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VANCOMYCIN AND DAPTOMYCIN SUSCEPTIBILITY PATTERN AMONG CLINICAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant cause of hospital- and community-acquired infections. Due to the emergence of vancomycin-intermediate and vancomycin-resistant strains, the effectiveness of vancomycin—traditionally the drug of choice—is being challenged. Daptomycin has emerged as a potent alternative for MRSA treatment. This study evaluates the minimum inhibitory concentrations (MICs) of vancomycin and daptomycin among MRSA clinical isolates.

Methods: A total of 209 *S. aureus* strains were isolated from various clinical specimens and identified using standard microbiological procedures. Methicillin resistance was determined via cefoxitin disc diffusion, while susceptibility to vancomycin and daptomycin was assessed using the E-test strip method, following CLSI guidelines.

Results: Out of 209 *S. aureus* isolates, 108 (52%) were identified as MRSA, and 101 (48%) as methicillin-sensitive *S. aureus* (MSSA). All MRSA isolates were susceptible to both vancomycin and daptomycin.

Conclusion: MRSA prevalence remains high, emphasizing the need for continued surveillance. Both vancomycin and daptomycin remain effective against MRSA isolates. However, given the global emergence of resistant strains, judicious antibiotic use and continuous monitoring are essential.

Keywords: *Staphylococcus aureus*, MRSA, VISA, VRSA, Vancomycin, Daptomycin

Introduction

Staphylococcus aureus is a Gram-positive, facultatively anaerobic coccus that colonizes human skin and mucous membranes and is a major cause of community-acquired and hospital-associated infections [1]. These infections can vary from simple skin and soft tissue infections to potentially fatal

illnesses like bacteremia, endocarditis, pneumonia, osteomyelitis, and device-related infections [2]. *S. aureus's* propensity to develop resistance to numerous antimicrobial treatments has made it a formidable pathogen in therapeutic settings [3]. One of the most significant resistance mechanisms found in *S. aureus* is methicillin resistance, which is imparted by the mecA gene, which encodes an altered penicillin-binding protein (PBP2a) with a lower affinity for beta-lactam antibiotics [4]. Methicillin-resistant *S. aureus* (MRSA) was originally identified in the 1960s, and it has now become a global public health concern. MRSA prevalence in India varies from 25% in the west to more than 50% in the south. The introduction and extensive spread of MRSA strains has considerably limited treatment choices and raised the burden on healthcare systems due to longer hospital stays, higher expenses, and higher morbidity and mortality rates [5]. Traditionally, vancomycin, a glycopeptide antibiotic, has been the primary treatment for significant MRSA infections. It inhibits bacterial cell wall synthesis and has been used for decades [6]. However, overreliance on vancomycin has resulted in the formation of strains with lower susceptibility, such as vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). The first report of VISA was from Japan in 1996, followed by VRSA cases in the United States in 2002 [7, 8]. Since then, several VISA and VRSA incidents have been documented worldwide, including in India. These strains frequently result from adaptive mutations or horizontal gene transfer, such as the acquisition of the vanA gene from vancomycin-resistant enterococci (VRE) [9]. The advent of heteroresistance, in which a subpopulation of a vancomycin-susceptible strain exhibits lower susceptibility, complicates treatment even further [10]. Due to vancomycin's limitations, other medicines such as daptomycin have been introduced to treat MRSA infections. Daptomycin is a cyclic lipopeptide antibiotic that comes from *Streptomyces roseosporus* [11]. It possesses a unique calcium-dependent method of action that involves binding to bacterial membranes, resulting in rapid depolarization, inhibition of protein, DNA, and RNA production, and, eventually, cell death [12]. Daptomycin is bactericidal, and the FDA has approved it for the treatment of complex skin and soft tissue infections, as well as *S. aureus* bacteremia, including right-sided endocarditis [13]. Its unique method of action and efficacy against multidrug-resistant Gram-positive bacteria make it an excellent alternative, particularly in cases of vancomycin treatment failure or intolerance [14]. However, there are growing fears that widespread usage of vancomycin and daptomycin will eventually result in the development of resistance, even to these last-resort medications. Reduced susceptibility to daptomycin, albeit uncommon, has been found in some clinical isolates and is frequently related with previous vancomycin therapy. This emphasizes the significance of continued antibiotic surveillance and susceptibility testing in order to provide appropriate patient care and avoid the spread of resistant strains [15, 16]. The aim of the present study was undertaken to determine the in vitro susceptibility patterns of vancomycin and daptomycin among clinical MRSA isolates in a tertiary care hospital. Determining the minimum inhibitory concentrations (MICs) of these critical antibiotics helps assess the current efficacy of treatment options and contributes to the broader understanding of resistance trends in the region. Early detection of reduced susceptibility is crucial for guiding empirical therapy, optimizing patient outcomes, and informing antibiotic stewardship policies.

Materials & Methods

Study Design and Ethical Approval

This was a prospective, cross-sectional study at a tertiary care hospital that lasted three months, from August to September 2022. The Institutional Scientific and Ethics Committee provided ethical permission before the investigation began.

Sample Size and Selection

The study included 100 consecutive clinical isolates of *Staphylococcus aureus*. These were collected from clinical specimens such as blood, urine, sputum, pus, and wound swabs sent to the microbiology laboratory during the study period. Patients who were already on antibiotics at the time of admission or who refused to provide consent were excluded from the trial.

Sample Collection and Culture
All clinical samples were collected under rigorous aseptic conditions and processed using conventional microbiological techniques. The specimens were inoculated into nutritional agar, MacConkey agar, and blood agar plates, then incubated aerobically at 37°C for 18 to 24 hours.

Identification of *Staphylococcus aureus*
Colony shape, pigmentation, and hemolytic pattern were used to presumptively identify *S. aureus*. Colonies on nutrient agar were smooth, round, and golden yellow. Pink colonies indicative of lactose fermentation were found on MacConkey agar, whereas a limited zone of beta-hemolysis was visible on blood agar. Mannitol fermentation on mannitol salt agar produced yellow colonies. Gram staining identified Gram-positive cocci clusters. The isolates were biochemically validated via the catalase and tube coagulase tests, both of which were positive.

Antimicrobial susceptibility testing
Antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) criteria for 2022. HiMedia Laboratories in India provided antibiotic discs containing penicillin (10 units), cefoxitin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), cotrimoxazole (1.25/23.75 µg), gentamicin (10 µg), and linezolid (30 µg).

Detection of Methicillin Resistance
Methicillin resistance in *S. aureus* was determined using cefoxitin (30 µg) as a surrogate marker for mecA-mediated resistance. Zone diameter interpretation was carried out as per CLSI 2022 guidelines. For quality control, *S. aureus* ATCC 43300 was used as the MRSA-positive control and ATCC 25923 as the methicillin-sensitive negative control.

Vancomycin Susceptibility Testing (E-test MIC Method)
Vancomycin minimum inhibitory concentrations (MICs) were determined for all MRSA isolates using the Epsilometer test (E-test). A 0.5 McFarland standard suspension of each isolate was prepared and lawn inoculated on Mueller–Hinton agar. Vancomycin E-test strips were applied and the plates incubated at 37°C for 24 hours. MIC values were interpreted at the point where the zone of inhibition intersected the E-strip. According to CLSI 2022 breakpoints, isolates were categorized as vancomycin-susceptible (≤2 µg/mL), vancomycin-intermediate (4–8 µg/mL), or vancomycin-resistant (≥16 µg/mL) [17].

Daptomycin Susceptibility Testing (E-test MIC Method)
Daptomycin MICs were determined using E-test strips on Mueller–Hinton agar supplemented with 50 mg/L of calcium, due to the calcium-dependent action of the drug. A 0.5 McFarland suspension of each MRSA isolate was prepared, lawn cultured, and the E-test strip applied. After incubation at 37°C for 24 hours, MICs were recorded. According to CLSI 2022 guidelines, isolates with MIC values ≤1 µg/mL were considered susceptible to daptomycin. *S. aureus* ATCC 29213 was used as a quality control strain [17].

Results
The study was conducted at the Department of Microbiology, Government Thoothukudi Medical College, over a period of three months from August 2022 to October 2022. A total of 209 *Staphylococcus aureus* isolates were obtained from various clinical specimens. Among these, 108 isolates (52%) were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), while the remaining 101 isolates (48%) were methicillin-sensitive *Staphylococcus aureus* (MSSA) (Figure 1).

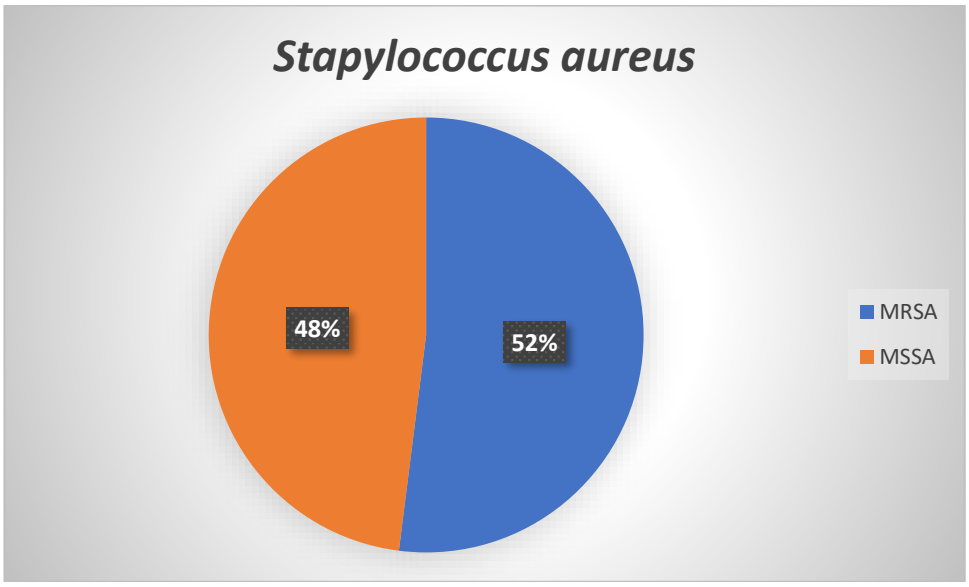

Figure 1. Percentage of MSSA and MRSA among *Staphylococcus aureus* isolates

Gender-wise Distribution

Among the 108 MRSA isolates, 64% were from male patients and 36% from female patients, resulting in a male-to-female ratio of 1.76:1 (Figure 2). Males showed a higher rate of MRSA isolation compared to females.

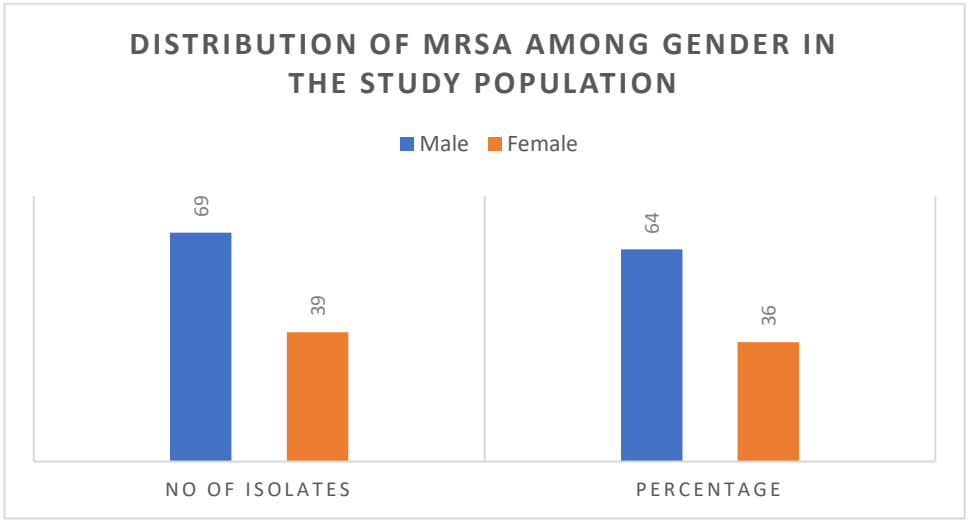

Figure 2. Distribution of MRSA among Gender in the study population

Age-wise Distribution

The age group with the highest MRSA isolation was patients older than 60 years (43%), followed by the 40–60 years age group (26%), 20–40 years (20%), and 0–20 years (11%) (Table 1). This indicates that elderly patients were more prone to MRSA infections.

Table 1. Distribution of MRSA among different age groups

Age	No of isolates (108)	Percentage
0-20 years	12	11%
20-40 years	22	20%
40-60 years	28	26%
> 60 years	46	43%
Total	**108**	**100%**

Distribution of MRSA by Clinical Specimen

MRSA was most frequently isolated from pus samples (70%), followed by urine (13%), blood (13%), and sterile fluids (4%) (Figure 3). The predominance in pus samples may be attributed to wound infections where *S. aureus* is a common pathogen.

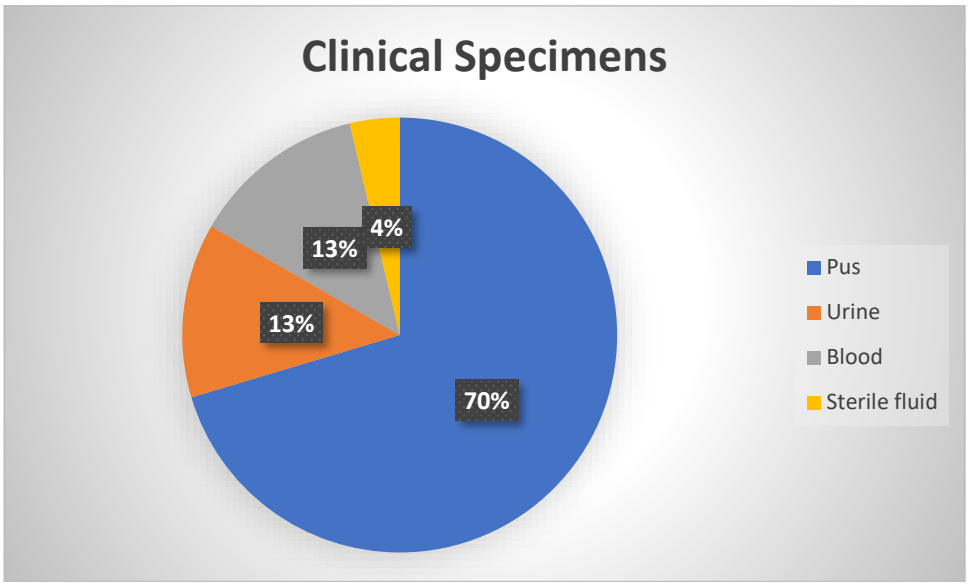

Figure 3. Distribution of MRSA isolated from the clinical specimen

Department-wise Distribution

Analysis of MRSA distribution among hospital departments showed the highest isolation from the Surgery department (53%), followed by Orthopaedics (19%), Intensive Medical Care Unit (IMCU) (14%), Paediatrics (8%), and Obstetrics & Gynaecology (6%) (Figure 4).

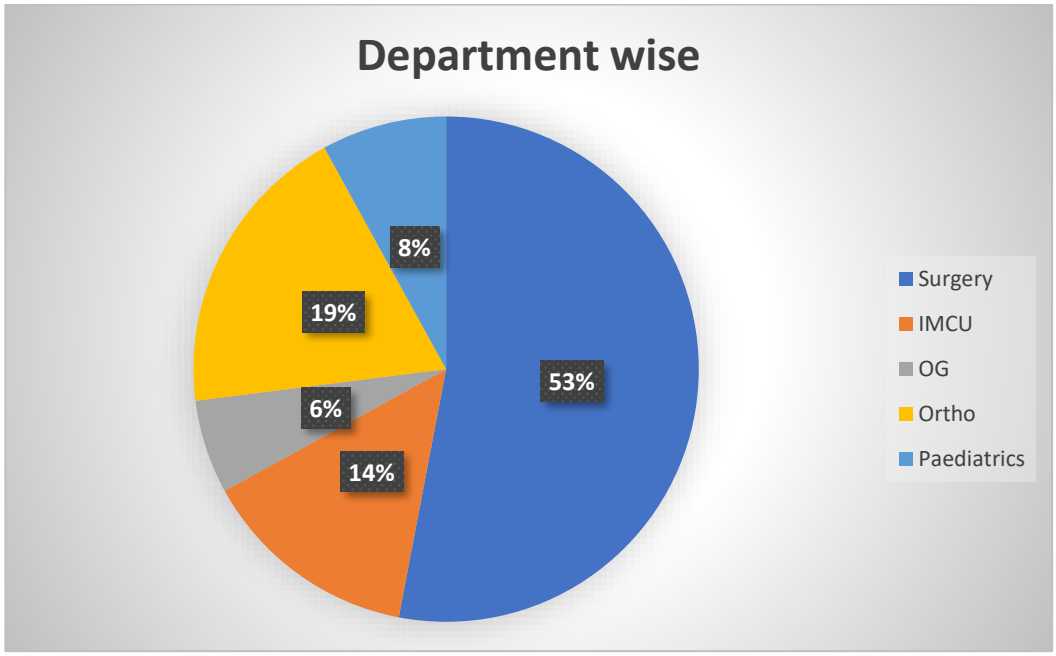

Figure 4. Percentage of distribution of MRSA in Department wise

Discussion

Emerging Antibiotic Resistance in *Staphylococcus aureus*

Antimicrobial resistance in *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), is an escalating global health concern. This organism has emerged as a prominent nosocomial

pathogen, frequently implicated in hospital-acquired infections due to its ability to acquire and disseminate resistance mechanisms [18]. Resistance patterns vary significantly between regions, hospitals, and time periods, emphasizing the need for localized surveillance. Early detection of methicillin resistance, along with assessment of vancomycin susceptibility, is crucial to effectively prevent and manage nosocomial outbreaks [19].

Prevalence of MRSA

The current investigation found that MRSA was detected in 52% of *S. aureus* isolates (108/209). This is consistent with findings by Majumder et al. (52.9%) [20] and Bouchiat et al. (52.4%) [21]. Other studies, however, reveal variability: Subedi et al. reported a lower incidence (15.4%) [22], Pandey et al. found an 18.1% rate [23], and Verma et al. reported a substantially higher prevalence (80.78%) [24]. Such discrepancies may be caused by institutional variances, referral status, patient population, infection control procedures, or antibiotic use trends. Referral hospitals frequently report greater MRSA rates due to patients' prior antibiotic exposure, which boosts selection pressure and resistance. Healthcare workers can be both vectors and reservoirs of MRSA, transmitting strains within the hospital and potentially into the community. Effective hand hygiene, screening and decolonization of MRSA carriers, and robust hospital hygiene are essential strategies to limit spread. Given the limited therapeutic options for MRSA, infections are often difficult to treat, leading to prolonged hospitalization, increased costs, and potential treatment failure.

Demographic Distribution of MRSA

The study found a predominance of MRSA among male patients, with a male-to-female ratio of 1.76:1. This pattern aligns with studies by Rao et al. [25] and Lohan K et al., [26] although contrasting findings were reported by Deotale V et al., who observed a higher prevalence among female patients (60.86%) [27]. Buzaid et al. noted no significant gender difference, suggesting that sex-related MRSA risk may vary depending on population or setting [28]. Age-wise, the most affected group was patients over 60 years, consistent with reports by Sharma S et al. [29] and Lohan K et al [26]. Age-related comorbidities, immunosenescence, and increased exposure to healthcare settings may explain this vulnerability among the elderly.

Clinical Specimen Distribution

MRSA was most typically identified from pus samples (71%), indicating a high prevalence of skin and soft tissue infections. This is consistent with results from Mallick and Basak (69.4%) [30], Tiwari et al. (68%) [31], and Rao and Srinivas (64%) [25]. The high pus recovery rate may be linked to *S. aureus*'s position as a skin commensal and opportunistic pathogen, especially in post-surgical wounds or open lesions. In contrast, Alli OT et al. found just 21.4% MRSA in pus samples, indicating potential variations in patient demographics or clinical characteristics [32].

Departmental Distribution

The highest MRSA isolation occurred in the Surgery department (53%), followed by Orthopaedics (19%), IMCU (14%), Paediatrics (8%), and Obstetrics & Gynaecology (6%). The predominance in surgical units reflects prolonged hospital stays, wound exposure, and broad-spectrum antibiotic use. Arora et al. similarly reported high MRSA prevalence (54.8%) in surgical wards [33]. Sharma et al. also noted 34% of MRSA cases in Orthopaedics and 18% in surgery [29]. The variation in departmental distribution highlights the importance of tailored infection control practices.

Antibiotic Resistance Profile

Resistance in *S. aureus*, particularly MRSA, is clinically significant due to its association with treatment failures, prolonged illness, and increased mortality. A meta-analysis by Cosgrove et al. found that MRSA bacteraemia is associated with double the mortality risk compared to MSSA [34]. In the present study, 96% of MRSA isolates were sensitive to linezolid, affirming its efficacy. Similar

findings were reported by Sireesha et al. [35] and Rajaduraipandi et al., [36] who observed 100% and 97.6% sensitivity, respectively. This reinforces linezolid as a vital therapeutic option for MRSA infections. Importantly, all of the MRSA isolates in this investigation were susceptible to vancomycin and daptomycin. These findings are consistent with previous research by Husain A et al. [37] and Niveditha N et al.,[38] which supports the continued use of these medicines in MRSA therapy. However, global reports of VISA and VRSA, particularly from the United States, France, and South Korea, have raised concerns. Although the strains frequently demonstrate low-level resistance (e.g., MIC 8 μg/mL), they underline the need for careful vancomycin administration and vigilant monitoring.

Study Limitations and Future Directions

The main limitation of this study is the absence of molecular characterization of isolates. Future studies should include genotyping, resistance gene profiling (e.g., mecA, vanA), and assessment of virulence factors to provide deeper epidemiological insights. Molecular surveillance would also help track clonal spread and inform more precise infection control strategies.

Conclusion

The current study found a significant frequency of methicillin-resistant *S.aureus* (MRSA), especially among older patients and those admitted to surgical and orthopedic departments. Pus was the most prevalent clinical specimen that produced MRSA. All MRSA isolates were shown to be sensitive to vancomycin and daptomycin, confirming their efficacy as essential treatment alternatives. However, the global rise of vancomycin-intermediate and resistant strains (VISA/VRSA) highlights the critical need for cautious antibiotic usage and strong infection control methods. The high resistance rate highlights the significance of regular monitoring of antibiotic susceptibility patterns in order to guide empirical therapy and reduce treatment failures. While phenotypic identification provides useful clinical data, future studies should include molecular characterization of resistance mechanisms for better epidemiological understanding. Regular antimicrobial surveillance, development of institutional antibiotic policies, and enhanced awareness among healthcare workers are essential to combat the rising threat of MRSA and improve patient outcomes.

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