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CLONAL DIVERSITY, VIRULENCE, AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS WITH SKIN AND SOFT TISSUE INFECTIONS FROM A PRIVATE TERTIARY TEACHING AND REFERRAL HOSPITAL IN KENYA

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# CLONAL DIVERSITY, VIRULENCE, AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS WITH SKIN AND SOFT TISSUE INFECTIONS FROM A PRIVATE TERTIARY TEACHING AND REFERRAL HOSPITAL IN KENYA

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## ABSTRACT

***Background:*** ***Staphylococcus aureus (S. aureus)*** **remains a significant global health concern because of its strong virulence factors and ability to develop antimicrobial resistance (AMR).**

***Objective*****: This study investigated the clonal diversity, virulence, and antimicrobial resistance of *S. aureus* isolated from patients with skin and soft tissue infections (SSTIs) at a Private Tertiary Teaching and Referral Hospital in Kenya.**

***Methods*****: This was a cross-sectional study conducted between October 2019 and November 2020. Wound specimens were cultured for *S. aureus* and tested for antimicrobial susceptibility using the VITEK® 2 platform. Deoxyribonucleic acid (DNA) was sequenced using a MinION Mk1B sequencer. Resistance genes were identified using ResFinder and ARIBA, and virulence genes were screened using Virulence Finder.**

***Results*****: Out of 327 specimens, 98 (30.0%) were positive for *S. aureus*. Among these, methicillin-resistant *S. aureus* (MRSA) accounted for 16 (16.3%) of the isolates. Resistance was highest to penicillin (90.8%), followed by trimethoprim/sulfamethoxazole (63.2%). Whole-genome sequencing confirmed the presence of key resistance and virulence determinants in MRSA, with a high concordance (≥87.5%) between genotypic and phenotypic profiles.**

**Regarding molecular characterization, isolate typing revealed four clonal complexes, nine sequence types (STs), ten staphylococcal protein A (spa) types, and three staphylococcal cassette chromosome *mec* (SCC*mec*) types.**
***Conclusion*: The study reveals the emergence of highly virulent and multidrug-resistant MRSA clones in Kenya. By uncovering the evolving genetic landscape of *S. aureus*, the study provides critical evidence to inform antimicrobial stewardship and regional genomic surveillance in East Africa.**

## INTRODUCTION

*Staphylococcus aureus (S. aureus)*, including drug-resistant forms such as methicillin-resistant *Staphylococcus aureus* (MRSA), constitutes a significant portion of global health problems as an agent of both nosocomial and community-associated infections (CAIs). Over the years, the bacteria have evolved to become a significant source of disease and death both in the community and in hospitals. MRSA specifically has remained a primary contributor to hospital-associated infections (HAIs), causing a substantial rise in healthcare costs [1]. While extensive genomic data exists in Europe, Asia, and North America, the molecular epidemiology of *S. aureus*, in sub-Saharan Africa —particularly MRSA— remains poorly described. Most studies characterizing MRSA have originated from North African countries like Tunisia, Algeria, and Egypt, and from other African countries, including Nigeria and South Africa [2]. In East Africa, only a few studies have described the molecular characteristics of *S. aureus*. In Kenya, limited hospital-based reports have documented MRSA prevalence ranging from 3.7% to 53.4%, with evidence of circulation of the multilocus sequence typing (MLST) ST239/spa type t037 clone, a hybrid of ST8 and ST30 that is widely distributed globally [3], [4]. This clone has been linked to multiple MRSA epidemics across various regions of the world [5]. However, little is known about the diversity of circulating clones, carriage of virulence genes, or resistance determinants in Kenya.

Globally, research suggests that approximately 20.0–30.0% of people have persistent asymptomatic *S. aureus* nasal colonization, and another 30.0% may experience intermittent *S. aureus* colonization [6]. As an opportunistic pathogen, it causes a range of illnesses, including skin and soft tissue infections **(**SSTIs). The implication of *S. aureus* as a causative agent of SSTIs has been acknowledged ever since Alexander Ogston initially discovered its role in pyogenic abscess development in the 1880s [7]. This clinically significant pathogen has several different virulence factors, including bacterial surface components and extracellular proteins such as toxic shock syndrome [TSS] toxin, exfoliants, and Panton-Valentine leukocidin [PVL], enterotoxins, hemolysins, and coagulase that help in adhesion, nutrition acquisition, and immune response evasion, all of which are necessary for it to successfully survive inside its hosts. Severe necrotic skin infections, cutaneous abscesses, and furuncles have all been linked to PVL production [8]. The development of antibiotic resistance in *S. aureus* has significantly contributed to its

global impact as a high-priority pathogen, particularly with the emergence of MRSA. Studies have shown that *S. aureus* has acquired resistance to multiple classes of antibiotics [9]. For instance, penicillin resistance emerged in the 1940s, driven by the *blaZ* β-lactamase gene. In the early 1960s, the first semi-synthetic anti-staphylococcal penicillin was introduced, and within a year, MRSA was detected [10]. Genomic data, however, indicates that methicillin resistance, mediated by the *mecA* gene, predated the clinical use of these antibiotics [11]. Staphylococcal cassette chromosome *mec* (SCC*mec*) is a genomic island that usually carries the *mecA* gene, a mobile extrinsic genetic element that is the primary cause of resistance.

There is great attention put into tracking, classifying, and understanding the diversity of *S. aureus* in different settings. Currently, molecular tools such as multilocus sequence typing (MLST) and spa gene typing have been widely used. MLST has confirmed that the most common infections in hospital settings caused by MRSA can be associated with a small set of families, clonal complexes (CC45, CC30, CC22, CC8, and CC5) [12, 2].

Research shows that the MRSA clones prevalent in various regions are being replaced by more diverse and fitter clonal types. This phenomenon has been observed in different countries, like Southeast Asia, yet data is insufficient regarding these developments in East Africa[13]. Additionally, several studies have shown that understanding the susceptibility patterns of *S. aureus*, particularly in the context of SSTIs, is crucial. Combined with insights into the genetic diversity of *S. aureus* clones, such understanding can shed light on the pathogen's evolutionary pathways. This study aimed to investigate the clonal diversity, virulence, and antimicrobial resistance of *S. aureus* isolated from patients with SSTIs.

## MATERIALS AND METHODS

### Study Design, Population, and Sampling

The study employed a cross-sectional design and was carried out over a fourteen-month period (October 2019- November 2020). It included wound specimens—specifically swabs, aspirates, and tissue biopsies—collected from patients with SSTIs presented at the Aga Khan University Hospital, Nairobi (AKUHN), and eight of its affiliated outpatient clinics located across various counties in Kenya. A non-probability convenience sampling method was utilized to recruit study participants. This approach involved selecting patients who presented with SSTIs during the study period based on their availability and willingness to participate at the time of sample collection, without any randomization or stratification. While this approach facilitated timely recruitment, it may limit the representativeness of the study population and restrict the generalizability of the findings.

### Sample Collection and Microbiology Culture

Consent was obtained from patients, and for minors under the age of eighteen, permission was sought from their guardians using informed written consent forms. The wound specimens from infection sites were collected

using sterile swabs and transported to the laboratory in Amies transport media. The collected specimens were then cultured on MacConkey and 5% blood agar and incubated at 37°C for 18-24 hours. *S. aureus* was identified by beta hemolysis on sheep blood agar, Gram stain, catalase enzyme, and presence of clumping factor, protein A, and staphylococcal capsule (Pastorex™ Staph Plus Latex Agglutination, Biorad, USA). The identity of *S. aureus* was confirmed with a VITEK®2 Compact gram-positive identification card (Biomérieux,Marcy-l'Étoile, France).

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility tests were performed using VITEK 2, which utilized the P580 antibiotic susceptibility card and the Gram-positive identification module. Thirteen antibiotics were tested, including tigecycline (TIG), vancomycin(VAN), tetracycline(TET),

trimethoprim/sulfamethoxazole(SXT), nitrofurantoin(NIT), gentamicin (GEN), clindamycin (CLI), erythromycin (ERY), oxacillin (OXA), penicillin (PEN), linezolid (LZD), levofloxacin (LEV), and cefoxitin (FOX). The quality control strains, *S. aureus* ATCC 25923 and ATCC 25913, were employed, and the findings were interpreted in compliance with the guidelines provided by the Clinical and Laboratory Standards Institute(CLSI) (CLSI M100-S29). All confirmed *S. aureus* organisms were stored at - 80°C pending more in-depth analysis. Whole-genome sequencing was done on all confirmed MRSA isolates.

**Deoxyribonucleic acid (DNA) Extraction**

Following the manufacturer's protocol, Zymo Research QuickDNA Fungal/Bacterial Miniprep Kit (Irvine, CA) was used to extract and purify genomic DNA from colonies of freshly cultured. A Nanodrop ND-1000 spectrophotometer was used to evaluate the quality of the extracted DNA, and the Qubit™ 4 Fluorometer was used to quantify the results. The flow cell was primed as per the Oxford Nanopore Technologies (ONT) guidelines, and the library was loaded for sequencing using a MinION MK1B sequencer and operated as directed by the manufacturer.

**Sequencing and Bioinformatics**

The reads were filtered and trimmed with a chopper, based on average read quality, minimum and maximum read lengths, and headcrop and tailcrop using default settings, while retaining reads longer than 500 bp. Genome assemblies were constructed using Flye. Polishing of assembled contigs was performed using Minimap2. Quast and BUSCO were used to evaluate genome completeness and assembly quality. De novo assemblies were annotated with Prokka, and Taxonomic content analysis to check for contaminating species was performed using Kraken2 with the bacteria database at default settings. Coverage was calculated by aligning the base-called and trimmed fastq reads to the genome assembly using Minimap2.

**Multilocus Sequence Typing**

The program MLST v.2.19.0 was used to confirm each isolate's sequence type (ST). It does this by extracting 7 housekeeping genes (yqiL, tpi, pta, gmk, glpF, aroE, and arc) from

the sequence contigs and comparing them with known alleles in the MLST database (http://saureus.mlst.net) hosted on the Center for Genetic Epidemiology, using eBURST, the related complexes' (CCs') clustering was identified.

**Detection of Antimicrobial Resistance Genes**

We identified resistance determinants resulting from horizontally acquired antimicrobial resistance genes using the ResFinder database and ARIBA v.2.14.5 [14, 15]. To assess pathogenic potential, we screened all genomes for known virulence genes using the Virulence Factor Database (VFDB) and Virulence Finder applying a minimum threshold of 60% for sequence coverage and 90% for sequence identity[16,17]. These thresholds were selected to balance sensitivity for detecting partial or divergent gene variants with specificity to minimize false positives and are consistent with thresholds applied in previous genomic studies [18]. We employed SCC*mec*-Finder v.1.2 to determine the presence and type of *mecA*-carrying staphylococcal chromosomal cassette (SCC*mec*)[19].

**Data Management**

The data was recorded in MS Excel 2007. For analysis, the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics Version 26) was utilized. Means and standard deviations (SD) were used to represent continuous variables, while proportions were used to summarize the microbiological, demographic data, and antimicrobial susceptibility testing (AST) results, classified as "susceptible," "intermediate," or "resistant", categorized according to Clinical and Laboratory Standards Institute(CLSI) interpretive criteria.

**Ethical Approval**

The Institutional Review Board (IRB) of JKUAT provided ethical approval, as well as the Ethics and Scientific Review Committee of the AKUHN (Version Ref. 2019/IERC-67(v2)).

**RESULTS**

A total of 327 specimens were collected from study sites across the country. Among these, 98 *S. aureus* were isolated, resulting in an overall prevalence of 30.0% (98/327). Wound swabs accounted for 65 out of 98 isolates (66.3%), abscess aspirates 28 (28.6%), and tissue samples 5(5.1%). Of the 98 positive *S. aureus* isolates, 16 (16.3%) were MRSA.

**Demographics Characteristics of Study Participants**

Out of the total samples, 169 (51.7%) were from female participants, and 158 (48.3%) were from males, with a female: male ratio of 1.07:1. Among 158 males, 56 (35.4%) tested positive for *S. aureus*, while among 169 females, 42 (24.9%) tested positive. The mean age (± SD) of the study subjects with positive *S. aureus* detected was 33.4 (±19.2) years. The highest proportion of *S. aureus* 42(42.9%) was found in adults aged 20-39 years.

**Antimicrobial Susceptibility Profile of *S. aureus***

Of the 98 *S. aureus*-positive isolates, (90.8%, n=89) were resistant to penicillin, resistance to clindamycin, erythromycin, gentamicin, levofloxacin, trimethoprim/sulfamethoxazole,

tetracycline, and tobramycin seen in 15 (15.3%), 23 (23.5%), 10(10.2%), 10(10.2%), 62 (63.3%) 16 (16.3%), and 14 (14.3%) isolates, respectively. No resistance was detected against linezolid, vancomycin, and tigecycline (Figure 1). Overall, 95 (96.9%) of the isolates were resistant to at least one of the drugs in the panel of 13 antibiotics analyzed, and 27 (27.6%) were found to be multidrug-resistant (MDR), defined as having resistance to more than three classes of antibiotics.

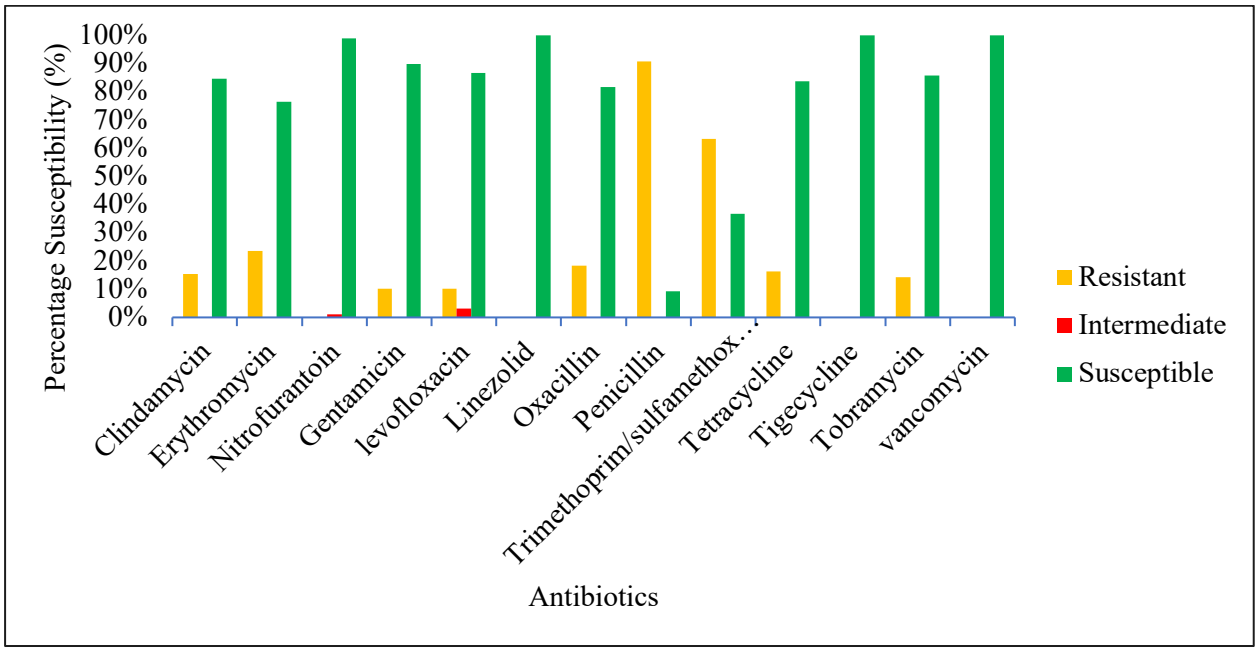


**Figure 1:** Antimicrobial susceptibility profile of *S. aureus* isolates (n=98)

The data represent the percentage of isolates classified as resistant, intermediate, or susceptible to the tested antibiotics.

Among the sixteen MRSA isolates, no resistance to nitrofurantoin, linezolid, tigecycline, and vancomycin was observed. The resistance against other antibiotic drugs was as follows: penicillin (100.0%), erythromycin (81.2%), trimethoprim/ sulfamethoxazole and tobramycin (75.0%), clindamycin and gentamicin (56.2%), tetracycline (43.8%), and levofloxacin (37.5%) (Table 1 and Supplementary Table S1)

**Table 1: Antimicrobial susceptibility profile of MRSA isolates (n=16)**

| **Antimicrobials tested** | **MRSA(n) (%)** | |
|---|---|---|
| | **Resistance n (%)** | **Susceptible n (%)** |
| Clindamycin | 9(56.2) | 7(43.8) |
| Erythromycin | 13(81.2) | 3(18.8) |
| Gentamicin | 9(56.2) | 7(43.8) |

| | | |
|---|---|---|
| Nitrofurantoin | 0(0.0) | 16(100.0) |
| Levofloxacin | 6(37.5) | 7(43.8) |
| Linezolid | 0(0.0) | 16(100.0) |
| Penicillin | 16(100.0) | 0(0.0) |
| Trimethoprim/sulfamethoxazole | 12(75.0) | 4(25.0) |
| Tetracycline | 7(43.8) | 9(56.3) |
| Tigecycline | 0(0.0) | 16(100.0) |
| Tobramycin | 12(75.0) | 4(25.0) |
| Vancomycin | 0(0.0) | 16(100.0) |

Methicillin-resistant *Staphylococcus aureus*(MRSA), *number(n)*

**Virulence Genes**

Among the 16 MRSA isolates, *hlg A/B/C* was the most detected gene, present in 100.0% of the isolates. This was followed by *aur, sak*, and *scn*, which were found in 15 (93.8%). The *splA/B/E* and *sek/seq* genes were each detected in 81.3% of isolates (n=13), while *lukD/E* was present in (68.8%; n=11). The *LukS/F-PVL* gene was identified in (50.0%; n=8), whereas ACME was much less common, being detected in 2 (12.5%) of the isolates. *edin* and *tst* were rare and found in only 1/16 (6.3%) of the isolates (Supplementary Table S2).

**Resistance Genes and Genotype–Phenotype Concordance of MRSA Isolates**

All the MRSA, 16(100.0%), exhibited resistance to penicillin and carried the *blaZ* gene. Resistance to erythromycin 13 (81.3%) and trimethoprim/sulfamethoxazole 12 (75.0%) was also common, although the corresponding resistance genes *ermA*, *msr*, and *dfrG* were inconsistently detected. For other antibiotics, moderate levels of phenotypic resistance were observed, ranging from 43.8% to 56.3% for gentamicin, clindamycin, and tetracycline, with associated resistance genes identified in varying proportions of the resistant isolates (Table 2). There was perfect concordance 16/16 (100%) between the *blaZ* gene and penicillin resistance, as well as between the *mecA* gene and resistance to both oxacillin and cefoxitin. High concordance was also observed between *aac'6'aph(2)* and gentamicin resistance (9/10; 90.0%), *tet* and tetracycline resistance (7/8; 87.5%), and *dfrG* and trimethoprim/sulfamethoxazole resistance (7/8; 87.5%) (Supplementary Table S3).

**Table 2: Phenotypic resistance patterns, and genotypic characteristics of MRSA isolates (n=16)**

| **Phenotype Antibiotics** | **Targeted gene** | Resistance gene **Present n (%)** | **Absent n (%)** |
|---|---|---|---|
| GEN-R (n=9) | *aac'6'aph (2)* | 9(100.0) | 0(0.0) |
| GEN-S(n=7) | | 1(14.3) | 6(85.7) |

| | | | |
|---|---|---|---|
| PEN-R(n=16) | *blaZ* | 16(100.0) | 0(0.0) |
| PEN-S(n=0) | | 0(0.0) | 0(0.0) |
| SXT-R(n=12) | *dfrG* | 7(58.3) | 5(41.7) |
| SXT-S(n=4) | | 1(25.0) | 3(75.0) |
| CLI-R(n=9) | *ermA* | 2(22.2) | 7(77.8) |
| CLI-S(n=7) | | 0(0.0) | 7(100.0) |
| ERY-R(n=13) | *ermA* | 1(7.7) | 12(92.3) |
| ERY-S(n=3) | | 1(33.3) | 2(66.7) |
| ERY-R(n=13) | *msr* | 4(30.8) | 9(69.2) |
| ERY-S(n=3) | | 0(0.0) | 3(100.0) |
| TET-R(n=7) | *tet* | 7(100.0) | 0(0.0) |
| TET-S(n=9) | | 1(11.1) | 8(88.9) |

Gentamicin **(**GEN), Penicillin **(**PEN), Trimethoprim/sulfamethoxazole (SXT), Clindamycin (CLI), Erythromycin (ERY), Tetracycline (TET), Resistant(R), Susceptible (S), Methicillin-resistant *Staphylococcus aureus*(MRSA)

**Clonal Complexes and SCC*mec* Types**

The 16 MRSA isolates had 4 distinct clonal complexes (CC8, CC15, CC22, CC30) and one unknown, 9 ST types (ST8, 612, 957, 960,1633,4618, 4803, 7635, 7760) and 10 spa types (t008, t024, t037, t148, t223, t355, t1130, t1476, t1971, t13194), and one unknown. Two SCC*mec* types (III and IV), and one divergent SCC*mec* type V (5C&5) were identified. A majority of the MRSA isolates belonged to CC8 (10/16; 62.5%), with most of them classified as ST4803 (4/16; 25.0%) and spa type t1476 (3/16; 18.8%). SCC*mec* IV was the most prevalent (12/16; 75.0%), followed by SCC*mec* III (2/16; 12.5%) and SCC*mec* type V (1/16; 6.3%). The PVL-negative ST8-IV (largely t1476) was the major clone (Table 3).

**Table 3: MRSA Clones**

| Isolate ID | Clonal Complex | ST | spa Type | SCC*mec* Type | Region | CAI/HAI |
|---|---|---|---|---|---|---|
| Staph 64 | 8 | 8 | 8 | IVa(2B) | Machakos | CAI |
| Staph 77 | 22 | 7760 | 223 | IVc(2B) | Embu | CAI |
| Staph78 | 8 | 4803 | 8 | IVa(2B) | Meru | CAI |
| Staph79 | 22 | 957 | Unknown | V(5C&5) | Nakuru | CAI |

| | | | | | | |
|---|---|---|---|---|---|---|
| Staph102 | 8 | 7635 | 37 | III (3A) | Nairobi | CAI |
| Staph104 | 8 | 612 | 1971 | IVd2B) | Machakos | CAI |
| Staph108 | 8 | 4803 | 1476 | IV(2B) | Nyahurur | CAI |
| Staph109 | 30 | 4618 | 1130 | IVa(2B) | Nairobi | CAI |
| Staph110 | 15 | 960 | 13194 | n/a | Kiambu | CAI |
| Staph113 | 30 | 4618 | 1130 | IVa(2B) | Nairobi | CAI |
| Staph114 | 8 | 4803 | 1476 | IV(2B&5) | Nyahurur | CAI |
| Staph141 | 8 | 8 | 148 | IVa(2B) | Machakos | CAI |
| Staph142 | Unknown | 1633 | 355 | IV(2B) | Nakuru | CAI |
| Staph145 | 8 | 4803 | 1476 | IV(2B) | Kiambu | CAI |
| Staph147 | 8 | 7635 | 37 | III (3A) | Nairobi | HAI |
| Staph148 | *8* | *8* | *24* | *IVa(2B)* | *Kisii* | CAI |

Methicillin-resistant *Staphylococcus aureus*(MRSA), sequence types(ST), staphylococcal protein A (spa), staphylococcal cassette chromosome *mec* (SCC*mec*) community-associated infections(CAI), Hospital-associated infections (HAI)

**Phenotypic Resistance by SCC*mec* Type**

All SCC*mec* type IV (12/12;100%) isolates showed resistance to erythromycin and were relatively resistant to other antibiotic agents, while all SCC*mec* type III isolates (2/2;100%) showed resistance to clindamycin, gentamycin, trimethoprim/ sulfamethoxazole, tetracycline, and tobramycin (Table 4).

**Table 4: Resistance profile of SCC*mec*-type**

| | | **SCC*mec* Type** | | |
|---|---|---|---|---|
| **Antibiotics** | **Resistance Profile** | **III** | **IV** | **V** |
| Gentamicin | (9) Resistant | 2(100.0%) | 6(50.0%) | 1(100.0%) |
| | (6) Susceptible | 0(0.0%) | 6(50.0%) | 0(0.0%) |
| SXT | (11) Resistant | 2(100.0%) | 9(75.0%) | 0(0.0%) |
| | (4) Susceptible | 0(0.0%) | 3(25.0%) | 1(100.0%) |
| Clindamycin | (9) Resistant | 2(100.0%) | 7(58.3%) | 0(0.0%) |
| | (6) Susceptible | 0(0.0%) | 5(41.7%) | 1(100.0%) |
| Erythromycin | (13) Resistant | 1(50.0%) | 12(100.0%) | 0(0.0%) |
| | (2) Susceptible | 1(50.0%) | 0(0.0%) | 1(100.0%) |
| Levofloxacin | (6) Resistant | 1(50.0%) | 5(41.7%) | 0(0.0%) |
| | (6) Susceptible | 0(0.0%) | 5(41.7%) | 1(100.0) |
| Tetracycline | (7) Resistant | 2(100.0%) | 5(41.7%) | 0(0.0%) |
| | (8) Susceptible | 0(0.0%) | 7(58.3%) | 1(100.0%) |
| Tobramycin | (12) Resistant | 2(100.0%) | 9(81.8%) | 1(100.0%) |

| | | | | |
|---|---|---|---|---|
| | (2) Susceptible | *0(0.0%)* | *2(18.2%)* | 0(0.0%) |
| trimethoprim/sulfamethoxazole(SXT),staphylococcal cassette chromosome *mec* (SCC*mec*) | | | | |

## DISCUSSION

The prevalence of *S. aureus* in our study was 30.0%. These findings are slightly higher but consistent with findings from the East African settings, such as a study conducted in Uganda, that reported an isolation rate of *S. aureus* at 28.7% in surgical site infections and closely comparable to a study conducted at a major referral hospital in Kenya, which reported 33.0% [20,21]. In our study, the proportion of MRSA among *S. aureus* isolates was 16.3%, which was distinctly lower than the 53.4% previously reported in a Kenyan hospital-based study [4].

We discovered a very high level of *S. aureus* resistance to penicillin (90.8%). This finding is consistent with a previous study in Kenya that reported 100.0% resistance and with another African study, which observed penicillin-resistance at (95.2%, n = 438) [22,23]. Although *S. aureus* strains from clinical infections in Africa have previously been shown to exhibit high resistance rates to this antibiotic, the findings remain concerning as penicillin remains commonly prescribed in many Kenyan healthcare facilities[23].

Furthermore, several studies demonstrated the occurrence of MDR-MRSA with cross-resistance to many antibiotics [24]. In this study, the phenotypic pattern of the tested antibiotics revealed a high susceptibility of 100.0% to tigecycline, vancomycin, linezolid, and nitrofurantoin, which is in line with results reported in a previous study [25]. These findings also indicate that these antibiotics remain highly effective against *S. aureus* in this setting, providing important therapeutic options where first-line agents, such as penicillin, are no longer reliable. Moreover, in the present study, MDR-MRSA isolates accounted for 27.6% of the total isolates, which aligns with findings reported in other African countries, and highlights the growing challenge of limited therapeutic options [5]. The continued efficacy of antimicrobials alongside the presence of MDR strains emphasizes the urgent need for antimicrobial stewardship, which directly reduces the selection pressure that causes bacteria to become resistant to multiple antimicrobial agents.

The study shows high concordance (≥87.5%) between specific resistance genes and their corresponding phenotypic resistance patterns of the MRSA isolates, for the majority of gene-drug pairs, except one pair with 1/2 (50.0%). This implies that detection of resistance genes is generally a reliable predictor of phenotypic resistance. However, alternative mechanisms—such as mutations outside the targeted genes, efflux pumps, or regulatory factors influencing gene expression—may explain the apparent discrepancy in which some phenotypically resistant isolates lacked detectable resistance genes [26].

All of the MRSA isolates in our investigation had the *hlg* gene in their genomes, which

encodes gamma-hemolysin, a major virulence factor and pore-forming cytotoxin that disrupts the localization and function of neutrophils, which in turn contributes to the progression of infections by *S. aureus*. This is consistent with data showing a high prevalence of *hlg* among MRSA isolates in Sudan [27]. This highlights how widely distributed the *hlg* gene is in various geographical areas. The frequent detection of the gene in MRSA strains underscores the necessity for additional study to fully comprehend its implications in local epidemiology and clinical outcomes, as well as its potential role in the virulence and pathogenicity of *S. aureus*.

In our study, 50.0% of MRSA isolates were PVL-positive and 75.0% carried SCC*mec* type IV, highlighting their potential role as priority pathogens in patients with SSTIs. Africa has been considered a PVL-endemic region with a high proportion of PVL-producing *S. aureus* strains (17-74%) suggesting widespread circulation of highly virulent clones across the continent [23].

MLST typing discovered high genetic diversity among the isolated MRSA strains, as demonstrated by the recovery of 10 different spa types. Most MRSA isolates belonged to clonal complex CC8, sequence type ST4803, and spa type t1476. These types are associated with local outbreaks in Kenya and correspond to some of the most common MRSA clones previously reported in Nairobi and surrounding areas [4]. Other well-known strains, such as USA300—a lineage linked to the acquisition of SCC*mec* IV, and presence of PVL, *seq*, and *sek* genes—were also observed. Overall, the MLST results confirm the heterogeneous nature of MRSA strains circulating in the region, emphasizing the need for ongoing surveillance to monitor the distribution of virulent and epidemiologically important clones. The study also underscores the presence of the ST8/CC8 and t008/spa strains, which have been connected to CAIs, demonstrating how easily *S. aureus* strains can spread across regional and international borders[22].

**Limitations of the study**

The study was limited by the small MRSA sample size (n = 16), which may reduce the statistical power and generalizability of the findings. Although isolates were obtained from multiple sites, the sample may not fully represent the overall *S. aureus* diversity and resistance patterns across the country. In addition, resource constraints limited the inclusion of detailed clinical and epidemiological data that could have provided deeper insight into transmission dynamics. Despite these limitations, the study offers important preliminary data on MRSA genetic diversity and resistance mechanisms, highlighting the need for larger, multicenter studies to validate and expand these observations.

## CONCLUSION

This study identified various *MRSA* clones, including novel spa types, suggesting that *S. aureus* has evolved and adapted well in the region. The agreement between resistance genes and phenotypic patterns, with virulence and resistance factors coexisting, underscores the potential of molecular diagnostics in clinical management. While *S. aureus* remains susceptible to certain

antibiotics, these results highlight the critical need for antimicrobial stewardship to prevent resistance and preserve antibiotic efficacy. Additionally, the findings underscore the need to strengthen national and regional surveillance systems to monitor *S. aureus* epidemiology, guide treatment strategies, and prevent the emergence and spread of resistant strains.

**Abbreviations:** AMR, antimicrobial resistance; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; CAIs, community-associated infections; CONS, coagulase-negative staphylococcus; DNA, deoxyribonucleic acid; HAIs, hospital-associated infections; HA-MRSA, hospital-associated methicillin-resistant *Staphylococcus aureus*; MDR, multi-drug resistant; MLST, multi-locus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*, MSSA, methicillin-susceptible *Staphylococcus aureus*

; PBP, penicillin-binding protein; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; PVL, panton-valentine leucocidin; SCC, staphylococcal cassette chromosome elements; spa, staphylococcus protein A gene; SSTIs, skin and soft tissue infections.

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**Conflict of Interest:** None

**Data Availability Statement:** Upon reasonable request, the corresponding author will make the dataset created during this study available.

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