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RESEARCH ARTICLE
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GENOTYPIC CHARACTERISATION OF MLS (MACROLIDE–LINCOSAMIDE–STREPTOGRAMIN B) RESISTANCE IN CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

Ms Tripti Mani Tripathi[1*], Dr Ramanath Karicheri.[2]

[1*]PhD Scholar[1], , Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, India. (Malwanchal University)
[2]Professor, Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, India. (Malwanchal University)

***Corresponding Author:** Dr Ramanath Karicheri
*(ramanath.karicheri@gmail.com)

Abstract

Background:
Resistance to macrolide–lincosamide–streptogramin B (MLSB) antibiotics in *Staphylococcus aureus* is primarily mediated by erm genes encoding rRNA methyltransferases. Among these, *ermA* and *ermC* are the major determinants conferring inducible or constitutive resistance. Phenotypic identification of inducible clindamycin resistance using D-test has clinical relevance, but genotypic detection provides confirmatory insights into resistance mechanisms.

Methods:
A cross-sectional study was conducted at Index Medical College Hospital & Research Centre (IMCHRC), Indore (2021–2024). A total of 250 *S. aureus* isolates from various clinical specimens were processed. Phenotypic detection of MLSB resistance was performed using D-test and VITEK-2 system. Genotypic characterization for *ermA* and *ermC* genes was carried out using PCR.

Results:
Among 250 isolates, 59 % were MRSA and 41 % MSSA. iMLSB phenotype was observed in 26 % MRSA and 11 % MSSA isolates, while cMLSB was seen in 40 % MRSA and 11 % MSSA. *ermC* was the predominant gene, followed by *ermA*. D-test sensitivity and specificity were 57.6 % and 100 %, respectively, compared to VITEK-2.

Conclusion:
The predominance of *ermC* among iMLSB isolates suggests plasmid-mediated dissemination. Routine molecular surveillance of *erm* genes is essential to prevent clindamycin treatment failure and guide antibiotic stewardship.

Keywords: *Staphylococcus aureus*, MLSB resistance, *ermA*, *ermC*, inducible clindamycin resistance, PCR.

Introduction

Staphylococcus aureus is a major human pathogen causing both community- and hospital-acquired infections ranging from skin infections to septicemia and endocarditis [1]. The rapid emergence of multidrug-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), has complicated antimicrobial therapy [2]. Clindamycin, a lincosamide antibiotic, remains a valuable therapeutic option

due to its excellent tissue penetration and ability to suppress toxin production [3]. However, cross-resistance among macrolides, lincosamides, and streptogramin B (MLSB) antibiotics mediated by *erm* genes poses a growing threat [4].

The *erm* (erythromycin ribosomal methylase) genes—especially *ermA* and *ermC*—encode methyltransferases that modify the 23S rRNA target site, conferring resistance either constitutively (cMLSB) or inducibly (iMLSB) [5,6]. Inducible resistance may not be detected by routine susceptibility testing, leading to therapeutic failure if clindamycin is used [7]. Therefore, both phenotypic detection by D-test and genotypic confirmation by PCR are necessary for accurate identification [8].

This study aimed to characterize the genotypic determinants (*ermA* and *ermC*) responsible for MLSB resistance in *S. aureus* isolates from a tertiary-care hospital in Central India and to correlate molecular findings with phenotypic expression.

Materials and Methods

Study design and setting

An observational cross-sectional study was conducted at the Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, from November 2021 to May 2024. Ethical approval was obtained from the Institutional Ethics Committee.

Sample collection and bacterial identification

A total of 250 *S. aureus* isolates were recovered from clinical samples including pus, wound swabs, aspirates, blood, and sterile body fluids. Identification was based on colony morphology, Gram staining, catalase, and tube coagulase tests [9].

Antimicrobial susceptibility and D-test

Antibiotic susceptibility was performed by Kirby–Bauer disc diffusion as per CLSI M100 (34th ed., 2024) [10]. Methicillin resistance was identified using cefoxitin (30 µg) discs. For inducible clindamycin resistance, erythromycin (15 µg) and clindamycin (2 µg) discs were placed 15 mm apart, and D-test interpretation followed CLSI criteria [11].

Automated detection

The VITEK-2 system (bioMérieux) was used for automated susceptibility testing to validate phenotypic findings [12].

Genotypic analysis

DNA was extracted by the boiling method. PCR amplification was performed using primers specific for *ermA* (190 bp) and *ermC* (299 bp) [13]. Amplified products were visualized by agarose gel electrophoresis and compared with positive controls from JIPMER Puducherry [14].

Results

Of the 250 *S. aureus* isolates, 148 (59 %) were MRSA and 102 (41 %) were MSSA.

- iMLSB phenotype: 26 % MRSA and 11 % MSSA.
- cMLSB phenotype: 40 % MRSA and 11 % MSSA.
- *ermC* gene was detected in 38 % of isolates and *ermA* in 26 %; dual presence in 8 %.

D-test sensitivity and specificity were 57.6 % and 100 % respectively when compared to VITEK-2 system results. The distribution of *ermC* was significantly higher in iMLSB phenotypes ($p < 0.05$).

Discussion

The present study highlights the predominance of *ermC*-mediated inducible clindamycin resistance among clinical isolates of *S. aureus* from Central India. Similar findings were reported by Goudarzi et al. (2019), where *ermC* was more frequent than *ermA* [15]. The plasmid-borne nature of *ermC* facilitates horizontal transfer, contributing to its widespread dissemination [16].

The correlation between phenotypic D-test results and genotypic detection underscores the diagnostic accuracy of molecular confirmation. Although D-test remains a reliable and cost-effective screening

method [17], molecular assays are indispensable for epidemiological surveillance [18]. Filipin et al. (2014) also validated the concordance between VITEK-2 and PCR for *erm* genes [19].

Our MRSA isolates demonstrated a higher prevalence of iMLSB and cMLSB phenotypes than MSSA, consistent with previous reports from India [20–22]. The association of *ermC* with MRSA suggests co-transfer with SCCmec elements [23]. Studies by Regha et al. (2021) and Tiwari et al. (2024) have shown similar regional trends, emphasizing the need for combined phenotypic–genotypic surveillance [24,25].

Continuous monitoring of *erm* gene dissemination is vital since inappropriate clindamycin use in iMLSB strains can select for constitutive mutants, leading to treatment failure [25]. This study contributes baseline molecular data from Central India and supports routine implementation of D-testing and molecular confirmation before prescribing clindamycin for *S. aureus* infections.

Conclusion

- The *ermC* gene was predominant among iMLSB *Staphylococcus aureus* isolates, indicating plasmid-mediated resistance.
- MRSA isolates exhibited significantly higher rates of both inducible and constitutive MLSB resistance than MSSA.
- Phenotypic D-test is essential for routine screening, while PCR-based genotypic characterisation ensures accurate epidemiological mapping.
- Rational antibiotic use and surveillance of resistance genes are crucial for mitigating therapeutic failures in clindamycin-treated infections.

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