Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu

RESEARCH ARTICLE

Phenotypic and Molecular Characterization of Mupirocin Resistance Mechanisms in Clinical Isolates of Staphylococcus aureus

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Article History
Received: 07.07.2025
Revised: 12.08.2025
Accepted: 17.09.2025
Published: 03.10.2025

Abstract: ***Background:*** Staphylococcus aureus remains a major cause of both community- and hospital-acquired infections, with methicillin-resistant S. aureus (MRSA) posing significant therapeutic challenges. Mupirocin is a critical topical agent used for decolonization, but the emergence of resistance threatens its effectiveness. Data on phenotypic and molecular characterization of mupirocin resistance in Indian hospitals are limited. ***Methods:*** A cross-sectional study was conducted over 18 months in a tertiary-care hospital in North India. A total of 246 clinical isolates of S. aureus were identified by standard microbiological tests. MRSA and MSSA were differentiated using cefoxitin disc diffusion. Antimicrobial susceptibility was assessed by the Kirby–Bauer method. Mupirocin resistance was screened phenotypically using 5 μg and 200 μg discs and categorized as low-level (LLMR) or high-level (HLMR). PCR was used to detect mecA and mupA genes. ***Results:*** Of the 246 isolates, 95 (38.6%) were MRSA and 151 (61.4%) MSSA. Mupirocin resistance was found in 46 isolates (18.7%), with 31 (67.4%) showing HLMR and 15 (32.6%) LLMR. PCR confirmed mecA in 92/95 (96.8%) MRSA isolates. The mupA gene was detected in 32/46 mupirocin-resistant isolates (69.6%). Among HLMR isolates, 30/31 (96.8%) carried mupA, whereas only 2/15 (13.3%) LLMR isolates harbored the gene. Phenotype–genotype concordance was excellent for HLMR. All isolates remained 100% susceptible to linezolid and vancomycin. ***Conclusion:*** Mupirocin resistance was observed in nearly one-fifth of S. aureus isolates, with high-level resistance predominating. The mupA gene showed strong correlation with HLMR, making PCR a reliable confirmatory tool for detection. The findings underscore the need for routine mupirocin resistance surveillance, prudent use of topical antimicrobials, and inclusion of mupirocin testing in hospital antibiograms to prevent decolonization failures.

Keywords: Staphylococcus aureus, MRSA, mupirocin resistance, mecA, mupA, PCR, antimicrobial resistance

INTRODUCTION

Staphylococcus aureus is a versatile pathogen responsible for a wide range of infections, from superficial skin and soft tissue infections to severe invasive diseases such as bacteremia, pneumonia, osteomyelitis, and endocarditis [1]. Its clinical significance is compounded by its ability to acquire resistance to multiple antibiotics. The emergence of methicillin-resistant S. aureus (MRSA), first reported in the 1960s, has become a global public health concern, with prevalence rates ranging from 20% to 50% in hospital settings worldwide [2,3]. In India, MRSA prevalence varies geographically, but multiple studies report rates between 30% and 40%, posing significant challenges to treatment and infection control [4,5].

Mupirocin is a topical antimicrobial agent widely used for the eradication of S. aureus nasal carriage, particularly in patients undergoing surgery, intensive care admissions, and among healthcare workers to prevent outbreaks [6]. It inhibits bacterial protein synthesis by binding to isoleucyl-tRNA synthetase (IleRS) [7]. However, excessive and indiscriminate use has led to the emergence of resistance, which can compromise decolonization strategies and facilitate nosocomial transmission [8].

Mupirocin resistance is phenotypically classified as low-level mupirocin resistance (LLMR), usually due to point mutations in the chromosomal ileS gene, and high-level mupirocin resistance (HLMR), most often mediated by the plasmid-borne mupA (or rarely mupB) gene that encodes an alternate IleRS enzyme [9,10]. LLMR typically allows partial efficacy of mupirocin in decolonization, whereas HLMR is strongly associated with treatment failure [11]. Studies from Europe, North America, and Asia have reported mupirocin resistance rates ranging from 5% to 25%, with HLMR predominating in settings where mupirocin use is widespread [12–14].

Molecular methods such as PCR are valuable in confirming resistance mechanisms, particularly for HLMR where the presence of mupA strongly correlates with resistance [15]. However, conventional PCR is limited in detecting LLMR caused by ileS mutations. Therefore, combined phenotypic and molecular

characterization provides a comprehensive understanding of mupirocin resistance epidemiology [16].

Given the limited Indian data integrating both phenotypic and molecular approaches, the present study aimed to investigate the prevalence of MRSA and MSSA isolates from clinical samples, assess their antibiotic susceptibility patterns, determine phenotypic mupirocin resistance (LLMR vs HLMR), and detect mecA and mupA genes by PCR.

MATERIAL AND METHODS

Study Design and Setting

A cross-sectional study was conducted in the Department of Microbiology, KM Medical College & Hospital, Mathura, Uttar Pradesh, over 18 months, following scientific and ethical committee approval.

Sample Size and Isolates

A total of 246 consecutive non-duplicate clinical isolates of S. aureus were included. Isolates were obtained from pus/wound swabs, blood, urine, respiratory samples, body fluids, and catheter tips. Only confirmed S. aureus isolates were included; non-S. aureus isolates were excluded.

Identification of Isolates

Standard microbiological procedures were followed:

- Gram stain: Gram-positive cocci in clusters.
- Catalase test: Bubble production confirming catalase activity.
- Coagulase tests: Both slide and tube methods for free and bound coagulase.
- DNase test: Hydrolysis of DNA on DNase agar.

Detection of MRSA

MRSA was identified using cefoxitin (30 µg) disc diffusion on Mueller–Hinton agar with 4% NaCl, as per CLSI guidelines. Zone diameter ≤21 mm was considered resistant (MRSA).

Antimicrobial Susceptibility Testing

Kirby-Bauer disc diffusion was performed for penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, gentamicin, cotrimoxazole, linezolid, and vancomycin, and results were interpreted as per CLSI standards.

Phenotypic Detection of Mupirocin Resistance

Disc diffusion with 5 µg and 200 µg mupirocin discs was used:

- Zone >14 mm: Susceptible
- Zone <14 mm with 5 µg but >14 mm with 200 µg: LLMR
- Zone <14 mm with both discs: HLMR
- Molecular Detection of Resistance Genes (PCR)
- DNA was extracted using a commercial kit (Qiagen, Germany). PCR was performed with published primers:
- mecA: forward 5′-AAAATCGATGGTAAAGGTTGGC-3′;
- mecA: reverse 5′-AGTTCTGCAGTACCGGATTTGC-3′ (~533 bp)
- mupA: forward 5′-AGTACAGAGAAATGGCTGAA-3′;
- mupA: reverse 5′-ATACAGGTCTTTAGCATTGC-3′ (~456 bp / 1.6 kb depending on primers)

PCR amplification was carried out in a Bio-Rad T100 Thermal Cycler under standard cycling conditions. Amplicons were visualized on 1.5% agarose gel with ethidium bromide. Positive and negative controls (ATCC strains) were included.

RESULTS AND OBSERVATIONS:

A total of 246 non-duplicate clinical isolates of *Staphylococcus aureus* were included in the study. The findings are presented in sequential order, beginning with the distribution of isolates as MRSA and MSSA, followed by their antibiotic susceptibility profiles, the prevalence of mupirocin resistance, and the molecular detection of resistance determinants. Correlation between phenotypic resistance and PCR-based detection of *mupA* was also assessed.

1. Prevalence of MRSA and MSSA

Of 246 isolates, 95 (38.6%) were MRSA and 151 (61.4%) MSSA.

Table 1: Distribution of *Staphylococcus aureus* Isolates as MRSA and MSSA

Isolate Type	Number (n)	Percentage (%)
MRSA	95	38.6
MSSA	151	61.4
Total	246	100

Table 1 shows the distribution of 246 clinical isolates into MRSA and MSSA categories. MRSA constituted 38.6% of all isolates, reflecting a significant burden of methicillin resistance in the hospital setting.

2. Antibiotic Susceptibility Pattern

MRSA isolates showed significantly higher resistance compared to MSSA, except for linezolid and vancomycin, which remained universally active.

Table 2: Antibiotic Susceptibility Pattern of MRSA vs MSSA

Antibiotic	MRSA Sensitive (%)	MRSA Resistant (%)	MSSA Sensitive (%)	MSSA Resistant (%)	p-value
Penicillin	5.2	94.8	13.2	86.8	<0.05
Erythromycin	31.6	68.4	66.2	33.8	<0.01
Clindamycin	57.9	42.1	79.5	20.5	<0.01
Ciprofloxacin	29.5	70.5	72.8	27.2	<0.001
Gentamicin	47.4	52.6	76.2	23.8	<0.001
Cotrimoxazole	36.8	63.2	71.5	28.5	<0.001
Linezolid	100	0	100	0	-
Vancomycin	100	0	100	0	-

Table 2 demonstrates the antibiotic resistance profile of MRSA compared to MSSA. MRSA isolates exhibited significantly higher resistance to multiple antibiotics, though all isolates remained 100% susceptible to linezolid and vancomycin.

3. Prevalence of Mupirocin Resistance

Among all isolates, 46 (18.7%) were mupirocin-resistant, comprising 31 HLMR (67.4%) and 15 LLMR (32.6%).

Table 3: Distribution of Mupirocin Resistance among *S. aureus* Isolates

Resistance Category	Number (n)	Percentage (%)
Sensitive	200	81.3
LLMR	15	6.1
HLMR	31	12.6
Total	246	100

Table 3 illustrates the overall prevalence of mupirocin resistance. A total of 18.7% isolates were resistant, with high-level resistance (HLMR) accounting for the majority compared to low-level resistance (LLMR).

4. PCR Detection of Resistance Genes

Table 4: Detection of *mecA* Gene among MRSA and MSSA Isolates

Isolate Type	Total (n)	*mecA* Positive (n, %)	*mecA* Negative (n, %)
MRSA	95	92 (96.8%)	3 (3.2%)
MSSA	151	0 (0%)	151 (100%)
Total	246	92 (37.4%)	154 (62.6%)

Table 4 shows the PCR-based detection of the mecA gene. Almost all MRSA isolates carried mecA, while none of the MSSA isolates were positive, confirming their methicillin susceptibility.

Table 5: Detection of *mupA* Gene among Mupirocin-Resistant Isolates

Resistance Category	Total Isolates (n)	*mupA* Positive (n, %)	*mupA* Negative (n, %)
LLMR	15	2 (13.3%)	13 (86.7%)
HLMR	31	30 (96.8%)	1 (3.2%)
Total Resistant	46	32 (69.6%)	14 (30.4%)

Table 5 demonstrates the detection of the mupA gene among mupirocin-resistant isolates. The gene was strongly associated with high-level resistance (HLMR), while most low-level resistant isolates lacked mupA, suggesting alternative mechanisms.

5. Phenotype–Genotype Correlation

Table 6: Correlation Between Phenotypic Mupirocin Resistance and *mupA* Detection

Phenotypic Category	Total Isolates (n)	*mupA* Positive (n, %)	*mupA* Negative (n, %)
Sensitive	200	0 (0%)	200 (100%)
LLMR	15	2 (13.3%)	13 (86.7%)
HLMR	31	30 (96.8%)	1 (3.2%)
Total	246	32 (13.0%)	214 (87.0%)

Table 6 presents the correlation between phenotypic resistance and molecular detection of mupA. There was excellent concordance for HLMR, while LLMR was mostly unexplained by mupA, indicating alternative genetic mechanisms.

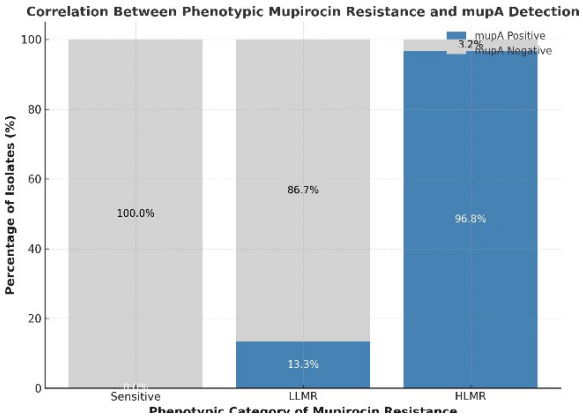

Figure 1. Correlation between phenotypic mupirocin resistance and mupA gene detection in Staphylococcus aureus. High-level resistant (HLMR) isolates showed strong mupA positivity (96.8%), while most low-level resistant (LLMR) isolates lacked the gene.

DISCUSSION

This study highlights the prevalence and molecular characterization of mupirocin resistance among S. aureus isolates in a tertiary-care hospital. The proportion of MRSA in our study (38.6%) was consistent with reports from other Indian centers, where MRSA prevalence has ranged between 30% and 40% [4,5,17]. Such rates reflect ongoing challenges of antimicrobial resistance in hospital settings and the necessity of robust infection-control measures.

The overall mupirocin resistance rate of 18.7% observed in our isolates is significant. Comparable rates have been documented in South India (15–20%) and Nepal (18%) [18,19]. Studies from Europe and North America, however, have reported higher prevalence in some centers (up to 25–30%) due to widespread mupirocin use in decolonization programs [12,14,20]. Our findings suggest that mupirocin resistance, though moderate, is emerging as an important concern in Indian hospitals.

Among resistant isolates, HLMR predominated (67.4%). This is clinically important, as HLMR is strongly associated with mupirocin decolonization failure [11]. A study from the UK demonstrated that patients colonized with HLMR MRSA strains had persistent carriage despite mupirocin therapy [12]. Similarly, in Spain and Canada, HLMR was linked with hospital outbreaks where mupirocin decolonization protocols failed [21,22].

Molecular analysis revealed mecA in nearly all MRSA isolates (96.8%), which is in agreement with global studies confirming mecA as the dominant methicillin resistance determinant [23]. Importantly, mupA was detected in 69.6% of mupirocin-resistant isolates, with excellent correlation to HLMR (96.8%). Similar correlations have been reported in the UK, where >95% of HLMR isolates carried mupA [12], and in Canadian and Spanish studies where concordance exceeded 90% [21,22].

In contrast, only 13.3% of LLMR isolates in our study carried mupA. This finding supports the role of chromosomal ileS mutations in mediating LLMR, as shown in earlier studies [9,24]. The absence of mupB in our isolates aligns with global reports indicating its rarity [10]. Thus, PCR-based detection of mupA remains highly reliable for confirming HLMR, though sequencing is required to fully elucidate LLMR mechanisms.

From an antimicrobial susceptibility standpoint, MRSA isolates in our study showed higher resistance rates compared to MSSA for commonly used antibiotics such as erythromycin, clindamycin, ciprofloxacin, and cotrimoxazole. These findings are consistent with prior Indian studies [4,25], underscoring the multidrug-resistant nature of MRSA. Encouragingly, all isolates remained susceptible to linezolid and vancomycin, reaffirming their role as last-line agents.

The clinical and epidemiological implications of these findings are noteworthy. The presence of mupirocin resistance, especially HLMR, threatens the success of decolonization protocols. This could lead to persistent carriage among patients and healthcare workers, facilitating nosocomial transmission [26]. Moreover, the strong association of mupirocin resistance with multidrug resistance, as observed in our isolates, compounds therapeutic challenges. These concerns echo global experiences where mupirocin resistance has undermined MRSA control programs [27].

Therefore, regular surveillance of mupirocin susceptibility, inclusion of mupirocin in hospital antibiograms, and stewardship of topical antibiotics should be prioritized. Restricting indiscriminate mupirocin use and reserving it for targeted decolonization regimens could help preserve its efficacy [28].

In summary, this study demonstrates that mupirocin resistance in our setting is predominantly mupA-mediated and strongly associated with high-level resistance. The integration of phenotypic testing with molecular confirmation enhances detection accuracy and provides a reliable framework for guiding infection-control strategies.

CONCLUSION

Mupirocin resistance was detected in nearly one-fifth of S. aureus isolates, with HLMR predominating. The mupA gene was strongly associated with HLMR and reliably detected by PCR. These findings underscore the need for routine mupirocin resistance screening and judicious use of topical antibiotics to prevent resistance-driven decolonization failure.

6. CONFLICT OF INTEREST: None

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