Comparative analysis of antibiotic resistance patterns and virulence genes in *Staphylococcus aureus* strains isolated from clinical samples at Hue University of Medicine and Pharmacy Hospital

Ung Thi Thuy, Nguyen Thi Khanh Linh, Dinh Thi Hai, Hoang Thi Minh Ngoc, Nguyen Hoang Bach*
Department of Microbiology - University of Medicine and Pharmacy, Hue University

Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) is a common pathogen associated with severe infections, and its antibiotic resistance is potentially associated with various virulence factors. This study explored the relationship between antibiotic resistance and virulence genes in *S. aureus* isolates from the clinical samples of Hue University of Medicine and Pharmacy Hospital. **Materials and Methods:** Between 2021 and 2022, 122 *S. aureus* strains were isolated from clinical samples, with 114 non-duplicate strains undergoing antibiogram and virulence gene analysis. The antimicrobial susceptibility was tested using the Kirby-Bauer method. Molecular genotyping of *Nuc*, *mecA*, *spa*, *pvl*, and *tsst-1* was performed using singleplex and multiplex PCR techniques. **Results:** Out of 122 isolated *S. aureus* strains, 114 non-duplicate strains were analyzed, with MRSA (Methicillin-resistant *Staphylococcus aureus*) comprising 49.1% and MSSA(Methicillin-sensitive *Staphylococcus aureus*) 50.9%. Carriage of *the mecA* and *spa* genes was significantly associated with MRSA infection ($p<0.05$). The *mecA* gene was associated with resistance to penicillin, erythromycin, clindamycin, and tetracycline ($p<0.05$), whereas the *spa* gene was associated with oxacillin resistance ($p=0.002$). tsst-1 was linked to resistance to penicillin and clindamycin ($p<0.001$). No correlation was found between the presence of *pvl* gene and antibiotic resistance. **Conclusion:** The presence of methicillin-resistant genes in MSSA poses significant challenges for its diagnosis and treatment. Investigating the virulence and antimicrobial resistance of MRSA and MSSA is crucial to improving patient treatment outcomes in the future.

Keywords: *Staphylococcus aureus*, *MRSA, MSSA, nuc, mecA, spa, pvl, tsst-1.*

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) has emerged as one of the most common bacteria associated with hospital- and community-acquired infections. It is associated with various conditions, including skin and soft tissue infections, osteomyelitis, invasive infections, and septicemia [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are known to have evolved through a combination of virulence and methicillin-resistant genes, enabling them to invade healthy individuals and spread quickly within populations. Therefore, understanding the molecular characteristics of MRSA isolates is crucial for effective infection control. While most studies have focused on MRSA, infections caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) may be even more clinically significant, as MSSA isolates often possess diverse virulence factors [2]. A previous study indicated that MSSA strains exhibit similar patterns of enterotoxin distribution, the methicillin-resistant gene *mecA*, and the virulence gene *tsst-1* (toxic shock syndrome toxin-1 gene) as MRSA strains. Consequently, MSSA isolates should be regarded as similar to MRSA strains because they can serve as potential sources of infection [3].

S. aureus produces several virulence factors, including toxins, enzymes, and adhesion factors, contributing to its pathogenicity. The Panton-Valentine leukocidin (PVL) toxin, encoded by the *pvl* gene, is linked to infections ranging from skin and soft tissue infections to severe conditions, such as necrotizing pneumonia. Additionally, Toxic Shock Syndrome Toxin-1 (TSST-1), encoded by tsst-1, can cause life-threatening toxic shock syndrome (TSS), such as rash, fever, and organ failure. Understanding the prevalence of the *tsst-1* gene and its link to antibiotic resistance is crucial for improving patient outcomes. Toxins from both the *pvl* and *tsst-1* genes can lead to severe infections, which are even more challenging to treat when associated with the *mecA* gene-mediated resistance to beta-lactam antibiotics [4]. The advancement of molecular biological techniques has been crucial for detecting genetic patterns in MRSA and MSSA. For example, the *nuc* gene is highly specific for identifying *S. aureus*, along with the presence of virulence genes such as *spa*

**Corresponding Author: Nguyen Hoang Bach, Email: nhbach@huemed-univ.edu.vn*
DOI: 10.34071/jmp.2025.2.14
Received: 8/11/2024; Accepted: 10/3/2025; Published: 28/4/2025

(Staphylococcal protein A), *pvl* (Panton-Valentine leukocidin), *mecA*, and *tsst-1*. Furthermore, these studies demonstrated a significant correlation between antibiotic resistance and virulence gene expression in *S. aureus* strains [5-7].

While MRSA is often recognized as the primary cause of moderate to severe staphylococcal infections, MSSA isolates are increasingly frequently isolated. The discussion on the role of pathogenesis in MRSA infections is complicated by the increasing prevalence of MSSA among patients with similar infections. Thus, investigating the virulence and antimicrobial resistance of both MRSA and MSSA populations is essential, as understanding the differences between them could enhance patient treatment outcomes [8]. Therefore, this study aimed to explore the relationship between the molecular characterization of virulence genes and antibiotic resistance phenotypes in MRSA and MSSA isolates collected from Hue University of Medicine and Pharmacy Hospital.

2. MATERIALS AND METHODS

2.1. Study design

Cross-sectional, descriptive, and laboratory experimental study.

2.2. Collection and species confirmation of *S. aureus* isolates

From April 2021 to November 2022, 122 strains were collected and confirmed to be *S. aureus* at the Hue University of Medicine and Pharmacy Hospital. The culture and isolation of *S. aureus* strains were performed according to routine microbiological procedures for phenotypic identification, which included Gram staining, catalase**,** and coagulase tests. Then, these strains were confirmed by conventional PCR with the *nuc* gene. 114 non-duplicate *S. aureus* strains will be analyzed for their antibiotic resistance patterns and virulence genes [6] [9]. These strains were stored at -20^{o}C, a retention medium in a cryovial (to reach an end concentration of 15% - 20%) at -80°C for long-term storage.

2.3. Antimicrobial susceptibility testing

Antibiograms were performed using the Kirby-Bauer disc diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines - Performance standards for antimicrobial susceptibility testing- M100 30th edition, 2020, on 114 non-duplicate *S. aureus* strains. A variety of eight antibiotics were tested: penicillin (10U), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), and doxycycline (30 µg). The isolates were phenotypically classified as MRSA or MSSA based on the antibiogram results from the cefoxitin (FOX) disk (30 µg). Oxacillin sensitivity and resistance were also interpreted according to the results of this antibiotic [10].

2.4. Preparation of DNA template

Staphylococcus aureus isolates were recovered on Brain Heart infusion agar (Merck KgaA, Germany) at 37°C for 24 h. Single colonies were picked and suspended in a 1.5 ml Eppendorf tube containing 200 µL of nuclease-free water. The cell suspension was boiled at 100°C for 10 min, and then the bacterial tubes were placed in an ice bath for 15 min. The samples were centrifuged at 14,000 rpm for 5 min at room temperature. A volume of 100 µL of the supernatant containing DNA was transferred to a new sterile tube and stored at -20°C until PCR amplification [11, 12].

2.5. *Nuc* gene PCR amplification

Nuc gene amplification was performed using a forward primer (5'-GCGATTGATGGTGATACGGTT-3') and reverse primer (5'-AGCCAAGCCTTGACGAACTAAAGC-3'). A total 25µL PCR reaction mixture consists of 12.5 µl Master Mix 2× Dream Taq™ (Phu Sa Biochem, Vietnam), 0.5 µl of each primer (10 µM), 11 µl nuclease-free water and 0.5 µl of DNA template. The cycling parameters consisted of 30 cycles of denaturation at 94^{0}C for 30 s, primer annealing at 50^{0}C for 1 min, and extension at 72^{0}C for 1 min 30 s in a Veriti® Thermal Cycler (Applied Biosystems, CA, USA) [6]. *S. aureus* ATCC 29213 strain was used as a positive control for the PCR reaction. The PCR products were separated by electrophoresis on a 2% agarose gel pre-colored with SafeView™ Classic in a VE100 vertical electrophoresis machine (Phu Sa Biochem, Can Tho, Vietnam). The PCR product was 279 bp [6].

2.6. Multiplex PCR for detecting resistant and virulent genes in *S. aureus*

2.6.1. Multiplex PCR for detecting mecA, spa, and pvl genes

A total of 114 S. *aureus* isolates were subjected to multiplex PCR targeting *mecA*, *spa*, and *pvl*. Briefly, PCR reactions were performed in a 25 µL volume containing 12.5 µl Master Mix 2× Dream Taq™ (Phu Sa Biochem, Can Tho, Vietnam) and 1 µL of each primer pair (Table 1).

7.5 µl nuclease-free water, and 2 µL DNA template. PCR was performed on a Veriti® Thermal

Cycler (Applied Biosystems, CA, USA). The reaction conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 59 °C for 1 min, and 72 for 1 minute [13]. Following PCR, 5 µl aliquots of each sample were subjected to electrophoresis on 2% agarose gel pre-colored with SafeView™ Classic to validate their identities. Bands were visualized using a VE100 vertical electrophoresis machine (Phu Sa Biochem, Can Tho, Vietnam).

Tabel 1. The primers for amplification of *mecA*, *spa*, and *pvl* genes in *S. aureus* [13]

Primers	Sequences	PCR product size (bp)
mecA_F	5'- TCCAGATTACAACTTCACCAGG - 3'	162
mecA_R	5'- CCACTTCATATCTTGTAACG - 3'	
spa_F	5'- TAAAGACGATCCTTCGGTGAGC - 3'	180-600
spa_R	5'- CAGCAGTAGTGCCGTTTGCTT - 3'	
pvl_F	5'- GCTGGACAAAACTTCTTGGAATAT-3'	83
pvl_R	5'- GATAGGACACCAATAAATTCTGGATTG-3'	

2.6.2. PCR amplification of tsst-1 gene

To amplify *tsst-1*, oligonucleotide primers *tsst-1* forward: 5'-CTGGTATAGTAGTGGGTCTG-3' and reverse: 5'-AGGTAGTTCTATTGGAGTAGG-3' were used. A volume of 0.5 µL of genomic DNA was amplified in 25 µL of a reaction mixture consisting of 12.5 µl Master Mix 2X Dream Taq™ (Phu Sa Biochem, Can Tho, Vietnam), 0.5 µl of each primer (10 µM), and 11 µL of nuclease-free water. PCR was conducted in a Veriti® Thermal Cycler (Applied Biosystems, CA, USA) with an initial denaturation step of 5 min at 94°C, 35 cycles of 1 min at 94°C, 2 min at 54°C, and 1 min at 72°C, followed by a 5 minutes final extension at 72°C [5]. To confirm the presence of the desired amplicon, electrophoresis was performed on a 2% agarose gel stained with SafeView™ Classic, and the products were visualized using a VE100 vertical electrophoresis machine (Phu Sa Biochem, Can Tho, Vietnam). The PCR product size for amplification of *tsst-1* was 271 bp [5].

2.7. Research methods

Data were entered and stored in Microsoft Excel. Data processing and analysis were performed using SPSS 25.0 software. The Chi-square test was applied to evaluate the relationship which was considered significant with $p<0.05$.

3. RESULTS

3.1. Species identification

All 122 *S. aureus* isolates were collected and identified from various clinical specimens, and > 86.9% were collected from pus samples. All *S. aureus* strains in our research identified by conventional biochemical tests were confirmed by singleplex PCR with the *nuc* gene (Figure 1). 114 non-duplicate strains were selected for antibiogram analysis and gene amplification.

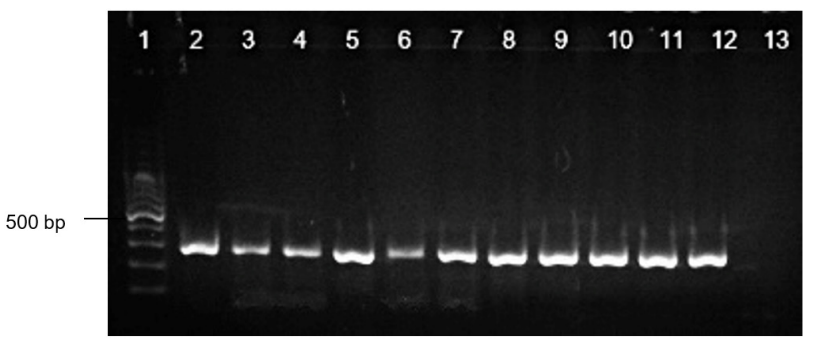

Figure 1. Electrophoresis of PCR products of *nuc* gene of *S. aureus*.

Lane 1:100 bp DNA ladder; lane 2: positive control (*S. aureus* ATCC 29213); lane 3, 4, 5, 6, 7, 8, 9, 10, 11, 12: amplified product of nuc gene (270 bp); lane 13: negative control.

3.2. Antimicrobial susceptibility testing and MRSA/MSSA classification

In total, 114 non-duplicate *S. aureus* strains were evaluated for antibiotic resistance. The antibiogram using a cefoxitin disk (30 µg) revealed that 56 of 114 strains (49.1%) were classified as MRSA, while

58 strains (50.9%) were identified as MSSA. Table 3 compares the activities of the eight antistaphylococcal antibiotics against MRSA and MSSA. Resistance frequencies were higher in MRSA than in MSSA for all antibiotics tested. Among the 56 MRSA isolates, the majority were resistant to penicillin (100.0%), clindamycin (92.9%), and erythromycin (89.3%). The resistance rates for other antibiotics were 48.2% for tetracycline, 39.3% for trimethoprim/sulfamethoxazole, 8.9% for chloramphenicol, and 1.8% for doxycycline. MSSA strains showed resistance to these agents at the following rates: 50%, 32.8%, 32.8%, 22.4%, 17.2%, 6.9%, and 0%, respectively. Our study showed a significant association between MRSA strains and resistance to penicillin, erythromycin, clindamycin, and tetracycline ($p<0.05$) (Table 2).

Table 2: Antibiotic susceptibility of *S. aureus* in MRSA and MSSA

Antibiotic	Resistant phenotype (%)				p
	MRSA (n = 56)		MSSA (n = 58)		
	n	%	n	%	
Penicillin	56	100	29	50	0.000
Oxacillin	56	100	0	0	0.000
Erythromycin	50	89.3	19	32.8	0.000
Clindamycin	52	92.9	19	32.8	0.000
Tetracycline	27	48.2	13	22.4	0.006
Doxycycline	1	1.8	0	0	0.249
Trimethoprim-sulfamethoxazole	19	33.9	10	17.2	0.121
Chloramphenicol	5	8.9	4	6.9	0.74

n = total isolates belonging to the same species with the same resistance to antibiotic

3.3. Gene profile of MRSA and MSSA

The *mecA*, *spa*, *pvl*, and *tsst-1* genes were analyzed in all 114 *S. aureus* strains, encompassing both MRSA and MSSA (Chart 2). Multiplex PCR detected the *mecA* gene in 48 out of 56 (85.7%) MRSA strains, whereas only 6.9% (4 out of 58) of MSSA strains possessed this gene. The *spa* gene was the most prevalent among the genes examined and was present in 55 (98.2%) MRSA strains and 44 (75.9%) MSSA strains. The *pvl* virulence gene was detected more frequently in the MRSA group (35.7%) than in the MSSA group (22.4%) (Figure 2). Only four *tsst-1* genes were identified across all 114 *S. aureus* strains in our study (Figure 3), with a notable predominance in the MSSA group (5.2%) compared to the MRSA group (1.8%) (Table 3).

Table 3. Frequency and distribution of *S. aureus* genotyping between MRSA and MSSA isolates

Gene	Genotype profile (%)			
	Total (n = 114)	MRSA (n = 56)	MSSA (n = 58)	*p*
MecA	52 (45.6%)	48 (85.7%)	4 (6.9%)	0.000
Spa	99 (86.8%)	55 (98.2%)	44 (75.9%)	0.002
Pvl	33 (28.9%)	20 (35.7%)	13 (22.4%)	0.149
Tsst-1	4 (3.5%)	1 (1.8%)	3 (5.2%)	0.619

Panton-Valentine leukocidin (*pvl*); Staphylococcus protein A (*spa*); Methicillin-resistant gene (*mecA*); toxic shock syndrome toxin (*tsst-1*).

The M-PCR products of *mecA*, *spa*, and *pvl* were analyzed by electrophoresis, revealing the anticipated bands: *mecA* at 162 bp, *pvl* at 83 bp, and the *spa* gene varying from 180 to 600 bp (Figure 2).

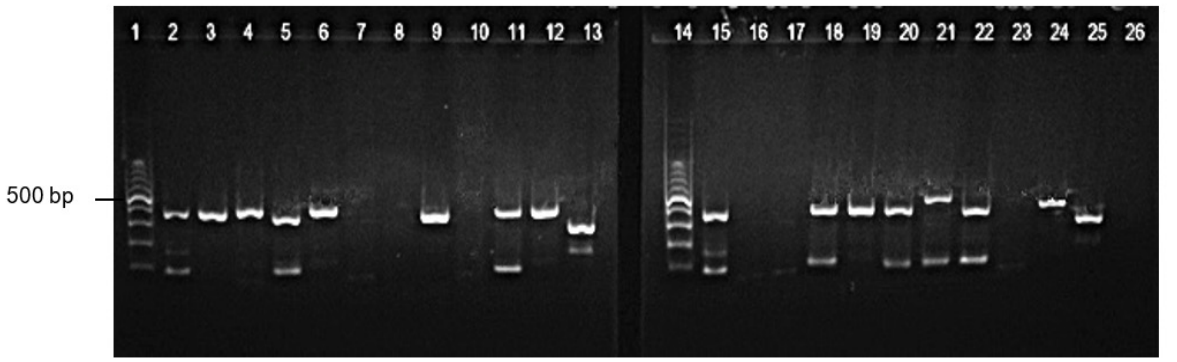

Figure 2. Agarose gel electrophoresis of the PCR-amplified products *spa*, *mecA*, and *pvl*
Lanes 1, 14: 100-bp ladder; Lanes 2, 15: positive control; lanes 3, 4, 6, 7, 24, 25: *spa* gene positive; lanes 5, 11, 18, 20, 21, 22: *spa* and *pvl* positive; lane 13: *spa* and *mecA* positive, lanes 7, 8, 10, 16, 17, 23: negative; lane 26: negative control.

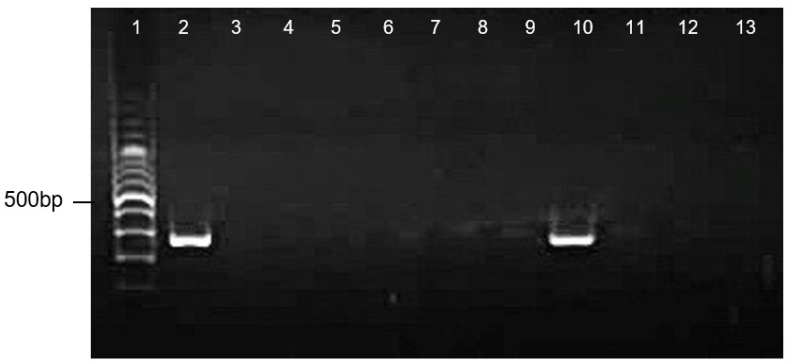

Figure 3. PCR for detecting *tsst-1***.** Lane 1:100-bp ladder; lane 2: positive control; lanes 3, 4, 5, 6, 7, 8, 11, 12: negative results of samples; lane 9: tsst-1 positive; Lane 13: negative control.

3.4. The correlation of resistance phenotype and genotype for *S. aureus* isolates

Results regarding the prevalence of *mecA*, *spa*, *pvl*, and *tsst-1* genes, along with the antibiotic resistance patterns of the isolates, are presented in Table 4. Resistance to penicillin, oxacillin, erythromycin, and clindamycin significantly increased in the presence of mecA (p<0.05). Notably, except for oxacillin (p=0.002), the distribution of the *spa* gene was not associated with resistance to the other antibiotics tested. *pvl* was only associated with resistance to tetracycline and doxycycline, with p-values of 0.001 and 0.015, respectively. Additionally, the *tsst-1* gene showed a significant correlation with resistance to penicillin and clindamycin (p<0.05), but no significant correlation was found with the other antibiotics in this study (Table 4).

Table 4: The Comparison of antibiotic resistance Pattern with the presence or absence of *mecA*, *spa*, *pvl*, and *tsst-1*

AB	*mecA*			*spa*			*pvl*			*tsst-1*		
	pos	neg	*p*	pos	neg	*p*	pos	neg	*p*	pos	neg	p
P	51 (98.1%)	34 (54.8%)	0.000	76 (76.8%)	9 (64.3%)	0.354	26 (78.8%)	59 (72.8%)	0.694	2 (50%)	83 (75.5%)	0.000
OX	48 (92.3%)	8 (12.9%)	0.000	55 (55.6%)	1 (7.1%)	0.002	20 (60.6%)	36 (44.4%)	0.149	1 (25.5%)	55 (50%)	0.619
E	45 (86.5%)	24 (38.7%)	0.000	64 (64.6%)	5 (35.7%)	0.139	19 (57.6%)	50 (61.7%)	0.089	1 (25%)	68 (61.8%)	0.177

CD	47 (90.4%)	24 (38.7%)	**0.000**	66 (66.7%)	5 (35.7%)	0.128	23 (69.7%)	48 (59.3%)	0.507	1 (25%)	70 (63.6%)	**0.000**
TE	25 (48.1%)	15 (24.2%)	**0.013**	36 (36.4%)	4 (28.6%)	0.059	20 (60.6%)	20 (24.7%)	0.001	1 (25%)	39 (35.5%)	0.845
DO	1 (1.9%)	0 (0%)	0.342	1 (1%)	0 (0%)	0.695	1 (3%)	0 (0%)	0.015	0 (0%)	1 (0.9%)	0.766
C	6 (11.5%)	3 (4.8%)	0.296	9 (9.1%)	0 (0%)	0.477	1 (3%)	8 (9.9%)	0.444	0 (0%)	9 (8.2%)	1.000
SXT	17 (32.7%)	12 (19.4%)	0.064	28 (28.3%)	1 (7.1%)	0.445	4 (12.1%)	25 (30.9%)	0.064	2 (50%)	27 (24.5%)	0.509
Total	52 (100%)	62 (100%)	-	99 (100%)	15 (100%)	-	83 (100%)	81 (100%)	-	4 (100%)	110 (100%)	-

AB: Antibiotics, P: penicillin; OX: oxacillin; E: erythromycin; CD: clindamycin; TE: tetracyclin; DO: doxycycline; C: chloramphenicol; SXT: trimethoprim-Sulfamethoxazole; Panton-Valentine leukocidin (PVL); Staphylococcus protein A (Spa); Methicillin-resistant gene (MecA), toxic shock syndrome toxin (TSST-1); pos: positive; neg: negative; P: p-value; A: antibiotic

4. DISCUSSION

Staphylococcus aureus (*S. aureus*) is a significant pathogen in nosocomial and community-acquired infections. Infections caused by *S. aureus*, including MRSA and MSSA, raise concerns regarding the acquisition of antibiotic resistance, which is linked to the dissemination of virulence genes as strains spread between environments. Consequently, monitoring the prevalence of antibiotic resistance, virulence genes, and associated phenotypes in *S. aureus* strains is crucial to predicting the potential risk of the spread of these traits [14].

One of the major findings of this project was that antibiotic resistance was higher in the MRSA group than in the MSSA group, with resistance rates for penicillin, erythromycin, and clindamycin exceeding 89%. This high resistance rate of *S. aureus* to penicillin, erythromycin, clindamycin, and tetracycline has been documented in previous cases of sepsis in Vietnam [15, 16]. Our study revealed a strong correlation between MRSA isolates and resistance to penicillin, erythromycin, clindamycin, and tetracycline ($P<0.05$), whereas MSSA isolates remained sensitive to these antibiotics. Trimethoprim/sulfamethoxazole, chloramphenicol, and doxycycline are effective in treating for both MRSA and MSSA infections. Among the 114 strains studied, only one isolate in the MRSA group was resistant to doxycycline, suggesting the drug's potential effectiveness for both populations. These findings may aid clinicians in selecting appropriate antibiotics for treating staphylococcal infections caused by MRSA and MSSA.

In our study, differences in the contribution of virulence genes were found between MSSA and MRSA strains. Of the MRSA isolates, 48/56 (85.7%) were *mecA-positive*, while eight (14.3%) did not detect the 162 bp band specific for the *mecA* gene. The *mecA* gene is known to encode penicillin-binding protein 2a (PBP2a), which significantly reduces susceptibility to beta-lactam antibiotics and molecular amplification of the *mecA* gene is a standard diagnostic tool for MRSA. However, the absence of this gene in the eight isolates may indicate other intrinsic factors contributing to resistance in areas with high MRSA prevalence. Additionally, the lack of *mecA* genes and PBP2a (Penicillin-biding protein 2a) suggests potential hyperproduction of β-lactamase or amino acid substitutions in these phenotypically MRSA isolates [17]. In our study, the methicillin-resistant gene *mecA* was significantly correlated with resistance to penicillin, oxacillin, erythromycin, clindamycin, and tetracycline. These findings are consistent with a previous study by Amira M. Sultan et al. conducted in 2019 [4]. The *mecA* gene is the central determinant of MRSA phenotype and may be regulated by two systems: *mecI-mecR1* and *blaI-blaR1*. The *mecI-mecR1* regulators can cause slow induction of *mecA* expression, potentially taking several hours, which may result in strains appearing phenotypically susceptible despite carrying the resistance gene [18]. This explains why our study identified 4 methicillin-susceptible mecA-positive isolates (6.9%). The emergence of such strains poses a diagnostic challenge for clinical microbiology laboratories, as they may go undetected and be misidentified as MSSA. Therefore, combining phenotypic determination with genotypic methods such as PCR is crucial for *mecA* gene detection, particularly in severe infections. Moreover, these

strains can revert to MRSA under selective pressure from beta-lactam treatment, leading to potential treatment failures [19].

Protein A is a virulence factor that does not adhere to the cell wall and is released into surrounding media. A previous report indicated that the *prevalence of spa* in MRSA and MSSA was 96.6% and 96%, respectively, which is higher than our findings of 55 (98.2%) for MRSA and 44 (75.9%) for MSSA [20]. In our study, the prevalence of *S. aureus* strains lacking protein A expression was 13.2%, significantly higher than the previously reported 3.8% [20]. The difference in *spa* gene prevalence between the two groups was statistically significant. In contrast, the distribution of the *pvl* gene was not statistically significant in this study. However, all the strains carrying the *pvl* gene in our study were isolated from pus and skin wound fluid specimens. This could be explained by the fact that *pvl* genes are commonly associated with furuncles, cutaneous abscesses, and severe necrotic skin infections. Epidemiologically, *pvl* is associated with MRSA infection and contributes to increased virulence [7]. This study identified the *tsst-1* gene as the least common toxin gene. The distribution of *tsst-1* genes in specimens from various departments showed a very low detection rate. This gene was either present in minimal quantities or undetected in Ethiopia in 2019 [7]. A higher proportion of MSSA isolates carried the tsst-1 gene compared to MRSA isolates, though this difference was not statistically significant. This could be due to the relatively low number of tsst-1 positive strains in both MRSA and MSSA in the study. MSSA strains with the tsst-1 gene may exhibit greater virulence. To better understand the gene's expression in both groups, further research with a larger sample size is needed. Additionally, our study identified a significant association between the presence of the tsst-1 gene and resistance to penicillin and clindamycin ($p<0.001$) [21]. Our study found an association between the presence of the *tsst-1* gene and resistance to penicillin and clindamycin ($p<0.001$). Clindamycin, a protein synthesis inhibitor, can suppress the expression of *S. aureus* virulence factors, such as PVL, TSST-1, and alpha-hemolysin (Hla). This may explain why the clindamycin-resistant strains in our study were more likely to express tsst-1. Elisabeth Hodille et al. recommended combining clindamycin with a bactericidal antibiotic to treat severe staphylococcal toxin-related infections [22].

5. CONCLUSION

In summary, our findings revealed diversity in the presence of toxins and resistance genes in MRSA and MSSA groups. Resistance to penicillin, oxacillin, erythromycin, and clindamycin was notably higher. While MRSA remains a significant concern in *S. aureus* infections, increasing attention is needed for MSSA isolates that harbor many virulence factors. Additionally, the emergence of *S. aureus* strains that are phenotypically susceptible, despite carrying the resistance gene, poses a diagnostic challenge for clinical microbiology laboratories, as they may go undetected and misidentified as MSSA. Therefore, comparative epidemiological studies of MSSA and MRSA populations will be essential to understand their co-evolution and to improve current treatment strategies.

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