacteremia was defined as bacteremia for ≥ 3 days while they were receiving appropriate antibiotics treatment or even if the period was less than 3 days, if a follow-up blood culture conducted within those 3 days was positive again. The comparator was defined as resolving bacteremia.[15,19]

In Asan Medical Center, routine infectious diseases consultation was conducted in patients with SAB with recommendations of follow-up blood cultures at 2-4 days interval until negative conversion. Clinical information, including demographics, the presence of metastatic infection, was reviewed for all patients with SAB a week after the first episode of SAB. The sources of primary infection were categorized as catheter-related bloodstream infection (CRBSI), skin & soft tissue infection (SSTI), infective endocarditis (IE), pneumonia, bone & joint infection (BJI), arteriovenous fistula graft infection, surgical site infection, peripheral venous catheter-related infection, urinary tract infection, unknown primary bacteremia, and others.

Of the patients with SAB those who excluded from the exclusion criteria and remained were analyzed in this study.

This study was approved by the Asan Medical Center Institutional Review Board (IRB number 20131002).

2. Data collection and definitions

The data obtained from all patients included demographics, presence of pre-existing underlying diseases or conditions, severity of the underlying diseases by the Charlson comorbidity score,[41] mode of acquisition,[42] the presence of indwelled device, sepsis severity, management, antibiotic therapy, and clinical outcomes. The system of McCabe and Jackson was used to classify prognosis of the underlying disease; rapidly fatal (expected death within several months), ultimately fatal (expected death within 4 years), and nonfatal (life expectancy was > 4 years). [43] The severity of bacteremia was identified based on the Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Pitt bacteremia score.[44] The presence of metastatic infection was defined as the newly developed infection in a new sterile site that was not clinically relevant at the time of the first blood culture and not identified at the initial diagnosis of SAB. Outcomes, including all-cause 30-day and 90-day mortality, SAB-related mortality, recurrence, were assessed during the 90 days after the first episode of SAB. Recurrence was defined as symptoms and signs of infection more than 7 days after clinical improvement with negative conversion of SAB. SAB-related mortality was defined as death occurring before the resolution of symptoms or signs within 7 days of SAB onset without other explanation.

3. Collection of *S. aureus* isolates

The *S. aureus* samples were plated on a blood agar plate. This sterile medium was streaked with a cotton swab and the plates were incubated overnight at 37℃. The isolate was grown to screen for and analysed for *S. aureus.* The strains were stored in 20% glycerol-tryptic soy broth at −80℃ (Becton Dickinson, Sparks, MD). The methicillin resistance of *S. aureus* isolates was determined based on the oxacillin minimal inhibitory concentration (MIC) and the presence of the *mecA* gene.

4. Microbiological data and analysis

1) Antimicrobial susceptibility tests

The antimicrobial susceptibility was determined using the standard criteria based on the MicroScan (Beckman Coulter, Brea, CA, USA) and the Clinical and Laboratory Standard Institute (CLSI)

guidelines.[45] Vancomycin MIC was determined with the broth microdilution (BMD) according to the CLSI guideline. For broth microdilution method, serial twofold dilutions were carried out in cation-adjusted Mueller-Hinton Ⅱ broth (Becton Dickinson, Sparks, MD) in microtiter plates following standard Criteria.[45] MIC is determined by the broth microdilution method and each spot is inoculated with 10 ^ 6 CFU. After 16-20 hours of incubation at 37 ℃, the MIC value is considered when the bacteria do not grow at the lowest concentration of antibiotics. Reference strain American Type Culture 4 Collection (ATCC) 29213 was used for quality control.

2) Deoxyribonucleic Acid (DNA) preparation Polymerase Chain Reaction (PCR)

A rapid lysis protocol for DNA extraction was used to prepare DNA for PCR amplification. Bacteria were harvested from overnight culture on sheep blood agar plates. Next day, Total DNA was isolated from 1-2ml of a Tryptic Soy Broth culture. Cells were pelleted by centrifugation for 1min at 13,000rpm. removed supernatant and Added 50 ㎕ of Pre-buffer [Before use, added 0.25 ㎕ RNase A stock solution (20mg/ml)] and 3 ㎕ of lysozyme solution (100mg/ml), mixed well and incubated at 37℃ for 15min. Added 250 ㎕ of lysis buffer solution [Before used, added 26.25 ㎕ of RNase A stock solution and added 5 ㎕ of Proteinase K stock solution (20mg/ml)]. Incubated at 65℃ for 15min and added 250 ㎕ of Binding buffer. Cell lysates loading on column and centrifugation at 13,000rpm for 1 min. To washed, added 500 ㎕ of Washing buffer to column and centrifugation at 13,000rpm for 1min. Placed the G-spin column in a clean 1.5ml micro centrifuge tube and added 40 ㎕ Elution buffer and at 13,000rpm for 1min. The DNA was used as the template in all PCRs described below.

3) Detection of *mecA* gene

The *mecA* gene sequence (532 bp) of all MRSA isolates was amplified by PCR. The amplification of *mecA* gene using *mecA1* (5 ′ AAA ATC GAT GGT AAA GGT TGG C 3′) and *mecA2* (3 ′ AGT TCT GCA GTA CCG GAT TTG C 5 ′) primers and sequence analysis. The PCR conditions were an initial

denaturation at 3min, followed by 30 cycles of 94℃ for 30s, 55℃ for 30s, and 72℃ 30s and final extension at 72℃ for 4min. PCR products (10 ㎕) were separated by 1% agarose gel in 0.5X Tris-borate-EDTA buffer at 100V and visualized with RedSafe (Figure 2, lane 1). The sequence information of each primer was as followed by Table 1.[46]

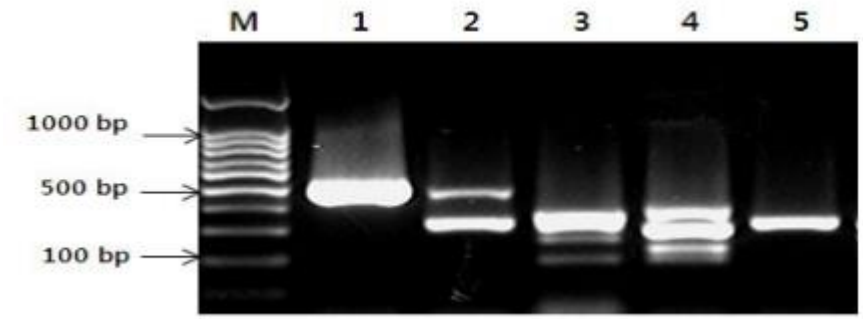


Figure 2. *mecA* gene and SCC*mec* types of MRSA isolates by multiplex PCR. *mecA* gene (lane 1); Lanes 2 and 5, SCC*mec* type I, II, III, and IV from 4 MRSA clinical isolates. Lane M, DNA molecular size marker (100 bp DNA Ladder, iNtRON)

Table 1. Primers used for amplification of the *mecA* genes from *S. aureus*

Gene	Primer	Primer sequence	Amplicon size (bp)
mec	*mecA*-F	5'-AAA ATC GAT GGT AAA GGT TGG C-3'	532
	mecA-R	5'-AGT TCT GCA GTA CCG GAT TTG C-3'	

4) Heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA)

The diagnosis of hVISA (heterogeneous Vancomycin-Intermediate Staphylococcus aureus) was conducted using the PAP-AUC (Population Analysis Profile - Area Under the Curve) method.[30]

The detailed procedure for this method is as follows.[47] The Staphylococcus aureus strain was cultured for 24 hours in brain heart infusion (BHI) broth (BLL, Becton Dickinson, MD). The cultured strain was adjusted to a 0.5 McFarland standard turbidity. This adjustment results in a bacterial count of 10^8 colony-forming units (CFU)/mL. The strain was inoculated in BHI agar containing various concentrations of vancomycin (0, 0.5, 1, 1.5, 2, 3, 4, 8 μg/mL) at dilutions ranging from 10^0 to 10^-6, with 20 μg inoculated per dilution. The inoculated plates were incubated at 37℃ for 48 hours. Colony-forming units on each plate were counted, and the results were plotted on a semi-logarithmic scale for analysis. The area under the curve (AUC) for the test strain was compared to the AUC of the standard strain Mu3 (ATCC 700698). If the ratio of the AUC of the test strain to the AUC of Mu3 exceeded 0.9, the strain was diagnosed as hVISA. If the result fell between 0.8 and 1, the test was repeated (Figure 3).

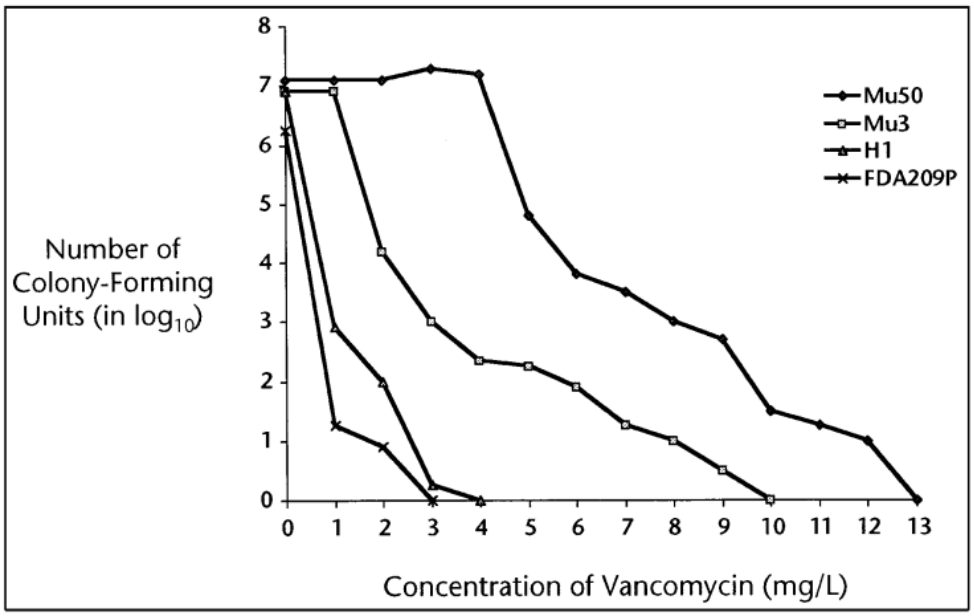


Figure 3. Population Analysis Profile - Area Under the Curve (PAP) Method for Screening hVISA: Schematic Diagram. Vancomycin resistance among subpopulations of the MRSA strains Mu3 and Mu50 and the MSSA strains H1 and FDA209P as determined by a population analysis for resistance.[48]

5) *Agr* functionality test

Delta-haemolysin activity was used to determine *agr* functionality by cross-streaking vertically to RN4220 and a test strain on a sheep blood agar plate (BAP). The beta-hemolysin (Traber & Novick, 2006) produced by RN4220 enables detection of delta-hemolysin.[49] Delta-hemolytic activity was indicated by an enhanced area of hemolysis at the intersection of the streaks (Figure 4).

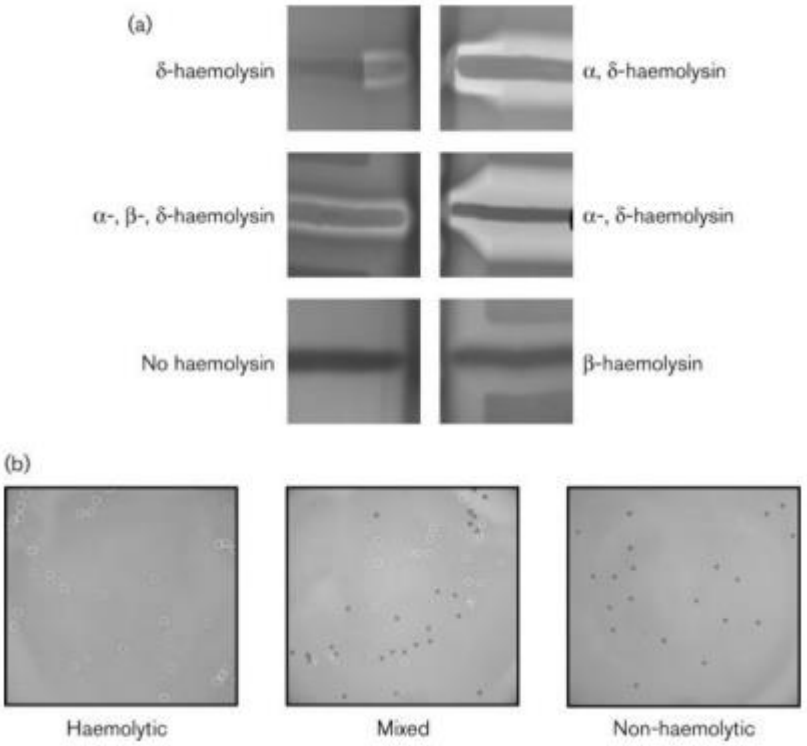


Figure 4. Haemolytic activities were determined by cross-streaking perpendicularly to RN4220, which produces only β-haemolysin (Traber & Novick, 2006), on a sheep blood agar (SBA) plate. This test can usually identify the three staphylococcal haemolysins active on SBA – α, β and δ – because of the interactions between them: β-haemolysin enhances lysis by δ - haemolysin, but inhibits lysis by α-haemolysin (Elek & Levy, 1950). To determine - haemolysin production by single colonies, SBA plates were prepared by spreading 400 µl of a sterile twofold-concentrated RN4220 supernatant before plating a suitable dilution of the strain to be tested. Note that the β-haemolysin produced by RN4220 enables detection of β-haemolysin.[49] (a) Strains were tested against RN4220; (b) analysis of single colonies for δ-haemolysin.

6) Accessory Gene Regulator (*agr*) Grouping

The *agr* gene of each MRSA isolate was amplified with primers Pan, *agr*1, *agr* 2, *agr* 3, and *agr* 4 as described previously.[50-53]

The *agr* region were amplified from 2 ㎕ of the purified nucleic acid solutions in a 25 ㎕ reaction mixture containing 1.25U of *Taq* DNA polymerase (*Taq* DNA polymerase in storage buffer A [Promega]), 200 μM dNTPs (Promega), 5 mM MgCl2, 50 mM KCl, 0.1% Triton X-100, 10 mM Tris•Cl (pH 9.0), and a 0.3 μM concentration of each of the following primers: Pan (5'-ATG CAC ATG GTG CAC ATG C-3'), *agr*1 (5'-GTC ACA AGT ACT ATA AGC TGC GAT-3'), *agr*2 (5'-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3'), *agr*3 (5'-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3'), and *agr*4 (5'-CGA TAA TGC CGT AAT ACC CG-3'). These primers allow the amplification of a 441-bp DNA fragment of the *agr* group I strains, of a 575-bp DNA fragment of the *agr* group II strains, of a 323-bp DNA fragment of the *agr* group III strains, and of a 659-bp DNA fragment of the *agr* group IV strains. Amplifications were carried out in an MJ Research thermocycler (PTC-100) through the following temperature program: 1 cycle of 5 min at 94℃; 30 cycles of 30 s at 94℃, 30 s at 55℃, and 60 s at 72℃; and finally 1 cycle of 72℃ for 10 min. PCR products (7~10 ㎕) were separated by 1.5% 6 agarose gel in 0.5X Tris-borate-EDTA buffer at 100V and visualized with ethidium bromide (Figure 5, Figure 6).

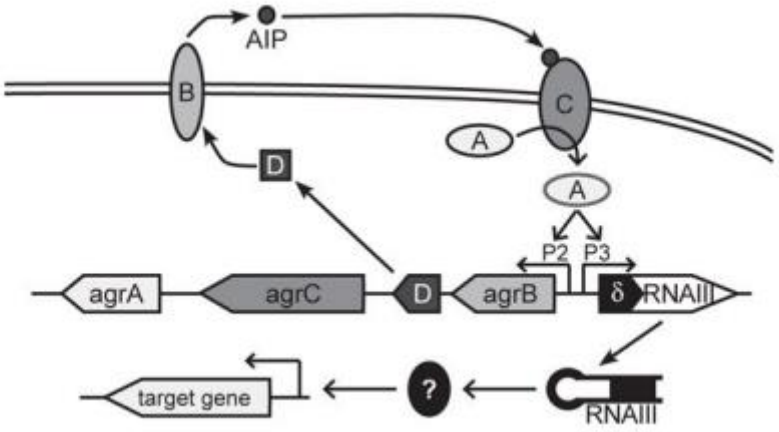


Figure 5. The *agr* locus.[49] It consists of two divergent transcription units driven by promoters P2 and P3. The P2 operon encodes a two-component signalling module, of which AgrC is the receptor and AgrA is the response regulator. It also encodes two proteins, AgrB and D, which combine to produce and secrete an autoinducing peptide (AIP) that is the ligand for AgrC. AgrA functions to activate transcription from its own promoter and from the agrP3 promoter, which drives the synthesis of RNAIII, the effector of target gene regulation. RNAIII also encodes delta-heomylsin, the expression of which serves as a surrogate for *agr* functionality.

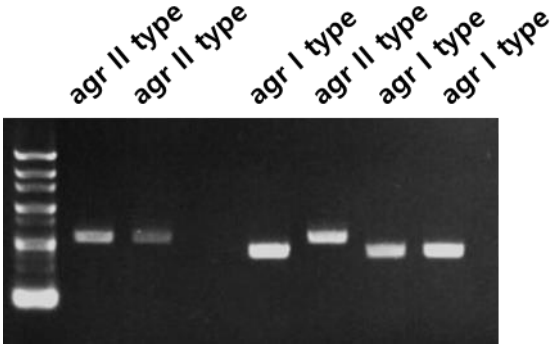


Figure 6. *agr* grouping. The primers (lane 1) allow the amplification of a 441-bp DNA fragment of the *agr* group I strains (labe 4, 6, 7), of a 575-bp DNA fragment of the *agr* group II strains (labe 2, 3, 5).

7) Multilocus sequence typing (MLST)

The MLST of the isolates was conducted by amplifying internal fragments of seven housekeeping genes of *S. aureus* as described previously.[54] The fragments were amplified by using the primers (reference): carbamate kinase (*arc*C), shikimate dehydrogenes e (*aro*E), glycerol kinase (*glp*F), guanylate kinase (*gmk*), phosphate acetyltransferas (*pta*), triosephophate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*). Following purification and sequencing of these genes, allele quantification and sequence typing were assigned using a well-characterized online database (https://pubmlst.org/). The sequence information of each primer was as followed by Table 2.

Table 2. Primers used in PCR for Multilocus sequence typing (MLST)

Locus	Primer	Primer sequence	Amplicon size (bp)
arc	*arc*-F	5'-TTG ATT CAC CAG CGC GTA TTG TC -3'	456
	arc-R	5'-AGG TAT CTG CTT CAA TCA GCG -3'	
aro	*aro*-F	5'-ATC GGA AAT CCT ATT TCA CAT TC -3'	456
	aro-R	5'-GGT GTT GTA TTA ATA ACG ATA TC -3'	
glp	*glp*-F	5'-CTA GGA ACT GCA ATC TTA ATC C -3'	465
	glp-R	5'-TGG TAA AAT CGC ATG TCC AAT TC -3'	
gmk	*gmk*-F	5'-ATC GTT TTA TCG GGA CCA TC -3'	429
	gmk-R	5'-TCA TTA ACT ACA ACG TAA TCG TA -3'	
pta	*pta*-F	5'-GTT AAA ATC GTA TTA CCT GAA GG -3'	474
	pta-R	5'-GAC CCT TTT GTT GAA AAG CTT AA -3'	
tpi	*tpi*-F	5'-TCG TTC ATT CTG AAC GTC GTG AA -3'	402
	tpi-R	5'-TTT GCA CCT TCT AAC AAT TGT AC -3'	
yqi	*yqi*-F	5'-CAG CAT ACA GGA CAC CTA TTG GC -3'	516
	yqi-R	5'-CGT TGA GGA ATC GAT ACT GGA AC -3'	

8) Staphylococcal cassette chromosome *mec* (SCC*mec*) typing

The SCC*mec* typing of MRSA isolates was performed using the multiplex polymerase chain reaction (PCR) method of Oliveira and de Lencastre.[55] The multiplex PCR includes eight loci (A through H) selected in the basis of previously described *mec* element sequences (Table 3). Locus A is located downstream of the *pls* gene and is specific for SCC*mec* type; locus B is internal to the *kdp* operon, which is specific for SCC*mec* type II; locus C is internal to the *mec* I gene present in SCC*mec* type II and III; locus D is internal the *dcs* region, present in SCC*mec* types I, II and IV; locus E is located in the region between integrated plasmid pI258 and transposon Tn*554*, specific for SCC*mec* type III; locus F, which is also specific for SCC*mec* type III, is located in the region between Tn*554* and the chromosomal right junction (*orfX*). Loci G and H were included to distinguish structural variants IA and IIIA, respectively. Locus G is the left junction between IS*431* and pUB110, and locus H is the left junction between IS*431* and pT181 (Figure2, lane 2 to 5 and Figure 7).

Primers were designed, and commercially obtained (COSMO Genetech, Korea). The multiplex PCR was performed in a total 50 ㎕ containing 5 ㎕ of DNA extract, 1X PCR buffer; 200μM (each) deoxy nucleoside triphate; 200nM of 5 primers KOP F1, KDP R1, RIF4 F3, and RIF4 R9; 400nM of primers ClF2 F2, ClF R2, MECI P2, MECI P3, RIF5 F10, RIF5 R13, pUB110 R1, and pT181 R1; 800nM concentrations of primers DCS F2, DCS R2, MECA P4, MECA P7, and IS431; and 1.25 U of I-Star *Taq* polymerase. The PCR conditions were a initial denaturation at 4min, followed by 30 cycles of 94℃ for 30s, 53℃ for 30s, and 72℃ 1min and final extension at 72℃ for 4min. PCR products (7~10 ㎕) were separated by 2% agarose gel in 0.5X Tris-borate-EDTA buffer at 100V and visualized with ethidium bromide. The sequence information of each primer was as followed by Table 3.

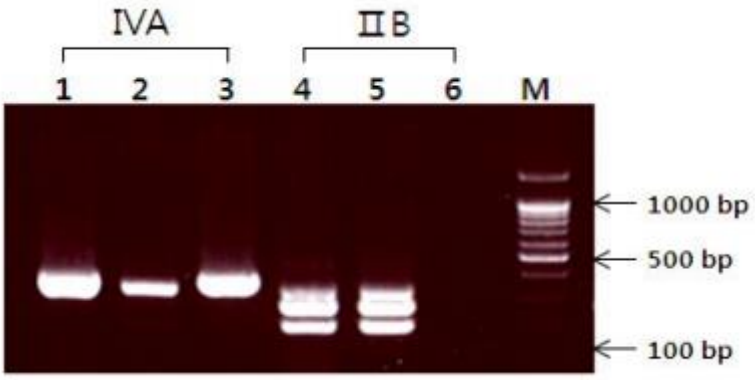


Figure 7. SCC*mec* subtypes of 2 MRSA clinical isolates by multiplex PCR. Two representative subtypes, IVA and IIB, are given at the top. SCC*mec* type IVA (lanes 1 to 3); lane 1, detection of D (342 bp) and G (381 bp) from locus A to H; lane 2, detection of D from locus A to D; lane 3, detection of G from locus E to H. SCC*mec* type IIB (lane 4 to 6), lane 4, detection of B (284 bp), C (209 bp), and D (342 bp) from locus A to H; lane 5, detection of B, C, and D from locus A to D; lane 6, no band from locus E to H. Lane M, DNA molecular size marker (100 bp DNA Ladder, iNtRON)

Table 3. Primers used in the multiplex PCR for SCC*mec* typing

Locus	Primer	Primer sequence	Amplicon size (bp)	SCC*mec* type
A	CIF2 F2	5’-TTCGAGTTGCTGATGAAGAAGG-3’	495	I
	CIF2 R2	5’-ATTTACCACAAGGACTACCAGC-3’		
B	KDP F1	5’-AATCATCTGCCATTGGTGATGC-3’	284	II
	KDP R1	5’-CGAATGAAGTGAAAGAAAGTGG-3’		
C	MECI P2	5’-ATCAAGACTTGCATTCAGGC-3’	209	II, III
	MECI P3	5’-GCGGTTTCAATTCACTTGTC-3’		
D	DCS F2	5’-CATCCTATGATAGCTTGGTC-3’	342	I, II, IV
	DCS R1	5’-CTAAATCATAGCCATGACCG-3’		
E	RIF4 F3	5’-GTGATTGTTCGAGATATGTGG-3’	243	III
	RIF4 R9	5’-CGCTTTATCTGTATCTATCGC-3’		
F	RIF5 F10	5’-TTCTTAAGTACACGCTGAATCG-3’	414	III
	RIF5 R13	5’-GTCACAGTAATTCCATCAATGC-3’		
G	IS431 P4	5’-CAGGTCTCTTCAGATCTACG -3’	381	
	pUB110 R1	5’-GAGCCATAAACACCAATAGCC-3’		
H	IS431 P4	5’-CAGGTCTCTTCAGATCTACG-3’	303	
	pT181 R1	5’-GAAGAATGGGGAAAGCTTCAC-3’		

9) Detection of Virulence Factors Genes

A multiplex PCR was performed to detect the presence of virulence genes. The virulence gene test was performed only on a subset of MRSA isolates. These factors include: 1) SEs (staphylococcal enterotoxin) of PTSAgs (pyrogenic toxin superantigene), SE-like toxins, TSST-1 (toxin shock syndrome-1) 2) ETs (exfoliative toxins) 3) Leukocidins (PVL, LUK gene, Hemolysin gene) 4) Edin (epidermal cell differentiation inhibitor) 5) Adhesion genes (*fnbA, clfA/B, cna, icaA*).[53,56,57] Primers were designed, and commercially obtained (COSMO Genetech, Korea). The PCR conditions were a initial denaturation at 4min, followed by 30 cycles of 94℃ for 1 min, 5 3℃ for 1 min, and 72℃ 1 min and final extension at 72℃ for 4 min. PCR 7 products (7~10 ㎕) were separated by 1~2% agarose gel in 0.5X Tris-borate-EDTA buffer at 100V and visualized with ethidium bromide. The sequence information of each primer was as followed by Table 4.

Table 4. Primers used in PCR for Virulence factors genes

Locus	Primer	Primer sequence	Amplicon size (bp)
bbp	BBP-1	5'-TCAAAAGAAAAGCCAATGGCAAACG-3'	500
	BBP-2	5'- ACCGTTGGCGTGTAACCTGCTG-3'	
clfA	CLFA-1	5'- GTAGGTACGTTAATCGGTT-3'	1,584
	CLFA-2	5'- CTCATCAGGTTGTTCAGG-3'	
clfB	CLFB-1	5'- TGCAAGATCAAACTGTTCCT-3'	596
	CLFB-2	5'- TCGGTCTGTAAATAAAGGTA-3'	
cna	CNA-1	5'- AGTGGTTACTAATACTG-3'	560
	CNA-2	5'- CAGGATAGATTGGTTTA-3'	
ebps	EBP-1	5'- GCAAGTAATAGTGCTTCTGCCGCTTCA-3'	550
	EBP-2	5'- CATTTTCCGGTGAACCTGAACCGTAGT-3'	
fnbA	FNBA-1	5'- CACAACCAGCAAATATAG-3'	1,362
	FNBA-2	5'- CTGTGTGGTAATCAATGTC-3'	
fnbB	FNBB-1	5'- CAGAAGTACCAAGCGAGCCGGAAA-3'	258
	FNBB-2	5'- CGAACAACATGCCGTTGTTTGTTGA-3'	
map/eap	MAP-1	5’-GCATGATAGAGGTATCGGGGAACGTG-3’	655
	MAP-2	5'-TCCCTTGATCATTTGCCATTGCTG-3'	
sdrC	SDRC-1	5'-CGCATGGCAGTGAATACTGTTGCAGC-3'	731
	SDRC-2	5'-GAAGTATCAGGGGTGAAACTATCCACAAATTG-3'	
sdrD	SDRD-1	5'-CCACTGGAAATAAAGTTGAAGTTTCAACTGCC-3'	467
	SDRD-2	5'-CCTGATTTAACTTTGTCATCAACTGTAATTTGTG-3'	
sdrE	SDRE-1	5'-GCAGCAGCGCATGACGGTAAAG-3'	894
	SDRE-2	5'-GTCGCCACCGCCAGTGTCATTA-3'	
eta	mpETA-1	5'-ACTGTAGGAGCTAGTGCATTTGT-3'	190
	mpETA-3	5'-TGGATACTTTTGTCTATCTTTTTCATCAAC-3'	
etb	mpETB-1	5'-CAGATAAAGAGCTTTATACACACATTAC-3'	612
	mpETB-2	5'-AGTGAACTTATCTTTCTATTGAAAAACACTC-3'	
lukDE	LUKDE-1	5'-TGAAAAAGGTTCAAAGTTGATACGAG-3'	269

	LUKDE-2	5'-TGTATTCGATAGCAAAAGCAGTGCA-3'	
PVL	PVL-1	5'-TGCCAGACAATGAATTACCCCCATT-3'	433
	PVL-2	5'-TCTGCCATATGGTCCCCAACCA-3'	
sea	SEA-1	5'-GAAAAAAGTCTGAATTGCAGGGAACA-3'	560
	SEA-2	5'-CAAATAAATCGTAATTAACCGAAGGTTC-3'	
seb	SEB-1	5'-ATTCTATTAAGGACACTAAGTTAGGGA-3'	404
	SEB-2	5'-ATCCCGTTTCATAAGGCGAGT-3'	
sec	mpSEC-1	5'-GTAAAGTTACAGGTGGCAAAACTTG-3'	297
	mpSEC-2	5'-CATATCATACCAAAAAGTATTGCCGT-3'	
sed	SED-1	5'-GAATTAAGTAGTACCGCGCTAAATAATATG-3'	492
	SED-2	5'-GCTGTATTTTTCCTCCGAGAGT-3'	
see	SEE-1	5'-CAAAGAAATGCTTTAAGCAATCTTAGGC-3'	482
	SEE-2	5'-CACCTTACCGCCAAAGCTG-3'	
seg	SEG-1	5'-AATTATGTGAATGCTCAACCCGATC-3'	642
	SEG-2	5'-AAACTTATATGGAACAAAAGGTACTAGTTC-3'	
seh	SEH-1	5'-CAATCACATCATATGCGAAAGCAG-3'	372
	SEH-2	5'-CATCTACCCAAACATTAGCACC-3'	
sei	SEI-1	5'-CTCAAGGTGATATTGGTGTAGG-3'	576
	SEI-2	5'-AAAAAACTTACAGGCAGTCCATCTC-3'	
sek	SEK-1	5'-GGTGTCTCTAATAGTGCCAG-3'	280
	SEK-2	5'-TCGTTAGTAGCTGTGACTCC-3'	
sel	SEL-1	5'-ATCAATGGCAAGCATCAAACAG-3'	250
	SEL-2	5'-TGGAAGACCGTATCCTGTG-3'	
sem	mpSEM-1	5'-CTATTAATCTTTGGGTTAATGGAGAAC-3'	300
	mpSEM-2	5'-TTCAGTTTCGACAGTTTTGTTGTCAT-3'	
sen	mpSEN-1	5'-ATGAGATTGTTCTACATAGCTGCAAT-3'	680
	mpSEN-2	5'-AACTCTGCTCCCACTGAAC-3'	
seo	mpSEO-1	5'-AGTTTGTGTAAGAAGTCAAGTGTAGA-3'	180
	mpSEO-2	5'-ATCTTTAAATTCAGCAGATATTCCATCTAAC-3'	

sep	SEP-1	5'-GACCTTGGTTCAAAAGACACC-3'	230
	SEP-2	5'-TGTCTTGACTGAAGGTCTAGC-3'	
seq	SEQ-1	5'-TCTAGCATATGCTGATGTAGG-3'	390
	SEQ-2	5'-CAATCTCTTGAGCAGTTACTC-3'	
tst	TST-1	5'-TTCACTATTTGTAAAAGTGTCAGACCCACT-3'	180
	TST-2	5'-TACTAATGAATTTTTTTATCGTAAGCCCTT-3'	
icaA	ICAA-1	5'-GATTATGTAATGTGCTTGGA-3'	770
	ICAA-2	5'-ACTACTGCTGCGTTAATAAT-3'	

10) Staphylococcus protein A (*spa*) typing

spa variable repeat region from each MRSA and MSSA isolate was amplified using simplex PCR oligonucleotide primers as described previously.[55,58] The purified *spa* PCR products were sequenced, and the typing of *spa* was performed using the public *spa* database website (http://spa.ridom.de/) for all *S. aureus* isolates. The sequence information of each primer for *spa* typing was as followed by Table 5.

Table 5. Primers used for amplification of the *spa* genes from *S. aureus*

Gene	Primer	Primer sequence	Amplicon size (bp)
spa	spa-F	5'- ACG GCA TCC TTC GGT GAG C -3'	532
	spa-R	5'- GCT TTT GCAATG TCA TTT ACT G-3'	

5. Statistical analysis

All statistical analyses were performed using SPSS software, version 29 (IBM, Armonk, New York, USA). Student t test or the Mann-Whitney U test was used to compare differences between continuous variables, and the Pearson chi-square test or Fisher's exact test was used for the corresponding categorical variables, as appropriate. The variables with *P* values < 0.10 in the univariate analysis and other variables of clinical importance were included in a multivariate logistic regression model to identify independent predictors for persistent SAB, persistent MRSA bacteremia or persistent MSSA bacteremia. Multicollinearity was considered to decide the variables. A two-tailed *P* value of less than 0.05 was considered statistically significant. To analyze the longitudinal changes in annual changes and longitudinal changes in the proportion of persistent SAB in total SAB and of major STs in persistent MRSA bacteremia over the study period, a linear regression analysis was conducted. This analysis identified whether the variables increased or decreased over time.

RESULTS

1. Study Population

During the study period, a total of 1974 patients with *S. aureus* bacteremia were observed, and patients with SAB those who excluded from the exclusion criteria and remained 1877 patients were analyzed. Of the 1877 patients, 953 (50.8%) had MRSA bacteremia and 924 (49.2%) had MSSA bacteremia. According to the definition of persistent bacteremia, 826 (44.0%) had resolving SAB and 1,051 (56.0%) had persistent SAB. 318 (33.3%) of the 953 MRSA bacteremia were resolving bacteremia, the remaining 635 (66.6%) were persistent MRSA bacteremia. 508 (55.0%) of the 924 MSSA bacteremia were resolving bacteremia, and the remaining 416 (45.0%) were persistent MSSA bacteremia.

2. Clinical, microbiological and genotypic characteristics and outcomes of *S. aureus* bacteremia

1) Overall *S. aureus* bacteremia

Comparisons of the clinical characteristics and outcomes of resolving SAB and persistent SAB are shown in Table 6 and the microbiologic and genetic characteristics are shown in Table 7. According to the McCabe and Jackson criteria, rapidly or ultimately fatal disease was more common in resolving SAB (51.8%, $P < 0.001$). Underlying diseases or conditions such as hematologic malignancy (10.5%, $P = 0.003$), neutropenia (8.5%, $P < 0.001$), chemotherapy and immunosuppressive therapy within 30 days prior to the first day of SAB were more common in resolving SAB (19.6%, $P < 0.001$ and 29.5%, $P = 0.036$). In indwelling devices, vascular grafts were more common in persistent MRSA bacteremia (9.4%, $P = 0.014$). The follow-up interval for the first blood culture was relatively shorter in persistent SAB (mean days, 2.79; interquartile range [IQR], 2.0-3.0; $P < 0.001$)) than resolving SAB (mean days, 3.06; IQR, 2.0-3.0). Previous antibiotic exposure and previous glycopeptide exposure within 30 days prior to the first day of SAB were more common in persistent SAB (44.1%, $P < 0.001$ and 13.7%, $P =$

0.013). Metastatic infection was more common in persistent SAB (24.9%, $P < 0.001$). SAB-related mortality was higher in the persistent SAB group (15.6%, $P = 0.002$), but there was no significant difference in 30-day and 90-day mortality between persistent SAB and resolving SAB.

Compared to resolving SAB, persistent SAB had significantly higher methicillin resistance rate than resolving SAB (60.4%, $P < 0.001$). In MLST type, ST5 was significantly higher in persistent SAB (38.2%, $P < 0.001$) while ST6 (4.5%, $P = 0.006$), ST15 (5.2%, $P = 0.018$), ST30 (5.6% $P = 0.024$) and ST188(10.7%, $P < 0.001$) was more common in resolving SAB.

In the results of the multivariate analysis, vascular graft (OR, 1.493; 95% CI, 1.036-2.153; $P = 0.032$), metastatic infection (OR, 3.447; 95% CI, 2.604-4.562; $P < 0.001$), methicillin resistance (OR. 2.582; 95% CI, 2.219-3.130; $P < 0.001$) were identified as independent risk factors for persistent SAB (Table 8).

Table 6. Comparison of clinical characteristics and outcomes of Persistent SAB (n = 1,051) and Resolving SAB (n = 826) [a]

Characteristic	Persistent SAB (n = 1,051)	Resolving SAB (n = 826)	*P* value
Age (years), median (IQR)	63.0 (53.0-71.0)	62.0 (51.0-72.0)	0.255
Male	654 (62.2)	507 (61.4)	0.713
Mode of acquisition			
Nosocomial	557 (53.0)	453 (54.8)	0.426
Healthcare-associated	317 (30.2)	253 (30.6)	0.827
Community-acquired	176 (16.7)	120 (14.5)	0.191
Charlson comorbidity index, median (IQR)	2.0 (1.0-4.0)	3.0 (2.0-5.0)	0.288
McCabe and Jackson criteria			
Rapidly or ultimately fatal disease	459 (43.7)	428 (51.8)	< 0.001
Underlying disease/condition			
Solid cancer	374 (35.6)	321 (38.9)	0.139
Hematologic malignancy	70 (6.7)	87 (10.5)	0.003
Diabetes mellitus	345 (32.8)	246 (29.8)	0.164
End-stage renal disease	131 (12.5)	88 (10.7)	0.229
Liver cirrhosis	153 (14.6)	140 (16.9)	0.153
Solid organ transplantation	66 (6.3)	41 (5.0)	0.225
Hematopoietic cell transplantation	23 (2.2)	14 (1.7)	0.447
Chronic lung disease	30 (2.9)	25 (3.0)	0.823
Rheumatologic disease	38 (3.6)	22 (2.7)	0.243
Ischemic heart disease	102 (9.7)	75 (9.1)	0.647
Heart failure	50 (4.8)	40 (4.8)	0.931
Neutropenia	41 (3.9)	70 (8.5)	< 0.001

Recent surgery[b]	205 (19.5)	137 (16.6)	0.104
Chemotherapy[b]	125 (11.9)	162 (19.6)	< 0.001
Immunosuppressive therapy[b]	265 (25.2)	244 (29.5)	0.036
Indwelling device			
Central venous catheter	419 (39.9)	306 (37.0)	0.220
Pacemaker or defibrillator	18 (1.7)	9 (1.1)	0.261
Prosthetic valve	39 (3.7)	25 (3.0)	0.418
Orthopedic device	43 (4.1)	31 (3.8)	0.709
Vascular graft	99 (9.4)	52 (6.3)	0.014
Sepsis grade			
Sepsis	619 (58.9)	483 (58.5)	0.854
Severe sepsis	109 (10.4)	90 (10.9)	0.714
Septic shock	103 (9.8)	87 (10.5)	0.601
Pitt bacteremia score			
Median (IQR)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	0.672
APACHE II score			
Median (IQR)	15.0 (11.0-20.0)	15.0 (11.0-20.0)	0.849
Management			
First blood culture follow up interval, median days (IQR)	3.0 (2.0-3.0)	3.0 (2.0-4.0)	< 0.001
Intensive care unit treatment	145 (13.8)	92 (11.1)	0.085
Mechanical ventilation	96 (9.1)	62 (7.5)	0.207
Antibiotic therapy			
Previous antibiotic exposure[b]	463 (44.1)	283 (34.3)	< 0.001
Previous glycopeptide exposure[b]	144 (13.7)	82 (9.9)	0.013
Days to appropriate antibiotic therapy, median (IQR)	-1.0 (-2.0-0.0)	0.0 (0.0-0.0)	0.996

Removal of eradicable focus[c]	519 (49.4)	371 (44.9)	0.067
Time to removal (d), median (IQR)	2.0 (0.0-5.0)	1.0 (0.0-3.0)	0.362
Focus still present at day 4[d]	140 (13.3)	99 (12.0)	0.389
Metastatic infection	262 (24.9)	78 (9.4)	< 0.001
30-day mortality	162 (15.7)	118 (14.3)	0.396
90-day mortality	284 (27.0)	213 (25.8)	0.547
SAB-related mortality	164 (15.6)	89 (10.8)	0.002
90-day recurrence	58 (5.5)	34 (4.1)	0.158

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

SAB, *Staphylococcus aureus* bacteremia; MRSA, methicillin-resistant *Staphylococcus aureus*; IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II

[a]This analysis included a total of 1877 SAB with different primary sites of infection, including catheter-related bloodstream infection (CRBSI) (n = 472), SAB pneumonia (n = 154), infective endocarditis (IE) (n = 74), skin & soft tissue infection (SSTI) (n = 174), bone & joint infection (BJI) (n = 184), unknown primary bacteremia (n = 303), and others (arteriovenous fistula graft infection, n = 57; surgical site infection, n = 105; peripheral venous catheter related, n = 118; urinary tract infection, n = 25; other sites of infection, n = 204).

[b]Within 30 days prior to the first day of *Staphylococcus aureus* bacteremia

[c]Percentage of patients with the eradicable focus.

[d]Day 1 represents the day of the index blood culture.

Table 7. Comparison of Microbiological and Genetic Characteristics of Persistent SAB (n = 1,051) and Resolving SAB (n = 826) [a]

Microbiological characteristic	Persistent SAB (n = 1,051)	Resolving SAB (n = 826)	*P* value
Methicillin resistance	635 (60.4)	318 (38.5)	< 0.001
MLST type[b]			
ST1	29 (2.8)	35 (4.2)	0.080
ST5	402 (38.2)	185 (22.4)	< 0.001
ST6	24 (2.3)	37 (4.5)	0.008
ST15	32 (3.0)	43 (5.2)	0.018
ST30	36 (3.4)	46 (5.6)	0.024
ST72	288 (27.4)	200 (24.2)	0.418
ST188	60 (5.7)	88 (10.7)	< 0.001
ST239	18 (1.7)	7 (0.8)	0.105

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; MLST, Multilocus sequence type; MIC, minimal inhibitory concentration; BMD, broth microdilution; hVISA, heteroresistant vancomycin-intermediate *Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator; ST, sequence type.

[a]This analysis included a total of 1877 SAB with different primary sites of infection, including catheter-related bloodstream infection (CRBSI) (n = 472), SAB pneumonia (n = 154), infective endocarditis (IE) (n = 74), skin & soft tissue infection (SSTI) (n = 174), bone & joint infection (BJI) (n = 184), unknown primary bacteremia (n = 303), and others (arteriovenous fistula graft infection, n = 57; surgical site infection, n = 105; peripheral venous catheter related, n = 118; urinary tract infection, n = 25; other sites of infection, n = 204).

[b]The major clones are shown. There were 39 isolates with STs not frequently detected, including ST1 (n = 64), ST6 (n = 61), ST8 (n = 56), ST15 (n = 75), ST30 (n = 82), ST59 (n = 18), ST88 (n = 3), ST89 (n = 6), ST97 (n = 25), ST101 (n = 15), ST188 (n = 148), ST121 (n = 21), ST199 (n = 3), ST254 (n = 6), ST291 (n = 17), ST509 (n = 4), ST728 (n = 1), ST834 (n = 1), ST3081 (n = 1), ST3558 (n = 1), and others.

Table 8. Multivariate Analysis of Risk Factors for Persistent SAB (n = 1,051)

Risk factor	No (%) of Patients		Univariate analysis		Multivariate analysis	
	Persistent SAB (n = 1,051)	Resolving SAB (n = 826)	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Underlying disease/condition						
Diabetes mellitus	345 (32.8)	246 (29.8)	1.150 (0.944-1.401)	0.164		
End-stage renal disease	131 (12.5)	88 (10.7)	1.193 (0.895-1.589)	0.229		
Indwelling device						
Central venous catheter	306 (37.0)	419 (39.9)	1.124 (0.932-1.357)	0.220		
Vascular graft	99 (9.4)	52 (6.3)	1.548 (1.092-2.193)	0.014	1.493 (1.036-2.153)	0.032
Antibiotic therapy						
Previous antibiotic exposure[a]	463 (44.1)	283 (34.3)	1.513 (1.253-1.827)	< 0.001		
Previous glycopeptide exposure[a]	144 (13.7)	82 (9.9)	1.439 (1.079-1.920)	0.013		
Metastatic infection	262 (24.9)	78 (9.4)	3.184 (2.426-4.179)	< 0.001	3.447 (2.604-4.562)	< 0.001
Microbiological characteristics						
Methicillin resistance	635 (60.4)	318 (38.5)	2.438 (2.023-2.940)	< 0.001	2.582 (2.129-3.130)	< 0.001

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; IQR, interquartile range; MLST, Multilocus sequence type; MIC, minimal inhibitory concentration;

BMD, broth microdilution; *Staphylococcus aureus*; ; *agr*, accessory gene regulator; SAB, *Staphylococcus aureus* bacteremia; OR, odds ratio; ICU, intensive care unit; ST, sequence type; NA, not applicable

[a]Within 30 days prior to the first day of *Staphylococcus aureus* bacteremia

2) MRSA bacteremia

Comparisons of the clinical characteristics and outcomes of resolving MRSA bacteremia and persistent MRSA bacteremia are shown in Table 9 and Table 10. According to the McCabe and Jackson criteria, rapidly or ultimately fatal disease was more common in resolving MRSA bacteremia (52.8%, $P = 0.003$). In underlying disease and condition, solid organ transplantation was more common in persistent MRSA bacteremia (8.5%, $P = 0.034$), while chemotherapy within 30 days prior to the first day of SAB was more common in resolving MRSA bacteremia (14.8%, $P = 0.040$). The days to appropriate antibiotic therapy after bacteremia onset were relatively shorter in persistent MRSA bacteremia (median days, -1.0; IQR, -2.0 – 0.0; $P < 0.001$). Metastatic infection was significantly higher in persistent MRSA bacteremia (22.5%, $P < 0.001$). There were no statistically significant differences in 30-day mortality, 90-day mortality, SAB-related mortality, or 90-day recurrence between the persistent MRSA bacteremia and the resolving MRSA bacteremia (Table 9).

In persistent MRSA bacteremia, *agr* dysfunction was significant more common than resolving MRSA bacteremia (64.4%, $P = 0.025$). *agr* type II (59.8%, $P = 0.008$) was more common in persistent MRSA bacteremia while *agr* type I (46.8%, $P = 0.018$) and SCC*mec* type IV (41.8%, $P = 0.024$) were more common in resolving MRSA bacteremia. ST5 was significantly more common in persistent MRSA bacteremia (59.5%, $P = 0.015$) while ST30 and ST72 were more common in resolving MRSA bacteremia (0.6%, $P = 0.045$ and 37.4%, $P = 0.038$). In virulence gene analysis, *sec* was common in persistent MRSA bacteremia (65.2%, $P = 0.033$), while *pvl* was common in resolving MRSA bacteremia (6.8%, $P = 0.012$). Additionally, no other virulence genes were identified as significantly higher in persistent MRSA bacteremia compared to resolving MRSA bacteremia (Table 10).

Multivariate analysis identified solid organ transplantation (adjusted OR, 1.975; 95% CI, 1.088-3.583), $P = 0.025$), metastatic infection (adjusted OR, 3.479; 95% CI, 2.227-5.435; $P < 0.001$), and ST5 (adjusted OR, 1.457; 95% CI, 1.103-1.925; $P = 0.008$) as independent risk factors for persistent MRSA bacteremia (Table 11).

Table 9. Comparison of Clinical Characteristics and Outcomes of Persistent MRSA bacteremia (n = 635) and Resolving MRSA bacteremia (n = 318)[a]

Characteristic	Persistent MRSA bacteremia (n = 635)	Resolving MRSA bacteremia (n = 318)	*P* value
Age (years), median (IQR)	64.0 (54.0-72.0)	65.0 (56.0-73.00)	0.116
Male	408 (64.3)	197 (61.9)	0.505
Previous colonization of MRSA	211 (33.2)	90 (28.3)	0.126
Mode of acquisition			
Nosocomial	422 (66.5)	210 (66.0)	0.897
Healthcare-associated	167 (26.3)	87 (27.4)	0.727
Community-acquired	46 (7.2)	21 (6.6)	0.715
Charlson comorbidity index, median (IQR)	300 (2.0-5.0)	3.0 (2.0-5.0)	0.509
McCabe and Jackson criteria			
Rapidly or ultimately fatal disease	271 (42.7)	168 (52.8)	0.003
Underlying disease/condition			
Solid cancer	236 (37.2)	134 (42.1)	0.128
Hematologic malignancy	40 (6.3)	21 (6.6)	0.847
Diabetes mellitus	213 (33.5)	107 (33.6)	0.948
End-stage renal disease	82 (12.9)	34 (10.7)	0.331
Liver cirrhosis	88 (13.9)	41 (12.9)	0.694
Solid organ transplantation	54 (8.5)	15 (4.7)	0.034
Hematopoietic cell transplantation	17 (2.7)	4 (1.3)	0.161
Chronic lung disease	22 (3.5)	15 (4.7)	0.340
Rheumatologic disease	26 (4.1)	7 (2.2)	0.134
Ischemic heart disease	70 (11.0)	38 (11.9)	0.659

Heart failure	26 (4.1)	21 (6.6)	0.089
Neutropenia	26 (4.1)	17 (5.3)	0.380
Recent surgery[b]	165 (26.0)	88 (27.7)	0.578
Chemotherapy[b]	62 (9.7)	47 (14.8)	0.040
Immunosuppressive therapy[b]	170 (26.8)	97 (30.5)	0.226
Indwelling device			
Central venous catheter	322 (50.7)	154 (48.4)	0.507
Pacemaker or defibrillator	10 (1.6)	4 (1.3)	0.701
Prosthetic valve	21 (3.3)	10 (3.1)	0.894
Orthopedic device	31 (4.9)	14 (4.4)	0.742
Vascular graft	59 (9.3)	23 (7.2)	0.285
Sepsis grade			
Sepsis	370 (58.3)	187 (58.8)	0.874
Severe sepsis	64 (10.1)	38 (11.9)	0.378
Septic shock	73 (11.5)	46 (14.5)	0.191
Pitt bacteremia score			
Median (IQR)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	0.455
APACHE II score			
Median (IQR)	16.0 (12.0-21.0)	16.0 (12.0-21.0)	0.525
Management			
First blood follow up interval, median days (IQR)	3.0 (2.0-4.0)	3.0 (2.0-4.0)	0.391
Intensive care unit treatment	121 (19.1)	64 (20.1)	0.694
Mechanical ventilation	86 (13.5)	43 (13.5)	0.993
Antibiotic therapy			
Previous antibiotic exposure[b]	388 (61.1)	175 (55.0)	0.081
Previous glycopeptide exposure[b]	120 (18.9)	62 (19.5)	0.807

Days to appropriate antibiotic therapy, median (IQR)	-1.0 (-2.0-0.0)	0.0 (-1.0-0.0)	< 0.001
Removal of eradicable focus[c]	346 (54.5)	147 (46.2)	0.479
Time to removal (d), median (IQR)	1.0 (0.0-4.25)	2.0 (0.0-3.0)	0.850
Focus still present at day 4[d]	95 (15.0)	42 (13.2)	0.467
Metastatic infection	143 (22.5)	27 (8.5)	< 0.001
30-day mortality	105 (16.5)	52 (16.4)	0.943
90-day mortality	179 (28.2)	95 (29.9)	0.588
SAB-related mortality	98 (15.4)	37 (11.6)	0.113
90-day recurrence	44 (6.9)	21 (6.6)	0.841

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; SAB, *Staphylococcus aureus* bacteremia;IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II.

[a]This analysis included a total of 953 MRSA bacteremia with different primary sites of infection, including catheter-related bloodstream infection (CRBSI) (n = 319), MRSAB pneumonia (n = 92), infective endocarditis (IE) (n = 26), skin & soft tissue infection (SSTI) (n = 58), bone & joint infection (BJI) (n = 69), unknown primary bacteremia (n = 131), and others (arteriovenous fistula graft infection, n =28; surgical site infection, n = 69; peripheral venous catheter related, n = 36; urinary tract infection, n = 14; other sites of infection, n = 110).

[b]Within 30 days prior to the first day of *Staphylococcus aureus* bacteremia

[c]Percentage of patients with the eradicable focus.

[d]Day 1 represents the day of the index blood culture.

Table 10. Comparison of Microbiological and Genetic Characteristics of Persistent MRSA bacteremia (n = 635) and Resolving MRSA bacteremia (n = 318)[a]

Microbiological characteristic	Persistent MRSA bacteremia (n = 635)	Resolving MRSA bacteremia (n = 318)	*P* value
Vancomycin MIC (mg/L) by BMD[b]			
≥1.5	57 (9.0)	19 (6.0)	0.105
Vancomycin trough level <15 mg/L[c]	308/51 (59.6)	140/232 (60.3)	0.842
hVISA[d]	155/398 (38.9)	53/148 (35.8)	0.503
***agr* dysfunction[e]**	409 (64.4)	181 (56.9)	0.025
***agr* type**			
I	242/624 (38.8)	147/314 (46.8)	0.018
II	373/624 (59.8)	159/314 (50.6)	0.008
III	8/624 (1.3)	8/314 (2.5)	0.158
IV	1/624 (0.2)	0/314 (0)	0.478
SCC*mec* type			
I	8/625 (1.3)	3/311 (1.0)	0.673
II	384/625 (61.4)	171/311 (55.0)	0.058
III	19/625 (3.0)	7/311 (2.3)	0.489
IV	214/625 (34.2)	130/311 (41.8)	0.024
MLST type[f]			
ST1	2 (0.3)	1 (0.3)	0.999
ST5	378 (59.5)	163 (51.3)	0.015
ST6	3 (0.5)	0 (0)	0.220
ST15	1 (0.5)	0 (0)	0.479
ST30	0 (0)	2 (0.6)	0.045
ST72	195 (30.7)	119 (37.4)	0.038

ST188	4 (0.6)	1 (0.3)	0.525
ST239	18 (2.8)	7 (2.2)	0.564
Virulence gene[g]			
Adhesin gene			
sdrC	301/351 (85.8)	100/118 (84.7)	0.788
sdrD	333/351 (94.9)	115/118 (97.5)	0.240
sdrE	335/351 (95.4)	116/118 (98.3)	0.161
clfA	351/351 (100)	118/118 (100)	N/A
clfB	351/351 (100)	118/118 (100)	N/A
can	0/351 (0)	0/118 (0)	N/A
map/eap	18/351 (5.1)	10/118 (8.5)	0.184
fnbA	298/298 (100)	97/97 (100)	N/A
fnbB	293/298 (98.3)	95/97 (97.9)	0.803
ebpS	344/351 (98.0)	116/118 (98.3)	0.838
Toxin gene			
sea	16/351 (4.6)	9/118 (7.6)	0.199
seb	0/351 (0)	0/118 (0)	N/A
sec	229/351 (65.2)	64/118 (54.2)	0.033
sed	0/298 (0)	0/97 (0)	N/A
see	0/351 (0)	0/118 (0)	N/A
seg	324/351 (92.3)	109/118 (92.4)	0.982
seh	0/298 (0)	0/97 (0)	N/A
sei	221/351 (91.5)	108/118 (91.5)	0.981
sej	0/351 (0)	0/117 (0)	N/A
sek	23/351 (6.6)	11/118 (9.3)	0.316
sel	273/351 (7.8)	86/118 (72.9)	0.277

sem	323/351 (92.0)	111/118 (94.1)	0.465
sen	325/351 (92.6)	109/118 (92.4)	0.937
seo	277/298 (93.0)	90/97 (92.8)	0.955
sep	13/297 (4.4)	4/97 (4.1)	0.915
seq	10/298 (3.4)	6/97 (6.2)	0.219
eta	2/351 (0.6)	0/118 (0)	0.411
etb	0/351 (0)	1/118 (0.8)	0.084
icaA	351/351 (100)	118/118 (100)	N/A
edin	4/351 (0.3)	118 (0.8)	0.417
bbp	337/351 (96.0)	117/118 (99.2)	0.093
lukDE	349/351 (99.4)	115/118 (97.5)	0.071
lukM	0/351 (0)	0/118 (0)	N/A
lukE	350/351 (99.7)	116/118 (98.3)	0.096
pvl	8/368 (2.2)	9/132 (6.8)	**0.012**
TSST1	206/298 (69.1)	58/97 (59.8)	0.090
Hemolysin gene			
hla	349/351 (99.4)	118/118 (100)	0.411
hlb	282/351 (80.3)	91/118 (77.1)	0.453
hld	277/298 (93.0)	90/97 (92.8)	0.955
hlg	0/298 (0)	0/97 (0)	N/A
hlg2	295/298 (99.0)	96/97 (99.0)	0.983

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; MLST, Multilocus sequence type; MIC, minimal inhibitory concentration; BMD, broth microdilution; hVISA, heteroresistant vancomycin-intermediate *Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator; NA, not applicable

[a]This analysis included a total of 953 MRSA bacteremia with different primary sites of infection,

including catheter-related bloodstream infection (CRBSI) (n = 319), MRSAB pneumonia (n = 92), infective endocarditis (IE) (n = 26), skin & soft tissue infection (SSTI) (n = 58), bone & joint infection (BJI) (n = 69), unknown primary bacteremia (n = 131), and others (arteriovenous fistula graft infection, n= 28; surgical site infection, n = 69; peripheral venous catheter related, n = 36; urinary tract infection, n = 14; other sites of infection, n = 110).

[b]BMD to determine vancomycin MIC was used in 952 patients, respectively.

[c]Percentage of patients who received vancomycin therapy and for whom vancomycin trough levels were monitored.

[d]Population analysing profiling (PAP) was performed in 601 MRSA isolates.

[e] *agr* dysfunction was performed in 953 MRSA isolates.

[f]The major clones are shown. There were 39 isolates with STs not frequently detected, including ST1 (n = 3), ST6 (n = 3), ST8 (n = 4), ST15 (n = 1), ST30 (n = 2), ST59 (n = 2), ST88 (n = 2), ST89 (n = 3), ST97 (n = 1), ST101 (n = 1), ST188 (n = 5), ST121 (n = 1), ST199 (n = 1), ST254 (n = 6), ST291 (n = 1), ST509 (n = 3), ST834 (n = 1), ST3081 (n = 1), ST3558 (n = 1), and others.

[g]MRSA isolates with performed gene tests were analyzed (MRSAB pneumonia, n = 92; CRBSI, n = 319; IE, n = 26; SSTI, n = 58; BJI, n = 69; unknown primary bacteremia, n = 99). 469 isolates (*sdrC, mapeap, sea, sec, seg, sei, sek, sel, sem, sen, seo, TSST1, hlb, hld*), 394 isolates (*sep),* 369 isolates (*seo)* and 395 isolates (*fnbA, fnbB, hld*) were analyzed. Genes found in > 95% or < 5% of the tested isolates were excluded in analysis; *fnbA* (100%, 395/395), *fnbB* (98.2%, 388/395), *bbp* (96.8%, 454/469), *ebps* (98.1%, 460/469), *sdrD* (95.5%, 448/469), *sdrE* (96.1%, 451/469), *clfA* (100%, 469/469), *clfB* (100%, 469/469), *can* (0%, 0/469), *icaA* (100%, 469/469), *seb* (0%, 0/469), *sed* (0%, 0/395), *see* (0%, 0/469), *seh* (0%, 0/395), *sej* (0%, 0/468), *sep* (4.3%, 17/392), *seq* (4.1%, 16/395), *eta* (0.4%, 2/469), *etb* (0.2%, 1/469), *lukD* and *E* (98.9%, 464/469), *lukE* (99.4%, 466/469), *hla* (99.6%, 467/469), *hlg* (0%, 0/395), *lukM* (98.9%, 464/469), *edin* (0.4%, 2/469).

Table 11. Multivariate Analysis of Risk Factors for Persistent MRSA bacteremia (n = 635)

Risk factor	No (%) of Patients		Univariate analysis		Multivariate analysis	
	Persistent MRSA bacteremia (n = 635)	Resolving MRSA bacteremia (n = 318)	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Underlying disease/condition						
Solid organ transplantation	54 (8.5)	15 (4.7)	1.781 (1.039-3.371)	0.037	1.975 (1.088-3.583)	0.025
End-stage renal disease	82 (12.9)	34 (10.7)	1.234 (0.807-1.887)	0.331		
Antibiotic therapy						
Previous antibiotic exposure[a]	388 (61.1)	175 (55.0)	1.275 (0.970-1.675)	0.081		
Indwelling device						
Central venous catheter	322 (50.7)	154 (48.4)	1.096 (0.837-1.434)	0.507		
Vascular graft	59 (9.3)	23 (7.2)	1.314 (0.795-2.170)	0.286		
Metastatic infection	143 (22.5)	27 (8.5)	3.133 (2.025-4.845)	< 0.001	3.479 (2.227-5.435)	<0.001
***agr* dysfunction**	409 (64.4)	181 (56.9)	1.370 (1.040-1.803)	0.025		
MLST type						
ST5	378 (59.5)	163 (51.3)	1.399 (1.067-1.834)	0.015	1.457 (1.103-1.925)	0.008

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; confidence interval; MLST, Multilocus sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator.

[a]Within 30 days prior to the first day of *Staphylococcus aureus* bacteremia

3) MSSA bacteremia

Comparisons of the clinical characteristics and outcomes of resolving MSSA bacteremia and persistent MSSA bacteremia are shown in Table 12 and Table 13. In mode of acquisition, nosocomial acquisition was more common in resolving MSSA bacteremia (47.8%, $P < 0.001$) while community-acquired acquired acquisition was more common in persistent MSSA bacteremia (31.3%, $P < 0.001$). In underlying disease and condition, hematologic malignancy (13.0%, $P = 0.004$), neutropenia (10.4%, $P < 0.001$), chemotherapy (22.6%, $P = 0.002$) and immunosuppressive therapy (28.9%, $P = 0.036$) within 30 days prior to the first day of SAB was more common in resolving MSSA bacteremia. In indwelling device, central venous catheter (CVC) was more common in resolving MSSA bacteremia (29.9%, $P = 0.023$) while vascular graft was more common in persistent MSSA bacteremia (9.6%, $P = 0.024$). The follow-up interval for the first blood culture was relatively shorter in persistent MSSA bacteremia (median days, 2.0; IQR, 2.0-3.0; $P < 0.001$)) than resolving MSSA bacteremia (median days, 3.0; IQR, 2.0-4.0). Time to removal of eradicable focus was significantly longer in persistent MSSA bacteremia (median days, 2.0; IQR, 1.0-5.0; $P = 0.026$) than resolving MSSA bacteremia (median days, 1.0; IQR, 1.0-2.0). Metastatic infection was significantly more common in persistent MSSA bacteremia (28.6%, $P < 0.001$). SAB-related mortality was higher in the persistent MSSA bacteremia (15.9%, $P = 0.011$), but there was no significant difference in 30-day, 90-day mortality and 90-day recurrence between persistent MSSA bacteremia and resolving MSSA bacteremia (Table 12).

In persistent MSSA bacteremia, ST72 was more common in persistent MSSA bacteremia (22.4%, $P = 0.013$) and other STs were no statistically significantly differences between resolving MSSA bacteremia and persistent MSSA bacteremia. *agr* type I was more common in resolving MSSA bacteremia (68.4%, $P < 0.001$)(Table 13).

The multivariate analysis identified community-acquired acquisition (adjusted OR, 1.637; 95% CI, 1.189-2.255; $P = 0.003$), vascular graft (adjusted OR, 1.990; 95% CI, 1.190-3.328; $P = 0.009$), metastatic infection (adjusted OR, 3.301; 95% CI, 2.286-4.767; $P < 0.001$), and ST72 (adjusted OR, 1.587; 95% CI, 1.127-2.236; $P = 0.008$) as independent risk factors for persistent MSSA bacteremia (Table 14).

Table 12. Comparison of Clinical Characteristics and Outcomes of Persistent MSSA bacteremia (n = 416) and Resolving MSSA bacteremia (n = 508)[a]

Characteristic	Persistent MSSA bacteremia (n = 416)	Resolving MSSA bacteremia (n = 508)	*P* value
Age (years), median (IQR)	61.0 (50.0-70.75)	59.0 (48.0-69.75)	0.209
Male	246 (59.1)	310 (61.0)	0.560
Mode of acquisition			
Nosocomial	135 (32.5)	243 (47.8)	< 0.001
Healthcare-associated	150 (36.1)	166 (32.7)	0.281
Community-acquired	130 (31.3)	99 (19.5)	< 0.001
Charlson comorbidity index, median (IQR)	2.0 (1.0-4.0)	2.5 (2.0-4.0)	0.185
McCabe and Jackson criteria			
Rapidly or ultimately fatal disease	188 (45.2)	260 (51.2)	0.070
Underlying disease/condition			
Solid cancer	138 (33.2)	187 (36.8)	0.249
Hematologic malignancy	30 (7.2)	66 (13.0)	0.004
Diabetes mellitus	132 (31.7)	139 (27.4)	0.147
End-stage renal disease	49 (11.8)	54 (10.6)	0.581
Liver cirrhosis	65 (15.6)	99 (19.5)	0.126
Solid organ transplantation	12 (2.9)	26 (5.1)	0.089
Hematopoietic cell transplantation	6 (1.4)	10 (2.0)	0.542
Chronic lung disease	8 (1.9)	10 (2.0)	0.960
Rheumatologic disease	12 (2.9)	15 (3.0)	0.961
Ischemic heart disease	32 (7.7)	37 (7.3)	0.806
Heart failure	24 (5.8)	19 (3.7)	0.143

Neutropenia	15 (3.6)	53 (10.4)	< 0.001
Recent surgery[b]	40 (9.6)	49 (9.6)	0.988
Chemotherapy[b]	60 (14.4)	115 (22.6)	0.002
Immunosuppressive therapy[b]	95 (22.8)	147 (28.9)	0.036
Indwelling device			
Central venous catheter	97 (23.3)	152 (29.9)	0.023
Pacemaker or defibrillator	8 (1.9)	5 (1.0)	0.228
Prosthetic valve	18 (4.3)	15 (3.0)	0.262
Orthopedic device	12 (2.9)	17 (3.3)	0.690
Vascular graft	40 (9.6)	29 (5.7)	0.024
Sepsis grade			
Sepsis	249 (59.9)	296 (58.3)	0.625
Severe sepsis	45 (10.8)	52 (10.2)	0.774
Septic shock	30 (7.2)	41 (8.1)	0.626
Pitt bacteremia score			
Median (IQR)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	0.796
APACHE II score			
Median (IQR)	14.0 (10.0-19.0)	15.0 (10.0-19.0)	0.125
Management			
First blood culture follow up interval, median days (IQR)	2.0 (2.0-3.0)	3.0 (2.0-4.0)	< 0.001
Intensive care unit treatment	24 (5.8)	28 (5.5)	0.866
Mechanical ventilation	10 (2.4)	19 (3.7)	0.246
Antibiotic therapy			
Previous antibiotic exposure[b]	75 (18.0)	108 (21.3)	0.235
Previous glycopeptide exposure[b]	24 (5.8)	20 (3.9)	0.189
Days to appropriate antibiotic therapy,	0.0 (-1.0-0.0)	0.0 (0.0-0.0)	0.434

median (IQR)			
Removal of eradicable focus[c]	173 (41.6)	224 (44.1)	0.990
Time to removal (d), median (IQR)	2.0 (1.0-5.0)	1.0 (1.0-2.0)	0.026
Focus still present at day 4[d]	45 (10.8)	57 (11.2)	0.846
Metastatic infection	119 (28.6)	51 (10.0)	< 0.001
30-day mortality	60 (14.4)	66 (13.0)	0.528
90-day mortality	105 (25.2)	118 (23.2)	0.477
SAB-related mortality	66 (15.9)	52 (10.2)	0.011
90-day recurrence	14 (3.4)	13 (2.6)	0.465

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II; SAB, *Staphylococcus aureus* bacteremia

[a]This analysis included a total of 953 MRSA bacteremia with different primary sites of infection, including catheter-related bloodstream infection (CRBSI) (n = 319), MRSAB pneumonia (n = 92), infective endocarditis (IE) (n = 26), skin & soft tissue infection (SSTI) (n = 58), bone & joint infection (BJI) (n = 69), unknown primary bacteremia (n = 131), and others (arteriovenous fistula graft infection, n = 28; surgical site infection, n = 69; peripheral venous catheter related, n = 36; urinary tract infection, n = 14; other sites of infection, n = 110).

[b]Within 30 days prior to the first day of *Staphylococcus aureus* bacteremia

[c]Percentage of patients with the eradicable focus.

[d]Day 1 represents the day of the index blood culture.

Table 13. Comparison of Microbiological and Genetic Characteristics of Persistent MSSA bacteremia (n = 416) and Resolving MSSA bacteremia (n = 508)[a]

Microbiological characteristic	Persistent MSSA bacteremia (n = 416)	Resolving MSSA bacteremia (n = 508)	*P* value
CC (ST)[b]			
CC1			
ST1	27 (6.5)	34 (6.7)	0.902
ST188	56 (13.5)	87 (17.1)	0.125
CC5			
ST5	24 (5.8)	22 (4.3)	0.317
ST6	21 (5.0)	37 (7.3)	0.163
CC7 (ST7)	7 (1.7)	9 (1.8)	0.918
CC8			
ST8	11 (2.6)	23 (4.5)	0.130
ST72	93 (22.4)	81 (15.9)	0.013
ST630	8 (1.9)	17 (3.3)	0.185
CC15 (ST15)	31 (7.5)	43 (8.5)	0.573
CC30 (ST30)	36 (8.7)	44 (8.7)	0.997
CC45 (ST45)	6 (1.4)	3 (0.6)	0.190
CC59 (ST59)	10 (2.4)	6 (1.2)	0.156
CC97 (ST97)	12 (2.9)	12 (2.4)	0.619
CC121 (ST121)	8 (1.9)	12 (2.4)	0.648
CC398 (ST291)	6 (1.4)	9 (1.8)	0.693
Vancomycin MIC (mg/L) by BMD[c]			
≥1.5	3 (0.7)	2 (0.4)	0.500
***agr* dysfunction**	131/412 (31.8)	152/500 (30.4)	0.650

***agr* type**			
I	140/399 (35.1)	335/490 (68.4)	< 0.001
II	57/399 (14.3)	70/490 (14.3)	1.000
III	50/399 (12.5)	77/490 (15.7)	0.177
IV	7/399 (1.8)	5/490 (1.6)	1.000

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MRSA, methicillin-resistant *Staphylococcus aureus*; MLST, Multilocus sequence type; MIC, minimal inhibitory concentration; BMD, broth microdilution; hVISA, heteroresistant vancomycin-intermediate *Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator; SAB, *Staphylococcus aureus* bacteremia; NA, not applicable

[a]This analysis included a total of 924 MSSA bacteremia with different primary sources, including CRBSI (n = 153), SSTI (n = 120), BJI (n = 115), MSSA pneumonia (n = 62), IE (n = 48), unknown primary bacteremia (n = 172), and others (peripheral venous catheter related, n = 82; surgical site infection, n = 36; arteriovenous fistula graft infection, n = 29; urinary tract infection, n = 11; other sites of infection, n = 96).

[b]The major clones are shown. There were 110 isolates with STs not frequently detected, including ST101 (n = 13), ST623 (n = 7), ST96 (n = 7), ST587 (n = 6), ST217 (n = 4), ST580 (n = 4) and others.

[c]BMD to determine vancomycin MIC was used in 924 patients.

Table 14. Multivariate Analysis of Risk Factors for Persistent MSSA bacteremia (n = 416)

Risk factor	No (%) of Patients		Univariate analysis		Multivariate analysis	
	Persistent MSSA bacteremia (n = 416)	Resolving MSSA bacteremia (n = 508)	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Mode of acquisition						
Community-acquired	130 (31.3)	99 (19.5)	1.878 (1.338-2.540)	< 0.001	1.637 (1.189-2.255)	0.003
Underlying disease/condition						
Diabetes mellitus	132 (31.7)	139 (27.4)	1.234 (0.929-1.639)	0.147		
End-stage renal disease	49 (11.8)	54 (10.6)	1.123 (0.745-1.692)	0.581		
Indwelling device						
Vascular graft	40 (9.6)	29 (5.7)	1.758 (1.070-2.889)	0.026	1.990 (1.190-3.328)	0.009
Metastatic infection	119 (28.6)	51 (10.0)	3.590 (2.507-5.141)	< 0.001	3.301 (2.286-4.767)	< 0.001
MLST type						
ST72	93 (22.4)	81 (15.9)	1.518 (1.090-2.114)	0.014	1.587 (1.127-2.236)	0.008

Data are presented as the number of patients (with the corresponding percentage shown in parentheses), unless otherwise specified.

MSSA, methicillin-susceptible *Staphylococcus aureus*; OR, odds ratio; CI, confidence interval; MLST, Multilocus sequence type; ST, sequence type.

3. Annual Changes and Longitudinal Changes in the Proportion of Persistent SAB in Total SAB

The results of the analysis of annual changes and longitudinal changes in persistent SAB, persistent MRSA bacteremia, and persistent MSSA bacteremia among total SAB, persistent MRSA bacteremia and persistent MSSA bacteremia patients are presented in Table 15 and Figure 8. Total number of SAB included overall SAB (n = 1,877) with excluded SAB patients (n = 92). Among total SAB (n = 1,969), an annual decreasing trend was observed in all persistent SAB (annual decrease 0.170%, *P* = 0.764). Among total MRSA bacteremia (n = 953) a statistically significant annual decreasing trend was observed in persistent MRSA bacteremia (annual decrease 1.782, *P* = 0.027). And among total MSSA bacteremia (n = 924) a statistically significant annual increasing trend was observed in persistent MSSA bacteremia (annual increase 2.160%, *P* = 0.001).

Table 15. Annual Changes in the Proportion of Persistent Bacteremia in Total SAB, Persistent MRSA bacteremia, Persistent MSSA bacteremia

Characteristic	No.(%) of patients	Annual change (%)	*P* value
Total SAB (n = 1,969)[a]			
Persistent SAB	1,051 (53.4)	-0.170	0.764
Persistent MRSA bacteremia	635/953[b] (64.1)	-1.782	0.027
Persistent MSSA bacteremia	416/924[c] (42.7)	2.160	0.001

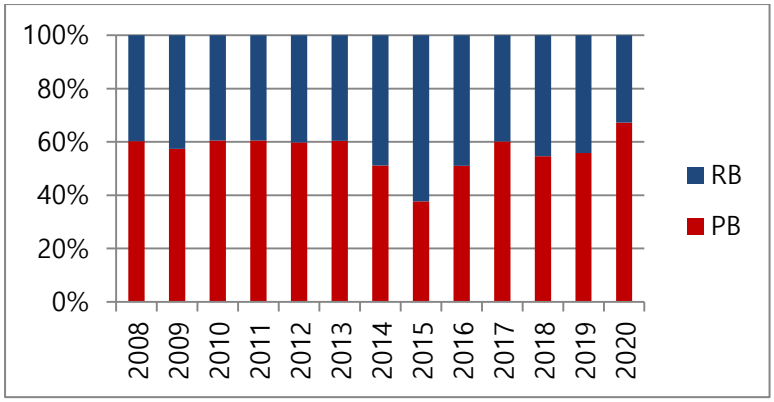
[a] This number included overall SAB (n = 1,877) with excluded SAB patients (n = 92).

[b]This number represents the proportion of persistent MRSA bacteremia among total MRSA bacteremia (n = 953).

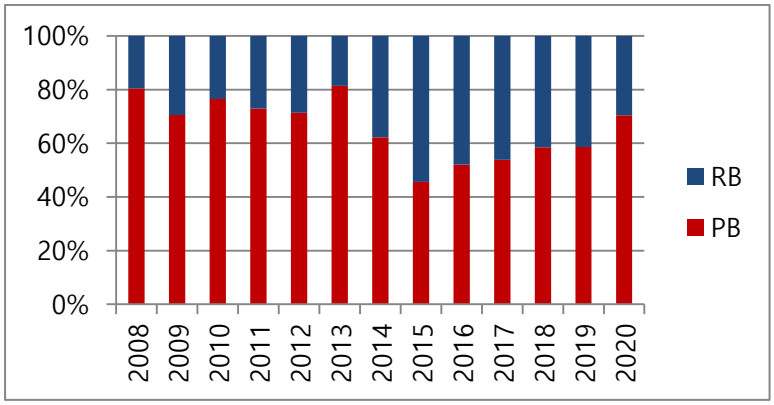
[c]This number represents the proportion of persistent MSSA bacteremia among total MSSA bacteremia (n = 924).

Figure 8. Longitudinal Changes in the Proportion of Persistent SAB in Total SAB

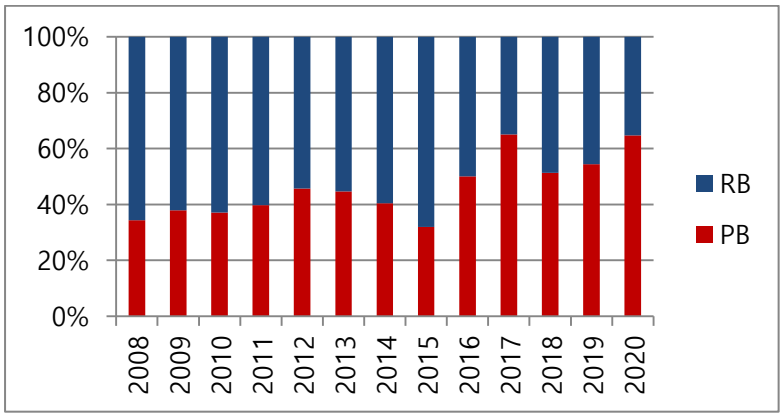
A. Overall SAB



B. MRSA bacteremia



C. MSSA bacteremia



RB, Resolving bacteremia; PB, persistent bacteremia.

4. Annual Changes and Longitudinal Changes in the Proportion of Major STs in Persistent MRSA bacteremia

The results of the analysis of annual changes and longitudinal changes in the proportion of major STs in persistent MRSA bacteremia are presented in Table 16 and Figure 9. Among total MRSA bacteremia, an increasing trend was observed in ST8 (annual increase 0.005%, $P = 0.001$), ST72 (annual increase 0.026%, $P < 0.001$) whereas a decreasing trend was observed in ST5 (annual decrease 0.034%, $P < 0.001$) and ST239 (annual decrease 0.003%, $P = 0.126$).

Table 16. Annual Changes in the Proportion of Major STs in Persistent MRSA bacteremia

Characteristic	No.(%) of patients	Annual change (%)	*P* value
Total Persistent MRSA (n = 635)			
ST5	378 (59.5)	-0.034	< 0.001
ST8	12 (1.9)	0.005	0.001
ST72	195 (30.7)	0.026	< 0.001
ST239	18 (2.8)	-0.003	0.126

Figure 9. Longitudinal Change in the Proportion of Major STs in Persistent MRSA bacteremia

ST, sequence type.

5. Molecular characteristics of MRSA bacteremia and MSSA bacteremia isolates

Molecular characteristics including MLST, *spa* type, SCC*mec* type, *agr* type, *PVL* positivity were analyzed in MRSA bacteremia and MSSA bacteremia (Table 17, Table 18).

As shown in Table 10, ST5 was the most common MRSA clone in both resolving (163/318, 51.3%) and persistent bacteremia (378/635, 59.5%) followed by ST72 in both resolving (119/318, 37.4%) and persistent bacteremia (195/635, 30.7%). In persistent MRSA bacteremia, the most common genotype was ST5-SCC*mec* type IIb-*agr* type II-t2460, followed by ST72-SCC*mec* type IVa-*agr* type I-t324. In persistent MSSA bacteremia, the most common genotype was ST5-SCC*mec* type IIb-*agr* type II-t002, followed by ST72-SCC*mec* type IVa-*agr* type I-t2460. *agr* type II and I correlated with ST5 (160/318) and ST72 (119/318) in resolving MRSA bacteremia, ST5 (368/635) and ST72 (193/635) in persistent MRSA bacteremia respectively. There were 2 isolates of PVL-positive in ST8-SCC*mec* typeIV-t008 and ST254-SCC*mec* type I-t2460 in resolving MRSA bacteremia group, respectively. And there were 7 isolates of PVL -positive in ST8-SCC*mec* typeIV-t008 in persistent MRSA bacteremia group. PVL-positive in ST8-SCC*mec* typeIV-t008 indicates USA300 or its genetically related ST8 genotype.

In Table 11, the high genetic diversity between isolates was identified in 508 resolving MSSA bacteremia isolates and 416 persistent MSSA bacteremia isolates, which included 12 distinct STs. ST72 was the most common clone (174/924, 18.8%), which accounted 15.9% (81/508) and 22.4% (93/416) in resolving MSSA bacteremia and persistent MSSA bacteremia, respectively. *Spa* type t126 was most common in ST72 (109/174, 62.6%) and *spa* type189 (122/143, 85.3%) was most common in ST188.

Table 17. Molecular Characteristics of Persistent MRSA bacteremia (n = 635) and Resolving MRSA bacteremia (n = 318)

MLST	Persistent MRSA bacteremia (n = 635)					Resolving MRSA bacteremia (n = 318)				
	n	*spa* type (n)	SCC*mec* type (n)	*agr* type (n)	PVL (+) /tested	n	*spa* type (n)	SCC*mec* type (n)	*agr* type (n)	PVL (+) /tested
ST5	378	t2460 (211), t9353 (45), t002 (35), t264 (11), t9363 (7), t601 (6), t463 (5), t535 (5), t18239 (5), t439 (4), t062 (2), t111 (2), t242 (2), t664 (2), t045 (1), t105 (1), t12704 (1), t148 (1), t18239 (1), t189 (1), t2461 (1), t3019 (1), t324 (1), t539 (1), t564 (1), t688 (1), t769 (1), unknown (23)	IIb (341), II (31), IIa (2), IIc (1), II or IIb (1)	II (368) I (3) unknown (7)	0/243	163	t2460 (97), t002 (19), t9353 (14), t264 (6), t604 (4), t111 (2), t148 (2), t463 (2), t010 (1), t105 (1), t12702 (1), t1560 (1), t17573 (1), t1784 (1), t18239 (1), t439 (1), t5076 (1), t688 (1), unknown (7)	II B (150), II (11), IIa (2)	II (160) I (1)	0/73
ST72	195	t324 (93), t664 (31), t148 (25), t2461 (6), t12699 (2), t4359 (2), t8578 (2), t10275 (1), t10555 (1), t1346 (1), t14576 (1), t14681 (1), t18196 (1), t1691 (1), t2431 (1), t3019 (1), t345 (1), t452 (1), t5123 (1), t5440 (1), t5553 (1), t693 (1), t901 (1), t9061 (1), unknown (17)	IVa (161), IV (32)	I (193) unknown (2)	0/82	119	t2460 (97), t002 (19), t9353 (14), t264 (6), t604 (4), t111(2), t148 (2), t463 (2), t010 (1), t105 (1), t12702 (1), t1560 (1), t17573 (1), t1784 (1), t1784 (1), t18239 (1), t439 (1), t5076 (1), t688 (1), unknown (7)	IVa (104), IV (13), II (1), IIa (1)	I (119)	0/35

ST239	18	t037 (16), unknown (2)	III (11), IIIa (6), unknown (1)	I (18)	0/13	7	t037 (4), t2029 (2), t148 (1)	IIIa (4), III (3)	I (7)	0/7
ST8	12	t008 (7), t1767 (2), t121 (1), t2460 (1), unknown (1)	IV (6), IVa (4), I (1), Ic(1)	I (12)	7/11	10	t008 (7), t211 (1), t334 (1), t986 (1)	IV (8), IVa (1), unknown (1)	I (10)	1/9
ST254	3	t2460 (2), t002 (1), t189 (1), unknown (1)	Ia (1), Ic(1)	I (2) unknown (1)	0/3	3	t2460 (1)	I (3)	I (3)	1/1
ST188	4	t189 (4)	I (1), IV (1), IVa (1), unknown (1)	I (4)	0/1	1	t189 (1)	unknown (1)	I (1)	
Unknown	3	t002 (1), t2947 (1), t375 (1)	IV (1), unknown (1)	I (1) II (1)	0/1	0				

MLST, multilocus sequence typing; ST, sequence type; spa, staphylococcus protein A; PVL, Panton-Valentine leucocidin; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator.

Table18. Molecular characteristics of Persistent MSSA bacteremia (n = 416) and Resolving MSSA bacteremia (n = 508)

MLST	Persistent MSSA bacteremia (n = 416)			Resolving MSSA bacteremia (n = 508)		
	n	*spa* type (n)	*agr* type (n)	n	*spa* type (n)	*agr* type (n)
ST72	93	t126 (63), t324 (6), t148 (4), t664 (3), t4727 (2), t8421 (2), t12229 (1), t127 (1), t18240 (1), t189 (1), t206 (1), t2453 (1), t2703 (1), t304 (1), t5026 (1), unknown (3)	I (90), unknown (2)	81	t126 (49), t324 (6), t189 (3), t2703 (2), t012 (1), t091 (1), t10756 (1), t17684 (1), t18240 (1), t2313 (1), t2461 (1), t345 (1), t4298 (1), t4727 (1), t6509 (1), t63 (1), t8421 (1), t967 (1), unknown (6)	I (79), unknown (1)
ST188	56	t189 (48), t126 (2), t2174 (2), t11978 (1), t363 (1), t387 (1), t4171 (1)	I (56)	87	t189 (74), t002 (1), t091 (1), t11978 (1), t126 (1), t17343 (1), t2284 (1), t2883 (1), t304 (1), t3887 (1), t8021 (1), t8275 (1)	I (86), unknown (1)
ST15	31	t085 (6), t084 (5), t346 (3), t491 (2), t2574 (1), t279 (1), t304 (1), t3345 (1), t358 (1), t4968 (1), t7200 (1), t774 (1), unknown (7)	I (5), II (25), unknown (1)	43	t084 (11) t085 (1), t360 (4), t7200 (3), t1038 (1), t14108 (1), t16983 (1), t2706 (1), t279 (1), t338 (1), t346 (1), t358 (1), t385 (1), t491 (1), t593 (1), t774 (1), t803 (1), unknown (4)	I (2), II (40), III (1)
ST6	21	t304 (10), t701 (3), t091 (1), t1627 (1), t164 (1), t243 (1), t4407 (1), t5593 (1), t8840 (1), unknown (1)	I (21)	37	t304 (21), t701 (2), t008 (1), t104 (1), t126 (1), t1627 (1), t18173 (1), t3209 (1), t4298 (1), t5133 (1), t648 (1), t711 (1), t8840 (1), unknown (3)	I (36), unknown (1)
ST97	12	t267 (4), t359 (4), t189 (1), t2802 (1), t3581 (1)	I (12)	12	t267 (4), t359 (3), t1200 (1),t12229 (1), t1247 (1), t2085 (1), t376 (1)	I (12)

ST5	24	t688 (5), t002 (3), t179 (3), t640 (2), t021 (1), t105 (1), t126 (1), t189 (1), t2049 (1), t242 (1), t264 (1), t270 (1), t4956 (1), t535 (1), t954 (1)	I (2), II (19), IV (2), unknown (1)	22	t688 (7), t179 (6), t002 (3), t062 (1), t148 (1), t1560 (1), t2302 (1), t2460 (1), t586 (1)	I (2), II (20)
ST1	27	t127 (21), t189 (2), t10269 (1), t128 (1), t330 (1), t693 (1)	I (4), III (21), unknown (2)	34	t127 (25), t189 (3), t12303 (1), t126 (1), t177 (1), t286 (1), t386 (1), t693 (1)	I (2), II (1), III (28), unknown (3)
ST30	36	t338 (9), t021 (6), t363 (6), t012 (2), t037 (2), t018 (1), t019 (1), t122 (1), t1577 (1), t189 (1), t1902 (1), t26 (1), t2868 (1), t633 (1), t688 (1), t7263 (1), t870 (1), unknown (2)	I (4), II (2), III (25), unknown (5)	44	t012 (8), t021 (8), t338 (7), t363 (3), t1577 (2), t238 (2), t017 (1), t019 (1), t084 (1), t127 (1), t1333 (1), t138 (1), t1649 (1), t189 (1), t274 (1), t2868 (1), t6361 (1), t8185 (1), t822 (1), unknown (1)	I (2), II (1), III (37), unknown (4)
ST8	11	t008 (6), t1767 (1), t267 (1), t2703 (1), t359 (1), t5554 (1)	I (11)	23	t008 (13), t091 (1), t126 (1), t1767 (1), t189 (1), t190 (1), t2078 (1), t267 (1), t7169 (1), t9723 (1), unknown (1)	I (23)
ST630	8	t377 (4), t16667 (1), t3388 (1), t5554 (1), unknown (1)	I (8)	17	t377 (9), t5554 (3), t189 (1), t3386 (1), t4047 (1), t4549 (1), unknown (1)	I (17)
ST121	8	t4956 (5), t3369 (1), t7641 (1), unknown (1)	I (3), IV (3), unknown (2)	12	t4959 (7), t008 (1), t012 (1), t3937 (1), unknown (2)	I (3), IV(8), unknown (1)
ST101	6	t2078 (3), t7760 (1), t814 (1), unknown (1)	I (6)	7	t2078 (5), t12946 (1), unknown (1)	I (7)

MLST, multilocus sequence typing; ST, sequence type; spa, staphylococcus protein A; *agr*, accessory gene regulator.

6. Relative Risk of SAB-related Mortality by Duration of SAB

To identify the incremental risk of SAB-related mortality with each day of bacteremia, 1 day of bacteremia was set as reference to analyze whether the SAB-related mortality rate increased each subsequent day of bacteremia. Table 19.A. was analyzed for all SAB, Table 19.B. for MRSA bacteremia, and Table 19.C. for MSSA bacteremia. As a result, the SAB-related mortality rate increased with each additional day of bacteremia, particularly in overall SAB and MSSA bacteremia. Notably, there was a statistically significant increase on day 3 in overall SAB (RR, 1.589; 95% CI, 1.060-2.355; $P = 0.024$) and on day 4 in MSSA bacteremia (Relative risk [RR], 2.035; 95% CI, 1.054-3.928; $P = 0.031$).

Table 19. Relative Risk of SAB-related Mortality by Duration of Bacteremia

A. Relative Risk of SAB related Mortality by Duration of *S. aureus* bacteremia

No. of Days of bacteremia	Total number	SAB related mortality, n (%)	Relative Risk (95% CI)	*P* value
1	673	72 (10.7)	Reference	Reference
2	331	34 (10.3)	0.956 (0.612-1.470)	0.836
3	289	46 (15.9)	1.580 (1.060-2.355)	0.024
4	166	22 (13.3)	1.275 (0.765-2.126)	0.350
5	114	12 (10.5)	0.982 (0.515-1.874)	0.956
6	52	9 (17.3)	1.747 (0.818-3.732)	0.145
7	42	9 (21.4)	2.277 (1.047-4.949)	0.033
8-10	75	18 (24.0)	2.636 (1.471-4.725)	0.001
11+	135	31 (23.0)	2.488 (1.556-3.980)	< 0.001

CI, confidence interval.

B. Relative Risk of SAB-related Mortality by Duration of MRSA Bacteremia

No. of Days of bacteremia	Total number	SAB related mortality, n (%)	Relative Risk (95% CI)	*P* value
1	229	27 (11.8)	Reference	Reference
2	176	16 (9.1)	0.748 (0.390-1.436)	0.382
3	167	28 (16.8)	1.507 (0.851-2.668)	0.157
4	91	8 (8.8)	0.721 (0.315-1.653)	0.438
5	66	7 (10.6)	0.888 (0.368-2.141)	0.791
6	27	4 (14.8)	1.301 (0.418-4.049)	0.649
7	25	5 (20.0)	1.870 (0.649-5.394)	0.240
8-10	54	15 (27.8)	2.877 (1.403-5.901)	0.003
11+	118	25 (21.2)	2.011 (1.107-3.653)	0.020

CI, confidence interval.

C. Relative Risk of SAB-related Mortality by Duration of MSSA Bacteremia

No. of Days of bacteremia	Total number	SAB related mortality, n (%)	Relative Risk (95% CI)	*P* value
1	444	45 (10.1)	Reference	Reference
2	155	18 (11.6)	1.165 (0.652-2.081)	0.606
3	122	18 (14.8)	1.535 (1.853-2.762)	0.151
4	75	14 (18.7)	2.035 (1.054-3.928)	0.031
5	48	5 (10.4)	1.031 (0.338-2.736)	0.951
6	25	5 (20.0)	2.217 (0.794-6.192)	0.120
7	17	4 (23.5)	2.728 (0.853-8.723)	0.079
8-10	21	3 (14.3)	1.478 (0.419-5.212)	0.541
11+	17	6 (35.3)	4.836 (1.707-13.702)	0.001

CI, confidence interval

7. Proportions, 90-day mortality, and SAB-related mortality of resolving bacteremia and persistent bacteremia according to major MLST types and corresponding spa types

Table 20 presents an analysis of the proportions and 90-day mortality rates of major MLST types and corresponding spa types in persistent bacteremia and resolving bacteremia. Among overall SAB, ST5-*spa*-t2460 (20.1%, $P < 0.001$), ST5-*spa*-t9353 (4.9%, $P < 0.001$) and ST239-*spa*-t037 (1.5%, $P = 0.030$) were more common in persistent SAB while ST6-*spa*-t304 (2.5%, $P < 0.022$), ST15-*spa*-t084 (1.3%, $P = 0.032$) ST188-*spa*-t189 (9.2%, $P < 0.001$) were more common in resolving SAB. Among MRSA bacteremia, ST5-*spa*-t9353 (8.2%, $P = 0.030$) was more common in persistent MRSA bacteremia compared with resolving MRSA bacteremia. Among MSSA bacteremia, ST72-*spa*-t126 (15.1%, $P = 0.011$) was more common in persistent MSSA bacteremia compared with resolving MSSA bacteremia.

The analysis of 90-day mortality rates for major MLST types and corresponding spa types revealed that ST5-*spa*-t9353 (2.1%, $P = 0.015$) in overall persistent SAB and ST72-*spa*-t126 (5.5%, $P = 0.012$) in persistent MSSA bacteremia had statistically significantly higher mortality rates compared with resolving bacteremia, while ST1-*spa*-t127 (0.7%, $P = 0.025$) in overall resolving SAB had statistically significantly higher mortality rates compared with persistent SAB.

The analysis of SAB-related mortality rates for major MLST types and corresponding *spa* types revealed that ST5-*spa*-t9353 (1.0%, $P = 0.013$) and ST72 with major *spa* (t324 [n=167], t664 [n=50], t148 [n=44], t126 [n=114]; 3.7%, $P = 0.015$) in overall persistent SAB had statistically significantly higher mortality rates compared with resolving bacteremia. And ST72-*spa*-t126 (3.8%, $P = 0.008$) in persistent MSSA bacteremia had statistically significantly higher mortality rates compared with resolving bacteremia.

Table 20. Proportions, 90-day mortality, and SAB-related mortality of persistent bacteremia and resolving bacteremia according to major MLST types and corresponding *spa* types

Characteristics	No (%) of patients		*P* value	No (%) of patients of 90- day mortality		*P* value	No (%) of patients of SAB-related mortality		*P* value
	Persistent bacteremia (n = 1,051)	Resolving bacteremia (n = 826)		Persistent bacteremia (n = 1,051)	Resolving bacteremia (n = 826)		Persistent bacteremia (n = 1,051)	Resolving bacteremia (n = 826)	
Major MLST type corresponding *spa* types									
SAB (n = 1,877)									
ST1 (t127 [n=48])	22 (2.1)	26 (3.2)	0.150	1 (0.1)	6 (0.7)	0.025	0 (0)	3 (0.4)	N/A
ST5 (t2460 [n=309], t002 [n=60], t9353 [n=66], t688 [n=14], t179 [n=9])	310 (29.5)	148 (17.9)	< 0.001	106 (10.1)	67 (8.1)	0.142	54 (5.1)	21 (2.5)	0.004
ST5/*spa*-t2460 [n = 309]	211 (20.1)	98 (11.9)	< 0.001	68 (6.5)	44 (5.3)	0.299	33 (3.1)	15 (1.8)	0.071
ST5/*spa*-t002 [n= 60]	38 (3.6)	22 (2.7)	0.246	12 (1.1)	10 (1.2)	0.890	7 (0.7)	3 (0.4)	0.371
ST5/*spa*-t9353 [n=66]	52 (4.9)	14 (1.7)	< 0.001	22 (2.1)	6 (0.7)	0.015	11 (1.0)	1 (0.1)	0.013
ST5/*spa*-t688 [n=14]	6 (0.6)	8 (1.0)	0.319	4 (0.4)	4 (0.4)	0.732	3 (0.3)	1 (0.1)	0.443
ST5/*spa*-t179 [n=9]	3 (0.3)	6 (0.7)	0.169	0 (0)	3 (0.3)	N/A	0 (0)	1 (0.1)	N/A
ST6 (t304 [n =33])	12 (1.1)	21 (2.5)	0.022	3 (0.3)	3 (0.3)	0.767	2 (0.2)	1 (0.1)	0.709

ST8 (t008 [n=33])	13 (1.2)	20 (2.4)	0.052	1 (0.1)	3 (0.3)	0.211	1 (0.1)	2 (0.2)	0.429
ST15 (t084 [n=16], t085 [n=14])	11 (1.0)	19 (2.3)	0.032	3 (0.3)	4 (0.4)	0.483	3 (0.3)	1 (0.1)	0.443
ST15/*spa*-t084 [n=16]	5 (0.5)	11 (1.3)	0.045	1 (0.1)	2 (0.2)	0.429	1 (0.1)	0 (0)	N/A
ST15/*spa*-t085 [n=14]	6 (0.6)	8 (1.0)	0.319	2 (0.2)	2 (0.2)	0.809	2 (0.2)	1 (0.1)	0.709
ST30 (t338 [n=17], t021 [n=14])	15 (1.4)	16 (1.9)	0.389	5 (0.5)	5 (0.6)	0.702	3 (0.3)	1 (0.1)	0.443
ST30/*spa*-t338 [n=17]	9 (0.9)	8 (1.0)	0.797	3 (0.3)	2 (0.2)	0.857	1 (0.1)	1 (0.1)	0.864
ST30/*spa*-t021 [n=14]	6 (0.6)	8 (1.0)	0.319	2 (0.2)	3 (0.3)	0.471	2 (0.2)	0 (0)	N/A
ST72 (t324 [n=167], t664 [n=50], t148 [n=44],t126 [n=114])	226 (21.5)	149 (18.0)	0.062	63 (6.0)	32 (3.8)	0.038	39 (3.7)	15 (1.8)	0.015
ST72/*spa*-t324 [n=167]	100 (9.2)	67 (8.1)	0.293	24 (2.3)	12 (1.5)	0.193	15 (1.4)	7 (0.8)	0.247
ST72/*spa*-t664 [n=50]	34 (3.2)	16 (1.9)	0.084	8 (0.8)	2 (0.2)	0.083	5 (0.5)	0 (0)	N/A
ST72/*spa*-t148 [n=44]	29 (2.8)	15 (1.8)	0.181	8 (0.8)	3 (0.3)	0.262	3 (0.3)	2 (0.2)	0.857
ST72/*spa*-t126 [n=114]	63 (6.0)	51 (6.2)	0.866	23 (2.2)	13 (1.6)	0.335	16 (1.5)	6 (0.7)	0.112
ST188 (t189 [n=127])	52 (4.9)	76 (9.2)	< 0.001	15 (1.4)	14 (1.7)	0.641	6 (0.6)	8 (0.9)	0.320
ST239 (t037 [n=20])	16 (1.5)	4 (0.5)	0.030	4 (0.4)	0 (0)	N/A	3 (0.3)	0 (0)	N/A

MRSA (n = 953)									
ST5 (t2460 [n=308], t9353 [n=59], t002 [n=54])	298/635 (46.9)	130/318 (40.9)	0.076	102/635 (16.1)	58/318 (18.2)	0.397	51/635 (8.0)	19/318 (6.0)	0.251
ST5/*spa*-t2460 [n=308]	211/635 (33.2)	97/318 (30.5)	0.396	68/635 (10.7)	43/318 (13.5)	0.202	33/635 (5.2)	15/318 (4.7)	0.749
ST5/*spa*-t t9353 [n=59]	52/635 (8.2)	14/318 (4.4)	0.030	22/635 (3.5)	6/318 (1.9)	0.174	11/635 (1.7)	1/318 (0.3)	0.064
ST5/*spa*-t002 [n=54]	35/635 (5.5)	19/318 (6.0)	0.771	12/635 (1.9)	9/318 (2.8)	0.351	7/635 (1.1)	3/318 (0.9)	0.820
ST8 (t008 [n=14])	7/635 (1.1)	7/318 (2.2)	0.184	1/635 (0.2)	1/318 (0.3)	0.618	1/635 (0.2)	0/318 (0)	N/A
ST72 (t324 [n=153], t664 [n=47], t148 [n=40])	149/635 (23.5)	91/318 (28.6)	0.084	33/635 (5.2)	16/318 (5.0)	0.913	17/635 (0.1)	9/318 (2.8)	0.891
ST72/*spa*-t324 [n=153]	93/635 (14.6)	60/318 (18.9)	0.094	20/635 (3.1)	11/318 (3.5)	0.800	9/635 (1.4)	7/318 (2.2)	0.374
ST72/*spa*-t664 [n=47]	31/635 (4.9)	16/318 (5.0)	0.920	8/635 (1.3)	2/318 (0.6)	0.367	5/635 (0.8)	0/318 (0)	N/A
ST72/*spa*-t148 [n=40]	25/635 (3.9)	15/318 (4.7)	0.571	5/635 (0.8)	3/318 (0.9)	0.803	3/635 (0.5)	2/318 (0.6)	0.752
ST239 (t037 [n=20])	16/635 (2.5)	4/318 (1.3)	0.200	4/635 (0.6)	0/318 (0)	N/A	3/635 (0.5)	0/318 (0)	N/A
MSSA (n = 924)									
ST1 (t127 [n=46])	21/416 (5.0)	25/508 (4.9)	0.935	1/416 (0.2)	6/508 (1.2)	0.101	0/416 (0)	3/508 (0.6)	N/A
ST5 (t688 [n=12], t179 [n=9])	8/416 (1.9)	13/308 (4.2)	0.069	3/416 (0.7)	7/508 (1.4)	0.337	2/416 (0.5)	2/508 (0.3)	0.841
ST5/*spa*-t688 [n=12]	5/416 (1.2)	7/508 (1.4)	0.811	3/416 (0.7)	4/508 (0.8)	0.908	2/416 (0.5)	1/508 (0.2)	0.450
ST5/*spa*- t179 [n=9]	3/416 (0.7)	6/508 (1.2)	0.477	0/416 (0)	3/508 (0.6)	N/A	0/416 (0)	1/508 (0.2)	N/A

ST6 (t304 =31)	10/416 (2.4)	21/508 (4.1)	0.145	2/416 (0.5)	3/508 (0.6)	0.821	2/416 (0.5)	1/508 (0.2)	0.450
ST8 (t008 [n=19])	6/416 (1.4)	13/508 (2.6)	0.232	0/416 (0)	2/508 (0.4)	N/A	0/416 (0)	2/508 (0.3)	N/A
ST15 (t084 [n=16], t085 [n=14])	11/416 (2.6)	19/508 (3.7)	0.350	3/416 (0.7)	4/508 (0.8)	0.908	3/416 (0.7)	1/508 (0.2)	0.227
ST15/*spa*-t084 [n=16]	5/416 (1.2)	11/508 (2.2)	0.262	1/416 (0.2)	2/508 (0.4)	0.684	1/416 (0.2)	0/508 (0)	N/A
ST15/*spa*-t085 [n=14]	6/416 (1.4)	8/508 (1.6)	0.867	2/416 (0.5)	2/508 (0.4)	0.841	2/416 (0.5)	1/508 (0.2)	0.450
ST30 (t338 [n=16], t021 [n=14])	15/419 (3.6)	15/508 (3.0)	0.591	5/4161.2)	5/508 (1.0)	0.758	3/416 (0.7)	1/508 (0.2)	0.227
ST30/*spa*-t338 [n=16]	9/416 (2.2)	7/508 (1.4)	0.365	3/416 (0.7)	2/508 (0.4)	0.500	1/416 (0.2)	1/508 (0.2)	0.887
ST30/*spa*-t021 [n=14]	6/416 (1.4)	8/508 (1.6)	0.867	2/416 (0.5)	3/508 (0.6)	0.821	2/416 (0.5)	0/508 (0)	N/A
ST72 (t126 [n=112])	63/416 (15.1)	49/508 (9.7)	0.011	23/416 (5.5)	12/508 (2.3)	0.012	16/416 (3.8)	6/508 (0.2)	0.008
ST188 (t189 [n=122])	48/416 (11.5)	75/508 (14.8)	0.148	15/416 (3.6)	14/508 (2.8)	0.461	6/416 (1.4)	8/508 (1.6)	0.870

MRSA, methicillin-resistant *Staphylococcus aureus*; SAB, *Staphylococcus aureus* bacteremia; ST, sequence type; MLST, multilocus sequence typing; ST, sequence type; *spa*, staphylococcus protein A; OR, odds ratio; CI, confidence interval; NA, not applicable

DISCUSSION

The purpose of this study was to identify clinical and microbiological differences in persistent SAB compared to resolving SAB based on a large cohort. To conduct this analysis, resolving bacteremia and persistent bacteremia were each divided into SAB, MRSA, and MSSA bacteremia, respectively. Clinical, microbiological, and genetic characteristics were comprehensively analyzed, and the mortality rate for each bacteremia period was examined to identify any significant increase in mortality rate with prolonged bacteremia duration.

This study identified several independent risk factors for persistent SAB, highlighting significant differences between MRSA and MSSA strains. The presence of a vascular graft as an indwelling device, metastatic infection, and methicillin resistance were notably associated with persistent SAB. Among persistent MRSA bacteremia, ST5 was the predominant strain and *agr* dysfunction, *agr* type II were more prevalent than resolving MRSA bacteremia. Furthermore, the virulence gene *sec* was more prevalent in persistent MRSA bacteremia. Multivariate analysis confirmed that solid organ transplantation, metastatic infection, and ST5 were independent risk factors for persistent MRSA bacteremia. Conversely, persistent MSSA bacteremia was associated with community-acquired acquisition, the presence of a vascular graft, metastatic infection, and ST72. In microbiologic and genotypic analysis, *agr* type I was more common in resolving MSSA bacteremia, while ST72 was more frequently seen in persistent MSSA bacteremia. These findings underscore the importance of specific clinical and microbiologic factors in the persistence of SAB, with distinct differences observed between MRSA and MSSA strains.

SAB-related mortality was significantly higher in persistent SAB compared to resolving SAB, while no significant differences were observed in 30-day and 90-day mortality rates. Additionally, in the MRSA and MSSA bacteremia groups, there were no significant differences in 30-day, 90-day, or SAB-related mortality rates.

In the analysis of the SAB-related mortality rate by bacteremia period, when analyzed by all persistent SAB, persistent MRSA bacteremia, and persistent MSSA bacteremia. There was a statistically significant increase on day 3 in overall SAB (RR, 1.589; 95% CI, 1.060-2.355; P = 0.024)

and on day 4 in MSSA bacteremia (Relative risk [RR], 2.035; 95% CI, 1.054-3.928; P = 0.031). And the analysis of 90-day mortality rates for major MLST types and corresponding *spa* types revealed that ST5-*spa*-t9353 (2.1%, *P* = 0.015) in persistent SAB and ST72-*spa*-t126 (5.5%, *P* = 0.012) in persistent MSSA bacteremia had significantly higher 90-day mortality rates compared to resolving cases, while ST1-*spa*-t127 (0.7%, *P* = 0.025) in resolving SAB had a higher mortality rate compared to persistent SAB.

Despite the use of appropriate antimicrobial agents in treating SAB patients, persistent SAB can occur and is often a problem that can be encountered in clinical practice. Several factors such as clinical factor,[1,2,7,20,40,53,58] microbiologic and genotypic factor,[22-27,59] and pharmacokinetic and pharmacodynamic characteristics of the antibiotic factor[10,60] are known to be the cause of persistent SAB. Although there are some differences between the results of the study, a recurring theme is the presence of retained intravascular devices or foreign bodies, which are independently associated with persistent SAB.[1,2,5,7,20,61] Similarly, metastatic infection including endocarditis, bone and joint infection, chronic renal failure, cirrhosis, and diabetes are associated with persistent SAB.[1,2,7,20] Our research showed results similar to those of studies. The presence of a vascular graft, metastatic infection was an independent risk factor for overall SAB, persistent SAB, and persistent MSSA bacteremia. In particular, in previous studies, CVC related infection is related to persistent SAB and metastatic infection.[1,2,5,7,20,61] However, unlike previous studies, multivariate analysis showed that CVC-associated infection was not significant in persistent SAB or MRSA and MSSA bacteremia in our study, which may be related to the definition of persistent bacteremia as more than 3 days in our study. And other possible causes may be related to the recent decrease in CVC infection due to active infection control and the gradual decrease in persistent SAB, which may be the hospital clones ST5 and ST239 have been replaced by community genotype ST72.[62]

Removal of eradicable source has been emphasized for the management of persistent SAB.[2,7,10] The mean time of removal of eradicable source was longer in a group with persistent bacteremia.[1,2] Chong et al. reported that delay (>3 day) in the removal of the eradicable focus was significant associated with persistent bacteremia (OR, 2.18; 95% CI, 1.05-4.55).[1] In this study, eradicable focus until day 4

was analyzed and it was not significant in all persistent SAB or MRSA and MSSA bacteremia group compared with resolving bacteremia groups. This finding suggests that the removal of an infection source itself is important, but other clinical and microbiological factors as mentioned above may also have had an effect in persistent bacteremia.

There has been a long-standing debate questioning whether MRSA with elevated vancomycin MIC (>1.5 mcg/mL) and hVISA are associated with worse clinical outcomes or not. The majority of data, including two systematic reviews and meta-analysis, indicates that MRSA bacteremia due to isolates with high vancomycin MIC (>1.5 mcg/mL) is associated with increased mortality compared to MRSA bacteremia due to isolates with low-vancomycin MIC (<1.5 mcg/mL).[18,47,63] However, this finding is not necessarily related to failure of vancomycin,[64] the systematic review and meta-analysis by van Hal et al. limited their analysis exclusively to studies that examined persistent MRSA bacteremia, the OR was 2.44 but was not significant (95% CI, 0.72-8.24).[47] And the recent study by Adani et al., Chong et al. reported that vancomycin MIC showed no significant difference in persistent SAB.[1,65] As for hVISA, too, some studies report worse clinical outcomes[66-72] and increased risk of persistent MRSA bacteremia,[66,68-70] with others, including one systematic review and meta-analysis, showing no significant difference in mortality or persistent MRSA bacteremia.[47,73-76] In our study, high vancomycin MIC (>1.5 mcg/mL) and hVISA were not statistically significantly higher in persistent MRSA bacteremia compared to resolving MRSA bacteremia.

In the study conducted by Chong et al. (2013) at the same center, when persistent SAB was defined as lasting over 7 days, vancomycin MIC, hVISA, genotype, and *agr* dysfunction was not associated with persistent SAB. On the other hand, several groups have demonstrated that specific *agr* genotypes are associated with persistent MRSA bacteremia.[13,33,34] In this study, *agr* dysfunction was found to be statistically significantly more prevalent in persistent MRSA bacteremia in univariate analysis. This discrepancy is likely due to the difference in persistent bacteremia definition, with Chong et al. defining it as lasting over 7 days, while this study defined it as lasting over 3 days. Furthermore, in a study comparing the characteristics of Community-associated MRSA strain ST72-SCC*mec*IV with other strains conducted by Part et al., *agr* dysfunction was found to be more prevalent in ST5-

SCC*mec*II (96.4%) compared to ST72-SCC*mec*IV (8.9%; $P < 0.001$) and ST239-SCC*mec*III (68.8%; $P = 0.001$). The frequency of the hVISA phenotype differed among ST5-SCC*mec*II (33.6%), ST72-SCC*mec*IV (13.9%), and ST239-SCC*mec*III (81.3%; $P < 0.001$).[73] This aligns with the statistically significantly higher prevalence of *agr* dysfunction in the persistent MRSA bacteremia group, as well as the significantly higher presence of ST5 in this group in our study.

Despite decades of research, the specific *S. aureus* virulence factors crucial for survival in bloodstream infections remain elusive, suggesting a complex interplay of factors across different infectious environments. Some studies have investigated virulence factor expression to specifically differentiate persistent MRSA bacteremia from resolving MRSA bacteremia. While some research suggests that specific virulence factors influence persistent MRSA bacteremia,[35] other studies have failed to find associations.[1,36] In this study, the virulence genes of the MRSA isolates were analyzed to identify the specific intrinsic virulence factors contribute to differences in persistent MRSA bacteremia. As a result, *sec* was found to be significantly more frequently in persistent MRSA bacteremia compared to resolving MRSA bacteremia. Additionally, *pvl* was significantly more frequent in resolving MRSA bacteremia compared to persistent MRSA bacteremia. In a study conducted by Chong et al. at the same center, no virulence genes associated with persistent bacteremia were identified.[1] However, in a study by Park et al., it was found that three staphylococcal superantigen genes, *sel, sec,* and *tst,* were associated to higher mortality. These genes were less prevalent in ST72-SCC*mec*IV isolates compared to ST5-SCC*mec*II isolates,[73] similar to our findings.

Staphylococcal superantigens are recognized for their ability to induce immune system dysregulation and toxic shock syndrome.[77] A recent experimental investigation revealed that these superantigens also play a critical role in the initiation and advancement of *S. aureus* infection.[27] It is presumed that the statistically significant abundance of virulence genes in persistent MRSA bacteremia, particularly in the ST5 group, is associated with poor clinical outcomes and high mortality. These inconsistencies between studies may highlight epidemiological, strain-specific virulence factors, vancomycin susceptibility and clinical factors differences between SAB isolates from different geographic centers. These diverse factors could interact with each other in a complex manner to impact persistent

bacteremia. In this study, the distribution analysis of major MLST types and corresponding *spa* types between persistent bacteremia and resolving bacteremia showed that ST5-*spa*-t2460 ($P < 0.001$), ST5-*spa*-t9353 ($P < 0.001$), and ST239-*spa*-t037 were more common in persistent SAB. Specifically, ST5-*spa*-t9353 ($P = 0.030$) was more prevalent in persistent MRSA bacteremia, while ST72-*spa*-t126 ($P = 0.011$) was more common in persistent MSSA bacteremia. Among these, ST5-*spa*-t9353 was statistically significantly associated with increased 90-day mortality and SAB-related mortality in overall SAB. ST72 with major *spa* types was significantly associated with SAB-related mortality. Additionally, in persistent MSSA bacteremia, ST72-*spa*-t126 was statistically significantly associated with increased 90-day mortality and SAB-related mortality. These results are consistent with the previous epidemiological distribution of major MLST types and corresponding spa types.[62] The association of these MLST types, particularly ST5, with frequently harboring virulence genes and leading to higher clinical poor outcomes and mortality aligns with previous studies.[27,77] The finding that ST72 with major *spa* types is associated with increased mortality in persistent SAB, and particularly that ST72-*spa*-t126 is associated with 90-day mortality and SAB-related mortality in PMSSA bacteremia, is noteworthy.

In recent epidemiological studies conducted in Korea, it has been observed that the hospital clones ST5 and ST239 are decreasing while community genotype ST72 and ST8 are increasing. However, ST5 still remains the most common in total SAB cases.[62,73] This phenomenon can be explained by clonal replacement, and the reduction of CVC-related SAB in critically ill patients may be one of major factors resulting in the reduction of these genotype replacements. Among MRSA isolates, ST5 and ST239 exhibited higher resistance to non-ß-lactam antimicrobial agents compared to ST72 and ST8 strains,[62] In the study by Kim et al., among 138 episodes of ICU-acquired MRSA bacteremia, ST5-MRSA-II-agr group II (87.4%) was identified as the major genotype, which was statistically significantly associated with recent surgery and hospital-acquired infection.[78] These findings could potentially explain the poor clinical outcome observed in persistent MRSA bacteremia of ST5 in this study. The longitudinal changes of MLST proportion in persistent MRSA bacteremia over the study period (Figure 9), our data showed a decreasing trend in ST5, while ST8, ST72 increased among

persistent MRSA bacteremia. Additionally, this study did not identify CVC-related infection as a significant risk factor. As mentioned above, ST5 was identified as an independent risk factor for persistent MRSA bacteremia, and the association of ST5 major *spa* types with increased mortality aligns with the findings of previous studies.

In recent years, the prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has been increased, becoming a significant contributor to healthcare-associated infections. USA300, a highly prominent PVL-positive CA-MRSA, has emerged as the predominant CA-MRSA strain in North America and is increasingly encountered in healthcare settings.[79,80] Meanwhile, in Korea, a notable CA-MRSA strain is PVL-negative ST72-SCC*mec* type IV MRSA.[81] In this study, all MRSA isolates of ST72-SCC*mec* type IV were PVL negative, and there were PVL-positive eight in ST8 and one isolate in ST254. These differences could serve as potential factors contributing to discrepancies between this study and other research findings.

In previous studies defining persistent SAB as lasting for 7 days or more, no significant difference in crude mortality compared to resolving SAB was observed.[1,5] In this study, persistent bacteremia was defined as bacteremia for $\geq$ 3 days while they were receiving appropriate antibiotics treatment or even if the period was less than 3 days, if a follow-up blood culture conducted within those 3 days was positive again. Similar to previous studies, we did not observe a statistically significant increase in 30-day and 90-day mortality in persistent bacteremia compared to resolving bacteremia. However, a significant increase in SAB-related mortality was identified in both persistent SAB and persistent MSSA bacteremia. Recent studies propose that the cutoff duration for persistent bacteremia should be shorter than 7 days, based on evidence indicating a significant increase in mortality within 2-3 days after bacteremia persists.[14,15] Despite applying a shorter duration of 3 days to define PB, consistent with recent research, no significant difference in crude mortality was evident. This discrepancy may be attributed to the substantial patient cohort size and their relatively higher severity of illness compared to other cohorts. This finding underscores the need for further research on bacteremia duration and mortality.

In this study, we analyzed whether there was a significant increase in SAB-related mortality with

each additional day of bacteremia, using bacteremia day 1 as the reference point, across all SAB, MRSAB, and MSSAB cases. Contrary to recent research findings,[14,15] we did not observe statistical significance in the increase in 30-day and 90-day mortality with each additional day of bacteremia. This study compared 1 day of bacteremia to other days under the same conditions. In contrast, Minejima et al. defined 1 day of bacteremia as patients without repeat blood cultures who experienced clinical success, setting them as the reference group.[15] Furthermore, among patients with resolving bacteremia, all cases of mortality occurring on day 1 of bacteremia were documented as such and occurred more than 3 days after the initiation of appropriate antimicrobial therapy. This implies that deaths attributable to rapidly or ultimately fatal diseases unrelated to SAB were excluded from the analysis. Consequently, it is plausible that the relatively patients with mild severity who achieved treatment success served as the reference group, which might have contributed to the statistically significant differences in mortality rates among patients with bacteremia lasting 2 days or more. Furthermore, the higher number of persistent SAB cases and the overall higher mortality rate in this study compared to previous research could also be potential factors contributing to the observed differences. However, there was a statistically significant increase observed on day 3 in overall SAB and day 4 in MSSA bacteremia. These findings support recent research indicating that the duration for defining persistent SAB should be shorter than 7 days.

The increasing proportion of cases caused by MSSA in the surveillance data from 2017 indicated approximately 120,000 cases of SAB occurred with a resultant mortality of nearly 20,000 patients is highly noteworthy.[82] While there is extensive research analyzing the characteristics of persistent MRSA bacteremia, there is a lack of studies analyzing the characteristics of persistent MSSA bacteremia compared to resolving MSSA bacteremia. Despite the availability of appropriate antibiotics for MSSA bacteremia, the increasing prevalence of MSSA bacteremia may be attributed to factors such as inadequate source control, treatment failure, or the occurrence of metastatic infections similar to MRSA bacteremia.[14,83] Furthermore, the majorities about MSSA bacteremia studies focus on comparing and analyzing different antibiotic treatment regimens and their clinical outcomes or are analyzed as subgroups within MRSA bacteremia research.[83-85] Minejima et al. reported that the risk of

mortality increased incrementally with each day of positive blood cultures, with a significant risk observed from day 3 onwards. Notably, approximately 70% of the participants in their study had MSSA bacteremia.[15] As far as I know, this study is the first to comprehensive analyze and compare the clinical, microbiological, genetic characteristics, and risk factors between resolving MSSA bacteremia and persistent MSSA bacteremia using a large cohort.

This study has several limitations. First, despite the use of a prospective cohort, patients with persistent bacteremia underwent more extensive diagnostic testing, potentially biasing towards the detection of increased infection sites and infective endocarditis. Second, as our study was conducted in a tertiary care center, the microbiologic and genotypic characteristics of MRSA isolates may vary from previous studies due to clonal and geographic factors. Therefore, there are limitations to generalizing the results of this study. Third, to analyze of genotypic characteristics of SAB isolates, this study tested only the presence of virulence genes, rather than their actual expression. The expression levels of specific virulence genes could be associated with persistent SAB. Fourth, daily blood culture repeats were not conducted in all patients. This could have impacted the duration of bacteremia and may be attributed to limitations in accurately grouping bacteremia duration. This might explain why, in MRSA bacteremia, when using bacteremia day 1 as the reference, an increase in bacteremia day did not correlate with an observed increase in SAB-related mortality rate, and why no increase in mortality rate was observed in major MLST types with corresponding *spa* types. Finally, despite the presence of metastatic infection was defined as the development of a new infection at a sterile site that was not clinically relevant during the initial blood culture and was not identified at the initial diagnosis of SAB, the possibility that metastatic infection could also result from persistent bacteremia cannot be entirely ruled out, which might have influenced the outcomes.

Despite these limitations, this study is valuable for the following reasons. First, by aligning with contemporary research trends, this study carefully defined the durations of SAB and persistent bacteremia, and analyzed relatively a large cohort. And confirmed that persistent SAB is associated with a significant increase in mortality rates and poor outcomes compared to resolving SAB. Second, previous studies divided MRSA bacteremia or MSSA bacteremia into subgroups for analysis, whereas

this study conducted a comprehensive analysis encompassing not only the entire spectrum of SAB but also dividing it into MRSA bacteremia and MSSA bacteremia, allowing for an extensive analyzation of microbiological, genotypic and clinical characteristics. As far as I know, this is the first study to do so. Therefore, this study has important clinical implications and provides useful information for preventive infection control measures based on the molecular epidemiology of SAB in South Korea.

CONCLUSION

In conclusion, vascular grafts, metastatic infection and methicillin resistance were identified independent risk factors for persistent SAB. Among these, solid organ transplantation, metastatic infection, and ST5 were significant independent risk factors for persistent MRSA bacteremia. Conversely, community-acquired acquisition, vascular grafts, metastatic infection, and ST72 were identified as significant risk factors for persistent MSSA bacteremia. In persistent MRSA bacteremia, *agr* dysfunction and *agr* type II were significantly more prevalent. *sec* was frequent virulent gene in persistent MRSA bacteremia. The most prevalent clone in persistent MRSA bacteremia was ST5-SCC*mec* II-t2460, while in persistent MSSA it was ST72-t126. These clones exhibited significantly higher mortality rates compared to resolving bacteremia. SAB-related mortality increase only occurred at day 3 for SAB-related mortality when using day 1 of bacteremia as the reference group. These factors may have contributed to the poor outcomes observed in persistent bacteremia. Furthermore, it may be necessary to reconsider the definition of persistent SAB as positive cultures for 3 or more days, or even if the period was less than 3 days, if a follow-up blood culture conducted within those 3 days was positive again, given the trend of increasing mortality with prolonged bacteremia duration. The findings of this study underscore the importance of early intervention, particularly in cases where bacteremia persists for 3 days or more despite the use of appropriate antibiotics, therapy optimization, daily blood culture follow-up, and infectious disease consultation to enhance treatment outcomes for SAB patients. Further studies are needed to confirm the optimal cutoff for persistent SAB, and genome-wide studies along with detailed functional analyses should be conducted to elucidate the role of virulence factors in the outcome of SAB.

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ABSTRACT IN KOREAN

배경 : *Staphyococcus aureus (S. aureus)* 균혈증은 적절한 항생제 치료에도 불구하고 지속될 수 있으며 이는 불량한 임상 결과 및 높은 사망률과 관련되어 있다. 이전 연구들은 지속성 *S. aureus* 균혈증의 위험 요인, 임상 결과 및 미생물학적 특성을 분석하였지만 제한적이다. 따라서 본 연구는 지속성 *S. aureus* 균혈증의 임상적 및 미생물학적 및 유전적 특성 및 불량한 임상 결과에 영향을 미치는 요인을 광범위하고 포괄적으로 분석하고자 하였다.

연구 대상 및 방법 : 이 전향적 코호트 연구는 대한민국의 2,700 병상 3 차 의료 기관인 서울아산병원에서 2008 년 8 월부터 2021 년 2 월까지 수행되었다. 등록된 환자 중 methicillin-resistant *S. aureus* (MRSA) 또는 methicillin-susceptible *S. aureus* (MSSA) 균혈증이 있는 환자의 임상적 특성, 치료, 결과, 미생물학적 및 유전적 특성을 비교하고 분석하였다. 지속성 *S. aureus* 균혈증은 균혈증이 확인되고 적절한 항균제가 투여된 시점부터 추적 배양결과 음전이 확인될 때 까지가 3 일 이상이거나, 그 기간이 3 일 미만이더라도 해당 기간동안 시행된 추적 혈액배양 결과가 다시 양성인 경우로 정의하였다. 미생물학적 및 유전적 특성은 Sequence type (ST), Staphylococcus protein A (*spa*), Staphylococcal cassette chromosome (*mec*), 및 독성 유전자를 포함한 미생물학적 자료를 사용하여 분석하였다. 또한, 지속성 MRSA 또는 MSSA 균혈증에서 주요 ST 와 다른 ST 간의 임상 및 미생물학적 특성 및 결과를 분석하였다.

연구 결과 : *S. aureus* 균혈증 환자 1,877 명 중 826 명은 비지속성 균혈증이었고, 1,051 명은 지속성 균혈증에 해당하였다. 이 중 953 명은 MRSA 균혈증이었으며(비지속성 MRSA 균혈증, n = 318 명; 지속성 MRSA 균혈증, n = 635 명), 924 명은 MSSA 균혈증이었다(비지속성 MSSA 균혈증, n = 508 명; 지속성 MSSA 균혈증, n = 416 명). 다변량 분석 결과, 인공 혈관, 전이 감염 및 메치실린 내성은 지속성 *S. aureus* 균혈증의, 고형장기이식, 전이감염 및

ST5 는 지속성 MRSA 균혈증의, 지역사회 획득 감염, 인공혈관, 전이감염 및 ST72 는 지속성 MSSA 균혈증의 독립적 위험 요인으로 확인되었다. ST5, *agr* dysfunction, *agr* type II 는 지속성 MRSA 균혈증에서 더 흔했으며, ST72 는 지속성 MSSA 균혈증에서 더 흔했다. 지속성 MRSA 균혈증에서 가장 흔한 클론은 ST5-SCC*mec* II-t2460 이었고, 지속성 MSSA 균혈증에서는 ST72-t126 이었다. 균혈증 1 일을 기준으로 하였을 때 균혈증 기간이 1 일씩 증가함에 따라 사망률이 유의하게 증가하였는지 분석한 결과, 균혈증 3 일째에서 SAB 관련 사망률이 전체 SAB 군에서 유의하게 확인되었다. 지속성 *S. aureus* 균혈증의 ST5-*spa*-t9353 와 ST72-*spa*-t126, 지속성 MSSA 균혈증의 ST72-*spa*-t126 은 90 일 사망률과 SAB 관련 사망률의 유의한 증가와 관련이 있었다.

결론 : 대규모 코호트를 기반으로 한 본 연구 결과, 지속성 *S. aureus* 균혈증 환자의 적절한 치료전략 수립 및 양호한 치료 결과를 얻기 위해 지속성 *S. aureus* 균혈증의 임상적 및 미생물학적, 유전적 특성과 주요 ST 의 특성을 이해하는 것이 도움이 될 수 있겠다.

중심 단어 : *Staphylococcus aureus*, persistent bacteremia, sequence type, virulence genes