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**Research Article**

# A study on Antibiotic Sensitivity Pattern among Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates

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**Article Info**

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## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major cause of healthcare- and community-associated infections worldwide. Its capacity to acquire multiple resistance determinants continues to challenge empirical management strategies, particularly in resource-limited settings. The aim of the study was to evaluate the antimicrobial sensitivity pattern of MRSA isolates from heterogeneous clinical samples. A cross-sectional study was conducted over six months in the Microbiology laboratory of a tertiary-care hospital in Central Tamilnadu, India. A total of 97 MRSA isolates were identified using standard biochemical tests and the cefoxitin disc diffusion test. Antimicrobial susceptibility testing was performed using the Kirby–Bauer method. D-testing was carried out for all erythromycin-resistant isolates. Data were analysed using descriptive statistics. A total of 120 *Staphylococcus aureus* were isolated, from which 97 MRSA isolates were identified. MRSA isolation was higher among inpatients (63.2%) and most common in individuals aged 42–72 years. The majority of isolates (70.10%) were recovered from pus and wound swabs. Abscesses and wound ulcers accounted for the largest proportion of clinical presentations. Inducible clindamycin resistance was detected in 32% of isolates. Penicillin (82.3%) and ciprofloxacin (76.0%) showed high resistance rates, whereas linezolid (85.4%), doxycycline (79.2%), and clindamycin (71.9% among D-test negative isolates) remained the most effective agents. MRSA isolates in this study exhibited multidrug-resistant patterns. The high proportion of inducible clindamycin resistance highlights the necessity of routine D-testing. Continued surveillance and antimicrobial stewardship are essential to guide effective therapy and limit MRSA transmission in healthcare settings.

**Keywords:** *Staphylococcus aureus*, methicillin resistant - Antibiotic Sensitivity Pattern, inducible resistance, Antimicrobial stewardship.

## Introduction

*Staphylococcus aureus* remains one of the most versatile and clinically important human pathogens. It is capable of producing a broad spectrum of infections, ranging from minor superficial skin lesions to deep-seated abscesses, pneumonia, bacteraemia, endocarditis, osteomyelitis, and device-associated infections [1]. The organism has adapted remarkably to selective pressure exerted

by antimicrobial agents, and its ability to acquire resistance determinants has transformed the management of staphylococcal infections over the past several decades. The emergence of methicillin-resistant *S. aureus* (MRSA) in the early 1960s marked a turning point in hospital infection control. Initially confined to healthcare settings, MRSA soon evolved into a major nosocomial pathogen associated with significant morbidity, prolonged hospitalization, and increased mortality [2].

Over time, strains possessing the mecA gene—which encodes the altered penicillin-binding protein PBP2a and confers resistance to all β-lactam antibiotics—began to appear outside hospitals, giving rise to community-associated MRSA (CA-MRSA) infections [3,4]. These strains, often linked to skin and soft-tissue infections, have increasingly been reported among healthy individuals without traditional risk factors, contributing to the changing epidemiology of MRSA worldwide.

Today, MRSA continues to be one of the most challenging multidrug-resistant pathogens encountered in clinical practice. In many regions, it accounts for a substantial proportion of S. aureus isolates recovered from healthcare settings, and in several countries, hospital-acquired MRSA (HA-MRSA) remains a leading cause of healthcare-associated infections [5]. Factors such as invasive procedures, extended hospital stays, injudicious antibiotic usage, and carriage among healthcare workers facilitate transmission within hospitals. The burden is particularly significant in low- and middle-income countries, where diagnostic resources and infection-control measures may be limited.

Early detection of MRSA and timely determination of its antimicrobial susceptibility profile are essential for optimizing clinical outcomes. With therapeutic options becoming increasingly restricted, the value of periodic surveillance studies cannot be overstated. Understanding local resistance patterns helps clinicians choose appropriate empirical therapy, assists microbiologists in revising laboratory protocols, and provides public health authorities with data required to formulate evidence-based antimicrobial stewardship policies [6]. The present study was undertaken to determine the antimicrobial susceptibility profile of MRSA isolates obtained from diverse clinical specimens in our institution, using standardized methods in accordance with the recent Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. By mapping current resistance trends, we aim to support rational antibiotic use, guide empirical therapy, and contribute to strengthened infection-control strategies.

## Material and Methods

**Ethics statement**: The present study was prepared as per the Institutional Research format and approved by the Institutional Ethics Committee (1170/TSRMMCH&RC/ME-1/2024 – IEC No: 147 dated 18.10.2024), and the ethical requirement for informed consent was waived.

**Study Design and samples**: This cross-sectional study was conducted in the Department of Microbiology of a tertiary-care hospital in central Tamil Nadu (India) over six months. All Microbiological procedures were performed in the Bacteriology laboratory following standard protocols.

**Inclusion Criteria:** All clinically significant MRSA *S. aureus* strains isolated from various clinical specimens were included in this study

**Exclusion Criteria:** Clinical specimens yielding growth of Gram-positive cocci other than *S. aureus*, duplicate isolates, and contaminants were excluded.

**Clinical sample processing:** Samples such as pus, wound swabs, urine, sputum, ET aspirates, and blood were collected aseptically and processed within 2 hours. Preliminary identification was done by Gram smear, which showed Gram-positive cocci arranged in clusters, catalase positive, oxidase negative, and slide and tube coagulase tests showing clump and clot formation at the bottom of the tube. Specimens were then inoculated on blood agar, MacConkey agar, nutrient agar, and mannitol salt agar plates, and incubated at 35–37°C for 18–24 hours. The nutrient agar plate showed 1-3mm, circular, smooth, convex, opaque, easily

emulsifiable colonies producing a golden yellow non-diffusible pigment. Blood agar showed a narrow zone of Beta-haemolytic colonies, while MacConkey agar showed small pink colonies due to lactose fermentation. Mannitol salt agar showed red to yellow colonies due to mannitol fermentation. This confirmed the organism to be *S. aureus*.

**Antibiotic sensitivity testing**: Antibiotic sensitivity testing was done to confirm *S. aureus* as MRSA or MSSA using the cefoxitin disc diffusion method (surrogate marker). Antibiotic susceptibility testing determines the in vitro susceptibility of *S. aureus* isolates to antimicrobial agents to guide appropriate therapy. In this study, the antibiotic disc diffusion method and MIC for vancomycin by E-test were performed and confirmed by an automated system (VITEK).

The *S. aureus* inoculum was suspended and adjusted to a 0.5 McFarland standard, then inoculated onto Mueller-Hinton agar for lawn culture using the Kirby–Bauer disc diffusion method. The following agents were tested: cefoxitin, penicillin, ciprofloxacin, gentamicin, erythromycin, clindamycin, doxycycline, trimethoprim–sulfamethoxazole, vancomycin, and linezolid. Plates were incubated at 35–37°C for 16 to 18 hours. MRSA was identified using the cefoxitin (30 µg) disc diffusion method, where a zone diameter of ≤22 mm was interpreted as methicillin resistance as per CLSI M100 35$^{th}$ edition (2025) guidelines [7]. Zones of inhibition for other agents were also measured using CLSI M100 35$^{th}$ edition (2025) guidelines, using appropriate ATCC controls for *S. aureus* (ATCC 25923) [7].

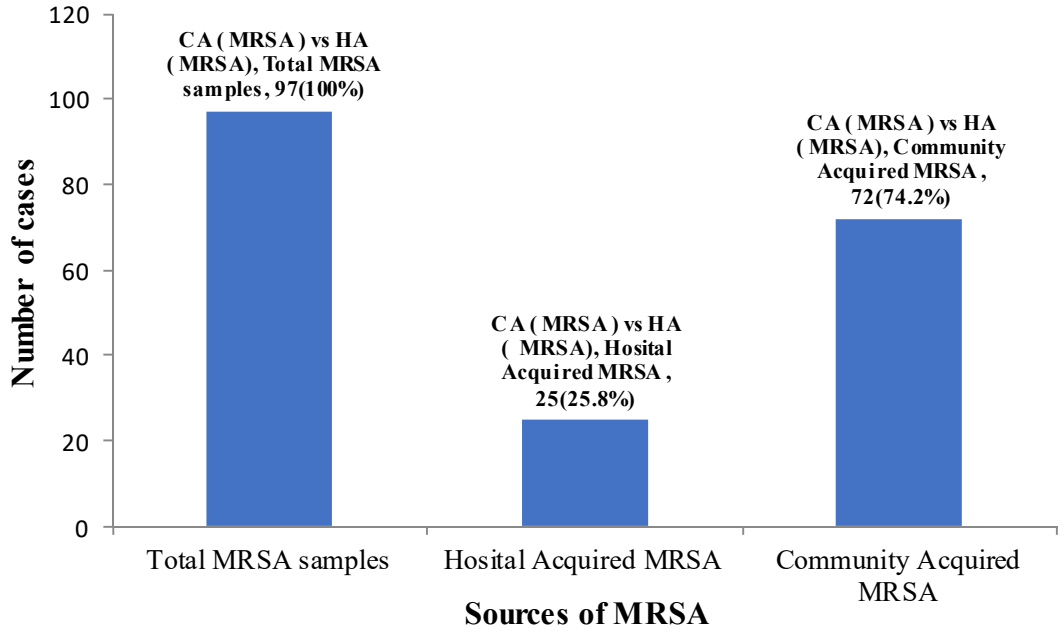
**MRSA screening:** Inducible clindamycin resistance was detected using the D-test with erythromycin and clindamycin discs placed 15 mm apart. MICs for vancomycin were performed using the E-test.

**Statistics**: Data analysis was performed using SPSS version 26.0. Continuous variables were assessed for normality; descriptive statistics were expressed as frequencies and percentages. The association between categorical variables was analyzed using the Chi-square test. The difference in the proportion of isolates between CA-MRSA and HA-MRSA sources was statistically significant ($p<0.005$). Similarly, the difference between D-test positive and negative isolates was statistically significant ($p<0.005$). A two-sided p-value <0.005 was considered statistically significant.

## Results

A total of 120 *Staphylococcus aureus* isolates were processed during the study period based on the preliminary identification. Among these, 97 samples yielded MRSA isolates, representing the confirmed MRSA-positive cases. This indicates that MRSA was isolated from a substantial proportion of the clinical specimens, reflecting its significant burden within the heterogeneous sample pool. Of the 97 MRSA samples analyzed, 25 isolates (25.8%) were identified as Hospital-Acquired MRSA (HA-MRSA), whereas the majority, 72 isolates (74.2%), were Community-Acquired MRSA (CA-MRSA). The findings indicate a predominance of community-associated MRSA compared to hospital-associated strains in the present study population (Figure 1).

**Figure 1:** CA (MRSA) vs HA (MRSA)




The distribution of isolates across the various age categories demonstrated a clear predominance among adults and middle-aged individuals. No isolates were recovered from the newborn (<28 days) or infant (1 to 23 months) age groups, indicating that the organism was not detected in the earliest stages of life within the study population. Among children (2 to 12 years), only 2 isolates (2.1%) were obtained, suggesting a relatively low burden in this age group. The prevalence increased progressively with age, with adolescents (13 to 18 years) accounting for 6 isolates (6.2%). A substantial rise in the number of isolates was observed among young adults (19 to 24 years), contributing 22 isolates (22.7%), highlighting early adulthood as a significant period of clinical presentation (Table 1).

**Table 1.** Age-wise Distribution of MRSA Isolates (MeSH Criteria)

| Category | Age Group (years) | No. of Isolates (n=97) |
|---|---|---|
| Child | 2-12 years | 2 (2.1) |
| Adolescent | 13-18 | 6 (6.2) |
| Young adult | 19-24 | 22 (22.7) |
| Adult | 25-44 | 24 (24.7) |
| Middle Aged | 45- 64 | 30 (30.9) |
| Aged | >65 | 13 (13.4) |

[Values in parenthesis represent percentages]

The adult age group (25 to 44 years) comprised 24 isolates (24.74%), while the middle-aged group (45–64 years) represented the highest proportion with 30 isolates (30.93%), indicating that middle-aged individuals formed the major affected demographic in this cohort. The aged population (>65 years) accounted for 13 isolates (13.40%), demonstrating a noticeable but comparatively lower proportion in the elderly. Overall, the distribution pattern shows minimal occurrence in pediatric groups with a steady rise in adolescence and young adulthood, peaking in middle age. This trend suggests possible age-related exposure, comorbidity patterns, or risk factors contributing to the isolation frequency across adult and older age groups.

The majority of isolates were obtained from pus/wound swabs (70.10%), followed by sputum (12.37%), urine (10.31%), and blood samples (7.22%) (Table 2). This indicates that most infections in the study were associated with skin and soft tissue sources.

**Table 2.** Specimen-wise Distribution of MRSA Isolates

| Specimen Type | No. of Isolates (n=97) |
|---|---|
| Pus / Wound Swab | 68 (70.1) |
| Sputum | 12 (12.4) |
| Urine | 10 (10.3) |
| Blood | 7 (7.2) |

[Values in parenthesis represent percentages]

D-test evaluation showed that 31 isolates (32.0%) demonstrated inducible clindamycin resistance (D-test positive), while 66 isolates (68.0%) were D-test negative (Table 3). Overall, inducible resistance was observed in nearly one-third of the isolates. While comparing the MRSA by D-test with HA and CA, no observable variations were recorded. Inducible clindamycin resistance detected by D-test was observed in 22 (30.6%) of CA-MRSA isolates and 8 (32.0%) of HA-MRSA isolates. D-test negativity, indicating true susceptibility, was noted in 69.4% and 68.0% of isolates, respectively. Although inducible resistance was marginally higher among hospital isolates, the difference between CA-MRSA and HA-MRSA was not statistically significant ($p > 0.005$).

**Table 3:** D-Test Findings

| D-Test Result | No. of Isolates (n=97) | |
|---|---|---|
| | CA-MRSA isolates (n=72) | HA-MRSA isolates (n=25) |
| Positive | 22 (30.6) | 08 (32) |
| Negative | 50 (69.4) | 17 (68) |

[Values in parenthesis represent percentages]

Further, the sensitivity pattern of MRSA was analyzed. The highest sensitivity was observed for linezolid (85.4%), followed by doxycycline (79.2%) and clindamycin (71.9%) (Table 4).

**Table 4.** Antimicrobial Susceptibility Profile of MRSA Isolates (n = 97)

| Antibiotic | Sensitive (%) | Resistant (%) |
|---|---|---|
| Cefoxitin | 0.0 | 100 |
| Penicillin | 17.7 | 82.3 |
| Ciprofloxacin | 22.9 | 76.0 |
| Erythromycin | 57.0 | 43.0 |
| Clindamycin* | 71.9 | 28.1 |
| Doxycycline | 79.2 | 20.8 |
| Linezolid | 85.4 | 14.6 |
| Vancomycin (E strip method) | 62.0 | 38.0 |

*Interpretation based on D-test negative isolates

Vancomycin susceptibility testing for *S. aureus* isolates was performed by MIC determination using the E-test method in accordance with CLSI M100 35th edition guidelines (2025). Disc diffusion results were not used for final interpretation due to the poor correlation between zone diameter and MIC values and the inability to reliably detect VISA (Vancomycin-intermediate *S. aureus*) and hVISA (heteroresistant) strains. MIC breakpoints were interpreted as susceptible (≤2 µg/mL), intermediate (4–8 µg/mL), and resistant (≥16 µg/mL).

## Discussion

In this study, MRSA accounted for a substantial proportion of the *Staphylococcus aureus* isolates processed, reflecting its sustained burden as a major healthcare challenge. The predominance of isolates in adults, particularly in the middle-aged group, is consistent with reports demonstrating higher MRSA prevalence among individuals with greater healthcare exposure, occupational risk, or evolving comorbidities

[2,8]. The minimal isolation from paediatric age groups further supports published observations that MRSA infections tend to increase with age due to cumulative exposure to healthcare environments and antimicrobial agents [2,5,9].

A significant number of MRSA samples came from pus or wound swabs, highlighting its known link to skin and tissue infections. Other research in India and abroad has shown comparable results, pointing to these infections as common sources of MRSA detection [10-12]. In areas where clinics handle many wound cases yet lack timely care measures, this pattern becomes even more noticeable.

Inducible clindamycin resistance appeared in 32% of MRSA samples, underlining why regular D-testing matters. Missing these strains can lead to treatment issues because of resistance activation. This rate aligns with past reports showing similar levels, between 20% [13] and 40% [14] in Indian MRSA cases. Results like these support the careful use of clindamycin, reserving it for when D-tests clearly show sensitivity. The proportion of D-test–positive isolates, though lower than the D-test negative group, still highlights the necessity of routine D-testing in diagnostic laboratories. Even a moderate prevalence of iMLSB resistance warrants caution, given the role of clindamycin as an important therapeutic agent for MRSA infections. Overall, these findings reinforce the importance of incorporating the D-test into routine antimicrobial susceptibility testing to prevent misclassification of clindamycin susceptibility and to guide more effective clinical management of HA-MRSA infection [8,13]. The antimicrobial resistance pattern observed in this study indicates that multidrug-resistant MRSA remains prevalent in our hospital setting. A high rate of penicillin and fluoroquinolone resistance matches nationwide trends linked to frequent use of β-lactams and fluoroquinolones, fuelling stronger resistance patterns [5,15]. On the other hand, sensitivity to linezolid and doxycycline aligns with results reported by several hospitals in India, where both drugs are still effective against MRSA infections [16,17]. Still, lower sensitivity to vancomycin seen in early tests is significant. Even though true VRSA (Vancomycin-Resistant *S. aureus*) cases are uncommon, rising numbers of VISA (Vancomycin-intermediate *S. aureus*) and elevated MIC levels call for close tracking of MIC results—alongside cautious antibiotic use—to avoid worsening trends [18,19].

The present study has certain limitations. First, it was a hospital-based cross-sectional study conducted in a single centre over a six-month period; therefore, the results may not fully represent the community-wide prevalence of MRSA in the region. Second, molecular characterization (such as SCCmec typing) was not performed due to resource constraints, which limits the ability to confirm the genetic relatedness of the strains.

Overall, these findings underscore the necessity for strengthened antimicrobial stewardship, rigorous infection-control measures, and continuous resistance surveillance. Integration of molecular epidemiology in future studies may provide further insights into strain dynamics, transmission pathways, and the distinction between community- and hospital-associated MRSA clones [20].

MRSA continues to be a significant clinical challenge, particularly in hospitalized adults and patients presenting with wound-related infections. The high levels of resistance observed to beta-lactams, fluoroquinolones, and other commonly used agents highlight the persistent burden of multidrug-resistant strains in the hospital setting. At the same time, the preserved activity of linezolid, doxycycline, and clindamycin (among D-test negative isolates), followed by vancomycin, offers viable therapeutic options for appropriate clinical scenarios. The identification of inducible clindamycin resistance in nearly one-third of isolates reinforces the need for routine D-testing in diagnostic laboratories. Overall, these findings underscore the importance of continuous local surveillance. Strict infection-control measures—including proper hand hygiene, environmental cleaning, and adherence to isolation precautions—are essential to

prevent the transmission of HA-MRSA and CA-MRSA. Robust antimicrobial stewardship is also critical to limit the spread of antimicrobial resistance and maintain the effectiveness of available therapeutic agents.

**Authors' contributions:**

**Kavya MS:** Investigation, writing, initial edition

**Diego Edwin:** Supervision, Conceptualization, methodology, data curation and final editing

**Chitra Rajalakshmi P**: Co-supervision, data management and analysis, initial editing

**Prabhusaran N**: Data curation, methodology, review writing, final drafting

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