Journal of Microbiology, Immunology and Infection 58 (2025) 251–257

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

journal homepage: www.e-jmii.com

Clinical characteristics and genomic changes of recurrent Methicillin-Resistant *Staphylococcus aureus* bacteremia

Tu-Hsuan Chang [a], Hung-Jen Tang [b], Chi-Chung Chen [c,d], Chih-Jung Chen [e,f,g,*]

[a] Department of Pediatrics, Chi Mei Medical Center, Tainan, Taiwan
[b] Department of Internal Medicine, Chi Mei Medical Center, Tainan, Taiwan
[c] Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan
[d] Department of Bioscience Technology, Chang Jung Christian University, Tainan, Taiwan
[e] Division of Pediatric Infectious Diseases, Chang Gung Memorial Hospital, 333, Taoyuan, Taiwan
[f] Molecular Infectious Diseases Research Center, Chang Gung Memorial Hospital, 333, Taoyuan, Taiwan
[g] Chang Gung University School of Medicine, 333 Taoyuan, Taiwan

ARTICLE INFO

Keywords:
Whole genome sequencing
MRSA
Bacteremia

ABSTRACT

Background: Recurrent or persistent methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia presents significant clinical challenges. Comprehensive genomic-scale studies on the genetic changes in MRSA that correspond to refractory bacteremia are lacking.

Method: From 2011 to 2019, MRSA blood isolates were collected from patients with persistent or recurrent bacteremia at a teaching hospital in southern Taiwan. Whole-genome sequencing (WGS) captured the genomic changes in strains responsible for refractory bacteremia, and the altered susceptibilities to specific antimicrobial agents were assessed through measurements of minimal inhibitory concentrations (MICs).

Result: A total of 35 MRSA blood isolates from 15 patients with recurrent or persistent bacteremia were analyzed. Reduced susceptibilities to at least one anti-MRSA agent developed in strains from seven (46.7 %) patients. Of them, a non-synonymous mutation on a global regulator *mgrA* was associated with reduced daptomycin susceptibility, while an increase in vancomycin MIC was linked to mutations in genes encoding LCP family protein. A 16-fold increase in MIC to fusidic acid was connected to a mutation in the elongation factor G. These recurrent strains commonly exhibited a loss or acquisition of adhesion genes that were involved in biofilm formation, including *fnbA*, *fnbB*, and *sdrD*, and *easG* series genes of type VII secretion system.

Conclusion: Changes in the susceptibility of successive strains to common anti-MRSA agents were frequently observed in recurrent MRSA bacteremia. These changes were linked to modifications in genes of regulatory cascade, peptidoglycan binding, adhesion, and type VII secretion system.

1. Introduction

Staphylococcus aureus is a bacterium that causes a wide range of infections in both community and healthcare settings. These infections can be serious and include diseases such as bacteremia, infective endocarditis, pneumonia, osteoarticular infections, and toxic shock syndrome. Of these, *S. aureus* bacteremia (SAB) is a commonly reported and well-understood disease, with an estimated incidence of 10–30 cases per 100,000 person-years in industrialized countries.[1] SAB can be a highly destructive disease, especially when caused by methicillin-resistant strains that carry the *mecA* gene, making them resistant to almost all beta-lactam antibiotics. Methicillin-resistant *S. aureus* (MRSA) infections can result in recurrent bloodstream infections, which are often associated with prolonged hospital stays and high mortality rates.[2–4]

Despite receiving appropriate therapy with anti-MRSA agents and infection control measures, a significant proportion of patients continue to experience recurrent MRSA bacteremia, which can lead to serious complications and even death.[5,6] A range of factors have been associated with recurrent MRSA bacteremia, including chronic health conditions, previous MRSA infections, indwelling medical devices, and prior use of antibiotics.[7] Studies have shown that certain emerging MRSA clones, such as USA300, were associated with recurrent MRSA bacteremia.[8]

* Corresponding author. Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital and Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, 5 Fu-Shin Street, Kweishan, 333, Taoyuan, Taiwan.
E-mail address: chinjung@cgmh.org.tw (C.-J. Chen).

https://doi.org/10.1016/j.jmii.2024.11.008
Received 10 January 2024; Received in revised form 25 October 2024; Accepted 22 November 2024
Available online 30 November 2024

Whole genome sequencing (WGS) is a powerful tool that can analyze the complete genetic makeup of microorganisms. It is highly effective in understanding the mechanisms of infectious diseases and offers valuable insights into identifying potential outbreaks and tracking the spread of specific strains. By examining the genetic relatedness of isolates, WGS can help to identify genetic markers associated with antimicrobial resistance, virulence, and pathogenesis.[9] This information can aid in identifying potential microbial factors for recurrent infections and guiding clinical decisions.[10]

The study aimed to gain insights into the mechanisms of persistent or recurrent MRSA bacteremia, which is a challenging disease to treat. We analyzed bacterial isolates from patients with MRSA that were repeatedly isolated from their bloodstream using WGS. Our findings provided a valuable understanding of the genomic evolution of MRSA during persistent or recurrent bacteremia, which can inform the development of better strategies to prevent and manage this disease.

2. Methods

2.1. Study design and population

In this study, we conducted a detailed analysis of the clinical MRSA isolates at a medical center located in southern Taiwan, over a period of eight years, from 2011 to 2019. Our primary focus was on patients who had experienced two or more distinct episodes of MRSA bacteremia. To collect relevant data, we carefully reviewed the medical charts of these patients, obtaining information related to their demographics, medical history, recent surgeries, metastatic complications, and outcomes. The identification of *S. aureus* was performed using the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) system (Bruker Daltonik GmbH, Bremen, Germany). The MRSA strains were confirmed by testing their resistance to oxacillin using the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) interpretation criteria.[11]

2.2. Antimicrobial susceptibility testing

The susceptibilities to clindamycin, daptomycin, fusidic acid, linezolid, rifampicin, teicoplanin, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin were determined. The minimum inhibitory concentrations (MICs) of MRSA to the antimicrobial agents were measured with the agar dilution method. The interpretation criteria for susceptibility tests were in accordance with the guidelines provided by CLSI.[11]

2.3. Definitions

Recurrent bacteremia was defined as the identification of MRSA in a blood culture taken at least four days after the prior episode.[12] Early recurrence refers to the reappearance of the same *S. aureus* blood strain within 30 days of the initial occurrence and during the same hospital admission. Late recurrence of bacteremia caused by the same *S. aureus* strain is considered if it occurred more than 30 days after the initial episode and with a complete resolution of symptoms from the previous episode. Persistent bacteremia is defined as the presence of *S. aureus* in blood cultures, despite the patient receiving at least 7 days of effective treatment.[13] Different isolates were considered to be the same strain if they were obtained from the same patient and possessed identical multilocus sequence types (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) types, and *spa* types.

2.4. Whole-genome sequencing (WGS) and analysis pipeline

The WGS was performed on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA), and the draft genomes were deposited at DDBJ/ENA/GenBank under Accession PRJNA953934. The bactopia pipeline v2.2.0 was used to analyze the genomes of 35 MRSA strains, including the determination of MLST, and *de novo* assembly by shovill pipeline with SPAdes as core assemble.[14,15] The Prokka database was used for annotation.[16] The resistance and virulence genes were detected by Ariba using the Comprehensive Antibiotic Resistance Database (CARD), and Virulence Factor Database (VFDB) database, respectively.[17,18] The assembly of the 35 strains generated the draft genome lengths ranging from 2,771,940 to 2,954,946 nucleotides. The number of assemblies contigs ranged from 23 to 183, with a median contig N50 of 96,033 nucleotides (range 37,505–463,095 nucleotides) (Table S1). The genome-wide nucleotide alterations between strains accounting for the recurrent infection episodes in the same patient were obtained by comparing the available data generated by the bactopia pipeline.

2.5. Statistical analysis

Descriptive statistics were employed to summarize the study variables. Categorical variables are presented as frequencies and percentages, while numerical variables are described using medians and ranges. To analyze categorical variables, the chi-square test and Fisher's exact test were applied. For numerical variables that deviated from a normal distribution, the Mann-Whitney *U* test was used to compare differences between two independent groups.

2.6. Ethical approval

This study was approved by the Institutional Review Board (IRB) of Chi Mei Medical Center (Reference number: 11206-011).

3. Results

WGS was performed on a total of 53 MRSA blood isolates in 24 patients with recurrent or persistent bacteremia. Four *S. aureus* strains collected from two patients were excluded from the study because of the absence of the SCC*mec* element. In another 7 pairs of isolates collected from 7 patients, the SCC*mec* and *spa* types were completely different for isolates in each pair, indicating repeated infections by different MRSA strains but not recurrent infections. The 14 isolates were also excluded. The remaining 35 MRSA isolates accounting for recurrent bacteremia in 15 patients were analyzed.

3.1. Features of patients with recurrent MRSA bacteremia

Table 1 shows the demographic and clinical characteristics of 15 patients diagnosed with recurrent and/or persistent (n = 8) MRSA bacteremia. Of them, 11 patients were female, and the median age was 76 years. All patients had chronic diseases, with diabetes being the most common comorbidity (46.7 %). Seven (46.7 %) patients had catheters or recent surgery prior to diagnosis. Four (26.7 %) patients had metastatic infections. The median time between the first and last positive blood cultures was 14 days (range, 4–170 days). In the early recurrence group, 4 out of 10 patients died while all 5 patients in the late recurrence group survived.

3.2. Characteristics of MRSA strains accounting for recurrent bacteremia

Table S2 summarizes the molecular characterizations and antimicrobial susceptibilities of 35 MRSA isolates from 15 patients with recurrent bacteremia. Three major clones - ST5, ST8, and ST239 - and four minor clones were identified. All isolates were susceptible to tigecycline and linezolid. However, ST5, ST239, and ST59 strains showed high-level resistance to clindamycin. Non-susceptibility to trimethoprim-sulfamethoxazole was identified in all ST239 and ST900 strains. Half of the ST239 isolates and all ST5 isolates were non-susceptible to rifampicin. All MRSA strains remained susceptible to glycopeptides, but the majority (77.1 %) had a vancomycin MIC of 2 μg/

Table 1
Demographic data and clinical characteristics of 15 patients with recurrent methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia.

Patient	Isolate	Age, years	Gender	Comorbidity	Permanent catheter or surgery	MRSA disease(s)	Fatal outcome	Number of blood cultures positive for MRSA	Early or late recurrence	Days between 1st and last positive culture	Anti-MRSA antibiotic treatment between bacteremia episodes
P1	CMSA01 CMSA02 CMSA03	88	Male	Cancer Tuberculosis	Y	Sepsis	Y	4	Early	8	Y (minocycline, vancomycin)
P2	CMSA07 CMSA08	77	Female	HTN	N	Pyomyositis, necrotizing fasciitis, sepsis	N	4	Early	14	Y (teicoplanin)
P3	CMSA17 CMSA20 CMSA23 CMSA24 CMSA25	57	Female	Hyper-IgE Syndrome	N	Osteomyelitis, sepsis, lung abscess	N	5	Late	60	Y (vancomycin, tigecycline, teicoplanin, daptomycin)
P4	CMSA47 CMSA48	53	Male	DM, HF	N	Sepsis	N	3	Early	9	Y (vancomycin, daptomycin)
P5	CMSA06 CMSA09	74	Female	CAD, DM, HTN	N	Infective endocarditis	N	2	Early	25	Y (vancomycin)
P6	CMSA49 CMSA50 CMSA51	49	Male	DM	N	Sepsis	N	3	Late	170	Y (minocycline, vancomycin)
P7	CMSA27 CMSA29	93	Female	CVA, HF	N	Sepsis	Y	2	Early	6	Y (vancomycin)
P8	CMSA44 CMSA46	63	Female	Cancer	Y	Sepsis	Y	2	Early	14	Y (teicoplanin)
P9	CMSA40 CMSA42	78	Female	CAD, DM, ESRD	Y	Sepsis	N	4	Late	130	Y (vancomycin)
P10	CMSA10 CMSA12	65	Female	DM, ESRD, HF, HTN	N	Sepsis	N	2	Early	4	N
P11	CMSA43 CMSA45	82	Female	COPD, HTN	Y	Sepsis	N	3	Early	17	Y (daptomycin, minocycline)
P12	CMSA11 CMSA15	40	Male	Cancer	Y	Sepsis	N	2	Early	8	N
P13	CMSA37 CMSA41	86	Female	COPD, CVA, DM, HF, HTN,	Y	Sepsis	N	2	Late	155	Y (teicoplanin, trimethoprim-sulfamethoxazole)
P14	CMSA31 CMSA32	96	Female	HF	N	Sepsis	N	3	Late	56	Y (teicoplanin)
P15	CMSA34 CMSA35	76	Female	Cirrhosis DM, ESRD, HF	Y	Sepsis, osteomyelitis, chest wall abscess	Y	3	Early	22	Y (vancomycin, teicoplanin)

Abbreviations: CAD, coronary artery disease; CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ESRD, end-stage kidney disease; HF, heart failure; HTN, hypertension.

mL. No statistically significant differences were found in the demographic data or antimicrobial susceptibilities between patients with two episodes of bacteremia and those with three or more episodes, as illustrated in Table S3.

3.3. Antimicrobial susceptibility changes in successive MRSA strains

Out of 15 patients, 7 (46.7 %) patients developed reduced susceptibilities to at least one antimicrobial agent in the MRSA strains (Table 2). Strains from four patients (P5, P6, P7, and P10) showed an increase in vancomycin MIC from 1 μg/mL to 1.5 or 2 μg/mL. Strains from two patients (P3, P8) showed an increase in daptomycin MIC. One strain from the patient (P9) developed fusidic acid resistance, with a MIC increase from 0.5 μg/mL to 8 μg/mL, even though he was not exposed to fusidic acid.

3.4. Genetic changes in the whole genome scale

The genetic changes in the MRSA isolates are detailed in Table S4. Out of the 20 successive isolates obtained from 15 patients, 26 genetic mutations were found in their genomes. These mutations included 16 missense mutations (61.5 %), 8 mutations in intragenic regions (30.8 %), and 2 (7.7 %) synonymous mutations. Among 10 isolates identified within an interval of 21 days or less, interestingly, only 1 isolate (CMSA03, Patient 1, Table S3) had a mutation. However, out of the 10 isolates identified with intervals greater than 21 days, 9 isolates (90 %) had mutations. These mutations occurred at intervals of 22–170 days and the number of mutations ranged from 1 to 11.

3.5. Genetic changes associated with reduced antimicrobial susceptibilities

Table 3 presents the nucleotide and amino acid alterations observed in strains that developed reduced antimicrobial susceptibility. In patient 3, the strains became resistant to daptomycin due to a missense point mutation on the *mgrA* gene, which encodes a transcriptional regulator. In patient 8, we detected reduced susceptibility to daptomycin, but no mutation was found. In three patients (Patients 5, 6, and 7), we identified an increase in vancomycin MICs in recurrent isolates. The isolate from patient 5 had a missense mutation in a GHKL domain-containing protein, while the two isolates from patient 6 had missense mutations in an LCP family protein and the 50S ribosomal protein L19, respectively. An isolate from patient 7 had a missense mutation in an AMP-binding protein. Lastly, a strain developing resistance to fusidic acid in patient 9 had a missense mutation in *fusA*.

Table 2
Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates developing reduced antimicrobial susceptibility changes during recurrent or persistent bacteremia.

Patient	Isolate	MLST	SCC*mec*	*Spa* type	Minimal inhibitory concentrations (μg/mL)								
					LZD	TGC	CLI	TMP/SXT	RIF	TEC	VAN	DAP	FUS
P3	CMSA17	5	IIa	t002	4	0.5	>128	0.06/1.14	4	2	2	1	0.5
	CMSA20											2	
	CMSA23											1	
	CMSA24											1	
	CMSA25											1	
P5	CMSA06	8	IVa	t008	4	0.5	0.25	0.06/1.14	0.06	0.5	1	1	0.5
	CMSA09										2		
P6	CMSA49	8	IVa	t008	4	0.5	0.25	0.06/1.14	0.06	1	1	1	0.5
	CMSA50										1.5		
	CMSA51										1		
P7	CMSA27	30	IVc	t019	4	0.5	0.25	0.06/1.14	0.06	0.5	1	1	0.5
	CMSA29										2		
P8	CMSA44	45	IVa	t026	4	0.5	0.125	0.06/1.14	0.06	1	2	0.5	0.5
	CMSA46											1	
P9	CMSA40	45	V or VII	t1081	4	0.5	0.25	0.06/1.14	0.06	1	2	1	0.5
	CMSA42						0.5						8
P10	CMSA10	59	IVa	t3736	4	0.5	>128	0.06/1.14	0.06	0.5	1	0.5	0.5
	CMSA12										2		

Abbreviations: CLI clindamycin, DAP daptomycin, FUS fusidic acid, LZD linezolid, MLST multilocus sequence typing, RIF rifampicin, TEC teicoplanin, TGC tigecycline, TMP/SXT trimethoprim-sulfamethoxazole, VAN vancomycin.
The MIC values for each patient are displayed for subsequent strains only if they differ from the first strain's value.

Table 3
The nucleotide changes and amino acid alterations in methicillin-resistant *Staphylococcus aureus* (MRSA) blood isolates developing reduced antibiotic susceptibilities during recurrent or persistent bacteremia.

Patient	Interval of recurrence (days)	Mutation type	Gene name/locus	Product	Nucleotide alteration and position	Amino acid alterations and position
Daptomycin						
P3	50	missense	*mgrA*	HTH-type transcriptional regulator MgrA	c.358T > A	Ser120Thr
Vancomycin						
P5	25	missense	USA300HOU_RS10970	GHKL domain-containing protein	c.637A > G	Asn213Asp
P6	170	missense	*rplS*	50S ribosomal protein L19	c.226T > A	Phe76Ile
		missense	USA300HOU_RS12505	LCP family protein	c.302C > T	Pro101Leu
P7	6	missense	C7M55_RS15490	AMP-binding protein	c.494C > T	Ala165Val
Fusidic acid						
P9	130	missense	*fusA*	elongation factor G	c.1211C > A	Pro404Gln

3.6. Acquisition or loss of carried virulence and resistant genes

In Fig. S1, we depicted the presence of virulence factors genes in the initial isolates identified in bacteremia. The changes in virulence factors after recurrence are summarized in Fig. 1. Changes in adhesion gene patterns were observed in certain MRSA strains during their persistent or recurrent infections. For instance, a ST59 and a ST5 strain acquired the *fnbA* gene, while a ST8 strain gained the *sdrD* gene. Conversely, one ST8 strain lost the *clfB* gene, and a ST45 strain lost the *fnbB* gene. The type VII secretion system is responsible for exporting virulence factors including enzymes and toxins that contribute to MRSA infections. During recurrent bacteremia episodes, acquisition or loss of the genes in the *easG* series of the type VII secretion system was observed in strains of all sequence types.

Fig. S2 presents an overview of the pattern of antibiotic resistance gene presence and acquisition or loss of the resistant genes during persistent or recurrent bacteremia. The pattern of resistant genes was generally related to the clonality of MRSA. An ST45 strain (CMSA46) lost both *ermC* and *erm-33* during recurrence, yet this did not result in a significant change in clindamycin susceptibility. Additionally, ST239 strains harboring *dfrG* (Patients 11, 12, 13, 14, 15) were linked to high-level resistance to trimethoprim-sulfamethoxazole (MIC >8/152 μg/mL). During recurrence, two ST239 strains (CMSA41, CMSA45) acquired *dfrK*, which also mediated trimethoprim-sulfamethoxazole resistance.

4. Discussion

Our study has provided critical insights into the genetic mechanisms behind antibiotic resistance and virulence factors of MRSA strains that cause recurrent bacteremia. Through our research, we have identified the primary strains responsible for recurrent or persistent bacteremia were ST5, ST8, and ST239 genotypes. We found that the antimicrobial susceptibilities of these strains were mainly dependent on the sequence type. However, we also noted that 66.7 % of the strains had an initial vancomycin MIC of 2 μg/mL, and in 26.7 % of them, the MIC levels increased after a recurrent bacteremia episode. Additionally, we have identified reduced daptomycin susceptibility in two patients. Finally, we have observed that some genes encoding virulence such as adhesion genes and type VII secretion systems, underwent changes during recurrent episodes. These findings are significant and provide crucial information for clinicians and researchers working in this field.

In our study, we identified three predominant sequence types (ST5, ST8, and ST239) among MRSA isolates from patients with recurrent bacteremia. However, it is important to note that the prevalence of these sequence types is relatively high in the general population, which complicates our ability to conclusively assert that they are more prone to cause recurrent bacteremia compared to other sequence types. The small

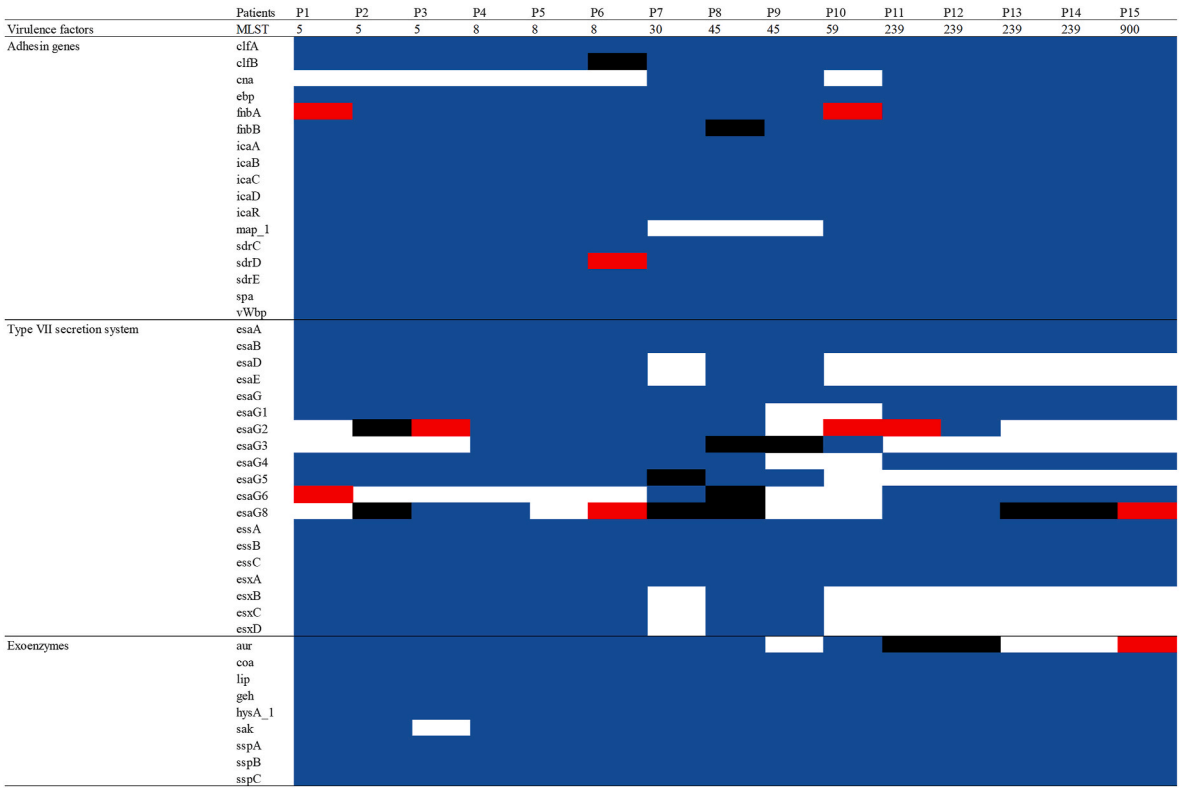

Fig. 1. The presence (blue color) and absence (white color) of virulence factors encoding genes in recurrent methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia isolates. Changes after recurrence were displayed as acquisition (red color) and loss of function (black color). The labels on top indicate patient ID, and sequence type (ST). The label on the left indicates the genes encoding virulence factors.

sample size of our cohort limits our capacity to draw definitive conclusions regarding the specific characteristics of these STs in relation to recurrent infections. In particular, ST239 was often linked to severe and long-lasting MRSA infections, especially in healthcare settings. In Taiwan, ST5 and ST239 were frequently reported as sequence types linked to healthcare-associated MRSA bacteremia. Our results were consistent with previous studies.[19,20] ST8 (USA300) was a common strain of MRSA associated with community-acquired infections and has recently emerged as a major clone in Taiwan.[21,22] Our study highlights the important role of ST8 in persistent MRSA bacteremia.

Antimicrobial resistance has been found to predict mortality in *S. aureus* infections. Daptomycin is effective against various *S. aureus* infections, including bacteremia and endocarditis. However, during the relapse of two patients, it was discovered that they had developed reduced susceptibility to daptomycin. Daptomycin resistance is known to be linked to mutations or changes in the expression of genes involved in surface charge, cell membrane phospholipids, and drug binding.[23] The *mgrA* is a regulatory gene that is known to play a crucial role in the development of daptomycin resistance. It functions as a global regulator of gene expression in *S. aureus*. In animal models, it has been observed that *mgrA* mutants exhibit lower levels of *mprF*, which was considered to be a possible pathway for the development of daptomycin resistance in clinical isolates.[24] Research suggests that the *mgrA* mutant *S. aureus* strain may play a role in persistent infections by requiring fewer generations to select for small colony variants.[25] However, further research is necessary to understand the implications.

Vancomycin MIC was significantly associated with mortality in MRSA infection, regardless of the source or MIC methodology.[26] An increase in vancomycin MIC was identified in strains causing recurrent bacteremia in four patients. In patient 6, we found a missense mutation in the gene responsible for LCP family proteins. LCP family proteins, such as LcpA, LcpB, and other variations, are essential to the synthesis of cell walls and the formation of cell envelopes in *S. aureus*. These proteins play a vital role in anchoring various cell wall components, especially peptidoglycan.[27] It is possible that they have a role in the development of resistance to vancomycin in cases of recurring infections. However, the process behind the increase in MICs of vancomycin is complex and may involve changes in bacterial cell wall structure or composition, as well as the presence of biofilms that prevent the antibiotic from penetrating. The mutations that were found in our isolates, such as GHKL domain-containing protein, 50S ribosomal protein L19, and AMP-binding protein, require further investigation to understand their roles.

Staphylococcal cells use adhesins to attach to host tissues and implanted medical devices, which is the first step in causing an infection. Adhesins also contribute to the formation of biofilms, which help the infection persist and become resistant to antimicrobial therapies. Among recurrent bacteremia isolates, two specific adhesins, *fnbA* and *SdrC*, have been identified. *fnbA* encodes for fibronectin-binding protein A, which works together with fibronectin-binding protein B to form biofilms.[28] During our investigation of recurrent bacteremia isolates, we discovered that *fnbA* was present along with previously identified *fnbB*. This indicates a potential synergistic effect, which could make treatment more challenging. *SdrC* is a protein anchored to the cell wall, and it is responsible for mediating bacterial adhesion to host cells. Its presence in recurrent infection strains also suggests that biofilm formation may be a contributing factor.[29] Understanding the role of adhesins in persistent staphylococcal bacteremia and their potential contribution to treatment failure is crucial.

Based on our analysis, we have found that the type VII secretion system (T7SS), specifically *EsaG*, is highly adaptable and diverse. This system plays a crucial role in the pathogenesis of *S. aureus* infections by enabling the secretion of virulence factors. One of these factors is *EsaD*, a large nuclease toxin that kills other bacterial strains and is involved in inter-species competition.[30] During its biosynthesis, *EsaD* exhibits toxic activity, which is neutralized through complex formation with an

antitoxin called EsaG. Even though some *S. aureus* strains lack EsaG, EsaD-secreting strains can still kill them, suggesting competition within S. aureus itself.[31] These findings indicate that *S. aureus* has evolved in environments with antibiotic selective pressure and other *S. aureus* strains during persistent or recurrent bacteremia. This provides further insight into the development of novel treatments that target bacterial biosynthesis rather than relying solely on traditional antimicrobial agents.

Our study has several limitations. First, the clinical isolates were randomly collected from a single center, and the sample size was small, which may limit the generalizability of our findings from an epidemiological perspective. Second, clinical factors such as residual artificial materials, catheters, and comorbidities could have influenced the persistence or recurrence of bacteremia, which were not fully analyzed in this study. Larger, prospective studies are needed to investigate specific sequence types and genomic changes in recurrent bacteremia more comprehensively. Moreover, we must consider the potential implications of our genome assembly approach. While we detected various genetic mutations and changes among the isolates, the gaps between contigs in our assembly may harbor significant coding sequences that could contribute to the observed phenotypic traits. This indicates the limitations of our current methodology, as critical genetic elements may not have been captured. The utilization of long-read sequencing technologies in future studies could help address these gaps, providing a more comprehensive view of the genomic landscape associated with recurrent MRSA bacteremia. Additionally, due to the retrospective nature of our study, we could only observe associations between antimicrobial susceptibility changes and certain mutations. Further experimental studies or animal models are needed to confirm or rule out causal relationships.

In conclusion, changes in the susceptibility of successive strains to common anti-MRSA agents were frequently observed in recurrent MRSA bacteremia. In our study, these changes were associated with modifications in genes involved in regulatory cascades, peptidoglycan binding, adhesion, and the type VII secretion system. Our findings highlight the need for a comprehensive evaluation of each sequence type and suggest the possibility of multiple evolutionary pathways contributing to recurrent episodes of bacteremia.

CRediT authorship contribution statement

Tu-Hsuan Chang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Hung-Jen Tang:** Resources. **Chi-Chung Chen:** Resources. **Chih-Jung Chen:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing.

Acknowledgment

This research was supported in part by a research grant from Chi Mei Medical Center (CMOR11401, CCFHR113062, CMNSTC11302) and the National Science and Technology Council (NSTC 113-2314-B-384-004), Taiwan. The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.11.008.

References

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28(3):603–661.
2. Wang JL, Chen SY, Wang JT, et al. Comparison of both clinical features and mortality risk associated with bacteremia due to community-acquired methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus*. *Clin Infect Dis*. 2008;46(6):799–806.
3. Camacho-Cruz J, Gutiérrez IF, Brand-López K, et al. Differences between methicillin-susceptible versus methicillin-resistant *Staphylococcus aureus* infections in pediatrics: multicenter cohort study conducted in Bogotá, Colombia, 2014-2018. *Pediatr Infect Dis J*. 2022;41(1):12–19.
4. Bai AD, Lo CK, Komorowski AS, et al. *Staphylococcus aureus* bacteremia mortality: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2022;28(8):1076–1084.
5. Choi SH, Dagher M, Ruffin F, et al. Risk factors for recurrent *Staphylococcus aureus* bacteremia. *Clin Infect Dis*. 2021;72(11):1891–1899.
6. Kim SH, Jeon M, Jang S, Mun SJ. Factors for mortality in patients with persistent *Staphylococcus aureus* bacteremia: the importance of treatment response rather than bacteremia duration. *J Microbiol Immunol Infect*. 2023;56(5):1007–1015.
7. Albertson J, McDanel JS, Carnahan R, et al. Determination of risk factors for recurrent methicillin-resistant *Staphylococcus aureus* bacteremia in a Veterans Affairs healthcare system population. *Infect Control Hosp Epidemiol*. 2015;36(5):543–549.
8. Moore CL, Hingwe A, Donabedian SM, et al. Comparative evaluation of epidemiology and outcomes of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 infections causing community-and healthcare-associated infections. *Int J Antimicrob Agents*. 2009;34(2):148–155.
9. Hassan RM, Elanany MG, Mostafa MM, Yousef RHA, Salem ST. Whole genome characterization of methicillin resistant *Staphylococcus aureus* in an Egyptian Tertiary Care Hospital. *J Microbiol Immunol Infect*. 2023;56(4):802–814.
10. Mason A, Foster D, Bradley P, et al. Accuracy of different bioinformatics methods in detecting antibiotic resistance and virulence factors from *Staphylococcus aureus* whole-genome sequences. *J Clin Microbiol*. 2018;56(9), e01815, 17.
11. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *J Clin Microbiol*. 2020;58(3), e01864, 19.
12. Woudt SHS, de Greeff SC, Schoffelen AF, Vlek ALM, Bonten MJM, Group IDSISARS. Antibiotic resistance and the risk of recurrent bacteremia. *Clin Infect Dis*. 2017;66(11):1651–1657.
13. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis*. 2011;52(3):e18–e55.
14. Petit RA, Read TD. Bactopia: a flexible pipeline for complete analysis of bacterial genomes. *mSystems*. 2020;5(4).
15. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 2012;19(5):455–477.
16. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068–2069.
17. Alcock BP, Huynh W, Chalil R, et al. Card 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res*. 2023;51(D1):D690–D699.
18. Liu B, Zheng D, Zhou S, Chen L, Yang J. Vfdb 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res*. 2022;50(D1):D912–D917.
19. Wang W-Y, Chiueh T-S, Sun J-R, Tsao S-M, Lu J-J. Molecular typing and phenotype characterization of methicillin-resistant *Staphylococcus aureus* isolates from blood in Taiwan. *PLoS One*. 2012;7(1), e30394.
20. Liao C, Lai C, Chen S, Huang Y, Hsueh P. Strain relatedness of meticillin-resistant *Staphylococcus aureus* isolates recovered from patients with repeated bacteraemia. *Clin Microbiol Infect*. 2010;16(5):463–469.
21. Huang YC, Chen CJ. USA300 (sequence type 8) has become a major clone of methicillin-resistant *Staphylococcus aureus* in northern Taiwan. *Int J Antimicrob Agents*. 2022;59(3), 106534.
22. Yu CH, Shen S, Huang KA, Huang YC. The trend of environmental and clinical methicillin-resistant *Staphylococcus aureus* in a hospital in Taiwan: impact of USA300. *J Microbiol Immunol Infect*. 2022;55(2):241–248.
23. Jones T, Yeaman MR, Sakoulas G, et al. Failures in clinical treatment of *Staphylococcus aureus* infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. *Antimicrob Agents Chemother*. 2008;52(1):269–278.
24. Li L, Wang G, Cheung A, Abdelhady W, Seidl K, Xiong YQ. MgrA governs adherence, host cell interaction, and virulence in a murine model of bacteremia due to *Staphylococcus aureus*. *J Infect Dis*. 2019;220(6):1019–1028.
25. Lee J, Carda-Diéguez M, Žiemytė M, et al. Functional mgrA influences genetic changes within a *Staphylococcus aureus* cell population over time. *J Bacteriol*. 2022;204(10), e00138, 22.
26. Van Hal S, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. *Clin Infect Dis*. 2012;54(6):755–771.
27. Hiramatsu K, Kayayama Y, Matsuo M, et al. Vancomycin-intermediate resistance in *Staphylococcus aureus*. *J Glob Antimicrob Resist*. 2014;2(4):213–224.
28. Shinji H, Yosizawa Y, Tajima A, et al. Role of fibronectin-binding proteins A and B in in vitro cellular infections and in vivo septic infections by *Staphylococcus aureus*. *Infect Immun*. 2011;79(6):2215–2223.

29. Barbu EM, Mackenzie C, Foster TJ, Höök M. SdrC induces staphylococcal biofilm formation through a homophilic interaction. *Mol Microbiol*. 2014;94(1):172–185.
30. Cao Z, Casabona MG, Kneuper H, Chalmers JD, Palmer T. The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. *Nat Microbiol*. 2016;2, 16183.
31. Wang Y, Zhou Y, Shi C, et al. A toxin-deformation dependent inhibition mechanism in the T7SS toxin-antitoxin system of Gram-positive bacteria. *Nat Commun*. 2022;13 (1):6434.