Tropical Biomedicine 43(2): 174-179 (2026)
https://doi.org/10.47665/tb.43.2.005

RESEARCH ARTICLE

Prospective surveillance of *Staphylococcus aureus* bacteremia (SAB) using *spa* typing to identify MRSA transmission in a tertiary care setting

Amelia, S.[1*], Santosaningsih, D.[2], Kusumawati, R.L.[1], Hamid, R.Z.[3], Zulham[4], Eyanoer, P.C.[5], Rozi, M.F.[6], Syamira, N.A.[7]

[1]Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[3]Department Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[4]Department of Histology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[5]Department of Public Health and Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[6]Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[7]Department of Biotechnology, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia
*Corresponding author: sriamelia@usu.ac.id

ARTICLE HISTORY

Received: 6 September 2025
Revised: 26 April 2026
Accepted: 28 April 2026
Published: 30 June 2026

ABSTRACT

The *spa* gene of *Staphylococcus aureus* encodes protein A, a key virulence factor contributing to molecular typing. *spa* typing provides valuable information encoding diversity and transmission, particularly for methicillin-resistant *S. aureus* (MRSA), which remains a major public health concern. A one-year prospective surveillance study was conducted involving *S. aureus* bacteremia (SAB) patients admitted to Haji Adam Malik General Hospital, Medan, Indonesia. SAB isolates were phenotypically analysed for cefoxitin resistance using the VITEK-2 system and genotypically characterised by PCR for the *mecA* and *spa* genes. *Spa* typing of the X region was performed at Apical Scientific Laboratory (Selangor, Malaysia), and clustering was analysed using the Based Upon Repeat Pattern (BURP) algorithm. A minimum spanning tree (MST) was constructed with Ridom SeqSphere to assess genetic relatedness. Clinical outcomes were compared between MRSA and MSSA clusters. A total of 120 SAB patients were included, equally divided into MRSA and MSSA groups. The dominant *spa* types included t852 (12.5%), t5422 (9.2%), and t4171 (7.5%). In-hospital mortality was 28.3%, significantly higher in MRSA compared with MSSA (*p-value* 0.015, OR 2.76). ICU admission occurred in 20.9% of cases. MRSA isolates predominantly clustered in a single group (Cluster 1), consistent with clonal transmission, whereas MSSA isolates displayed greater genetic heterogeneity across multiple clusters. This prospective surveillance highlights the clonal expansion of MRSA within a tertiary care setting and supports the utility of *spa* typing as a practical molecular tool for monitoring transmission and guiding infection control strategies.

Keywords: Antimicrobial resistance; virulence; typing; protein A; bacteremia.

INTRODUCTION

Staphylococcus aureus is a ubiquitous bacterial species capable of simultaneously functioning as a commensal and pathogen due to the presence of multiple virulence factors encoded within its genome (Tong *et al.*, 2015). This bacterium can cause several infections, including *S. aureus* bacteremia (SAB). SAB incidence ranges from 10 to 30 cases per 100,000 individuals annually, with higher rates reported in low-income countries, almost 48 cases per 100,000 individuals (Hassoun *et al.*, 2017; Loftus *et al.*, 2022). The emerging challenge of antimicrobial resistance (AMR), particularly with methicillin-resistant *S. aureus* (MRSA), galvanizes the burden on healthcare systems (Lee *et al.*, 2013). As novel antimicrobial development has stagnated, infection prevention and control remain the most effective strategies to curb MRSA transmission (Humphreys *et al.*, 2009; Salge *et al.*, 2017; Shlaes & Bradford, 2018).

Molecular epidemiology provides critical insights into the dynamics of MRSA transmission and clonal expansion (Kumar *et al.*, 2021). *Spa* typing methods are used as first-line tools for *S. aureus* surveillance in hospital settings by identifying polymorphic regions of the *Staphylococcus* protein A (*spa*) gene, a key virulence determinant (Chadi *et al.*, 2022). Protein A, encoded by the *spa* gene, contributes to immune evasion by binding to the Fc region of immunoglobulin G (IgG), thereby inhibiting opsonization and phagocytosis. In addition, protein A can interact with B-cell receptors, acting as a B-cell superantigen that disrupts adaptive immune responses and promotes bacterial persistence (Bear *et al.*, 2023).

In comparison with other extensive methods, such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole genome sequencing (WGS), *spa* typing offers a practical, cost-effective, and reproducible method for outbreak investigation, particularly in resource-limited settings (Ruppitsch *et al.*, 2006). Beyond its utility in strain differentiation, variations in *spa* repeats

are also linked to virulence and host immune evasion (Ruppitsch, 2016; Strommenger *et al.*, 2008).

In Indonesia, molecular typing studies are highly scarce despite a reported MRSA prevalence rate approaching 20% in previous surveillance (Amelia *et al.*, 2021). This paucity of data poses significant challenges for outbreak investigation and the containment of resistant strains, particularly in tertiary healthcare facilities with diverse patient populations. Previous studies from other regions have shown that *spa* typing not only reveals the distribution of predominant lineages but also provides valuable context for clinical outcomes and transmission pathways (Asadollahi *et al.*, 2018; Mollerup *et al.*, 2022; Özdemir, 2022). Therefore, this study aimed to perform prospective surveillance of SAB using *spa* typing to characterise MRSA transmission and compare clonal dynamics between MRSA and MSSA in a tertiary care hospital. Therefore, this study aimed to characterize the clonal distribution of SAB using *spa* typing and to evaluate the association between methicillin resistance, clonal clustering, and clinical outcomes.

MATERIAL AND METHODS

Study design and isolate collection

This was a prospective surveillance study conducted at H. Adam Malik General Hospital, Medan, Indonesia, a tertiary referral center. All consecutive patients with confirmed SAB from various infection sources (respiratory, urinary, gastrointestinal, and others) were included over 12 months (September 2022–September 2023). Patient outcomes were recorded prospectively, including in-hospital mortality (primary outcome) and intensive care unit (ICU) admission. In-hospital mortality was defined as death attributable to sepsis or its related complications. Blood samples were collected in duplicate using BACTEC culture bottles (10 mL per bottle) and processed in an automated blood culture system (BD BACTEC™, USA). *S. aureus* isolates were identified and phenotypically classified as MRSA or MSSA based on cefoxitin resistance using the VITEK-2 Compact system (bioMérieux, France). Confirmed isolates were subcultured on blood agar to ensure purity before molecular analysis.

mecA and *spa* gene detection

Genomic DNA was extracted using the QIAamp DNA Microbiome Kit (QIAGEN, Germany) according to the manufacturer's instructions. The presence of the *mecA* gene, indicative of methicillin resistance, was assessed by PCR using specific primers (F: AAAATCGATGGTAAAGGTTGGC; R: AGTTCTGGAGTACCGGATTTGC), yielding a 533 bp product. The thermal cycling protocol consisted of an initial denaturation at 95°C for 3 minutes, followed by 33 cycles of denaturation at 92°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 3 minutes. A final extension step was carried out at 72°C for 3 minutes. The amplified products were then analyzed to confirm the presence of the *mecA* gene, indicative of methicillin resistance in *S. aureus* (Amelia *et al.*, 2021). Subsequently, the polymorphic X region of the *spa* gene was amplified using primers 1095F and 1517R. PCR conditions followed previously established protocols (Harmsen *et al.*, 2003). Amplicons were analyzed using 2% agarose gel electrophoresis to confirm expected product sizes. All procedures were conducted at the Multidisciplinary Laboratory, Faculty of Medicine, Universitas Sumatera Utara.

Spa typing and BURP procedure

Spa typing was performed based on the sequencing of the polymorphic X region to characterize clonal relationships and identify potential transmission patterns in the Apical Scientific Laboratory, Selangor, Malaysia. Sequence data were analyzed using Ridom SeqSphere+ software (version 9.0.10), which assigns *spa* types based on repeat patterns. Clonal relatedness among isolates was assessed using the Based Upon Repeat Pattern (BURP) algorithm, which groups *spa* types into *spa* clonal complexes (*spa* CC) according to repeat similarity. Highly divergent types were classified as singletons. A minimum spanning tree (MST) was constructed to visualize genetic relationships and infer potential transmission clusters. This approach enabled the identification of dominant lineages and assessment of clonal expansion within the hospital setting.

Data analysis and ethical approval

Molecular characteristics of *Staphylococcus aureus* isolates were analyzed to determine methicillin resistance status and clonal relationships. The presence of the *mecA* gene was used to classify isolates as MRSA or MSSA. *Spa* typing results were used to assign isolates into specific *spa* types and further grouped into *spa* clonal complexes (*spa* CC) using the BURP algorithm. Clonal relatedness was visualized using a minimum *spa*nning tree (MST). The distribution of MRSA and MSSA isolates across *spa* clonal complexes was evaluated to identify potential clustering patterns suggestive of clonal transmission. Clinical outcomes, including in-hospital mortality and ICU admission, were compared between the MRSA and MSSA groups and across *spa* clusters. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. The strength of association between methicillin resistance and clinical outcomes was expressed as odds ratios (ORs) with 95% confidence intervals (CIs). This study was approved by the Local Ethical Committee for Medical Research, Faculty of Medicine, Universitas Sumatera Utara (Reference No: 540/KEPK/USU/2022).

RESULTS

Bacterial isolates and patient characteristics

During a 12-month surveillance period, a total of 171 *S. aureus* isolates were collected from various clinical specimens, including blood, sputum, mucocutaneous, urine, and other body fluids. Of these, 125 non-duplicate isolates from blood cultures were confirmed as cases of SAB and included for further analysis. Phenotypic classification using cefoxitin screening identified 60 isolates as MRSA and 65 as MSSA. In contrast, genotypic analysis detected the *mecA* gene in 65 isolates, while 60 isolates were negative. Five isolates showing discordant results (phenotypically MSSA but *mecA*-positive) were excluded to ensure consistency between phenotypic and genotypic classification. After exclusion, a total of 120 isolates with concordant results were included, comprising 60 MRSA and 60 MSSA, and were subsequently subjected to *spa* typing and sequencing.

The isolates included in this study were obtained from hospitalized patients within a tertiary healthcare facility. Most patients were admitted to non-intensive care units (n=95, 79.1%), while a smaller proportion were admitted to intensive care units. Regarding diagnoses, 72.5% of patients (n=87) had non-surgical conditions. The final part of our study revealed that the overall in-hospital mortality rate among the study population was 28.3% (n=34 patients). This data was observed throughout the hospitalization period.

Spa type analysis

A total of 114 samples underwent successful *spa* typing analysis, consisting of 60 MRSA and 54 MSSA isolates, while 6 MSSA samples were categorized as unknown. The most frequently identified *spa* gene type was t852 (12.5%), followed by t5422 (9.2%) and t4171 (7.5%). *Spa* type t4171 was the predominant *spa* type among MSSA isolates, which accounted for nine isolates, followed by t1171 and t159, each of which was borne by five isolates. In MRSA isolates, *spa* type was more general, grouping into four prevalent *spa* types, such as t852 (15 isolates), t5422 (11 isolates), t657 (5 isolates), and t315 (5 isolates). Two novel *spa* gene types were detected in MRSA bacteria, t21423 and t21425, represented by 1 and 2 samples, respectively. Among the MSSA isolates, three novel *spa* gene types were identified: t21449, t21450, and t21456, with each type represented by 1 sample.

Spa type classification

Each node represents a unique *spa* type, with node size corresponding to the number of isolates sharing that type in a minimum *spa*nning tree (MST) (Figure 1). The lines connecting nodes indicate genetic similarity, with shorter distances reflecting closer relationships in their repeat sequences. This analysis demonstrates the genetic distribution and clustering of the *spa* types within the study isolates. Fifteen clusters and several singletons were identified (10 *spa* types) in the study (Figure 1). Cluster 1 is the largest, comprising the largest *spa*-type groups, including t852 (15 isolates) and t5422 (11 (isolates). Other clusters, such as Cluster 11-15, have fewer *spa* types. Several *spa* types did not belong to significant clusters and were categorized as singletons, including t019, t11016, t13046, t133, t21423, t258, t3632, t4723, t5729, and t878. These were defined as singletons due to the absence of significant genetic similarity with the dominant *spa* types commonly found in the study location.

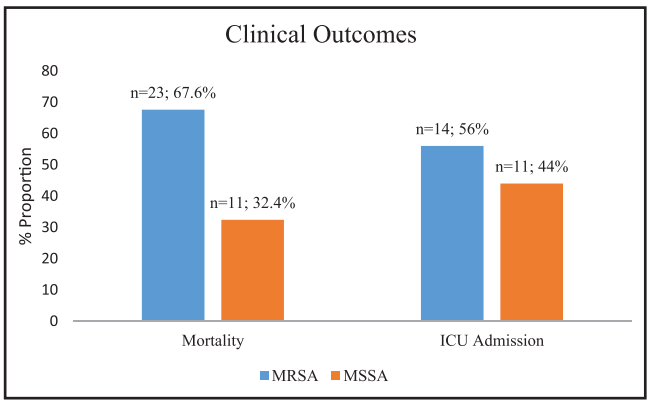

Figure 1. Comparison of clinical outcomes, including in-hospital mortality and ICU admission, between MRSA and MSSA isolates.

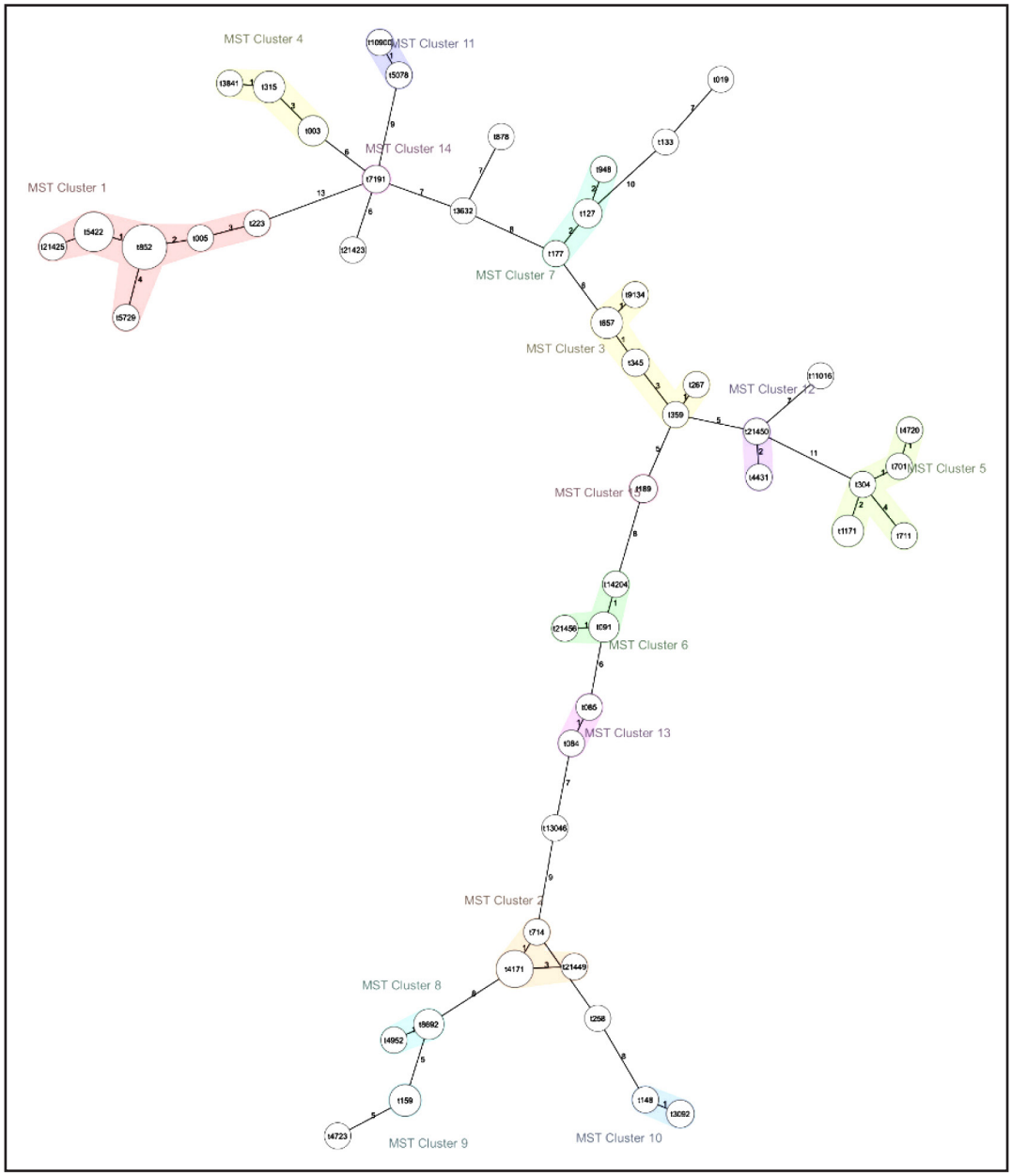

Figure 2. Minimum *spa*nning tree (MST) of Staphylococcus aureus *spa* types isolated from bacteremia cases. Each node represents a distinct *spa* type, with node size proportional to the number of isolates. Node colors indicate methicillin resistance status (red: MRSA; blue: MSSA). Edges reflect genetic relatedness based on *spa* repeat patterns, with shorter distances indicating closer relationships. Distinct clusters represent *spa* clonal complexes. Notably, Cluster 1 is dominated by MRSA isolates, suggesting a potential clonal transmission within the hospital setting.

Table 1. *Spa* clonal complex (*spa* CC) and resistance status

Spa CC	MSSA n (%)	MRSA n (%)	Mortality n (%)	ICU admission n (%)	Total
Cluster 1	0 (0.0)	30 (50.5)	10 (29.4)	8 (32.0)	30 (25.0)
Cluster 2	10 (16.7)	0 (0.0)	1 (2.9)	2 (8.0)	10 (8.3)
Cluster 3	1 (1.7)	9 (15.0)	3 (8.8)	2 (8.0)	10 (8.3)
Cluster 4	3 (5.0)	7 (11.7)	3 (8.8)	0 (0.0)	10 (8.3)
Cluster 5	6 (10.0)	4 (6.7)	5 (14.7)	4 (16.0)	10 (8.3)
Cluster 6	5 (8.3)	1 (1.7)	1 (2.9)	1 (4.0)	6 (5.0)
Cluster 7	5 (8.3)	0 (0.0)	0 (0.0)	1 (4.0)	5 (4.2)
Cluster 8	4 (6.7)	1 (1.7)	0 (0.0)	1 (4.0)	5 (4.2)
Cluster 9	5 (8.3)	0 (0.0)	0 (0.0)	2 (8.0)	5 (4.2)
Cluster 10	3 (5.0)	0 (0.0)	1 (2.9)	1 (4.0)	3 (2.5)
Cluster 11	2 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)
Cluster 12	2 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)
Cluster 13	2 (3.3)	0 (0.0)	1 (2.9)	2 (8.0)	2 (1.7)
Cluster 14	0 (0.0)	2 (3.3)	1 (2.9)	0 (0.0)	2 (1.7)
Cluster 15	2 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)
Singleton	4 (6.7)	6 (10.0)	6 (17.6)	1 (4.0)	10 (5.0)
Unknown	6 (10.0)	0 (0.0)	2 (5.9)	0 (0.0)	6 (5.0)

***Spa* clonal complex and outcomes**

In Table 1, the data demonstrate the distribution of *S. aureus* strains across *spa* clusters, divided into MSSA and MRSA. Clusters 2, 7, 9, 10, 11, 12, 13, and 15 consist entirely of MSSA strains (100.0%), while clusters 1 and 14 consist entirely of MRSA strains (100.0%). Clusters 5, 6, and 8 comprise both MSSA and MRSA strains, with varying proportions of each. Clusters 3 and 4 have a higher proportion of MRSA strains (90.0% and 70.0%, respectively) than MSSA (10.0% and 30.0%). Conversely, clusters 5, 6, and 8 have a higher proportion of MSSA strains (60.0%, 83.3%, and 80.0%, respectively) than MRSA. This data demonstrates the variable distribution of MSSA and MRSA across different *spa* clusters.

In-hospital mortality among SAB patients was stratified based on *spa* clonal complex or cluster (Table 1). Clusters 1 and 3 contributed to more than 30% of the mortality in the study, while fewer mortality cases were demonstrated among clusters 2, 6, 10, 13, and 14, each presenting one case of mortality. Nevertheless, clusters 7, 8, 9, 11, 12, and 15 showed no mortality. Cluster 1 had the highest mortality rates compared to the other clusters, while predominantly MSSA clusters demonstrated better outcomes (Clusters 2, 6, 7, 8, 9, 11, 12, and 15). MRSA isolates were concentrated in some Clusters, focusing on Clusters 1 and 3, while other clusters shared similarities with their MSSA counterparts (Clusters 4, 5, 6, and 8). Further analysis demonstrated that resistance status and mortality were significantly associated (p-value 0.015, OR 2.769, 95% CI 1.201-6.387).

The last outcome was ICU admission rates, stratified based on *spa*-type clusters. Clusters 4, 11, 12, 14, and 15 demonstrated no ICU admissions. Meanwhile, clusters 2, 3, 6, 7, 8, 9, 10, 13, and the singleton have low ICU admission rates, ranging from 4-8%. In contrast, clusters 1 and 5 contributed to a significant number of ICU patients. Cluster 13 has the highest ICU admission rate, with 100.0% of patients requiring intensive care. High rates of ICU admission were dominated by the MSSA clusters (Clusters 5, 9, 10, and 13), with varying reports of ICU admission on dispersed clusters for no ICU admission (Clusters 4 and 14 were predominantly MRSA clusters; Clusters 11, 12, and 15 were MSSA-only clusters). Bivariate analysis did not show any significant relationship between resistance status and ICU admission (p-value 0.5, OR 0.738, 95% CI 0.304-1.790).

DISCUSSION

Based on prospective surveillance of SAB, this study demonstrates that MRSA isolates were predominantly concentrated in *spa* Cluster 1, which was associated with higher mortality (>30%) and ICU admission rates. In contrast, MSSA isolates were dispersed across multiple clusters and displayed greater genetic diversity, with lower associated mortality. Cluster 1 exclusively comprised MRSA isolates and accounted for 29.4% of all deaths, underscoring its clinical importance. This cluster also harboured the most frequent *spa* types, particularly t852 and t5422. These findings suggest clonal expansion of MRSA strains within the hospital environment, in line with previous evidence of clonal dominance from large-scale WGS studies of healthcare-associated MRSA (Zhou *et al.*, 2025).

Staphylococcal protein A is a crucial virulence determinant of *S. aureus*, playing a significant role in immune evasion and persistence (Gordon & Lowy, 2008; Pidwill *et al.*, 2021). Genotyping the tandemly repeated DNA sequence in the variable X region of the *spa* gene can help stratify *S. aureus* isolates, particularly in the context of local epidemiology. While this region produces short sequence repeats that are generally stable, genomic rearrangements via replication slippage or the repair of double-strand breaks (DSBs) can generate variability. These mechanisms regulate the contraction and expansion of *spa* types observed in various contexts, which is useful in identifying nosocomial transmission patterns and emerging clones (Verstrepen *et al.*, 2005).

Global comparisons demonstrate geographic variation in *spa* type distribution. A comprehensive review identified t032, t008, and t002 as the dominant *spa* types in Europe, while t037 and t002 were predominant in Asia. In contrast, our study revealed unique findings, with t852, t5422, and t417 emerging as the dominant *spa* types in the healthcare facility. *spa* types t032 and t037 predominated in global surveillance, while being absent or less significant in our study. Our study identified *spa* type t084, typically associated with MRSA (Fasihi *et al.*, 2017; Hadyeh *et al.*, 2019; Jogenfors *et al.*, 2024), in MSSA isolates, suggesting that MSSA strains may serve as precursors capable of acquiring the *mecA* gene via SCCmec elements (Wielders *et al.*, 2002). Conversely, instability of SCCmec

through cassette recombinases may also generate MSSA strains from MRSA lineages. Cassette-specific recombinases, such as ccrA and ccrB, as well as the activity of various internal mobile genetic elements, drive this instability (Scharn *et al.*, 2022). Such dynamics, shaped by antibiotic pressure, infection control policies, and clonal expansion, highlight the complex evolutionary pathways of *S. aureus* in healthcare settings (Cheng *et al.*, 2011; Grundmann *et al.*, 2014; McVicker *et al.*, 2014).

This study identifies several novel *spa* types and a broad singleton (n = 16 isolates). Novel *spa* type includes t21423 and t21425 in MRSA isolates; meanwhile, t21449, t21450, and t21456 are among the MSSA isolates. *Spa* type t21423 (35-17-34-02-17-17-16), which circulated in our study, has an almost identical repeat pattern to t4430 (35-17-34-17-17-16) and t5829 (35-17-34-16) in 2009 Swedish isolates (Petersen *et al.*, 2021). Another phenomenon captured by the study was the presence of an unidentified *spa* type in six MSSA isolates. This could be associated with several mutations that could not be stratified based on the Ridom database. The presence of untypeable MSSA isolates further supports the need for WGS in future studies to characterise emerging lineages.

The predominance of MRSA in specific clusters, coupled with its association with worse clinical outcomes, strongly suggests ongoing clonal transmission within the tertiary hospital environment. The inclusion of SAB patients from tertiary referral hospitals with varying sources of infection may also indicate broader patterns of MRSA dissemination in this setting. In a study conducted in Malaysia, a few dominant *spa* types (t032 and t304) emerged as the most prevalent *spa* types associated with MRSA infections; these shifting trends are suspected of contributing to clonal expansion but require further investigation using a larger sample size (Jones *et al.*, 2021). Antibiotic exposure is critical in driving MRSA transmission, as evidenced by increased relative risk (RR) values associated with specific antibiotics: 3.0 for quinolones, 2.9 for glycopeptides, and 2.2 for cephalosporins (Tacconelli *et al.*, 2008). The emergence of MRSA in this study is hypothesized to be driven by several contributing factors, with irrational antibiotic use exerting significant selective pressure. This pressure facilitates the survival and proliferation of MRSA strains within their hosts, leading to clonal expansion (Kennedy *et al.*, 2008; McVicker *et al.*, 2014; Bal *et al.*, 2016). In *spa* typing analysis, this dynamic is reflected in the dominance of MRSA isolates associated with Cluster 1. Within this context, *spa* typing provides a practical and cost-effective tool for monitoring transmission in resource-limited settings where advanced molecular typing methods such as MLST or WGS may not be readily available.

The clustering of MRSA in specific *spa* types and associated adverse outcomes highlights the need for effective infection control strategies. As the study was conducted in a tertiary referral hospital, these findings underscore the importance of molecular surveillance in monitoring the spread of dominant MRSA strains in high-risk environments. This study also provides molecular and clinical insights into *S. aureus* infections within such a setting. A limitation of this study is the absence of advanced typing techniques, such as MLST and WGS, which could have offered a deeper understanding of the genetic background and evolutionary dynamics of MRSA strains. Nonetheless, *spa* typing was sufficient to identify clonal relationships and dominant MRSA clusters.

CONCLUSIONS

This prospective surveillance study demonstrates that MRSA isolates in SAB were predominantly clustered within a single clonal complex (Cluster 1), indicating potential clonal transmission within the tertiary care setting. MRSA infection was significantly associated with higher in-hospital mortality compared to MSSA, while no significant association was observed with ICU admission. In contrast, MSSA isolates exhibited greater genetic diversity, being distributed across multiple *spa* clonal complexes. The presence of MRSA-associated *spa* types among MSSA isolates further suggests possible evolutionary exchange between susceptible and resistant lineages. These findings support the utility of *spa* typing as a practical molecular tool for identifying transmission patterns and guiding infection control.

REFERENCES

Amelia, S., Wahyuni, D.D., Yunita, R. & Rozi, M.F. (2021). The active surveillance of Staphylococcus aureus using polymerase chain reaction-based identification method among hospitalized-patient of Haji Adam Malik General Hospital, Medan, Indonesia. *Open Access Macedonian Journal of Medical Sciences* **9**: 622-625. https://doi.org/10.3889/oamjms.2021.6646

Asadollahi, P., Farahani, N.N., Mirzaii, M., Khoramrooz, S.S., van Belkum, A., Asadollahi, K., Dadashi, M. & Darban-Sarokhalil, D. (2018). Distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and-susceptible Staphylococcus aureus around the world: a review. *Frontiers in Microbiology* **9**: 163. https://doi.org/10.3389/fmicb.2018.00163

Bal, A., Coombs, G., Holden, M., Lindsay, J., Nimmo, G., Tattevin, P. & Skov, R. (2016). Genomic insights into the emergence and spread of international clones of healthcare-, community-and livestock-associated meticillin-resistant Staphylococcus aureus: blurring of the traditional definitions. *Journal of Global Antimicrobial Resistance* **6**: 95-101. https://doi.org/10.1016/j.jgar.2016.04.004

Bear, A., Locke, T., Rowland-Jones, S., Pecetta, S., Bagnoli, F. & Darton, T.C. (2023). The immune evasion roles of Staphylococcus aureus protein A and impact on vaccine development. *Frontiers in Cellular and Infection Microbiology* **13**: 1242702. https://doi.org/10.3389/fcimb.2023.1242702

Chadi, Z.D., Dib, L., Zeroual, F. & Benakhla, A. (2022). Usefulness of molecular typing methods for epidemiological and evolutionary studies of Staphylococcus aureus isolated from bovine intramammary infections. *Saudi Journal of Biological Sciences* **29**: 103338. https://doi.org/10.1016/j.sjbs.2022.103338

Cheng, V., Chan, J., Lau, E., Yam, W., Ho, S., Yau, M., Tse, E., Wong, A., Tai, J., Fan, S. *et al.* (2011). Studying the transmission dynamics of meticillin-resistant Staphylococcus aureus in Hong Kong using *spa* typing. *Journal of Hospital Infection* **79**: 206-210. https://doi.org/10.1016/j.jhin.2011.03.024

Fasihi, Y., Kiaei, S. & Kalantar-Neyestanaki, D. (2017). Characterization of SCC mec and *spa* types of methicillin-resistant Staphylococcus aureus isolates from health-care and community-acquired infections in Kerman, Iran. *Journal of Epidemiology and Global Health* **7**: 263-267. https://doi.org/10.1016/j.jhin.2011.03.024

Gordon, R.J. & Lowy, F.D. (2008). Pathogenesis of methicillin-resistant Staphylococcus aureus infection. *Clinical Infectious Diseases* **46**: S350-S359. https://doi.org/10.1086/533591

Grundmann, H., Schouls, L.M., Aanensen, D.M., Pluister, G.N., Tami, A., Chlebowicz, M., Glasner, C., Sabat, A.J., Weist, K., Heuer, O. *et al*. (2014). The dynamic changes of dominant clones of Staphylococcus aureus causing bloodstream infections in the European region: results of a second structured survey. *Eurosurveillance* **19**: 20987. https://doi.org/10.2807/1560-7917.ES2014.19.49.20987

Hadyeh, E., Azmi, K., Seir, R.A., Abdellatief, I. & Abdeen, Z. (2019). Molecular characterization of methicillin-resistant Staphylococcus aureus in west bank-palestine. *Frontiers in Public Health* **7**: 130. https://doi.org/10.3389/fpubh.2019.00130

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D. & Vogel, U. (2003). Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for *spa* repeat determination and database management. *Journal of Clinical Microbiology* **41**: 5442-5448. https://doi.org/10.1128/jcm.41.12.5442-5448.2003

Hassoun, A., Linden, P.K. & Friedman, B. (2017). Incidence, prevalence, and management of MRSA bacteremia across patient populations – a review of recent developments in MRSA management and treatment. *Critical Care* **21**: 1-10. https://doi.org/10.1186/s13054-017-1801-3

Humphreys, H., Grundmann, H., Skov, R., Lucet, J.-C. & Cauda, R. (2009). Prevention and control of methicillin-resistant Staphylococcus aureus. *Clinical Microbiology and Infection* **15**: 120-124. https://doi.org/10.1111/j.1469-0691.2009.02699.x

Jogenfors, A., Mernelius, S., Löfgren, S., Bengnér, M., Bengnér, J., Ingemansson, F., Wetterbrandt, S. & Matussek, A. (2024). Substitution of the Empirical Antibiotic Regimen Eliminated an Outbreak of Tobramycin-Resistant Staphylococcus aureus of *spa* type t084 in a Swedish Neonatal Intensive Care Unit – We recommend an Early Warning System for Antibiotic Resistance. https://doi.org/10.21203/rs.3.rs-4693322/v1

Jones, S.U., Chua, K.H., Chew, C.H., Yeo, C.C., Abdullah, F.H., Othman, N., Kee, B.P. & Puah, S.M. (2021). *spa* diversity of methicillin-resistant and-susceptible Staphylococcus aureus in clinical strains from Malaysia: a high prevalence of invasive European *spa*-type t032. *PeerJ* **9**: e11195. https://doi.org/10.7717/peerj.11195

Kennedy, A.D., Otto, M., Braughton, K.R., Whitney, A.R., Chen, L., Mathema, B., Mediavilla, J.R., Byrne, K.A., Parkins, L.D., Tenover, F.C. *et al.* (2008). Epidemic community-associated methicillin-resistant Staphylococcus aureus: recent clonal expansion and diversification. *Proceedings of the National Academy of Sciences* **105**: 1327-1332. https://doi.org/10.1073/pnas.0710217105

Kumar, S., Anwer, R., Yadav, M., Sehrawat, N., Singh, M. & Kumar, V. (2021). Molecular typing and global epidemiology of Staphylococcus aureus. *Current Pharmacology Reports* **7**: 179-186. https://doi.org/10.1007/s40495-021-00264-7

Lee, B.Y., Singh, A., David, M.Z., Bartsch, S.M., Slayton, R.B., Huang, S.S., Zimmer, S.M., Potter, M.A., Macal, C.M., Lauderdale, D.S. *et al.* (2013). The economic burden of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). *Clinical Microbiology and Infection* **19**: 528-536. https://doi.org/10.1111/j.1469-0691.2012.03914.x

Loftus, M.J., Young-Sharma, T.E., Wati, S., Badoordeen, G.Z., Blakeway, L.V., Byers, S.M., Cheng, A.C., Jenney, A.W., Naidu, R., Prasad, A. *et al.* (2022). Epidemiology, antimicrobial resistance, and outcomes of Staphylococcus aureus bacteraemia in a tertiary hospital in Fiji: A prospective cohort study. *The Lancet Regional Health–Western Pacific* **22**: 100438. https://doi.org/10.1016/j.lanwpc.2022.100438

McVicker, G., Prajsnar, T.K., Williams, A., Wagner, N.L., Boots, M., Renshaw, S.A. & Foster, S.J. (2014). Clonal expansion during Staphylococcus aureus infection dynamics reveals the effect of antibiotic intervention. *PLoS Pathogens* **10**: e1003959. https://doi.org/10.1371/journal.ppat.1003959

Mollerup, S., Worning, P., Petersen, A. & Bartels, M.D. (2022). *Spa* typing of methicillin-resistant Staphylococcus aureus based on whole-genome sequencing: the impact of the assembler. *Microbiology Spectrum* **10**: e02189-22. https://doi.org/10.1128/spectrum.02189-22

Özdemir, F. (2022). Antimicrobial Resistance, multilocus sequence, and *spa* typing of Staphylococcus aureus isolated from retail raw meat products. *BioMed Research International* **2022**: 6035987. https://doi.org/10.1155/2022/6035987

Petersen, A., Larssen, K.W., Gran, F.W., Enger, H., Hæggman, S., Mäkitalo, B., Haraldsson, G., Lindholm, L., Vuopio, J., Henius, A.E. *et al.* (2021). Increasing incidences and clonal diversity of methicillin-resistant Staphylococcus aureus in the Nordic countries: results from the Nordic MRSA surveillance. *Frontiers in Microbiology* **12**: 668900. https://doi.org/10.3389/fmicb.2021.668900

Pidwill, G.R., Gibson, J.F., Cole, J., Renshaw, S.A. & Foster, S.J. (2021). The role of macrophages in Staphylococcus aureus infection. *Frontiers in Immunology* **11**: 620339. https://doi.org/10.3389/fimmu.2020.620339

Ruppitsch, W. (2016). Molecular typing of bacteria for epidemiological surveillance and outbreak investigation/Molekulare Typisierung von Bakterien für die epidemiologische Überwachung und Ausbruchsabklärung. *Die Bodenkultur: Journal of Land Management, Food and Environment* **67**: 199-224. https://doi.org/10.1515/boku-2016-0017

Ruppitsch, W., Indra, A., Stöger, A., Mayer, B., Stadlbauer, S., Wewalka, G.N. *et al.* (2006). Classifying *spa* types in complexes improves interpretation of typing results for methicillin-resistant Staphylococcus aureus. *Journal of Clinical Microbiology* **44**: 2442-2448. https://doi.org/10.1128/JCM.00113-06

Salge, T.O., Vera, A., Antons, D. & Cimiotti, J.P. (2017). Fighting MRSA infections in hospital care: how organizational factors matter. *Health Services Research* **52**: 959-983. https://doi.org/10.1111/1475-6773.12521

Scharn, C., Tickler, I., Tenover, F. & Goering, R. (2022). Characterization of SCC mec instability in methicillin-resistant Staphylococcus aureus affecting adjacent chromosomal regions, including the gene for Staphylococcal Protein A (*spa*). *Antimicrobial Agents and Chemotherapy* **66**: e02374-21. https://doi.org/10.1128/aac.02374-21

Shlaes, D.M. & Bradford, P.A. (2018). Antibiotics – from there to where?: how the antibiotic miracle is threatened by resistance and a broken market and what we can do about it. *Pathogens & Immunity* **3**: 19. https://doi.org/10.20411/pai.v3i1.231

Strommenger, B., Braulke, C., Heuck, D., Schmidt, C., Pasemann, B., Nubel, U. & Witte, W. (2008). *spa* typing of Staphylococcus aureus as a frontline tool in epidemiological typing. *Journal of Clinical Microbiology* **46**: 574-581. https://doi.org/10.1128/jcm.01599-07

Tacconelli, E., De Angelis, G., Cataldo, M.A., Pozzi, E. & Cauda, R. (2008). Does antibiotic exposure increase the risk of methicillin-resistant Staphylococcus aureus (MRSA) isolation? A systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy* **61**: 26-38. https://doi.org/10.1093/jac/dkm416

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L. & Fowler Jr, V.G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews* **28**: 603-661. https://doi.org/10.1128/cmr.00134-14

Verstrepen, K.J., Jansen, A., Lewitter, F. & Fink, G.R. (2005). Intragenic tandem repeats generate functional variability. *Nature Genetics* **37**: 986-990. https://doi.org/10.1038/ng1618

Wielders, C., Fluit, A., Brisse, S., Verhoef, J. & Schmitz, F. (2002). *mecA* gene is widely disseminated in Staphylococcus aureus population. *Journal of Clinical Microbiology* **40**: 3970-3975. https://doi.org/10.1128/jcm.40.11.3970-3975.2002

Zhou, W., Jin, Y., Chen, P., Ge, Q., Dong, X., Chen, Y., Jiang, M. & Xiao, Y. *et al.* (2025). Reshaping the battlefield: A decade of clonal wars among Staphylococcus aureus in China. *Drug Resistance Updates* **78**: 101178. https://doi.org/10.1016/j.drup.2024.101178