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Phenotypic and Genotypic Characterization of Macrolide-Lincosamide-Streptogramin B Resistance in *Staphylococcus aureus* Isolated from Skin and Soft Tissue Infections

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Abstract

The usefulness of clindamycin in the management of staphylococcal skin and soft tissue infections (SSTIs) is threatened by the increasing prevalence of macrolide-lincosamide-streptogramin B (MLS_B) resistance. Routine susceptibility testing may miss inducible clindamycin resistance and falsely classify a resistant strain as clindamycin susceptible. Such major errors can lead to treatment failure. The present study aimed to investigate the frequency of MLS_B-resistant phenotypes among *Staphylococcus aureus* isolates from SSTIs, as well as the distribution of erythromycin ribosome methylase genes (*erm*) in these isolates. A total of 233 *S. aureus* isolates were prospectively obtained from SSTIs over the course of a year. An automated identification system, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), was used to identify the isolates. Antimicrobial susceptibility testing was performed using the VITEK 2 system. The D-test was performed for all erythromycin-resistant isolates. Erythromycin ribosome methylation genes *ermA*, *ermB*, and *ermC* were analyzed by multiplex PCR. Of the 233 isolates, 91.8% exhibited erythromycin resistance, and 39.5% showed clindamycin resistance. Inducible MLS_B ($iMLS_B$), constitutive MLS_B ($cMLS_B$), and MS phenotypes accounted for 36.9%, 6.07%, and 57.0% of the isolates, respectively. *erm* genes were detected in 42.9% of isolates. *ermC* was predominant (89.1%), followed by *ermA* (10.9%); *ermB* was not detected. *erm* genes were significantly found in $iMLS_B$ strains ($P < 0.001$). High frequencies of inducible clindamycin resistance support routine double-disk diffusion testing for erythromycin-resistant *S. aureus* isolates. The widespread prevalence of *ermC* underscores the importance of combining genetic and phenotypic surveillance to optimize treatment and preserve antibiotic efficacy. It also guides stewardship practices.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Skin and Soft Tissue Infections, Inducible Macrolide-Lincosamide-Streptogramin B, Erythromycin Ribosome Methylation Genes

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INTRODUCTION

Staphylococcus aureus is one of the ESKAPE pathogens that urgently warrants new treatment approaches. It is a major cause of skin and soft tissue infections. Except for more recent drugs such as ceftaroline, almost all β-lactam antibiotics are ineffective against these strains owing to methicillin resistance, conferred by the *mecA* gene encoding penicillin-binding protein 2a. These therapeutic constraints have prompted the clinical use of macrolides, lincosamides, and streptogramin B (MLS_B) as substitutes. Owing to its good oral absorption and wide tissue penetration, clindamycin has emerged as a favoured empirical option for methicillin-resistant *S. aureus* (MRSA) skin and soft tissue infections.[1] However, the prevalence of staphylococcal strains resistant to MLS_B antibiotics has increased because of the extensive use of such antibiotics. Erythromycin ribosome methylation (*erm*) genes usually confer resistance to erythromycin and clindamycin.[2]

MLS_B resistance can be expressed inducibly ($iMLS_B$) or constitutively ($cMLS_B$). Inducible clindamycin resistance is of particular concern in the clinical setting. Isolates may appear susceptible to clindamycin on standard antimicrobial susceptibility testing yet develop resistance during therapy, resulting in treatment failure. However, classifying all erythromycin-resistant *S. aureus* strains as clindamycin-resistant could prevent the use of clindamycin in situations where it would be a useful treatment.[3,4]

MLS_B resistance prevalence varies globally (10%-60%). Inducible resistance is frequently underreported due to the lack of routine D-testing. However, there is a dearth of comprehensive information regarding the distribution of MLS_B phenotypes and *erm* gene carriage in *S. aureus*. This study aimed to characterize specific clindamycin-resistant phenotypes in *S. aureus* isolates and analyze the prevalence and distribution of *erm* determinants across them.

MATERIALS AND METHODS

A prospective observational study was conducted in a tertiary healthcare institution in southern India from October 2025 to June 2026. The study was approved by the institutional ethics committee. *S. aureus* isolates from SSTIs were analyed in this study. *S. aureus* repeatedly isolated from the same patient with similar susceptibilities during the study period and isolates from other sources (blood, respiratory, or urinary specimens) were excluded.

Exudate specimens such as pus, wound swabs, tissue, and aspirates were cultured on MacConkey agar and 5% sheep blood agar, as per standard laboratory operating procedures. *S. aureus* was identified using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF-VITEK MS) system. Antimicrobial susceptibility testing was performed using the VITEK 2 system (BioMérieux, Inc., Durham, NC, USA). Antibiotics such as cefoxitin, oxacillin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin, doxycycline, clindamycin, vancomycin, teicoplanin, and linezolid were tested. *S. aureus* was reported as MRSA after minimum inhibitory concentration (MIC) testing for cefoxitin and oxacillin using the VITEK 2 automated system. Isolates were considered methicillin resistant if their MICs for oxacillin and cefoxitin were >4 µg/mL and the cefoxitin screen was flagged positive. Isolates exhibiting intermediate resistance or resistance to erythromycin were subjected to the double-disk approximation test (D-test),[5] and multiplex PCR was used to detect the presence of *erm* genes.[1] Other resistance determinants associated with the MLS_B phenotype were not evaluated.

Statistical analysis

Statistical evaluation was performed using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). The chi-square test was used to examine differences between unordered categorical variables, which were expressed as percentages. P-values below 0.05 were considered statistically significant, and all tests were two-tailed.

RESULTS

Antibiotic susceptibility

Antibiotic susceptibility tests were performed on 233 non-repetitive *S. aureus* isolates. Among these, 125 (53.6%) were MRSA isolates. Doxycycline susceptibility was the highest in *S. aureus* isolates (216, 92.7%). All the strains

were 100% susceptible to reserved antibiotics such as vancomycin, teicoplanin, and linezolid. Of the 233 isolates, only 8.2% (n = 19) were susceptible to erythromycin, whereas 91.8% (n = 214) were resistant to it. Only 39.5% (n = 92) of *S. aureus* isolates exhibited clindamycin resistance (Figure). All 214 erythromycin-resistant isolates were further examined for inducible MLS_B resistance using the D-test.

Prevalence of MLS_B-resistant phenotypes

Among the 214 erythromycin-resistant *S. aureus* isolates, an inducible MLS_B ($iMLS_B$) resistance phenotype was detected in 36.9% (79/214). The percentage of isolates exhibiting constitutive resistance ($cMLS_B$) was 6.07% (n = 13). The prevalence of the MS phenotype was 57% (n = 122). Both erythromycin and clindamycin-susceptible *S. aureus* accounted for 8.2% (19/233) of the isolates.

A large number of the MRSA isolates were resistant to erythromycin (n = 110). Among the 110 MRSA isolates, the incidence of inducible resistance to MLS_B was 40.9% (45), constitutive resistance was 5.5% (6), and the MS phenotype was 53.6% (59) Table 1.

Both erythromycin-and clindamycin-susceptible MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates accounted for 10.6% (n = 15) and 2.8% (n = 4) of the isolates, respectively (P = 0.013). Isolates resistant to clindamycin alone were not observed in this study.

Table 1. Frequency of Macrolide-Lincosamide-$Streptogramin_B$ resistant phenotypes

	MRSA (110)	MSSA (104)	Total (214)	P-value
$iMLS_B$	45 (40.9%)	34 (32.7%)	79 (36.9%)	0.468
$cMLS_B$	6 (5.5%)	7 (6.7%)	13 (6.07%)	0.577
MS	59 (53.6%)	63 (60.6%)	122 (57%)	0.090

Table 2. *erm* genes in MRSA and MSSA

erm genes	MRSA	MSSA	Total (n = 92)	P-value
ermC	46 (56.1%)	36 (43.9%)	82 (89.1%)	0.581
ermA	5 (50%)	5 (50%)	10 (10.9%)	0.813

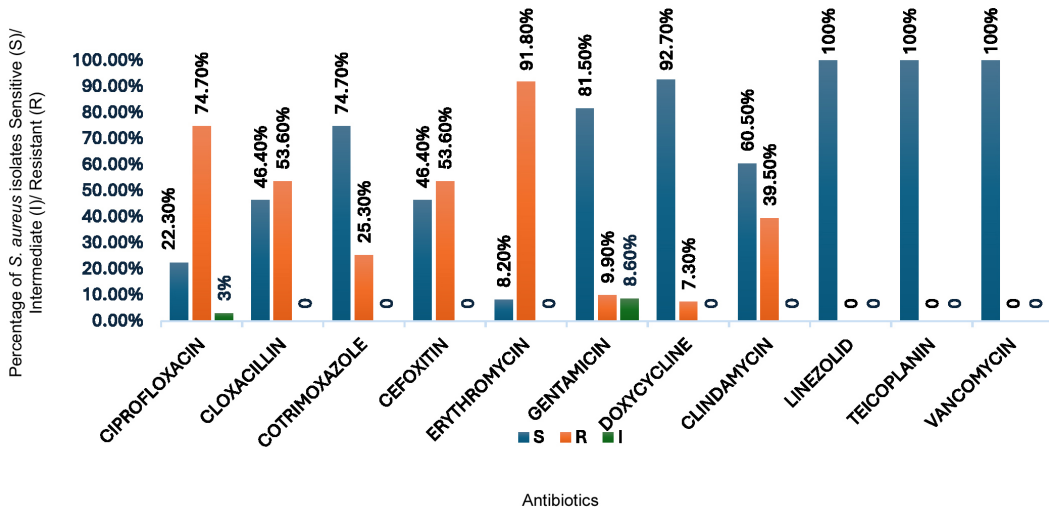


Figure. Antibiotic Susceptibility pattern of *S. aureus* (%) (N = 233)

Table 3. Distribution of *erm* genes in MLS_B-resistant phenotypes

Resistant phenotypes	*ermC*	P-value	*ermA*	P-value
$cMLS_B$	12 (14.6%)	<0.001	1 (10%)	0.534
$iMLS_B$	70 (85.4 %)	<0.001	9 (90%)	<0.001
Total (n = 92)	82 (89.1%)	-	10 (10.9%)	-

Prevalence of *erm* genes

The prevalence of *erm* genes in 214 *S. aureus* isolates was 42.9% (n = 92). *erm* genes were present in 55.4% of the MRSA isolates (n = 51/92) and 44.6% of the MSSA isolates (n = 41/92) (P = 0.659). Table 2 illustrates the distribution of various *erm* genes in the MRSA and MSSA isolates. The occurrence of *erm*C (89.1%; n = 82/92) was significantly higher than that of *erm*A (10.9%; n = 10/92) (P = 0.017). The *erm*B gene was not detected. Overall, the prevalence of *erm* genes was higher in $iMLS_B$ (85.9%; n = 79) than in $cMLS_B$ phenotypes (14.1%; n = 13, P <.001). Table 3 shows the distribution of different *erm* genes in diverse resistant phenotypes.

DISCUSSION

S. aureus infections remain a significant global public health concern owing to their rising prevalence, diverse clinical presentations, and escalating antimicrobial resistance. Skin and soft-tissue infections associated with this bacterium are particularly critical because of their clinical complexity and potential for treatment failure. With the increasing prevalence of MRSA infections, MLS_B antibiotics, particularly clindamycin, serve as important therapeutic alternatives. However, the indiscriminate use of the MLS_B group of antibiotics has led to an increase in resistance among staphylococcal isolates.

In this study, 233 clinical isolates of *S. aureus* were analyzed for antibiotic susceptibility patterns, MLS_B resistance phenotypes, and *erm* gene prevalence. MRSA was found at a frequency of 53.6% in our study population. A similar percentage of MRSA in SSTIs has been documented globally.[1,2,6] Among the skin and soft tissue infections, chronic diabetic ulcers are especially prone to harboring multidrug-resistant organisms owing to repeated empirical antibiotic courses and inadequate source control.[7] MRSA is also predominate in surgical site infections.[8,9] The MRSA isolates remained susceptible to vancomycin, teicoplanin, and linezolid. This result is consistent with those of earlier studies that recognized glycopeptides and oxazolidinones as potent treatments for MRSA infections.[10] Among the non-beta-lactam antibiotics tested, ciprofloxacin demonstrated the highest rate of resistance (74.7%). Suboptimal ciprofloxacin exposure widens the mutant selection window and favors the emergence of ciprofloxacin resistance.[11,12] Doxycycline showed the highest susceptibility (92.7%), followed by gentamicin (81.5%), cotrimoxazole (74.7%), glycopeptides, and oxazolidinones. Doxycycline and cotrimoxazole can be prescribed as oral alternatives for uncomplicated SSTIs. One of the most important findings of the present study was the increased frequency of clindamycin resistance (39.5%, 92/233), although clindamycin is known to be the preferred empirical choice for skin and soft tissue infections caused by MRSA. Resistance to MLS_B antibiotics is clinically relevant in *S. aureus* because these antibiotics share an overlapping binding site on the 50S ribosomal subunit. The 214 isolates in this study had the following MLS_B phenotype distribution: constitutive MLS_B, 6.07%; inducible MLS_B, 36.9%; and the MS phenotype, 57%. This distribution is consistent with what has been reported in several studies.[13-15]

The molecular underpinnings of MLS_B resistance are well documented. *erm* genes encode methyltransferases that simultaneously decrease the binding affinity of macrolides, lincosamides, and streptogramin B by adding one or two methyl groups to 23S rRNA. These *erm* genes are typically located on transposons and plasmids. They facilitate horizontal transfer across staphylococcal populations and explain the rapid dissemination of MLS_B resistance. When *erm* expression is constitutive, the organism is

resistant to all three classes ($cMLS_B$ phenotype) of antibiotics. When the expression is inducible, the organism appears susceptible to clindamycin by standard disc diffusion but can rapidly upregulate *erm*-mediated methylation in the presence of a macrolide inducer such as erythromycin, leading to in vivo treatment failure.[16] Our genotypic analysis revealed that *erm* genes were present in 42.9% (92/214) of all isolates. *erm*C (89.1%) was overwhelmingly more common than *erm*A (10.9%), and *erm*B absent. This *erm*C dominance is consistent with the results of several recent studies.[2,17] The presence of significant numbers of *erm* genes in the $iMLS_B$ ($P < 0.001$) and $cMLS_B$ ($P = 0.004$) phenotypes confirms the link between *erm*-mediated ribosomal methylation and MLS_B resistance.

The predominance of the MS phenotype (52.4%) in our study is noteworthy. It suggests that efflux-mediated macrolide resistance is the dominant mechanism in our *S. aureus* population rather than ribosomal methylation. A distinct pathway involves the *msrA*/*msrB* genes, which encode ATP-dependent efflux pumps that actively export macrolides and streptogramin B, but not lincosamides, from bacterial cells. Isolates harboring only *msrA*/*msrB* display the MS phenotype (erythromycin-resistant, clindamycin-susceptible, D-test negative) and remain genuinely susceptible to clindamycin.

The distinct distribution of MLS_B resistance traits between MRSA and MSSA isolates is an important finding of this study. Most reported studies have consistently found that MRSA exhibits a higher prevalence of $iMLS_B$ and $cMLS_B$ phenotypes than MSSA, although the extent of the difference varies greatly.[17,18] The preponderance of MLS_B resistance in MRSA over MSSA is not coincidental. MRSA strains tend to accumulate multiple resistance determinants because of the selective pressure exerted by the heavy antibiotic exposure that these organisms encounter in hospital environments. Patients infected with MRSA are more likely to have received multiple courses of antibiotics, including macrolides. The overall prevalence of $iMLS_B$ and $cMLS_B$ resistance among MRSA was lower than that reported in other studies.[15,17] This may reflect geographic variations in circulating clonal lineages or differences in local macrolide prescription pressure.

erm genes were found in both MRSA and MSSA isolates but did not differ significantly between MRSA (55.4%) and MSSA (44.6%) ($P = 0.659$). This lack of association contradicts the assumption that *erm* genes are preferentially enriched in MRSA.[18] One possible explanation for this is the independent genetic location of *erm*C. Because *ermC* is carried on small, autonomously replicating plasmids rather than on the SCC*mec* element itself, its acquisition and maintenance are not genetically linked to *mec*A. MSSA strains can acquire *erm*C-carrying plasmids as readily as MRSA strains through conjugation, transduction, or transformation. This contrasts with *erm* A, which in some lineages is located on the transposon Tn554 integrated within or adjacent to the SCC*mec* element.[16] The complete absence of *erm*B in our isolates is consistent with its known association with enterococci and streptococci rather than staphylococci.

This study highlights the increasing prevalence of MLS_B resistance and the important role of *erm* genes in conferring clindamycin resistance in *Staphylococcus aureus* isolates. It is crucial to continuously monitor the spread of resistance and employ both phenotypic and molecular detection techniques to guide appropriate antibiotic therapy.

CONCLUSION

The present study of *S. aureus* isolated from various skin and soft tissue infections highlights several clinical and microbiological findings. Regardless of the methicillin resistance status, routine D-testing should be mandated for all erythromycin-resistant *S. aureus* isolates, given the high prevalence of the inducible MLS_B phenotype (36.9%). Without routine D-testing, these isolates can be falsely reported to be susceptible to clindamycin. The dominance of the MS phenotype (57%) suggests that clindamycin can be safely prescribed for a substantial proportion of erythromycin-resistant isolates, only after inducible resistance is excluded. Genotypic analysis revealed that *erm*C (35.2%) was the predominant *erm* gene in our study, with *erm*A detected at a

much lower frequency (4.3%) and *erm*B absent. The lack of a significant difference in *erm* gene carriage between MRSA and MSSA isolates (P = 0.659) implies that MLS_B resistance can emerge independently of methicillin resistance and that MSSA isolates should not be assumed to be free of MLS_B resistance determinants. In conclusion, the high prevalence of MRSA, the significant burden of inducible MLS_B resistance, and the predominance of *erm*C-mediated resistance in our *S. aureus* isolates highlight the need for an integrated approach incorporating phenotypic detection (D-test), molecular surveillance of *erm* genes, and effective antimicrobial stewardship to optimize treatment outcomes and limit the further spread of resistance.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BB and MV conceptualized and designed the study. BB performed data analysis. BB and JSJ performed data interpretation. BB, MV and JSJ contributed to manuscript writing. MV reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Kasturba Medical College, Manipal, India, under reference number IEC2:543/2025.

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