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Original Article

Antimicrobial Susceptibility Profiles of Clinical Staphylococcus Isolates: A Focus on Methicillin and Vancomycin Resistance in a Tertiary Care Setting

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ABSTRACT

Introduction: Staphylococcus species, particularly S. aureus, are major nosocomial pathogens. The escalating prevalence of methicillin-resistant Staphylococcus aureus and the potential for vancomycin resistance present substantial challenges for empirical antimicrobial therapy. This research aimed to evaluate the drug sensitivity profiles of staphylococcal isolates, with a specific focus on resistance to methicillin and vancomycin.

Material& Methods: A 12-month cross-sectional analysis was performed at a tertiary hospital in Lucknow, India. One hundred non-duplicate Staphylococcus isolates were collected and identified through standard laboratory protocols. Identification was done using standard microbiological techniques (Gram stain, culture on Mannitol salt & blood agar, catalase, coagulase tests). Antimicrobial susceptibility was performed by Kirby-Bauer disc diffusion. Methicillin resistance was detected using a cefoxitin (30µg) disc, and vancomycin Minimum Inhibitory Concentration (MIC) was determined using the Ezy MIC™ strip test.

Results: Of 100 isolates, 51% were methicillin-resistant. Pus (64%) was the most common source. Overall sensitivity was highest for linezolid (97%), vancomycin (94%), and doxycycline (89%). Penicillin (25%), erythromycin (35%), and azithromycin (48%) showed the lowest sensitivity. Phenotypic vancomycin resistance was detected in 6% of isolates—all of which were MRSA. Multidrug resistance was markedly more prevalent in MRSA (86.3%) compared to methicillin-sensitive isolates (40.8%; $p<0.001$).

Conclusion: The high incidence of MRSA (51%) and emerging vancomycin resistance (6%) highlight a critical clinical threat. Linezolid and vancomycin remain highly effective, but the significant MDR burden mandates robust antimicrobial stewardship and routine surveillance to preserve last-resort antibiotics.

Keywords: Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus (MRSA), Vancomycin Resistance, Antimicrobial Susceptibility, Multidrug Resistance.

INTRODUCTION

Staphylococcus species, particularly *Staphylococcus aureus*, remain among the most critical pathogens isolated from hospitalized patients worldwide. Their high prevalence, capacity for invasive diseases (bacteraemia, endocarditis, skin and wound infections), and remarkable propensity to acquire antimicrobial resistance (AMR) pose a significant global health threat [1, 2]. The development of methicillin-resistant *Staphylococcus aureus* has severely constrained therapeutic options, as these strains are typically resistant to nearly all -lactam antibiotics due to the acquisition of the *mecA* gene encoding PBP2a, over the past five decades [3]. While vancomycin has long been the last-resort drug for serious MRSA infections, the recent emergence of vancomycin-intermediate (VISA) and vancomycin-resistant *S. aureus* (VRSA) threatens even this final line of defense [4].Antimicrobial resistance patterns vary significantly by geography, clinical setting, and even

between hospital wards, making local surveillance data indispensable for guiding empirical therapy, infection control policies, and antimicrobial stewardship [5]. Several Indian studies report MRSA prevalence ranging from 30-70%, but data on vancomycin susceptibility and resistance in coagulase-negative staphylococci (CoNS) remain less comprehensive. This study was therefore designed to investigate the antimicrobial susceptibility of clinical *Staphylococcus* isolates in a Lucknow-based tertiary care facility, specifically targeting methicillin and vancomycin resistance.

MATERIAL AND METHODS

Study Design: This cross-sectional study was conducted over one year within the Department of Microbiology at TS Misra Medical College & Hospital, Lucknow.

Ethical Considerations: The Institutional Ethics Committee provided approval, and all patient information was anonymized to ensure privacy.

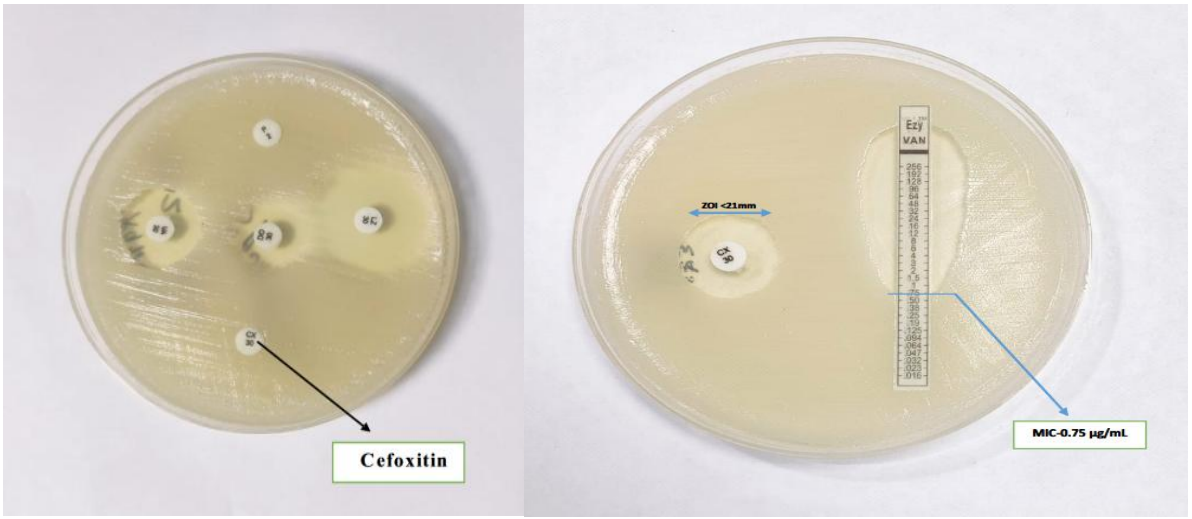
Reporting Guidelines: This study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies.

Sample Selection: Clinical samples (pus, blood, urine, high vaginal swabs, body fluids) received in the central laboratory from both inpatients and outpatients were included if they were positive for *Staphylococcus* species. Samples with incomplete antibiotic susceptibility testing were excluded.

Sample Size Calculation: Based on a previous study reporting a 30% resistance rate to common antibiotics, with a 10% margin of error and 95% confidence interval, the minimum sample size was calculated as 81. After a 25% contingency provision, a target sample size of 100 consecutive *Staphylococcus* isolates was enrolled.

Study Methodology:

1. **Isolation & Identification:** Specimens were inoculated onto Mannitol salt agar, chromogenic agar, and blood agar. *Staphylococcus* species were identified by Gram staining (Gram-positive cocci in clusters), catalase test (positive), and coagulase test (slide and tube) to differentiate *S. aureus* (coagulase-positive) from CoNS (coagulase-negative) [6].
2. **Antimicrobial Susceptibility Testing (AST):** Disc diffusion was performed according to CLSI standards across various antibiotic classes. Antibiotics tested included azithromycin, erythromycin, ciprofloxacin, norfloxacin, levofloxacin, gentamicin, clindamycin, chloramphenicol, penicillin, doxycycline, tetracycline, and linezolid.
3. **Detection of Methicillin Resistance:** Methicillin resistance was defined by a cefoxitin zone diameter of ≤ 24 mm. [7].
4. **Vancomycin MIC:** Vancomycin MICs were determined using Ezy MIC™ strips and interpreted using CLSI thresholds (Sensitive: ≤ 2 µg/ml for MRSA; Resistant: ≥16 µg /ml for MRSA).
5. **Statistical Analysis:** Data were entered in Microsoft Excel and analyzed using IBM SPSS version 25.0. The Chi-square test was used to compare categorical variables. A p-value <0.05 was considered statistically significant.



RESULTS

A total of 100 *Staphylococcus* isolates were analysed. The overall prevalence of *Staphylococcus* among total clinical specimens (n=4312) was 8.3%. The prevalence of methicillin resistance among the 100 isolates was 51% (n=51) (Table 1). Coagulase-negative staphylococci (CoNS) comprised 16% of isolates, with a non-significant higher proportion in the MRSA group (19.6%) vs. MSSA (12.2%) (p=0.315).

Table 1: Baseline Characteristics and Methicillin Resistance Association (N=100)

Characteristic	Methicillin-Resistant (n=51)	Methicillin-Sensitive (n=49)	Total (N=100)	p-value
Mean Age (years ± SD)	40.45 ± 14.86	34.67 ± 17.66	37.62 ± 16.46	0.079
Sex (Male)	32 (62.7%)	32 (65.3%)	64 (64%)	0.790
Specimen Type (Pus)	34 (66.7%)	30 (61.2%)	64 (64%)	0.141
Coagulase Negative	10 (19.6%)	6 (12.2%)	16 (16%)	0.315

Pus (64%) was the most common specimen, followed by blood (17%) and urine (8%). No significant association was found between methicillin resistance and age, sex, or specimen type.

Antimicrobial Susceptibility Pattern:

Overall, the highest sensitivity was observed for linezolid (97%), vancomycin (94%), and doxycycline (89%), while penicillin (25%), erythromycin (35%), and azithromycin (48%) were the least effective (Table 2). Methicillin-resistant isolates showed significantly lower sensitivity to most antibiotics compared to MSSA ($p<0.05$), except for norfloxacin, doxycycline, and linezolid. Vancomycin resistance was seen only in the MRSA group (11.8% of MRSA isolates; 6% overall). Linezolid resistance was minimal (3% overall).

Table 2: Antibiotic Susceptibility Pattern (Proportion of Sensitive Isolates)

Antibiotic Class	Antibiotic	MRSA (n=51)	MSSA (n=49)	Total (N=100)	p-value
Oxazolidinone	Linezolid	48 (94.1%)	49 (100%)	97 (97%)	0.085
Glycopeptide	Vancomycin	45 (88.2%)	49 (100%)	94 (94%)	**0.013**
Tetracycline	Doxycycline	43 (84.3%)	46 (93.9%)	89 (89%)	0.127
Aminoglycoside	Gentamicin	31 (60.8%)	41 (83.7%)	72 (72%)	**0.011**
Lincosamide	Clindamycin	30 (58.8%)	40 (81.6%)	70 (70%)	**0.013**
Fluoroquinolone	Ciprofloxacin	17 (33.3%)	41 (83.7%)	58 (58%)	**<0.001**
Macrolide	Azithromycin	15 (29.4%)	33 (67.3%)	48 (48%)	**<0.001**
Penicillin	Penicillin	6 (11.8%)	18 (36.7%)	24 (24%)	**0.003**

Multidrug Resistance (MDR): The overall prevalence of MDR (resistance to ≥3 antibiotic classes) was 64%. MDR was significantly higher in MRSA (86.3%) compared to MSSA (40.8%) ($p<0.001$) (Table 3).

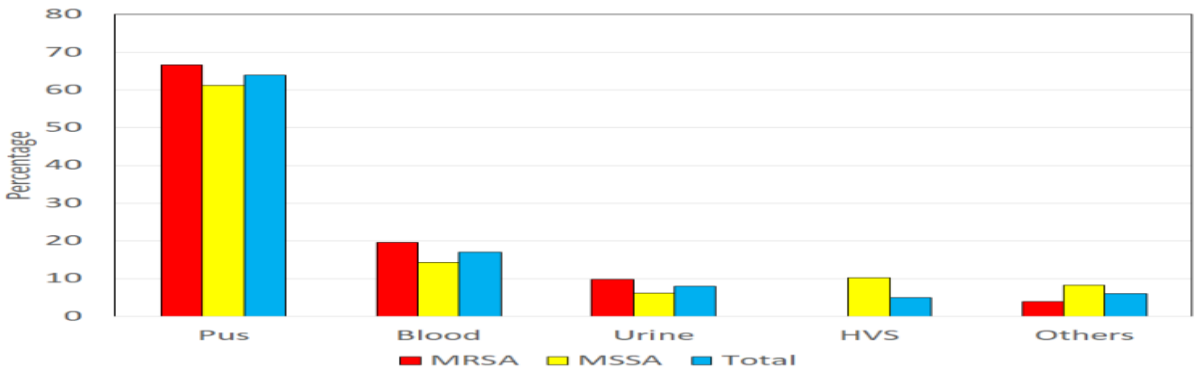
Table 3: Multidrug Resistance Pattern

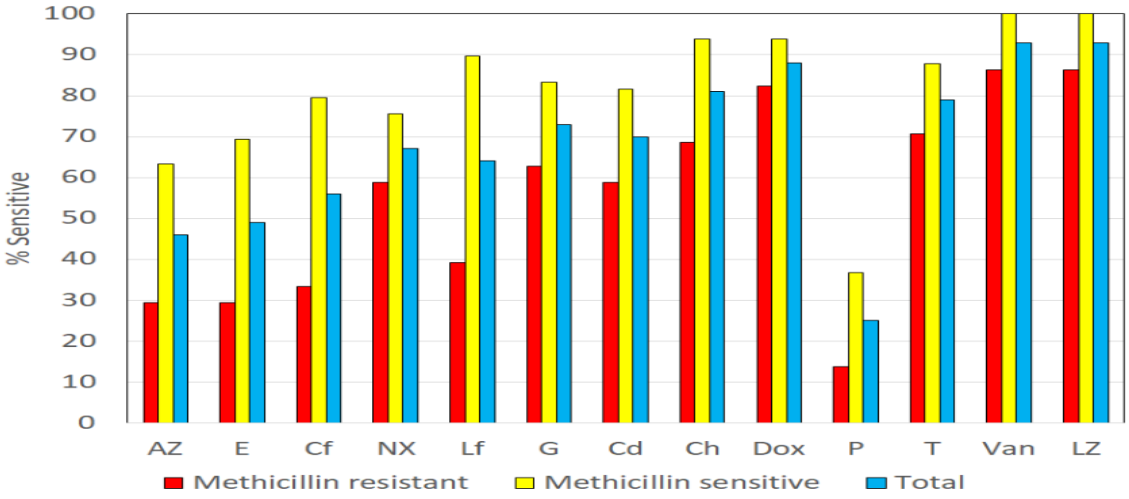
MDR Status	MRSA (n=51)	MSSA (n=49)	Total (N=100)	p-value
MDR Present	44 (86.3%)	20 (40.8%)	64 (64%)	**<0.001**
No MDR	7 (13.7%)	29 (59.2%)	36 (36%)	

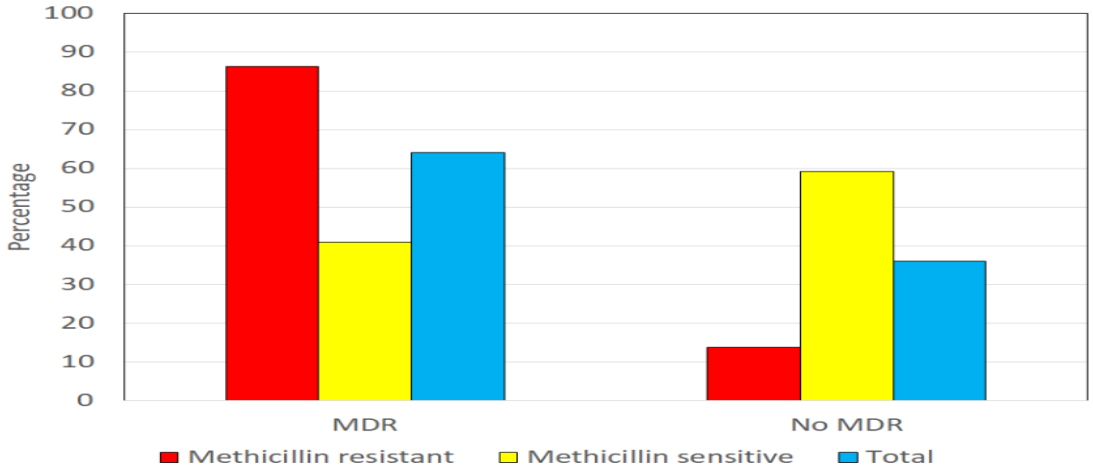
Vancomycin MIC Distribution: Of the 6 isolates with vancomycin resistance (MIC ≥16 µg/ml), all were MRSA. No VISA (MIC 4-8 µg/ml) or VRSA with *vanA* gene were specifically genotyped, but phenotypic resistance was confirmed (Table 4).

Table 4: Vancomycin Susceptibility by MIC (Phenotypic)

Vancomycin Interpretation	MRSA (n=51)	MSSA (n=49)	Total (N=100)
Sensitive (MIC ≤2 µg/ml)	45 (88.2%)	49 (100%)	94 (94%)
Resistant (MIC ≥16 µg/ml)	6 (11.8%)	0 (0%)	6 (6%)







DISCUSSION

These results provide essential local insights into staphylococcal resistance in North India. Our MRSA prevalence of 51% aligns closely with findings from other Indian tertiary care centers, such as 54.1% in Karnataka [8] and 48.1% in Rajasthan [9]. This high burden underscores the failure of routine infection control practices in many Indian hospitals and necessitates urgent intervention. The finding of 16% CoNS, with a higher proportion in the MRSA group, is concerning as CoNS are often reservoirs for resistance genes like *mecA* that can transfer to *S. aureus* [10]. A key finding is the 6% overall vancomycin resistance, which manifested exclusively in the MRSA group (11.8% of MRSA). While this is lower than some reports from Nigeria (45%) [11], it is higher than many Indian studies that report 0% resistance [12]. This emergence of vancomycin resistance in a setting where it is a last-resort drug is a significant red flag. It could be due to the selection pressure from injudicious vancomycin use or the circulation of hVISA strains that later develop full resistance. The continued high efficacy of linezolid (97% sensitive) offers an alternative, but resistance to linezolid is also emerging globally and was noted in 5.9% of MRSA in our study.The antibiotic susceptibility pattern reveals a classic divide. Traditional, cheaper drugs like penicillin (24% sensitive) and macrolides (35-48% sensitive) are largely ineffective. Even fluoroquinolones like ciprofloxacin showed poor activity against MRSA (only 33.3% sensitive). This renders them

unsuitable for empiric therapy of suspected staphylococcal infections. In contrast, doxycycline showed good activity (89% overall), which may be a useful oral option for non-severe MRSA infections post-discharge, aligning with other studies [8]. The significantly higher MDR burden in MRSA (86.3%) compared to MSSA (40.8%) is expected, as the *SCCmec* cassette often carries genes for multiple resistances. This transforms a simple infection into a complex, costly therapeutic challenge.

Strengths & Limitations: The strength of this study is its focus on a high-priority clinical problem with standard methodology. Limitations include its single-center design, small sample size limiting generalizability, and the absence of molecular confirmation (e.g., *mecA*, *vanA/B*) to definitively characterize the resistance mechanisms. Clinical outcomes and prior antibiotic exposure were not correlated.

CONCLUSION

This study confirms a high burden of MRSA (51%) and a concerning 6% rate of phenotypic vancomycin resistance in our clinical setting. Linezolid and vancomycin remain the most effective agents, but the significant multidrug resistance, especially in MRSA, limits therapeutic options. Routine surveillance, antimicrobial stewardship to preserve last-resort drugs, and strengthened infection control practices are urgently needed. Molecular surveillance for *vanA* and *mecA* genes should be integrated into future hospital protocols.

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