

RESEARCH ARTICLE

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## Antimicrobial Susceptibility and Prevalence of MRSA in a Riyadh Tertiary Care Hospital

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### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections of soft tissues and deep and superficial wounds affect morbidity, mortality, and healthcare resources in Saudi Arabia. The growing frequency of these infections underscore the need for effective antimicrobial stewardship projects and robust infection control. This study included patients from various age groups and was conducted in a tertiary care facility in Riyadh. Clinical specimens from different body parts were cultured, as part of the study protocol. Samples showing significant *Staphylococcus aureus* (*S. aureus*) growth were identified phenotypically and tested for microbial susceptibility. Antibiotics were evaluated and analyzed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. MRSA isolates were screened using cefoxitin discs. Data was gathered and analyzed over one year, from January to December 2023. This study included 11,837 culture specimens, of which 3,795 yielded positive cultures. *S. aureus* was isolated in 333 cases, accounting for 2.8% of all specimens, and 8.8% of positive cultures. Most patients were male, comprising 121 study participants (36%). The highest MRSA prevalence was observed between 12 and 40 years. The prevalence of MRSA in the local healthcare environment was 3.3%. Hospitalized patients showed a slightly higher incidence of MRSA infection. MRSA prevalence was significantly associated with the specimen type ( $p < 0.05$ ). Vancomycin and linezolid were 100% effective against all *S. aureus* strains, followed by tetracycline and gentamicin. The D-test was used to assess 307 *S. aureus* strains to assess inducible clindamycin resistance, 28 tested positive. The high MRSA infection load and new MRSA strains highlight the need for ongoing surveillance, stringent infection control, and targeted antibiotic stewardship programs. Our findings could be used to guide antibiotic treatment and combat antibiotic resistance in local hospitals.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*, Antimicrobial Susceptibility Testing, Resistance

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## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that develops resistance to methicillin and other beta-lactam antibiotics.<sup>1</sup> The emergence of MRSA dates back to the early 1960s, shortly after the introduction of methicillin, when certain strains of *S. aureus* began exhibiting resistance to this antibiotic.<sup>2</sup> MRSA quickly became a significant concern in healthcare settings because of its ability to cause infections that are challenging to treat. Despite the introduction of methicillin and other semisynthetic penicillins as advancements in the treatment of *S. aureus* infections, the emergence of methicillin-resistant strains, such as MRSA, within just a couple of years has highlighted a major setback in combating these bacteria.<sup>2</sup> Over time, MRSA resistance mechanisms have evolved, resulting in strains resistant to methicillin and other classes of antibiotics, posing a significant challenge in clinical management.<sup>3</sup>

Distinguishing healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) is crucial for understanding the epidemiology and transmission dynamics of these strains.<sup>4</sup> While HA-MRSA is often linked to healthcare settings and nosocomial infections, CA-MRSA has emerged as a distinct entity that affects otherwise healthy individuals in community settings.<sup>5</sup> The rise of CA-MRSA has raised concerns about its potential to spread rapidly among the general population, as it can be transmitted through close contact or shared items in schools, gyms, and households. Unlike HA-MRSA, which is often acquired in healthcare facilities, CA-MRSA infections can occur in individuals without any previous healthcare exposure. This shift in MRSA epidemiology highlights the importance of targeted prevention strategies and surveillance efforts to control the spread of resistant bacteria in healthcare and community settings.

Globally, the spread of MRSA remains a serious concern, with studies reporting rising prevalence across diverse populations and environments.<sup>6</sup> MRSA's ability to colonize different niches, including the nasal cavity, skin, and wounds, has contributed to its persistence and dissemination.<sup>7</sup> Its evolutionary history is shaped by the acquisition of resistance determinants and

genetic elements that confer survival advantages under antibiotic pressure. The antimicrobial susceptibility patterns of MRSA strains vary geographically, necessitating region-specific strategies for antimicrobial stewardship and prevention of infection. MRSA is a serious issue in Saudi Arabia, where reports of its prevalence vary by geography and hospital setting.<sup>8-10</sup> Some studies have reported MRSA prevalence as high as 82.5% in certain healthcare facilities in Saudi Arabia.<sup>8</sup> Furthermore, studies have highlighted the emergence of new clonal complexes and MRSA strains in the country.<sup>11,12</sup> This highlights the dynamic nature of MRSA epidemiology in Saudi Arabia and emphasizes the need for continuous surveillance and monitoring.<sup>12</sup> MRSA is a significant concern in Saudi Arabia, with varying prevalence rates and the emergence of new strains. This dynamic nature emphasizes the importance of ongoing surveillance and monitoring to combat the spread of MRSA in healthcare facilities effectively. Region-specific antimicrobial stewardship and infection prevention are crucial for addressing this serious issue and reducing the impact of MRSA on public health in Saudi Arabia.

Saudi Arabia has a high rate of methicillin-resistant *S. aureus* (MRSA) infection, which has a major effect on healthcare costs, morbidity, and mortality.<sup>13,14</sup> MRSA infections have been linked to several clinical disorders such as hospital-acquired infections and infections of soft tissues, wounds, and skin.<sup>14</sup> In addition, the frequency of MRSA infections has steadily increased in the country.<sup>13</sup> These findings highlight the importance of implementing effective infection control measures and antimicrobial stewardship programs to mitigate the spread of MRSA in healthcare settings.<sup>9,15</sup> These programs can help reduce the incidence of MRSA infections, improve patient outcomes, and reduce healthcare costs. To combat the rising trend of MRSA in Saudi Arabia, it is crucial for healthcare facilities to prioritize infection control practices such as proper hand hygiene, disinfection protocols, and appropriate use of antibiotics. By proactively addressing these issues, healthcare providers can improve the overall health and well-being of the Saudi Arabian population.

Continued surveillance and monitoring of antimicrobial resistance patterns is essential

for guiding treatment decisions and preventing the spread of resistant strains. Healthcare professionals should prioritize infection prevention measures such as hand hygiene and proper disinfection practices to minimize the risk of MRSA transmission in healthcare settings. Collaborative efforts among healthcare providers, public health authorities, and researchers are crucial for developing and implementing effective strategies to address the growing threat of MRSA and other antimicrobial-resistant infections in Saudi Arabia. Moreover, studies have identified distinct antimicrobial resistance patterns of MRSA, highlighting the need for comprehensive, coordinated responses to this public health challenge.<sup>14,16,17</sup> The emergence of MRSA strains with reduced susceptibility to glycopeptides further complicates clinical management of MRSA infections.<sup>18</sup>

## MATERIALS AND METHODS

### Study design and settings

This retrospective study included patients from our 450-bed tertiary care hospital. The hospital established standards for culture, sensitivity of sample collection, and aseptic transport to the laboratory. All patient data were entered into the hospital's electronic system. Microbiological and antimicrobial susceptibility testing were performed by the Microbiology Department of MD Lab, Riyadh, Saudi Arabia.

### Ethics approval

This study was approved by the Research Committee of Dr. Sulaiman Al Habib Medical Group Research Center, Riyadh, Saudi Arabia, vide reference number RC24.05.13.

### Data collection

Data was collected and analyzed retrospectively from January to December 2023.

### Inclusion criteria

This study included all non-duplicate samples submitted for sensitivity and culture from patients of all ages who were registered inpatients or outpatients at this institution.

### Exclusion criteria

This study excluded all duplicate, repeat, or follow-up cultures performed within 7 days of the initial culture request.

### Statistical analysis

Statistical analysis was performed using Excel and IBM SPSS version 20. Statistical significance was set at  $p < 0.05$  significant.

### Study protocol

Clinical samples from various body sites, including pus, blood, urine, sputum, body fluids, wound swabs, tracheal aspirates, were plated on appropriate media following the standard operating procedures (SOPs) to process each sample. Culture plates were incubated at 37 °C for 24 to 48 hours. Using VITEK 2, all cultures with significant *S. aureus* growth were phenotyped and tested for antibiotic susceptibility. Antimicrobial susceptibility testing was performed using the Standard Kirby-Bauer disc diffusion method as per the M100 CLSI criteria. All *S. aureus* isolates were screened for MRSA using a ceftioxin (FOX 30 µg) disc. This was further confirmed by measuring oxacillin resistance using the VITEK 2 system.

Antibiotics tested were penicillin (PG 10 units), oxacillin (OX 1 µg), ciprofloxacin (CIP 5 µg), levofloxacin (LEV 5 µg), erythromycin (E 15 µg), clindamycin (CD 2 µg), trimethoprim/sulfamethoxazole (TS 1.25/23.75 µg), gentamicin (GM 10 µg), fusidic acid (FC 10 µg), tetracycline (T 30 µg), vancomycin (VA 30 µg), and linezolid (LZD 30 µg).

## RESULTS

During the study period, 11,837 culture specimens were collected. Of these, 3795 (32%) yielded positive bacterial cultures with susceptibility results. *S. aureus* was detected in 333 cases, representing 2.8% of culture requests and 8.8% of positive cultures. The overall frequency of *S. aureus* isolation in males was 180 (54.1%), whereas in females it was 153 (45.9%). Among the 333 cases, 259 (77.8%) were recorded as outpatients in various OPD clinics, while the remaining 74 patients (22.2%) were admitted

**Table 1.** Association of MSSA and MRSA isolates (n = 333) with gender, age, patient registration and specimen type

Variables		MSSA Count (%)	MRSA Count (%)	P-value
<b>Gender</b>	Male	118 (65.5%)	62 (34.4%)	0.258
	Female	91 (59.4%)	62 (40.5%)	
<b>Age group</b>	Less than 12 years	40 (65.5%)	21 (34.4%)	0.475
	12 to 40 years	73 (60.3%)	48 (39.6%)	
	41 to 60 years	41 (57.7%)	30 (42.2%)	
	More than 61 years	55 (68.7%)	25 (31.2%)	
<b>Patient reg.</b>	OP	164 (63.3%)	95 (36.6%)	0.685
	IN	45 (60.8%)	29 (39.1%)	
<b>Specimen</b>	Blood	12 (75%)	4 (25%)	<0.001
	Body fluids	8 (72.7%)	3 (27.2%)	
	Conjunctival swabs	1 (100%)	0 (0%)	
	Ear swabs	26 (83.8%)	5 (16.1%)	
	HVS	1 (100%)	0 (0%)	
	Nasal swabs	8 (57.1%)	6 (42.8%)	
	Sputum/tracheal aspirates	13 (50%)	13 (50%)	
	Pus and wound	122 (58.9%)	85 (41%)	
	Urine	18 (69.2%)	8 (30.7%)	

MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, Patient reg: Patient registration, OP: Outdoor patients, IN: Indoor patients HVS: High vaginal swab

as inpatients. Patient ages ranged from 1 day to 105 years, with a mean of  $39.5 \pm 25.41$  standard deviation (SD). The highest frequency of *S. aureus* isolation pertained to individuals with ages 12 to 40 years, with 121 cases (36%), followed by those older than 61 years (80 cases; 24%). The isolates were most commonly detected in pus and wound specimens (n = 207, 62.2%), followed by ear swabs (n = 31, 9.3%), sputum/tracheal aspirates, and urine (n = 26, 7.8% each).

The prevalence of MRSA was found to be 3.3% in our local healthcare settings. Among the 333 *S. aureus* samples, the isolation of MRSA was 124 (37.2%), while the isolation of MSSA accounted for 209 (62.8%). Table 1 shows the association of MRSA with patients' sex, age, registration in the hospital, and the type of specimen collected. The prevalence of MRSA in male patients was 34.4% compared to 40.5% in female patients, with a p-value = 0.258. No significant correlation was observed between MRSA isolation and sex. MRSA isolation was highest in 12-40 year olds (48 cases). This was followed by 30 cases in the 41-60-year age group. MRSA isolation was not significantly associated with age (P = 0.475).

Among cases occurring outdoors, 95 (36.7%) were reported as MRSA, whereas among

indoor patients, there were 29 (39.2%) cases of MRSA. The frequency of MRSA infection has somewhat increased among hospitalized patients. However, there is no statistically significant link between the isolation of MRSA and patient hospitalization, as indicated by a p-value >0.05 (0.685), as shown in Table 1.

However, the presence of *S. aureus* exhibited a robust correlation with specimen type. A total of 207 cases were recorded from pus and wound cultures, with a statistically significant p-value <0.001. MRSA isolation was found to be significantly correlated with the specimen type. The maximum isolation was observed in pus and wounds (85 of 124 cases), with a p-value of 0.021 (<0.05), as shown in Table 1.

Table 2 presents the antibiograms of all the tested antibiotics. Vancomycin and linezolid demonstrated 100% effective against all *S. aureus* isolates, resulting in a 0% occurrence of VRSA or VISA isolation in our clinical settings. Tetracycline and gentamicin ranked second highest in efficacy against *S. aureus* infections, with susceptibility rates of 95.8% and 94.9%, respectively. Clindamycin, co-trimoxazole, levofloxacin, ciprofloxacin, erythromycin, and fusidic acid followed with susceptibility rates of 86.3%, 84.4%, 82.3%, 81.7%,

**Table 2.** Antimicrobial susceptibility pattern of *S. aureus* (n = 333) to the tested antibiotics

Antibiotics	Antibiogram	Count	Percentage
Penicillin	susceptible	13	3.9%
	resistant	320	96.1%
Oxacillin	susceptible	209	62.8%
	resistant	124	37.2%
*Erythromycin	susceptible	203	66.1%
	resistant	104	33.8%
*Clindamycin	susceptible	265	86.3%
	resistant	42	13.6%
Gentamicin	susceptible	319	95.8%
	resistant	14	4.2%
Ciprofloxacin	susceptible	272	81.7%
	resistant	61	18.3%
Levofloxacin	susceptible	274	82.3%
	resistant	59	17.7%
Tetracycline	susceptible	316	94.9%
	resistant	17	5.1%
Co-trimoxazole	susceptible	281	84.4%
	resistant	52	15.6%
Vancomycin	susceptible	333	100.0%
	resistant	0	0.0%
Linezolid	susceptible	333	100.0%
	resistant	0	0.0%
Fusidic acid	susceptible	143	42.9%
	resistant	190	57.1%

\*Erythromycin and clindamycin were not tested for urinary isolates

66.1%, and 42.9%, respectively. All gentamicin-resistant cases were MRSA isolates, whereas no resistance was observed against gentamicin in the MSSA isolates, as shown in Figure.

Based on their multidrug-resistance patterns, 33.87% (n = 42/124) of MRSA isolates were classified as HA-MRSA, while the majority of isolates i.e., 66.12% (n = 82/124) were CA-MRSA. The D-test was used to assess 307 *S. aureus* samples for inducible clindamycin resistance. However, urinary isolates were not included in this test. A total of 28 isolates tested positive in the D-test, with 12 cases of MSSA and 16 cases of MRSA. A statistically significant correlation was observed between the *S. aureus* resistant phenotype and the D-test, with a p-value of 0.039 (Table 3).

**Table 3.** Inducible clindamycin resistance (D-test) association with MSSA and MRSA phenotypes of *S. aureus* (n = 307)

	D-test		Total
	Negative n (%)	Positive n (%)	
MSSA	179 (93.7%)	12 (6.2%)	191
MRSA	100 (86.2%)	16 (13.7%)	116
Total	279 (91.1%)	28 (9.1%)	307

P-value = 0.039

## DISCUSSION

The study period involved the analysis of 11,837 culture specimens, of which 3795 (32%) tested positive for bacterial growth and susceptibility. The most common specimens yielding *S. aureus* were pus and wound specimens (62.2%). Our result is supported by another study, which reported 64.8% isolation of *S. aureus* from pus and wound cultures.<sup>19</sup> Consistent with our findings, Naimi et al. found no statistically significant association between MRSA isolation age and patient sex.<sup>20</sup>

Several studies have examined the frequency of local MRSA infections. In our setting, MRSA accounted for 3.3% of all isolates. A literature survey showed that the prevalence rates vary by time and place. Studies have reported MRSA prevalence rates ranging from 4% in a hospitals in western Saudi Arabia<sup>21</sup> to as high as 39.5% in the western region.<sup>22</sup> Moreover, a meta-analysis found 38% MRSA prevalence in Saudi Arabia.<sup>23</sup> Variations in prevalence rates were also evident within different cities, with MRSA prevalence ranging from 5.97% to 94% in Dhahran and Riyadh, respectively.<sup>24</sup> Additionally, one study found that CA-MRSA infections increased from 9.9 per 10,000 admissions in 2001 to 67 in 2008.<sup>13</sup>

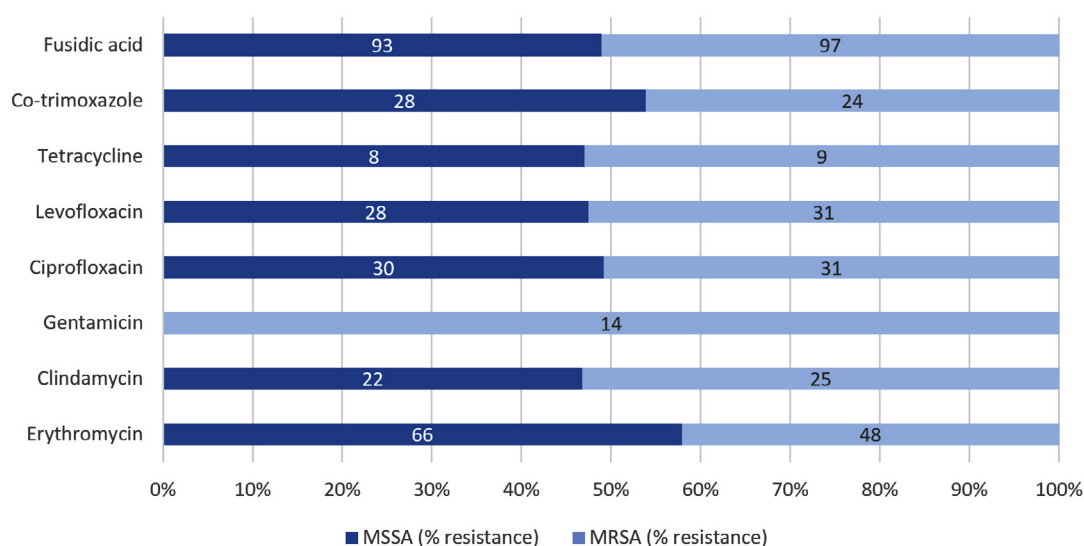
The prevalence of MRSA in Saudi Arabia was investigated in a specific healthcare setting. Similarly, a study conducted in Jeddah reported a prevalence of community-acquired MRSA at 25.2%.<sup>25</sup> Similarly, a study from a tertiary hospital in Riyadh found the prevalence of MRSA to be

39%.<sup>15</sup> These findings highlight the importance of understanding MRSA prevalence in various healthcare settings in Saudi Arabia. Notably, the prevalence of MRSA has varied over time. For instance, a study reported a significant increase in the prevalence of MRSA from 29.9% to 38% over a certain period.<sup>23</sup> Similarly, another study found that the prevalence of MRSA in Saudi Arabia ranged from 7.5% to 33%.<sup>26</sup> These variations underscore the dynamic nature of the MRSA prevalence and the need for ongoing surveillance and research. In conclusion, the prevalence of MRSA in Saudi Arabia varies across regions and healthcare settings. The reported prevalence rates range from 4% to 39.5%, with significant increases observed over time. These findings emphasize the importance of continued monitoring and research to better understand the epidemiology of MRSA in Saudi Arabia.

The presence of *S. aureus* showed a significant correlation with the type of specimen, particularly pus and wound cultures, indicating a strong association between the presence of *S. aureus* and these types of specimens.<sup>27</sup> Moreover, MRSA isolation showed a statistically significant correlation with specimen type, with the highest number of isolates recovered from pus and wound samples.<sup>28</sup> The study found a significant increase in MRSA in 2012 compared to 2004-

2005.<sup>28</sup> This study supports these findings by highlighting the isolation of *S. aureus* from various clinical specimens, with the highest isolation rate observed in pus samples.<sup>29</sup> Methicillin-resistance in *S. aureus* can be traced to the acquisition of the exogenous gene *mecA*, which is part of the staphylococcal cassette chromosome *mec* (SCC*mec*). This *mecA* gene encodes an additional penicillin-binding protein, PBP2a. PBP2a is a peptidoglycan transpeptidase that can confer resistance to all  $\beta$ -lactam antibiotics, including penicillins, cephalosporins, and carbapenems. Other *S. aureus* isolates may exhibit a broader range of resistance owing to the presence of additional resistance genes within specific SCC*mec* types, such as types II and III. The presence of PBP2a or *mecA* is the basis for the identification of MRSA or oxacillin-resistant *S. aureus* (ORSA).<sup>30</sup>

Based on the provided references, it is evident that vancomycin and linezolid have consistently demonstrated complete susceptibility against *S. aureus*.<sup>31-34</sup> This aligns with our findings that there was a 0% occurrence of vancomycin-resistant *S. aureus* (VRSA) or vancomycin-intermediate *S. aureus* (VISA) isolation in our clinical settings. Additionally, gentamicin and tetracycline have shown high efficacy in treating *S. aureus* infections, with susceptibility rates of 95.8% and 94.9%, respectively.



**Figure.** Comparison of antimicrobial percent resistance of MSSA and MRSA to the different antibiotics



The majority of MRSA isolates were classified as CA-MRSA, while HA-MRSA accounted for 33.87%. This distribution is consistent with findings from another region of Saudi Arabia, where 80% of MRSA isolates were reported as CA-MRSA.<sup>35</sup> Inducible clindamycin resistance has been found in many *S. aureus* isolates, with a statistically significant correlation between the resistance phenotype and D-test.<sup>33,36-38</sup> The D-test remains a reliable method for detecting inducible clindamycin resistance in *S. aureus*.<sup>39</sup> Among other antibiotics tested, clindamycin, co-trimoxazole, levofloxacin, ciprofloxacin, erythromycin, and fusidic acid showed varying susceptibility levels, with clindamycin demonstrating a susceptibility rate of 86.4%.<sup>36,40</sup> These findings have important clinical implications, especially in light of the limited therapeutic options available for the treatment of MRSA infections and the known limitations of vancomycin.<sup>38</sup>

## CONCLUSION

In conclusion, the introduction of methicillin in the 1960s marked a turning point in the battle against *Staphylococcus aureus* infections, but also led to the emergence of MRSA strains that pose significant challenges to healthcare systems worldwide. The evolution of MRSA, from early detection to the development of multidrug-resistant strains, underscores the urgent need for comprehensive strategies to combat the spread of these bacteria. Understanding the epidemiology, transmission dynamics, and resistance mechanisms of MRSA is essential for guiding infection control practices, antimicrobial stewardship efforts, and for developing novel treatment options to address infections caused by resilient pathogens. By remaining informed and proactive in managing MRSA infections, healthcare professionals can help prevent the further spread of these antibiotic-resistant bacteria. Collaboration among healthcare facilities, researchers, and public health agencies is crucial to effectively address the challenges posed by MRSA. Through collective effort and focus on evidence-based practices, we can work to reduce the impact of MRSA on patient outcomes and healthcare systems worldwide.

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None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

FK conceptualized and designed the study. FK, IA, AE and CP performed data collection. FK, AA and HA performed analysis and results interpretation. FK and OTK wrote the manuscript. All authors read and approved the final manuscript for publication.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This study was approved by the Research Committee of Dr. Sulaiman Al Habib Medical Group Research Center, Riyadh, Saudi Arabia, vide reference number RC24.05.13.

## INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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