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RESEARCH ARTICLE
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A CROSS-SECTIONAL STUDY ON PHENOTYPIC DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND INDUCIBLE CLINDAMYCIN RESISTANCE IN A TERTIARY CARE HOSPITAL IN WESTERN INDIA

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major cause of hospital-acquired infections, with increasing concerns over resistance to clindamycin due to inducible mechanisms. Phenotypic detection of such resistance is critical for appropriate antibiotic therapy.

Objectives: To determine the prevalence of MRSA and inducible clindamycin resistance among clinical isolates of *S. aureus* and to compare resistance patterns between MRSA and MSSA strains in a tertiary care setting.

Methods: This cross-sectional study was conducted over eight months in a tertiary care hospital in Western India. A total of 100 *S. aureus* isolates from various clinical specimens were identified and tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method. MRSA detection was performed using the cefoxitin disc method, and erythromycin-resistant isolates were subjected to D-testing to detect inducible clindamycin resistance. Data were analyzed using descriptive statistics, chi-square tests, and odds ratios.

Results: MRSA was identified in 38% of isolates. Inducible clindamycin resistance (iMLSB) was detected in 30.4% of erythromycin-resistant isolates, with cMLSB in 10.7%, and MS phenotype in 26.8%. Constitutive resistance was exclusively observed in MRSA ($p = 0.02$). Resistance to clindamycin, erythromycin, ciprofloxacin, and gentamicin was significantly higher in MRSA compared to MSSA ($p < 0.001$). All isolates were susceptible to vancomycin and linezolid.

Conclusion: A high prevalence of MRSA and inducible clindamycin resistance underscores the necessity of routine phenotypic screening, particularly the D-test, for guiding effective antibiotic therapy. These findings support the need for ongoing surveillance and robust antimicrobial stewardship programs.

Keywords: *Staphylococcus aureus*, MRSA, Inducible Clindamycin Resistance, D-test, Antibiotic Resistance

Introduction

Infectious diseases remain a major global health concern, contributing significantly to morbidity and mortality, particularly in resource-limited and hospital settings [1]. The rapid emergence and spread of antimicrobial-resistant organisms, particularly Gram-positive cocci such as *Staphylococcus aureus*, have exacerbated these challenges. *Staphylococcus aureus* is a highly versatile pathogen responsible for a broad spectrum of infections ranging from skin and soft tissue infections to life-threatening conditions like bacteraemia, pneumonia, and endocarditis [2].

Of particular concern is Methicillin-resistant *Staphylococcus aureus* (MRSA), a formidable nosocomial and community-acquired pathogen that exhibits resistance to nearly all β-lactam antibiotics. MRSA has become a prominent cause of healthcare-associated infections in tertiary care centers, long-term care facilities, and community hospitals, largely due to its ability to colonize hospital personnel and persist in the environment [3]. According to global surveillance data, the prevalence of MRSA has steadily increased since its first detection in the 1960s, with some countries reporting resistance rates as high as 44% among *S. aureus* isolates [4].

In India, the burden of MRSA is significant and growing, with prevalence rates ranging from 26% to over 50% across various regions and hospitals [5]. The emergence of MRSA strains resistant to multiple classes of antibiotics has led to therapeutic limitations and higher clinical failure rates, especially when clindamycin is used without proper susceptibility testing. Clindamycin, a lincosamide antibiotic, is frequently employed to treat *S. aureus* infections due to its excellent tissue penetration and effectiveness in penicillin-allergic patients. However, its use is complicated by inducible resistance mediated by the *erm* gene, which may not be detected by standard susceptibility tests, leading to treatment failure [6].

To overcome this diagnostic gap, the Clinical and Laboratory Standards Institute (CLSI) recommends the D-test—a simple disc diffusion method that detects inducible clindamycin resistance in *S. aureus* strains resistant to erythromycin but apparently sensitive to clindamycin [5,7]. This phenotypic differentiation is critical for appropriate antibiotic selection, especially in resource-limited settings. Given the clinical importance and therapeutic implications of MRSA and inducible clindamycin resistance, this study was conducted to determine the prevalence of MRSA and assess clindamycin resistance patterns among clinical *S. aureus* isolates in a tertiary care setting. Accurate detection and reporting of resistance patterns will help guide antibiotic stewardship and infection control strategies.

Aims and Objectives

Aim

The study aimed to evaluate the prevalence and resistance patterns of Methicillin-resistant *Staphylococcus aureus* (MRSA), with special reference to inducible clindamycin resistance, among clinical isolates obtained from patients at a tertiary care hospital in Western India.

Objectives

1. To isolate and identify *Staphylococcus aureus* from various clinical specimens collected at a tertiary care hospital.
2. To determine the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and evaluate their antimicrobial susceptibility patterns using phenotypic methods.
3. To detect inducible clindamycin resistance among erythromycin-resistant *S. aureus* isolates using the D-test and compare resistance patterns between MRSA and MSSA strains.

Materials and Methods

Study Design and Setting

This was a cross-sectional observational study conducted in the Department of Microbiology at a tertiary care hospital in Rajkot, Western India. The study was carried out over a period of eight months, from July 2019 to February 2020.

Sample Collection and Inclusion Criteria
A total of 100 non-duplicate clinical isolates of *Staphylococcus aureus* were obtained from various clinical specimens, including pus, blood, urine, respiratory samples, and body fluids. Only samples from patients with suspected bacterial infections submitted for routine culture and sensitivity testing were included. Environmental and non-clinical samples were excluded.

Isolation and Identification of *Staphylococcus aureus*
Specimens were cultured on blood agar and mannitol salt agar and incubated at 35–37°C for 24–48 hours. Colonies suspected to be *S. aureus* based on morphology and mannitol fermentation were further subjected to Gram staining, catalase test, and slide/tube coagulase tests for confirmation.

Antimicrobial Susceptibility Testing (AST)
Antimicrobial susceptibility was assessed using the **Kirby-Bauer disc diffusion method** on Mueller-Hinton agar following **CLSI 2019** guidelines. Antibiotics tested included cefoxitin (30 μg), erythromycin (15 μg), clindamycin (2 μg), ciprofloxacin, gentamicin, vancomycin, and linezolid. The zone of inhibition was measured after 24 hours of incubation at 35°C.

Detection of MRSA
Cefoxitin (30 μg) disc diffusion was used for the phenotypic detection of MRSA. A zone of inhibition ≤21 mm was interpreted as methicillin-resistant, in accordance with CLSI standards.

Detection of Inducible Clindamycin Resistance (D-test)
Erythromycin-resistant isolates were subjected to the D-test to detect inducible resistance to clindamycin. On a Mueller-Hinton agar plate, erythromycin and clindamycin discs were placed 15–20 mm apart. Following overnight incubation at 35°C, isolates showing blunting of the clindamycin zone adjacent to the erythromycin disc (D-shaped zone) were recorded as D-test positive, indicating inducible MLS_B resistance.

Isolates resistant to both drugs were labelled as constitutively resistant, while isolates resistant to erythromycin but susceptible to clindamycin without D-zone formation were categorized as MS phenotype.

Quality Control
Staphylococcus aureus ATCC 25923 was used as the control strain for antimicrobial susceptibility testing.

Data Analysis
Data were compiled in Microsoft Excel and analyzed using descriptive statistics. Frequencies and percentages were calculated for categorical variables. Chi-square test was used to assess associations between MRSA and clindamycin resistance phenotypes. A p-value of <0.05 was considered statistically significant.

Results:
1. Distribution of *Staphylococcus aureus* Isolates by Clinical Sample Type
A total of 100 non-duplicate *Staphylococcus aureus* isolates were obtained from a variety of clinical specimens submitted for routine bacteriological examination. The most common source of isolates was pus samples, accounting for more than half of the total, followed by blood, urine, respiratory specimens, and body fluids.

Table 1. Distribution of *S. aureus* Isolates by Sample Type

Sample Type	Number of Isolates (n)	Percentage (%)
Pus	52	52%
Blood	18	18%
Urine	15	15%
Respiratory	10	10%
Body Fluids	5	5%
Total	**100**	**100%**

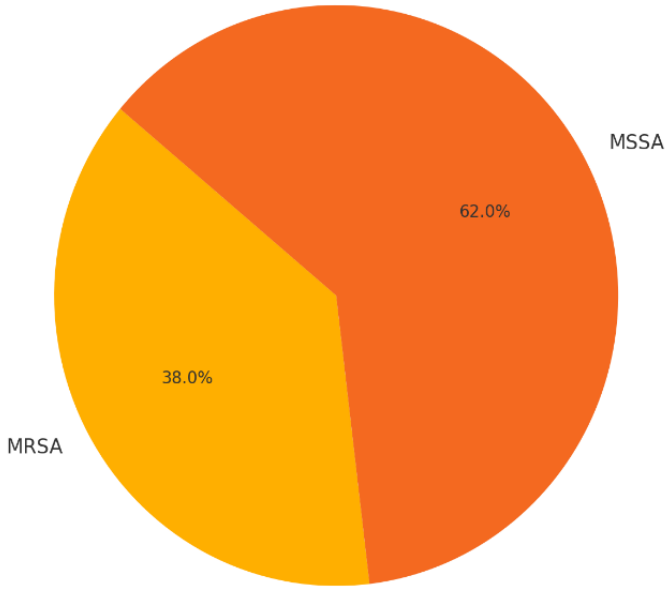
2. Prevalence of MRSA and MSSA

Out of the 100 confirmed *Staphylococcus aureus* isolates, 38 were identified as Methicillin-resistant *Staphylococcus aureus* (MRSA) based on cefoxitin disc diffusion testing, while the remaining 62 were Methicillin-sensitive *S. aureus* (MSSA). This reflects a relatively high prevalence of MRSA in the hospital setting during the study period.

Table 2. Prevalence of MRSA and MSSA Among Clinical Isolates

Strain Type	Number of Isolates (n)	Percentage (%)
MRSA	38	38%
MSSA	62	62%
Total	**100**	**100%**

Figure 1. *Proportional distribution of MRSA and MSSA among clinical isolates (n = 100).*



3. Antibiotic Susceptibility Patterns of MRSA vs MSSA

The antimicrobial susceptibility patterns of MRSA and MSSA isolates were compared using the disc diffusion method in accordance with CLSI 2019 guidelines. Vancomycin and linezolid showed 100% sensitivity across all isolates. In contrast, MRSA strains demonstrated significantly higher resistance rates to erythromycin, clindamycin, gentamicin, and ciprofloxacin compared to MSSA.

Table 3. Antibiotic Resistance Profile of MRSA vs MSSA Isolates

Antibiotic	MRSA Resistant (n=38)	MSSA Resistant (n=62)	p-value (Chi-square)
Erythromycin	30 (78.9%)	26 (41.9%)	< 0.001
Clindamycin	16 (42.1%)	5 (8.1%)	< 0.001
Ciprofloxacin	28 (73.7%)	21 (33.9%)	< 0.001
Gentamicin	25 (65.8%)	16 (25.8%)	< 0.001
Vancomycin	0 (0%)	0 (0%)	–
Linezolid	0 (0%)	0 (0%)	–

Figure 2. Bar Chart Comparing Resistance Rates in MRSA vs MSSA

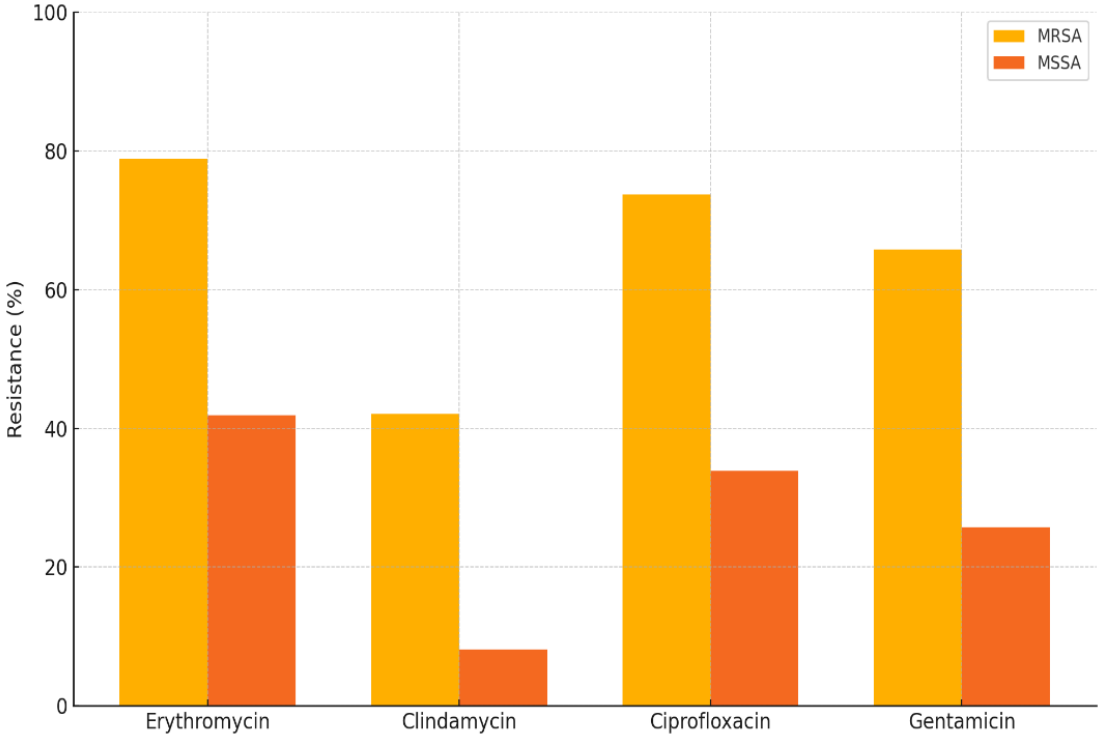


Table 4. Distribution of Clindamycin Resistance Phenotypes Among Erythromycin-Resistant Isolates (n = 56)

Phenotype	Number of Isolates (n)	Percentage (%)
Inducible MLS_B (iMLSB)	17	30.4%
Constitutive MLS_B (cMLSB)	6	10.7%
MS Phenotype	15	26.8%
Clindamycin Susceptible	18	32.1%
Total	56	100%

4. Clindamycin Resistance Phenotypes (D-Test Results)

Among the 56 erythromycin-resistant *Staphylococcus aureus* isolates, the D-test was used to differentiate clindamycin resistance phenotypes. The majority of isolates exhibited **inducible clindamycin resistance (iMLSB)**, followed by **MS phenotype** and **constitutive clindamycin resistance (cMLSB)**.

Table 4. Distribution of Clindamycin Resistance Phenotypes Among Erythromycin-Resistant Isolates (n = 56)

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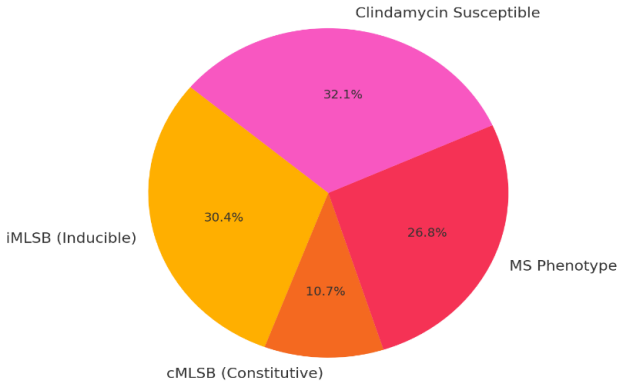
MS Phenotype	15	26.8%
Clindamycin Susceptible	18	32.1%
Total	**56**	**100%**

Figure 3. Pie Chart of Clindamycin Resistance Phenotypes



5. Comparative Analysis of Clindamycin Resistance Between MRSA and MSSA

Among the erythromycin-resistant isolates, inducible and constitutive clindamycin resistance phenotypes were more frequently observed in MRSA strains compared to MSSA. A comparative analysis was performed using the Chi-square test, and **odds ratios (OR)** were calculated to assess the strength of association between MRSA status and clindamycin resistance.

Table 5. Clindamycin Resistance Phenotypes in MRSA vs MSSA (n = 56)

Phenotype	MRSA (n = 30)	MSSA (n = 26)	p-value	Odds Ratio (95% CI)
iMLSB (Inducible)	10 (33.3%)	7 (26.9%)	0.62	1.36 (0.42–4.37)
cMLSB (Constitutive)	6 (20.0%)	0 (0%)	0.02*	– (undefined, rare event)
MS Phenotype	6 (20.0%)	9 (34.6%)	0.22	0.47 (0.15–1.51)
Clindamycin Susceptible	8 (26.7%)	10 (38.5%)	0.34	0.58 (0.19–1.78)

*$p < 0.05$ considered statistically significant

6. Summary of Key Findings

This section provides a consolidated view of major results, highlighting key prevalence values, resistance phenotypes, and statistically significant associations.

Table 6. Summary of Major Findings in the Study

Parameter	Result	Comment
Total *S. aureus* isolates	100	From pus, blood, urine, respiratory, fluids
MRSA prevalence	38%	High burden in hospital setting

Parameter	Result	Comment
MSSA prevalence	62%	
Most common sample type	Pus (52%)	Reflects soft tissue infections
Vancomycin and Linezolid resistance	0%	All isolates susceptible
Most resistant antibiotic (MRSA)	Erythromycin (78.9%)	Followed by ciprofloxacin (73.7%)
iMLSB phenotype among erythromycin-resistant isolates	30.4%	Detected via D-test
cMLSB phenotype (constitutive)	10.7%	All cMLSB found in MRSA only
MS phenotype	26.8%	More common in MSSA
Statistically significant difference (cMLSB in MRSA)	p = 0.02	MSSA showed no constitutive resistance
Odds ratio for iMLSB (MRSA vs MSSA)	1.36 (95% CI: 0.42–4.37)	Not statistically significant

Discussion

In this study, the prevalence of MRSA was found to be 38%, indicating a substantial methicillin resistance burden in the study setting. Among erythromycin-resistant *S. aureus* isolates, inducible clindamycin resistance (iMLSB) was observed in 30.4%, constitutive resistance (cMLSB) in 10.7%, and MS phenotype in 26.8%. Notably, cMLSB was exclusively observed in MRSA, with a statistically significant association ($p = 0.02$). MRSA strains showed significantly higher resistance to ciprofloxacin (73.7% vs 33.9%) and clindamycin (42.1% vs 8.1%) compared to MSSA, with $p < 0.001$ in both cases. All isolates remained susceptible to vancomycin and linezolid, confirming their continued reliability for treatment.

Our MRSA prevalence (38%) is consistent with reports from Nepal and India. Maharjan et al. (2022) found a prevalence of 35.1% MRSA among clinical isolates in Kathmandu, supporting our findings [6]. Similarly, Adhikari et al. (2017) reported a comparable MRSA burden in their study from a tertiary care hospital [7]. Deotale et al. (2010) observed high resistance to erythromycin and ciprofloxacin in MRSA isolates, consistent with our study where over 70% of MRSA strains were resistant to both [8].

The observed 30.4% inducible resistance aligns closely with previous studies. Lyall et al. (2013) reported 32.3% iMLSB phenotype, while Baiu & Al-Abdli (2016) found 28.4% in MRSA isolates [9,10]. The exclusive detection of cMLSB in MRSA mirrors findings by Regmi et al. (2020), who emphasized this phenotype as a marker of multidrug resistance [11]. Additionally, Baral & Khanal (2017) emphasized the clinical importance of detecting inducible resistance to prevent therapeutic failures [12]. Our use of the D-test, as endorsed by CLSI and widely applied in South Asian hospitals, remains critical for accurate clindamycin use [6,13].

The odds ratio for iMLSB in MRSA vs MSSA was 1.36 (95% CI: 0.42–4.37), suggesting a higher likelihood of inducible resistance in MRSA, though not statistically significant. However, the exclusive presence of cMLSB in MRSA (20%, $p = 0.02$) demonstrates a noteworthy pattern, supported by findings from Thapa et al. (2021) and Assefa (2022), who reported similar links between methicillin and constitutive clindamycin resistance [14,15].

Vancomycin and linezolid susceptibility across all isolates is encouraging but warrants continuous surveillance, as increasing reports of VISA and VRSA globally threaten these last-line therapies [16]. The high prevalence of inducible resistance among MRSA reinforces recommendations by Gupta et al. (2009) and Lall & Sahni (2014) to integrate D-testing into routine susceptibility testing workflows to improve clinical outcomes [17,18].

Limitations

This study was limited by its single-centre design and relatively small sample size, which may restrict the generalizability of findings. Molecular confirmation of resistance mechanisms (e.g., *mecA*, *erm* genes) was not performed, which could have provided deeper insights into genetic

determinants. Additionally, clinical outcome data were not included, limiting assessment of treatment impact related to resistance phenotypes.

Conclusion

This study highlights a substantial prevalence of MRSA (38%) and a high rate of inducible clindamycin resistance (30.4%) among erythromycin-resistant *Staphylococcus aureus* isolates in a tertiary care hospital setting. MRSA strains exhibited significantly higher resistance to multiple antibiotics compared to MSSA, with clindamycin resistance being notably more prevalent.

The exclusive detection of constitutive clindamycin resistance in MRSA and the statistically significant association underscore the importance of phenotypic testing, particularly the D-test, in guiding effective antibiotic therapy. Given the continued susceptibility to vancomycin and linezolid, these agents remain reliable options, though their use must be preserved through strict antimicrobial stewardship.

Routine implementation of D-testing and continuous local surveillance are essential for optimizing treatment outcomes, preventing therapeutic failures, and curbing the spread of multidrug-resistant *S. aureus* strains in clinical settings.

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