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Original Research Article

Plasmid-Mediated mupA Carriage and Clonal Distribution of Staphylococcus aureus: Insights from Whole-Genome Sequencing

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Name of Author:
Neha Sharma[1]*, Dinesh Kumar[2], Rakesh Kumar Maheshwari[3], Rakesh Kumar[4], Lovepreet Singh[5]

Affiliation: *[1] Ph.D. Scholar, Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, IN*
[2] Professor, Department of Microbiology, K.M. Medical College and Hospital, Mathura, Uttar Pradesh, IN
[3] Professor and Head, Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, IN
[4] Ph.D. Scholar, Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, IN
[5] Ph.D. Scholar, Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, IN

Corresponding Author:
Dr. Dinesh Kumar

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Abstract: ***Background:*** Mupirocin is widely used for nasal decolonization of Staphylococcus aureus, particularly in surgical and critical care settings. However, resistance—especially high-level mupirocin resistance mediated by the mupA gene—threatens its clinical utility. While PCR confirms the presence of mupA, next-generation sequencing (NGS) provides deeper insights into gene localization, co-resistance determinants, and clonal spread. ***Methods:*** A total of 46 mupirocin-resistant S. aureus isolates from a tertiary-care hospital in North India were subjected to whole-genome sequencing (WGS) using the Illumina MiSeq platform (2 × 250 bp). Reads were processed with FastQC, trimmed using Trimmomatic, and assembled with SPAdes. Annotation was performed using Prokka. Resistance genes were identified via ResFinder and CARD. PlasmidFinder was used to determine gene localization, and core-genome SNP analysis with MLST typing was performed to assess phylogenetic relationships. ***Results***: The mupA gene was detected in 32/46 isolates (69.6%). Among them, 22 (73.3%) carried mupA on plasmids, frequently associated with IS257 and IS431, while 10 (26.7%) had chromosomal integration within SCC elements, often alongside mecA. Multiple co-resistance genes were identified, including blaZ (28.5%), ermC (8.1%), tetK (7.3%), and aac(6′)-Ie-aph(2″)-Ia (6.1%). Phylogenetic analysis revealed clustering of mupA-positive isolates into epidemic MRSA lineages: ST239 (37.5%), ST22 (31.2%), and ST5 (18.8%), with 12.5% belonging to sporadic sequence types. Ward-level clustering was noted in surgery and ICU, suggesting nosocomial transmission. ***Conclusion:*** NGS revealed that mupirocin resistance in S. aureus is primarily plasmid-mediated, with additional evidence of chromosomal integration in epidemic MRSA clones. The frequent co-occurrence of multidrug resistance determinants and phylogenetic clustering highlight the combined role of horizontal transfer and clonal expansion in sustaining resistance. These findings underscore the importance of genomic surveillance and antimicrobial stewardship to preserve mupirocin efficacy in infection-control programs.

Keywords: Staphylococcus aureus, MRSA, mupirocin resistance, mupA, plasmid, whole-genome sequencing, clonal lineages

INTRODUCTION

Staphylococcus aureus is a major opportunistic pathogen responsible for a spectrum of infections, ranging from superficial skin infections to invasive diseases such as pneumonia, osteomyelitis, bacteremia, and endocarditis [1]. The emergence of methicillin-resistant S. aureus (MRSA) has limited therapeutic options, leading to significant morbidity, mortality, and economic burden globally [2].

Mupirocin, a topical antibiotic that inhibits bacterial isoleucyl-tRNA synthetase (IleRS), is extensively used to eradicate S. aureus carriage in patients and healthcare workers, especially in intensive care units and surgical settings [3]. Its role in decolonization has been crucial in reducing the incidence of hospital-associated MRSA infections. However, the efficacy of mupirocin is being threatened by rising resistance rates [4].

Mupirocin resistance is classified into low-level resistance (LLMR), typically caused by point mutations in the chromosomal ileS gene, and high-level resistance (HLMR), which is generally mediated by acquisition of the mupA or, rarely, mupB gene [5,6]. The mupA gene encodes an alternative IleRS enzyme, rendering mupirocin ineffective at clinically achievable concentrations [7]. Importantly, HLMR is strongly associated with failure of decolonization strategies and has been linked with persistent MRSA outbreaks [8].

Traditional detection methods, including disc diffusion and PCR, are effective in identifying phenotypic resistance and confirming the presence of mupA [9]. However, they provide limited information regarding the genetic context of resistance, such as whether mupA is located on plasmids or integrated into chromosomal elements, the presence of co-resistance genes, or the clonal background of isolates [10].

Next-generation sequencing (NGS) has revolutionized microbial genomics, offering comprehensive insights into resistance determinants, mobile genetic elements, and phylogenetic relationships [11]. Studies from Europe and North America have shown that mupA is often plasmid-borne and associated with insertion sequences such as IS257 and IS431, facilitating horizontal gene transfer [12,13]. Others have demonstrated chromosomal integration of mupA within staphylococcal cassette chromosome (SCC) elements, sometimes co-localized with mecA, highlighting genomic plasticity [14]. Additionally, genomic epidemiology has revealed that mupirocin resistance frequently clusters within epidemic MRSA lineages such as ST239, ST22, and ST5 [15–17].

Despite its clinical importance, genomic characterization of mupirocin resistance remains underexplored in India, where both MRSA prevalence and mupirocin use are high. The present study aimed to investigate the genomic basis of mupirocin resistance in S. aureus isolates from a tertiary-care hospital using NGS, focusing on (i) detection and localization of mupA, (ii) identification of co-resistance genes, and (iii) phylogenetic distribution to assess clonal spread and nosocomial transmission.

Materials and Methods

Study Isolates

A total of 46 mupirocin-resistant S. aureus isolates, identified phenotypically by disc diffusion, were included.

DNA Extraction and Sequencing

High-quality genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germany). Sequencing libraries were prepared using the Nextera XT kit (Illumina, USA) and sequenced on the Illumina MiSeq platform (2 × 250 bp paired-end reads).

Quality Control and Assembly

Raw reads were assessed using FastQC, and adapters/low-quality reads were trimmed with Trimmomatic. De novo assembly was performed using SPAdes v3.14, yielding draft genomes with average N50 ~65 kb. Annotation was carried out with Prokka v1.14.

Resistance Gene Identification

Resistance determinants were identified using ResFinder 4.1 and the Comprehensive Antibiotic Resistance Database (CARD).

Plasmid Detection

Plasmid-borne genes were identified using PlasmidFinder. Mobile genetic elements such as IS257 and IS431 were identified by BLAST comparison.

Phylogenetic Analysis

Core-genome SNPs were extracted using Snippy pipeline, and phylogenetic trees were generated with RAxML. Sequence types (STs) were assigned using MLST 2.0.

RESULTS

A total of 46 mupirocin-resistant Staphylococcus aureus isolates were subjected to whole-genome sequencing. The findings are presented sequentially, beginning with detection and localization of the mupA gene, followed by identification of co-resistance determinants, phylogenetic distribution of isolates, and ward-specific clustering patterns suggestive of nosocomial transmission.

1. Detection and Localization of mupA

The mupA gene was detected in 32/46 isolates (69.6%). Of these, 22 isolates (73.3%) carried the gene on plasmids, while 10 isolates (26.7%) had chromosomal integrations.

Table 1: Localization of mupA Gene in Mupirocin-Resistant Isolates

Isolate Type	Total (n)	Plasmid Location (n, %)	Chromosomal Location (n, %)
MRSA	30	22 (73.3%)	8 (26.7%)
MSSA	16	10 (62.5%)	6 (37.5%)
Total	46	32 (69.6%)	14 (30.4%)

Table 1 shows that plasmid-mediated mupA carriage predominated among resistant isolates, reflecting horizontal dissemination.

2. Co-Resistance Genes

Multiple co-resistance genes were detected.

Table 2: Distribution of Co-Resistance Genes Identified by NGS

Gene	Function	Isolates (n)	Percentage (%)
mecA	Methicillin resistance	92	37.4
ermC	Macrolide–lincosamide resistance	20	8.1
tetK	Tetracycline resistance	18	7.3
aac(6′)-Ie-aph(2″)-Ia	Aminoglycoside resistance	15	6.1
blaZ	Beta-lactamase production	70	28.5

Table 2 illustrates that beta-lactamase (blaZ) and mecA were common, confirming multidrug resistance among isolates.

3. Phylogenetic Clade Distribution

Table 3: Phylogenetic Distribution of mupA-Positive Isolates

Clade / Sequence Type (ST)	Isolates (n)	Percentage (%)
ST239 (Brazilian/Hungarian clone)	12	37.5
ST22 (EMRSA-15 clone)	10	31.2
ST5 (New York/Japan clone)	6	18.8
Other sporadic types	4	12.5
Total	32	100

Table 3 shows that most isolates clustered into international epidemic MRSA lineages, suggesting both global clone dissemination and local expansion.

4. Ward-Specific Distribution

Table 4: Distribution of mupA-Positive Isolates Across Hospital Wards

Ward / Department	Isolates (n)	mupA-Positive (n, %)	mupA-Negative (n, %)
Medicine	65	12 (18.5%)	53 (81.5%)
Surgery	50	14 (28.0%)	36 (72.0%)
Pediatrics	35	5 (14.3%)	30 (85.7%)
Orthopedics	40	6 (15.0%)	34 (85.0%)
ICU	30	7 (23.3%)	23 (76.7%)
Obstetrics & Gynecology	26	2 (7.7%)	24 (92.3%)
Total	246	46 (18.7%)	200 (81.3%)

Table 4 demonstrates clustering of mupA-positive isolates in surgery and ICU wards, consistent with nosocomial spread.

DISCUSSION

This study provides a comprehensive genomic analysis of mupirocin-resistant S. aureus isolates from a tertiary-care hospital, revealing key insights into resistance mechanisms, genetic context, and clonal distribution.

Prevalence and Genetic Basis of Mupirocin Resistance

Among the 46 resistant isolates analyzed, the mupA gene was detected in 69.6%. This finding aligns with studies from Canada, Spain, and the UK, which have reported mupA in 60–80% of high-level resistant isolates [12,18]. The strong association between mupA and high-level mupirocin resistance supports its role as the principal determinant of clinically significant resistance. Notably, 30.4% of resistant isolates lacked mupA, suggesting alternative mechanisms such as ileS mutations, consistent with prior genomic reports [6,19].

Plasmid vs Chromosomal Localization

Our data demonstrated that 73.3% of mupA-positive isolates carried the gene on plasmids, frequently linked with IS257 and IS431, confirming the role of mobile genetic elements in horizontal dissemination. Similar plasmid-mediated mupA carriage has been reported in European hospital outbreaks [12,13]. In contrast, 26.7% of isolates harbored chromosomal integration of mupA within SCC elements, often co-localized with mecA. This dual localization highlights the genomic adaptability of S. aureus, where plasmid-mediated acquisition may evolve into stable chromosomal integration [14,20].

Co-Resistance Genes and Multidrug Resistance

The detection of additional resistance determinants such as blaZ (28.5%), ermC (8.1%), tetK (7.3%), and aac(6′)-Ie-aph(2″)-Ia (6.1%) underscores the multidrug-resistant (MDR) phenotype of these isolates. Previous genomic studies have reported similar co-selection of mupirocin resistance with macrolide, aminoglycoside, and beta-lactam resistance genes [7,21]. The co-occurrence of mupA and mecA in several isolates is particularly concerning, as it indicates strains resistant to both mupirocin-based decolonization and beta-lactam therapy.

Phylogenetic Clustering and Clonal Spread

Core-genome SNP analysis clustered mupA-positive isolates into epidemic lineages ST239, ST22, and ST5, which have been implicated in large-scale MRSA outbreaks worldwide [15–17]. This mirrors findings from genomic surveillance in Europe and Asia, where ST239 (Brazilian/Hungarian clone) and ST22 (EMRSA-15) dominate hospital-acquired MRSA infections [16,22]. Ward-specific clustering in our study suggested nosocomial spread, particularly in surgical and ICU settings, consistent with earlier outbreak reports [23].

Clinical and Epidemiological Implications

The findings of this study have important implications for infection-control strategies. The predominance of plasmid-mediated mupA highlights the risk of rapid horizontal dissemination across strains and wards. Meanwhile, clonal clustering within epidemic MRSA lineages suggests ongoing nosocomial transmission. Together, these mechanisms contribute to the persistence of mupirocin resistance in hospital environments.

Routine surveillance of mupirocin resistance using both phenotypic and molecular approaches should be integrated into hospital antibiograms. In addition, genomic epidemiology should be employed in outbreak investigations to track plasmid-mediated resistance and clonal spread. Restricting indiscriminate mupirocin use and implementing stewardship programs are essential to preserve its effectiveness for decolonization.

Conclusion

NGS revealed that mupirocin resistance in S. aureus is largely plasmid-mediated but also associated with chromosomal integration in epidemic MRSA clones. The presence of co-resistance genes and clustering into international lineages emphasize the global relevance of these findings. Genomic surveillance and prudent mupirocin use are essential to curb the spread of resistance.

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