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A Molecular characterization and virulence profiles of Staphylococcus aureus bloodstream infection and their associations with clinical prognosis: a comparative analysis of MSSA/MRSA and predominant ST188/ST398

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Research Article

Keywords: MRSA, Biofilm-forming, ST188, ST398, Bloodstream infection

Posted Date: November 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10797440/v1

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Additional Declarations: No competing interests reported.

Abstract

Objective

The study explores correlations of molecular epidemiology and virulence with *Staphylococcus aureus* bloodstream infection (SA-BSI), informing pathogenesis and clinical risk management.

Methods

A retrospective cohort study enrolled 81 patients diagnosed with SA-BSI admitted to a tertiary hospital in Ningbo from January 2021 to December 2025. Whole-genome sequencing, antimicrobial susceptibility testing, semi-quantitative biofilm assay, and in vitro hemolytic activity test were performed on all clinical isolates.

Results

Multilocus sequence typing (MLST) showed that ST188 (14 isolates) and ST398 (10 isolates) were the predominant clones. All ST188 isolates belonged to methicillin-susceptible *Staphylococcus aureus* (MSSA), while 50% of the ST398 isolates were methicillin-resistant *Staphylococcus aureus* (MRSA), showing a statistically significant difference in the MRSA proportion between the two groups. The multivariate analysis revealed that MRSA was an independent risk factor for poor prognosis in patients with SA-BSI. In vitro virulence gene profiling showed that the ST188 isolates possessed markedly higher biofilm-forming ability but lower hemolytic activity than the ST398 isolates; when stratified by MRSA/MSSA, no statistically significant difference was noted in biofilm-forming ability and hemolytic activity between the two groups. The drug resistance gene analysis showed that the resistance gene *mecA* was a key molecular marker for distinguishing MSSA from MRSA. IV and V were the main SCCmec elements. The virulence factor analysis revealed that MRSA isolates often harbored significant deletions of genes encoding the extracellular serine protease and exotoxin genes, such as splABCDEF, set17, set25, set34, lukD, and lukE. Compared with ST188 isolates, most of the ST398 isolates also showed deletions of the gene cluster splABCDEF that encoded the extracellular serine protease. In addition, the exotoxin gene clusters significantly differed between the two STs: ST398 isolates mainly carried the exotoxin genes set1 and set3-set6, while ST188 isolates predominately carried set16-set19, set21-set26, set34, as well as genes such as lukD and lukE. All MRSA isolates within the ST398 clonal lineage belonged to cgST24977.

Conclusion

MRSA is an independent influential factor for unfavorable prognosis in patients with SA-BSI. ST188 and ST398 exhibit differentiated virulence strategies, which are significant for clinical anti-infective treatment

decisions and the development of hosital infection control strategies.

Introduction

Staphylococcus aureus is a ubiquitous Gram-positive bacterium that colonizes the human body as a commensal bacterium and a major pathogenic bacterium inducing a wide array of disorders spanning skin and soft tissue infections to life-threatening conditions, such as bacteremia and pneumonia [1]. Even with advanced therapeutic and diagnostic strategies, the 90-day mortality of patients with *Staphylococcus aureus* bacteremia (SAB) remains as high as 27% [2].

SAB is typically categorized into three categories based on the site of infection acquisition: community-acquired, healthcare-associated, and hospital-acquired. The consensus of earlier investigations was that SAB infections in hospitalized patients are mainly hospital-acquired. However, accumulating studies in recent years have verified that community-acquired infections account for a considerable proportion even within the hospitalized populations. A research report shows that community-acquired SAB infections occupy 71% of all cases. Community-acquired methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) display prominent heterogeneity in epidemiological characteristics, resistance profiles, and clinical prognosis [3]. Surveillance data issued from more than 200 medical center across 45 counters further demonstrate an evolving epidemiological trend of community-acquired MRSA bloodstream infections (BSI) [4]. Genomic evidence additionally reveals that a large proportion of MRSA outbreaks in hospitals may actually stem from community transmission [5].

Mechanistically, the drug resistance and persistent infective capacity of *Staphylococcus aureus* are predominantly attributed to its ability to form biofilms [6]. Biofilms not only effectively resist antibiotics and host immunity, but also build a defensive barrier to support bacterial survival and subsequent dissemination. In addition, α-hemolysin (Hla/α-toxin) produced by *Staphylococcus aureus* is a pore-forming toxin that also plays a key role in the pathogenicity of *Staphylococcus aureus* [7, 8]. MRSA and MSSA differ significantly in virulence gene profiles, biofilm formation, and hemolytic activity, which are tightly associated with their carriage of toxin genes (such as Panton–Valentine leukocidin [PVL]) [9] and regulatory networks [10]. However, it remains to be elucidated whether these discrepancies are independent of the genetic lineage background of the strains and whether the correlation between drug resistance and virulence phenotype is conserved across various clones. An in-depth comparison of phenotypic and genomic characteristics among strains with distinct resistance statuses and different genetic lineages is of great significance for elucidating the pathogenic mechanisms of SAB from different sources and guiding clinical experience and data.

Multilocus sequence typing (MLST) represents a typing technique relying on differences in multiple housekeeping gene sequences, which is suitable for tracing long-term epidemiological trends and dissecting population genetic structure. However, when the research issue shifts from population-level epidemiological dynamics to identification of homologous transmission events or recent evolutionary

relatedness among strains, the discriminative ability of the seven housekeeping gene loci may be insufficient to support precise strain identification. The core genome MLST (cgMLST) typing method can provide high-resolution identification by profiling the sequence variations in hundreds to thousands of core genes and is suitable for analyzing genetic differentiation disparities between strains belonging to an identical clone [11]. By comparing the resistance characteristics and virulence gene profiles, cgMLST can help reveal the genomic differences between resistant and non-resistant strains belonging to an identical clone, providing a more refined insight into understanding the genetic association between resistance and virulence.

At present, despite extensive studies on the clinical and molecular characteristics of *Staphylococcus aureus* BSI, there remains a paucity of systematic evidence regarding differences in virulence phenotypes and genomics between MSSA and MRSA isolates from community-acquired *Staphylococcus aureus* BSI. Notably, with the ongoing infiltration of community-acquired MRSA clones into hospitals, traditional single-marker binary methods, such as *Staphylococcal* cassette chromosome mec (SCCmec) typing, are facing challenges [12]. In this article, we retrospectively analyzed the clinical characteristics of *Staphylococcus aureus* BSI in hospitalized patients at a tertiary comprehensive hospital from 2021 to 2025. The isolates were sequenced using the Illumina NovaSeq platform and subjected to multidimensional genomic analyses, including sequence type (ST) identification, SCCmec typing, virulence gene profiling, resistance genomic mapping, and high-resolution phylogenetic analysis. Accordingly, we intended to systematically characterize the genetic profiles, virulence phenotypes, and prognostic associations of *Staphylococcus aureus* bloodstream isolates. Regional epidemiological surveillance helps to uncover region-specific epidemic patterns overlooked by global population data, laying a foundation for precise prevention and control.

Methods

Samples and bacterial strains

From January 2021 to December 2025, we enrolled 81 patients with a first SA-BSI and new cinical data from The Affiliated Lihuili Hospital of Ningbo University in China were collectd. *Staphylococcus aureus* ATC29213 and ATCC 25923 were used as quality control strains for microbial identification, antimicrobial susceptibility testing and semi-quantitative biofilm assays.

Main instruments and reagents

Columbia blood agar plates were procured from Autobio Co., Ltd. (Zhengzhou, China). The AST-GP67 antimicrobial susceptibility test cards were sourced from bioMérieux (France). Biological safety cabinets were supplied by Haier Biomedical Co., Ltd. (Qingdao, China), and constant-temperature incubators were acquired from Shanghai Yiheng Scientific Instruments Co., Ltd. (Shanghai, China). The mass spectrometer for microbial species identification was sourced from bioMérieux. The Covaris ultrasonic disruptor was purchased from Covaris (Woburn, MA, USA). The Agilent 5400 Bioanalyzer System was

obtained from Agilent Technologies (Santa Clara, CA, USA). Illumina NovaSeq X Plus platform (paired-end reads of 150 bp, PE150) was provided by Illumina (San Diego, CA, USA)

Bacterial resuscitation, identification, and antimicrobial susceptibility testing

A total of 81 clinical *Staphylococcus aureus* isolates preserved at an ultra-low temperature freezer (-80°C) were resuscitated. Single colonies were isolated using the three-region streaking method on Columbia blood agar plates and incubated at 35°C for 16–20 hours in a constant-temperature incubator. The single colonies with typical characteristics were selected for purification and passage to acquire genetically homogeneous pure cultures. Species identification was performed using a VITEK MS mass spectrometer. All isolates were subjected to gram-positive susceptibility test on the VITEK 2 COMPACT machine according to the instructions of the AST-GP67 card. The minimum inhibitory concentrations (MICs) of diverse antimicrobial agents, including clindamycin, penicillin G, tigecycline, vancomycin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, oxacillin, and linezolid, were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI). The cefoxitin screening test and clindamycin induction test (D test) were also carried out. The interpretation of drug susceptibility test results strictly followed the 35th edition of CLSI document M100, Performance Standards for Antimicrobial Susceptibility Testing.

Next-generation sequencing (NGS)

DNA from isolates was extracted and purified using the STE method for whole-genome sequencing (WGS). The DNA library was established using Illumina NEBNext Ultra II FS DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) as per the manufacturer's instructions. After that, the Agilent 5400 system was employed to detect the integrity of the library DNA fragments and the size of the inserted fragments. After confirming that the insertion fragment size matched the expected range, the quantitative polymerase chain reaction (qPCR) method was adopted to accurately determine the effective concentration of the library (target: 1.5 nM) for library quality validation. Subsequently, paired-end sequencing was conducted on the Illumina NovaSeq platform, with a read length of 150 base pairs. All the aforementioned operations were completed by Novogene (Beijing, China). The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive [13] in National Genomics Data Center[14], China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA048045) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Bioinformatics analysis

Genome assembly and annotation of *Staphylococcus aureus* isolates were implemented using the online visualization platform for microbial genome analysis (https://cloud.mimazi.net), including SCCmec element identification and MLST typing. Isolates with unassigned STs were submitted to the pubMLST database, and new ST IDs were successfully designated. Fastp v0.23.1 software (minimum read length = 70 nt, minimum quality score = 20) was utilized to perform quality control and statistical analysis on the obtained sequencing data. Genomic K-mer frequency assessment was achieved using Jellyfish

v2.3.1 (a k-mer size of 17). GC-Depth distribution analysis was completed using SAMtools, BWA, and BED tools (default parameters). Further, de novo genome assembly was conducted with the utilization of SPAdes v4.2.0 (a k-mer range of 35–115), with multiple sets of K-mer parameters tested to achieve optimal assembly. With coverage ≥ 80% and sequence identity ≥ 80% as screening thresholds, drug resistance genes were screened among the predicted results from the Comprehensive Antibiotic Resistance Database (CARD) and the ResFinder database. Genes related to stress response and virulence and uncharacterized variant mutations were eliminated from the predicted results of the CARD database, and prediction outputs from the ResFinder database were subsequently supplemented for a comprehensive analysis. In addition, the *blaI* and *blaR1* genes were verified through independent BLAST alignment. With the identical input parameters, virulence genes were retrieved from the VFDB database. SCCmec typing was performed *via* comparative alignment using the *Staphylococcus aureus* SCCmec element identification tool (batch processing; v1.0). The CgMLST typing scheme was publicly available from the PubMLST.org platform. CoreSNP evolutionary tree was constructed using the kSNP3(https://doi.org/10.1093/bioinformatics/btv271) and the maximum likelihood method (ML). The beautification peocess was employed via the iTOL (https://itol.embl.de/).

Semi-quantitative biofilm assay

First, 81 clinical isolates were cultured in Tryptic Soy Broth (TSB) at 37°C for 16 hours on a shaker (220 rpm). The next day, the cultured bacterial suspension was then inoculated into fresh biofilm-inducing medium at a ratio of 1:100 and incubated until the logarithmic growth phase. The bacterial suspension was next diluted to 1×10^7 CFU/mL using fresh biofilm-inducing medium. Then, 200 μL bacterial suspension was added to a sterile 96-well plate and incubated at 37°C for 24 hours. Subsequently, the precipitate was aspirated, and the supernatant was discarded. The harvested precipitate was then gently rinsed once with sterile PBS. After drying, the biofilm was fixed with 99% methanol for 15 minutes, cleaned, and dried. The precipitate was stained with 100 μL 0.1% crystal violet per well for 30 minutes, gently rinsed under running water, dried, and photographed. Finally, 30% acetic acid was added, and the optical density (OD) was detected at 600 nm to assess the crystal violet-stained biofilm. *Staphylococcus aureus* ATCC 25923 served as a positive control, and sterile TSB supplemented with glycerol (TSBG) was used as a negative control. Experiments were run in triplicate.

Hemolytic activity assay

The bacterial culture was cultured in TSB for 24 hours and then centrifuged at 8000 rpm for 2 minutes to collect the supernatant. Then 100 μL bacterial supernatant was mixed with 900 μL PBS containing 3% defibrinated rabbit blood cells, and the mixture was incubated in a water bath at 37°C for 1 hour. Triton X-100 was chosen as the positive control, and 0.9% (w/v) NaCl solution was selected as the negative control. The supernatant was harvested after centrifugation. Ultimately, the OD value was detected at 600 nm, and the hemolysis rate was calculated using the formula: hemolysis rate (%) = $(OD_{isolate} - OD_{negative\ control})/(OD_{positive\ control} - OD_{negative\ control}) \times 100\%$. Experiments were run in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS 25.0 software. The chi-square test and Fisher's exact test were applied for comparation, and a $P < 0.05$ was considered statistically significant. The plots in Figue 2 were generated using the CNSknowall platform (https://cnsknowall.com), a comprehensive web service for data analysis and visualization. To control the false positive risk brought by multiple comparisons, the false discovery rate (FDR) correction was performed online in BioLadder(bioladder.cn)[15]. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

Results

Core genome phylogenetic tree construction

In this research, a core genome phylogenetic analysis was conducted on 81 Staphylococcus aureus BSI isolates collected between January 2021 and May 2025. The phylogenetic tree constructed based on coreSNP is depicted in Fig. 1. The patient samples were sourced from multiple clinical departments, mainly distributed in the Intensive Care Unit (15 isolates, 18.5%) and the Department of Nephrology (15 isolates, 18.5%). These isolates exhibited high genetic diversity and belonged to 27 STs, including 23 known STs and 4 newly discovered STs (ST11287, ST11320, ST11321, ST11322). ST188 and ST398 constituted the predominant clones among all isolates, accounting for 17.3% (14/81) and 12.3% (10/81), respectively (Fig. 1).

Analysis of risk factors for MSSA, MRSA, ST188, and ST398 BSI

This retrospective analysis enrolled 81 individuals between January 2021 and December 2025, consisting of 55 males and 26 females. The median Charlson Comorbidity Index adjusted for age (aCCI) was 5.0, with an interquartile range [IQR] of 3.0–7.0. Among all cases, 57 patients were diagnosed with community-acquired infections and 24 with hospital-acquired infections. Regarding clinical prognosis, 57 patients achieved improvement, whereas 24 patients had poor prognosis, including 3 deaths. The median length of stay was 15.0 days (IQR, 9.5–27.0). Univariate analysis was conducted on the clinical data of MSSA and MRSA BSI groups. The results showed a statistically significant difference in the proportion of community-acquired infections and clinical efficacy between the two groups ($P < 0.05$; Table 1). In addition, we observed a suggestive upward trend in the proportion of MRSA in the ST398 group with a limited sample size (0/14 *vs*. 5/10, $P = 0.006$); the median length of stay (IQR) was high in the ST188 group than in the ST398 group (18 days *vs*. 8 days; $P = 0.009$). However, this difference may be related to the more severe clinical status of patients in the ST398 group with active hospitalization demand. No statistically significant difference was noted between the two groups in terms of aCCI, source of infection (community-acquired or hospital-acquired), and clinical efficacy ($P > 0.05$; Table 2).

Table 1
Univariate analysis of variance for MSSA and MRSA bloodstream infection

Item		**Total**	**MRSA**	**MSSA**	***P***
Sex	Male	55	16	39	0.375
	Female	26	6	20	
aCCI		5.0 (3.0–7.0)	5.0 (3.3-6.0)	5.0 (3.0–7.0)	0.971
Source of Infection	CA	57	18	39	**0.013**
	HA	24	4	20	0.261
Clinical outcomes	Improvement	57	11	46	**0.038**
	Poor prognosis (including death)	24(3)	11(3)	13(0)	
Length of hospital stay		15.0 (9.5–27.0)	14.5 (8.3–25.5)	15.0 (10.0-30.5)	0.873
aCCI: Age-Adjusted Charlson Comorbidity Index, CA: Community-acquired, HA: Hospital-acquire					

Table 2
Univariate analysis of variance for bloodstream infection Caused by *Staphylococcus aureus* ST188 and ST398 Clones

Item		**Total**	**ST188**	**ST398**	***P***
Sex	Male	15	8	7	0.678
	Female	9	6	3	
aCCI		5.2 ± 1.9	5.3 ± 2.2	5.1 ± 1.4	0.802
Source of Infection	CA	16	8	8	0.388
	HA	8	6	2	
Clinical outcomes	Improvement	18	12	6	0.192
	Poor prognosis	6	2	4	
MRSA		5	0	5	**0.006**
Length of hospital stay		14.5(8.0-29.5)	18.0(12.0-33.3)	8(3.8–16.8)	**0.009**

The bold font in the table indicates statistical significance.

In order to explore the determinants of outcomes after infection, a prognostic model was established with clinical efficacy (poor prognosis = 1, good prognosis = 0) as the dependent variable. The results exhibited that MRSA infection (*OR* = 1.430, 95%*CI* 1.390-13.245, *P* = 0.012) was an independent influencing factor for poor prognosis of patients with *Staphylococcus aureus* BSI, as shown in Fig. 2.

ST188 isolates exhibit stronger biofilm-forming ability but lower hemolytic activity than ST398 isolates

In an attempt to dissect the virulence characteristics of the 81 clinical isolates collected in this study, we tested the biofilm-forming ability and hemolytic activity of all isolates. The results of the Mann-Whitney U test showed no statistical difference between MSSA and MRSA isolates (28.7% [3.0% -59.1%] *vs*. 38.7% [2.2% -64.9%], *P* = 0.545 > 0.05). Interestingly, the two predominant subtypes, ST188 and ST398, showed distinct results. We observed significantly stronger biofilm-forming ability of the ST188 isolates biofilm-forming ability than the ST398 isolates (1.423 [0.998–1.677] *vs*. 0.941 [0.747–1.153], *P* = 0.026 < 0.05), while the hemolysis rate of the ST188 isolates was dramatically lower than that of ST398 isolates (12.8% [2.2% -30.5%] *vs*. 65.0% [64.0% -65.8%], *P* < 0.0001)(Fig. 7)

Discussion

Staphylococcus aureus is a common and important pathogen responsible for BSI. Although the mortality of SAB has declined over the past three decades, over 25% of patients still die within 3 months, highlighting an urgent need for continuous optimization of therapeutic strategies [16]. Although MRSA has received much attention, increasing evidence suggests that MSSA isolates also have significant clinical importance and constitute a major contributor to the burden of BSI. For instance, the MSSA ST398 lineage, with an incidence of about 20% in China and France, has high virulence and causes severe BSI, endocarditis, and osteoarticular infections [17]. Moreover, the predominant clonal lineages exhibit significant geographical heterogeneity. For example, the abundance of SA-BSI isolates in Shandong, China, varies over time: ST398 was the dominant ST from 2016 to 2017, while ST59 was the dominant ST from 2018 to 2020 [18]; the incidence of ST398 BSI continues to increase in countries, such as France and Switzerland [19]; ST152-MSSA lineage serves as its characteristic indigenous clone in Nigeria [20]. In this article, a comprehensive investigation of SA-BSI in the Ningbo region over the past 5 years showed that ST188 and ST398 were the main dominant STs, accounting for a total of 29.7%. This distribution pattern differs from the global dominant clones (ST59, ST398, ST239, and ST5) [21, 22]. Therefore, localized surveillance can delineate region-specific epidemic characteristics that are obscured by global data.

SCCmec types IV and V have been the dominant types among MRSA isolates in patients with BSI, highly consistent with multiple epidemiological studies based on clinical isolates in Japan and the United States [23], which documented that SCCmec type IV replaced SCCmec type II as the most dominant type in patients with BSI. HA-MRSA isolates typically harbor SCCmec types I, II, and III but rarely carry *pvl*; CA-

MRSA isolates usually carry SCCmec types IV and V and are frequently *pol*-positive [24]. In this research, although 5 MRSA isolates were identified as hospital-acquired infections from the site of infection, their SCCmec elements were mainly community-related types, accounting for 80%. This finding suggests that MRSA isolates from hospital settings probably originate from persistent input *via* community transmission networks. The dynamic transmission network of MRSA in the community actually drives in-hospital dissemination, which progressively blurs the traditional epidemiological boundary between hospital- and community-acquired MRSA. These evolutionary patterns are of great significance for infection prevention and control strategies, and in the future, it is necessary to integrate community public health interventions with hospital infection prevention and control.

Furthermore, this study revealed a complicated and interesting relationship between virulence profiles and clinical prognosis in patients with SA-BSI through a comprehensive multi-level grouping analysis.

Firstly, the multivariate analysis in this article validated MRSA infection as an independent risk factor for poor prognosis, which is consistent with the findings in the previous research [25]. However, when stratified by the predominant clonal lineages ST188 and ST398, the rate of poor prognosis showed insignificant differences between the two groups (ST188 *vs*. ST398: 14.3% *vs*. 40%). This phenomenon is highly correlated with the distribution of MRSA within both groups, with ST188 isolates all belonging to MSSA and 50% of ST398 isolates belonging to MRSA. Owing to the high correlation between clonal lineage and MRSA status in the isolates of our study, simultaneous inclusion of both variables in the multivariate regression model leads to confounding bias such as collinearity, which precludes the effective differentiation of their independent effects. Therefore, when interpreting the prognostic effects of MRSA, it is necessary to consider the confounding effects caused by uneven distribution of MRSA isolates across different clonal lineages. The numerically higher incidence of poor prognosis in the ST398 group may be largely attributable to the high proportion of MRSA isolates within this clonal lineage.

In vitro phenotype experiments showed that biofilm-forming ability and hemolytic activity were independent of MRSA and MSSA status, but closely related to the genetic lineage (ST) of the isolates. Our study suggested that the ST188 clonal lineage exhibited stronger biofilm-forming ability (1.423 [0.998–1.677] *vs*. 0.941 [0.747–1.153], $P < 0.05$). This phenotypic characteristic may be related to its frequent carriage of the gene cluster encoding extracellular Serine protease, *splABCDEF*. The core function of these extracellular enzymes is to control the infection process by selectively regulating the stability of virulence factors [26]. Cao et al. Conducted a transcriptome analysis and found that upregulation of *splA* and *splF* expression exerts a crucial role in biofilm formation and pathogenicity [27]. The *in vitro* hemolytic activity of *Staphylococcus aureus* is mainly driven by α-hemolysin (Hla) [28]. In the present study, the ST398 clonal lineage exhibited higher hemolytic activity (12.8% [2.2% -30.5%] *vs*. 65.0% [64.0% -65.8%]; $P < 0.0001$), which may be attributed to high α-hemolysin levels. Although the ST188 isolates carry more *LukED*, this toxin gene mainly targets immune cells [29, 30]instead of erythrocytes. Moreover, *LukED*-mediated lysis of erythrocytes relies on Duffy antigen receptor for chemokines (DARC) in the host [31]. However, the *in vitro* hemolysis experimental system used in this

study did not express DARC; therefore, it cannot effectively demonstrate the hemolytic function of *LukED*. There was no statistically significant difference in both phenotypes between MRSA and MSSA groups. These findings suggest that differences in virulence phenotypes are more strongly associated with the genetic lineage of isolates, whereas their association with drug tolerance is relatively weak. The virulence gene profiles varied among different groups, but genotype and phenotype were not fully concordant. The WGS analysis in this study showed differences in virulence genes between MRSA and MSSA isolates, as well as between ST188 and ST398 isolates. This phenomenon suggests that no simple correspondence exists between virulence gene profiles and final phenotypic output, which may be influenced by transcriptional or translational regulation. ST188 and ST398 may represent two distinct virulence evolution strategies. However, further experimental verification is needed to determine whether the molecular mechanisms responsible for these differences are attributed to differences in virulence genes themselves or other genetic factors. Overall, ST188 and ST398 have distinctly divergent virulence evolution strategies. This finding further implies that incorporating both the resistance profiles and clonal lineages of isolates into microbiology reports may help to more accurately assess the prognostic risk of infected patients.

MLST is a typing method based on sequence differences in multiple housekeeping genes, suitable for analyzing long-term epidemiological trends. However, when the problem shifts from the population-level epidemic structure to the identification of homologous transmission events, the discriminative ability of the seven MLST loci may be insufficient to support more accurate identification. To further investigate the genetic heterogeneity within the same clonal lineage, we introduced the cgMLST typing method based on the core genome [11]. The results of cgMLST analysis revealed that MRSA and MSSA isolates could be distinguished at the core genome level within the ST398 clonal lineage, suggesting that these isolates may belong to different sublineages. This finding is consistent with previous studies reporting that MSSA lineages such as ST398 can evolve into corresponding MRSA lineages through the acquisition of SCCmec elements [32]. Of note, ST188 and ST398 clonal lineages in this work were also reported in *Staphylococcus aureus* isolates from different sources (frozen pork) in Hangzhou, China, which were also the dominant types [33]. This cross-source distribution suggests that these clonal lineages may have the potential to disseminate across clinical settings, animal hosts, and food chains. Based on the findings in this study that MRSA and MSSA subgroups within ST398 could be distinguished at the cgMLST level, future research needs to include more isolates from animal, food, and environmental sources for a comparative phylogenetic analysis and further explore whether different subgroups within the ST398 clonal lineage have different ecological niche adaptations or host preferences. From the perspective of One Health, characterizing the distribution patterns of distinct sublineages within the same clonal lineage across different ecological niches is of great value for accurately formulating cross-domain prevention and control strategies against drug-resistant bacteria.

Our research still has some limitations. First, the sample sizes of ST188 and ST398 isolates are limited, making it impossible to conduct multivariate calibration analysis. Second, zero MRSA events in the ST188 group resulted in statistical model instability. Third, the length of stay is influenced by non-clinical factors, such as patient-initiated discharge, making it difficult to distinguish the true effects of different

types. Therefore, the results of this study only suggest that ST398 may be associated with an increased proportion of MRSA, but further validation is needed in studies with larger sample sizes.

In summary, these findings highlight the complex relationship between virulence characteristics, drug resistance, and clinical prognosis in *Staphylococcus aureus* infection. MRSA, as an independent risk factor for poor prognosis, is confounded by clonal-background factors. The two predominant clones ST188 and ST398 show differentiated virulence phenotypes, which are more closely linked to genetic lineage than to antimicrobial-resistance status. However, the inconsistency between virulence gene profiles and phenotypes, as well as the distinguishability of MRSA and MSSA isolates within ST398 at the genomic level, suggest a more complicated genetic association between virulence and drug resistance in *Staphylococcus aureus* isolates, which warrants further elucidation through functional experiments and large-scale studies.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Ningbo Medical Center Lihuili Hospital (Approval #: KY2025SL010-01).

Inform consent

All participants provided written informed consent before recruitment.

Funding

This work was supported by Zhejiang Provincial Natural Science Foundation of China(LKLY25H200006); Ningbo Major Research and Development Plan (2024Z215); Zhejiang Province Medical and Health Planning(2024KY293)

Author Contribution

Y.Z. , F.W. and H.L. designed the study and methodologies. Y.C. and X.W. conducted epidemiological investigation and collected the data. C.H. performed the bioinformatics analysis and figure plot. Y.Z. and Y.C. completed the in vitro experiments. Y.Z., Y.C., C.H., X.W., F.W., and H.L. collected the data and revised manuscript. Y.Z. wrote the original draft with contributions from all the authors.

Acknowledgement

We thank the CNSknowall platform (https://cnsknowall.com) for providing data analysis services.

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Figures

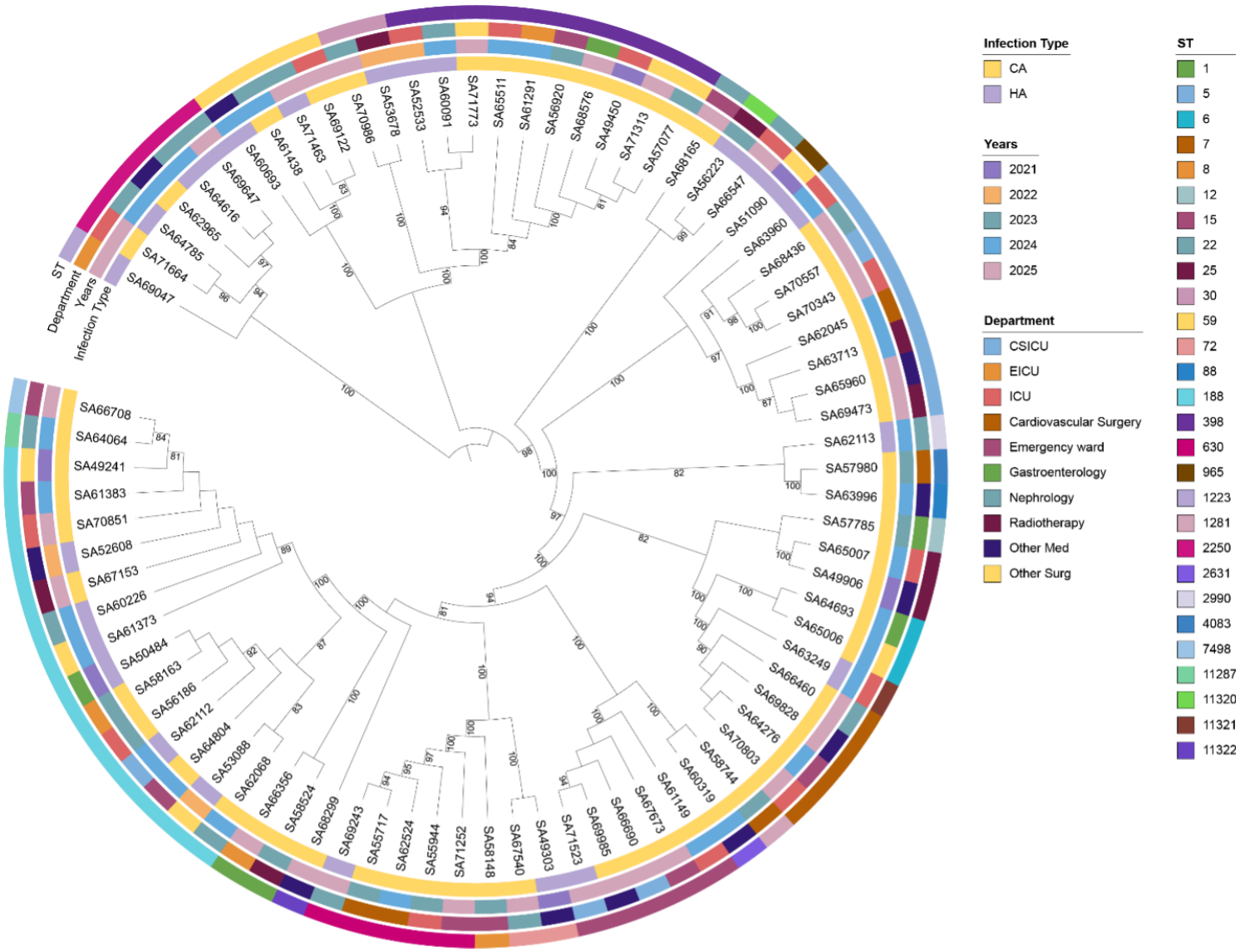


Figure 1

A phylogenetic tree of 81 *Staphylococcus aureus* BSI isolates. The numbers at the center of the phylogenetic tree represent bootstrap values, used to assess the reliability of the tree branches; only results from 70% to 100% are shown in the figure. The outer ring is labeled sequentially from the inside outward with information such as infection type, years, department and ST type (sequence typing) of isolation.

Variables	β	S.E	Z	P	OR(95%CI)
MRSA					
No					1.000(Reference)
Yes	1.430	0.569	2.511	0.012	4.178(1.390~13.245)
Gender					
Female					1.000(Reference)
Male	-0.793	0.561	-1.413	0.158	0.453(0.147~1.359)
CA					
Yes					1.000(Reference)
No	-0.632	0.652	-0.969	0.332	0.531(0.132~1.809)
aCCI	0.159	0.128	1.242	0.214	1.172(0.918~1.525)

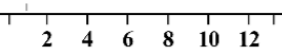


Figure 2

Forest plot of multivariate analysis of factors associated with prognosis following bloodstream Infection.

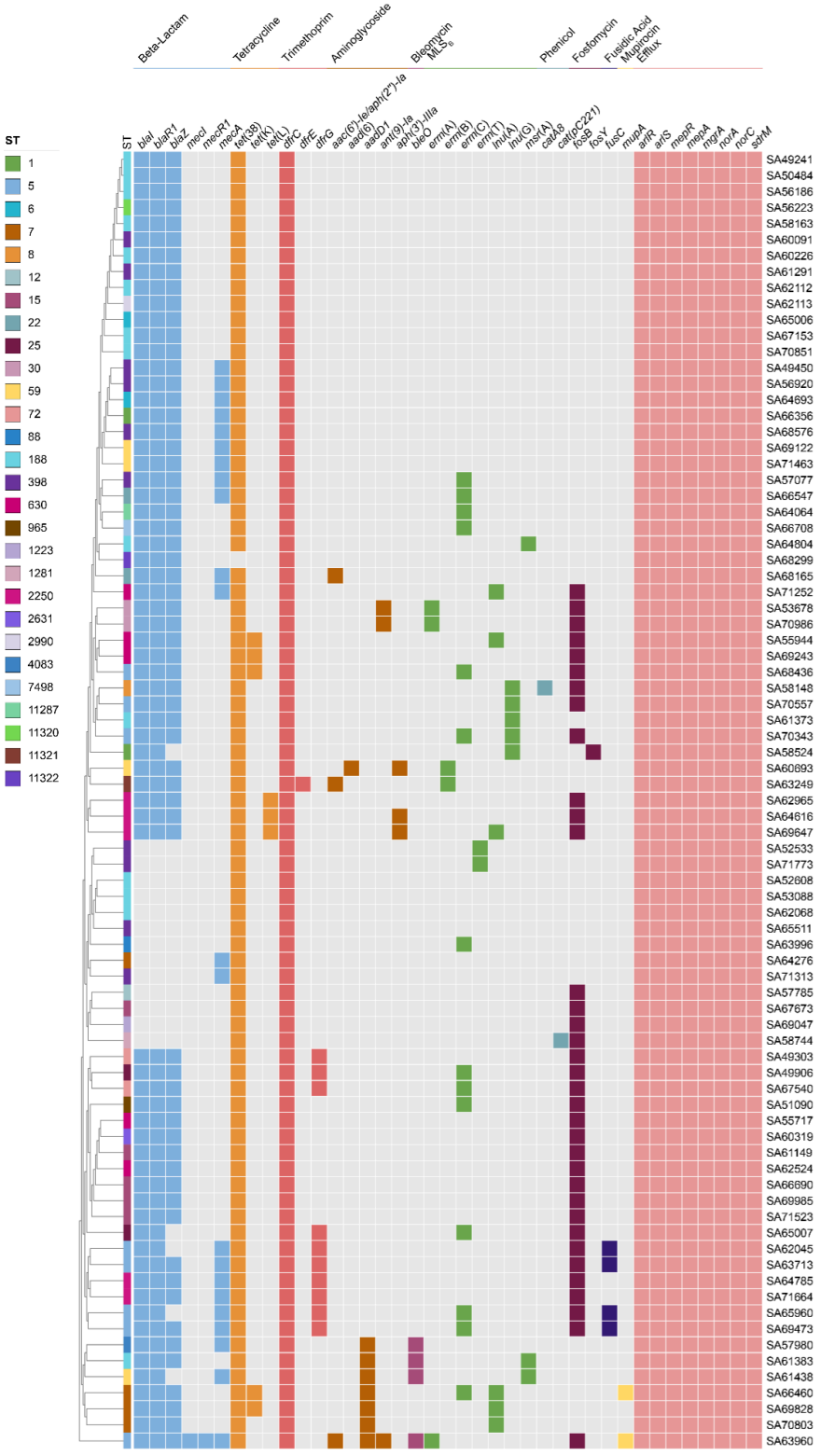


Figure 3

Virulence gene profiles of the 81 *Staphylococcus aureus* isolates. Coloured blocks represent the presence of genes and gray blocks present absence. The horizotal colour bar (from left to right) represents genes associated with adherence, biofilm, effector delivery system, exoenzyme, exotoxin, hemolysin, PVL, immune modulation, capsule, nutritional/metabolic factor.

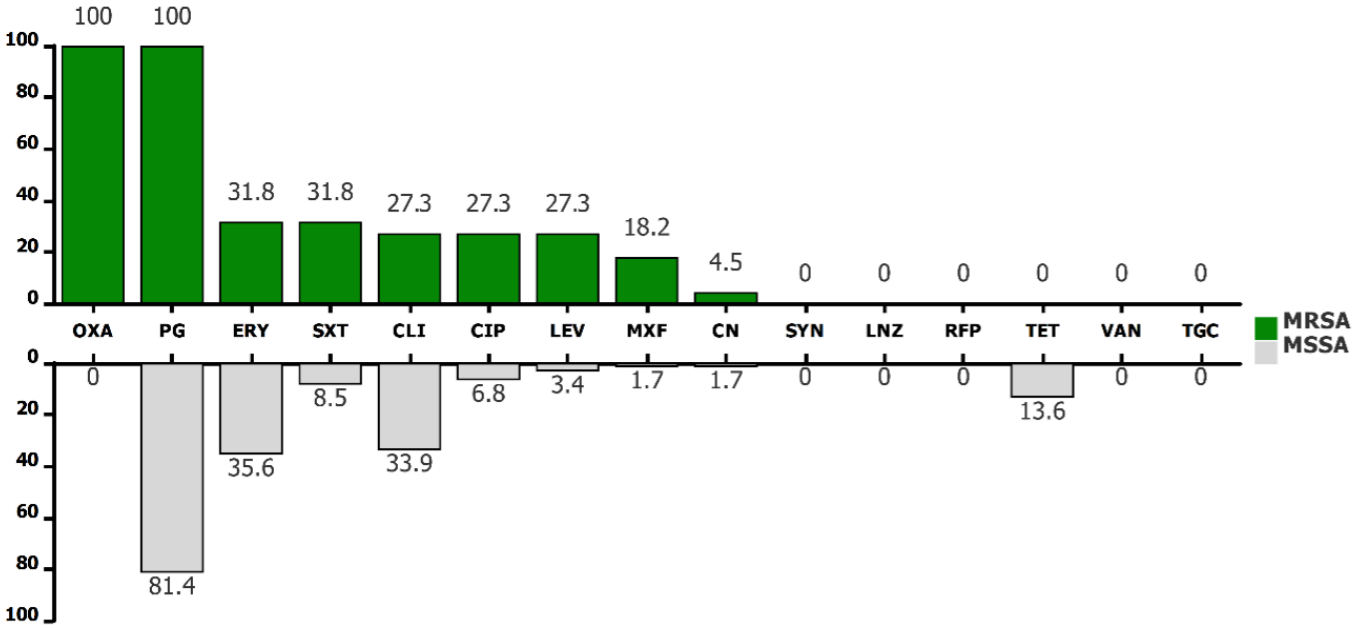


Figure 4

Antibiotics susceptibility test. MRSA: MSSA: OXA: oxacilin, PG: penicilin, ERY: eryhtromycin, SXT: cotrimoxazole, CLI: clindamycin, CIP: ciprofloxacin, LEV: levofloxacin, MXF: moxifloxacin, CN:gentamycin, SYN: Quinupristin/Dalfopristin, LNZ: linezolid RFP: rifampicin / rifampin, TET: tetracycline, VAN: vancomycin, TGC: tigecycline

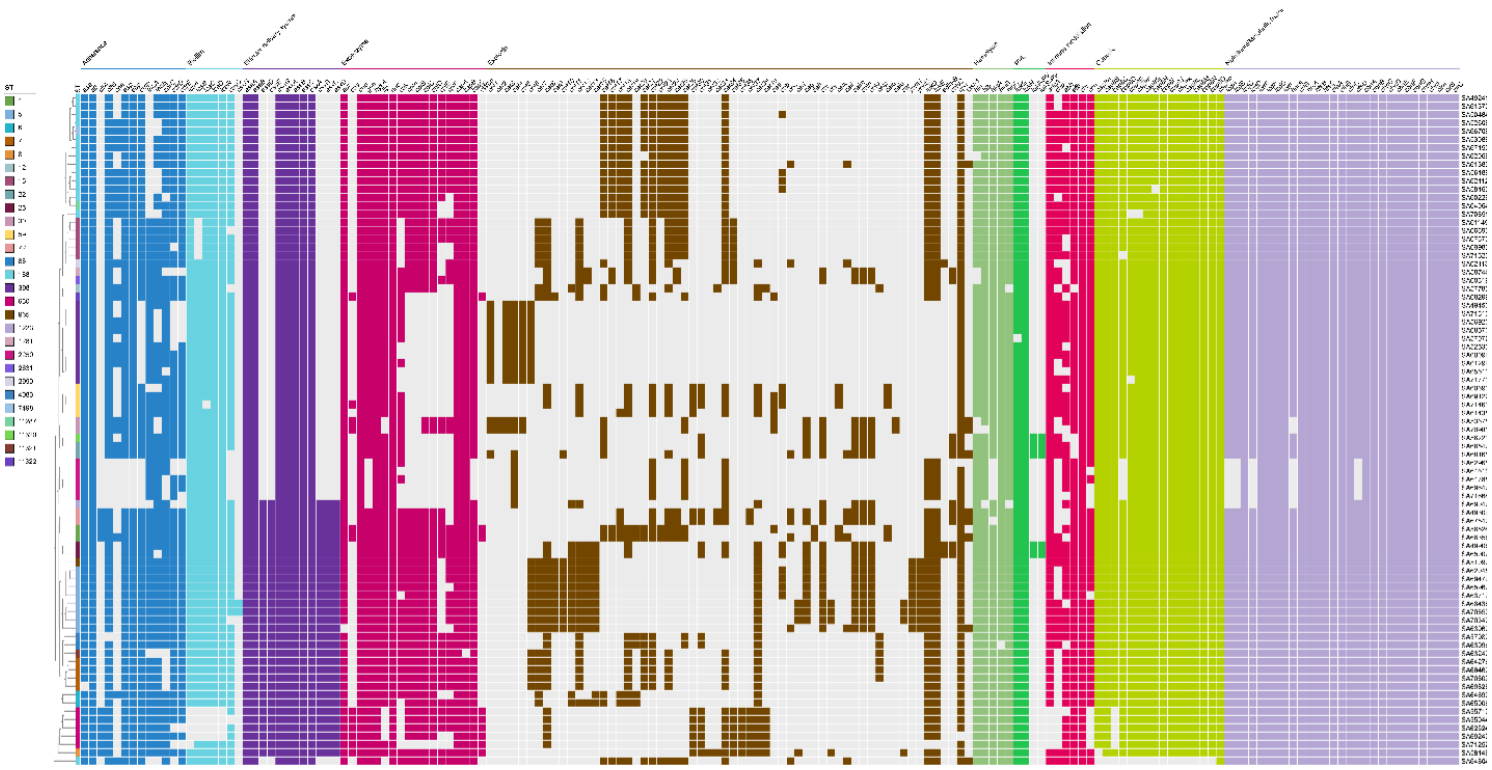


Figure 5

Virulence gene profiles of the 81 *Staphylococcus aureus* isolates. Coloured blocks represent the presence of genes and gray blocks present absence. The horizotal colour bar (from left to right) represents genes associated with adherence, biofilm, effector delivery system, exoenzyme, exotoxin, hemolysin, PVL, immune modulation, capsule, nutritional/metabolic factor.

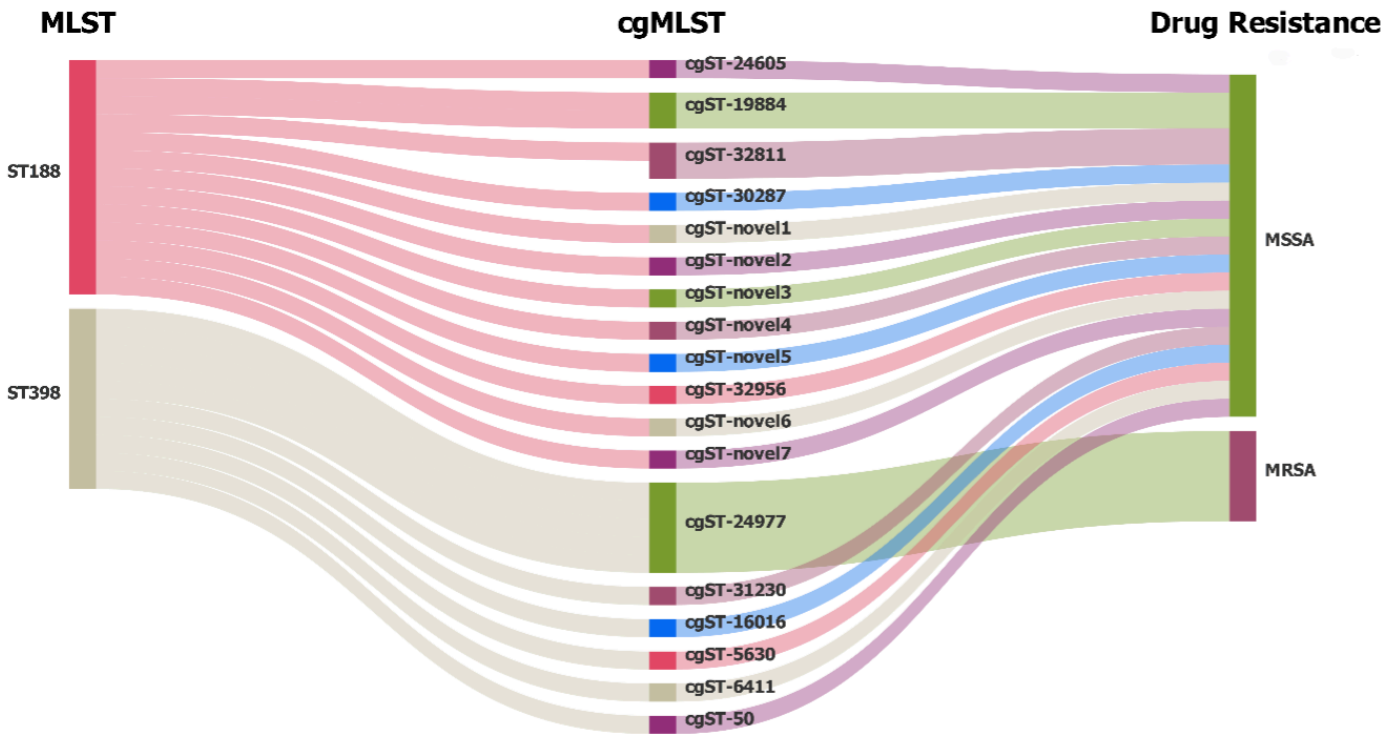


Figure 6

The Sankey diagram showing hierarchical distribution of bacterial clones stratified by ST typing, cgST, and MRSA/MSSA phenotypes. Node width represents the number of isolates; arrow direction denotes the trajectory of hierarchical classification. CgST-novel1-1-7 correspond to 7 unclassified subtypes.

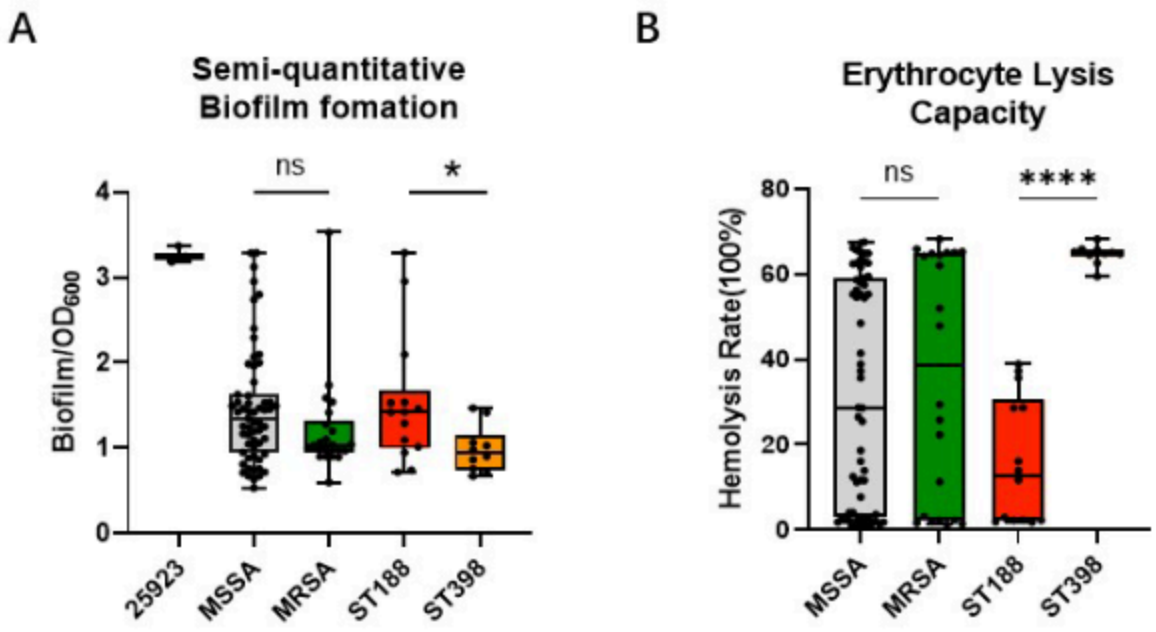


Figure 7

Phenotypic characterization of Staphylococcus aureus isolates. (A) Semi-quantitative assessment of biofilm formation capacity across 81 Staphylococcus aureus isolates, stratified by antimicrobial resistance profile and sequence type(ST). (B) Hemolytic activity, expressed as hemolysis rate, measured for the same 81 Staphylococcus aureus isolates. Hemolysis ratio(%)=(ODsample-ODnegative)/(ODpositive-ODnegative)*100%.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx
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