Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/ttaahe39

PHENOTYPIC CHARACTERISATION OF MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN B (MLSB) RESISTANCE IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL IN CENTRAL INDIA

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Abstract

Background: *Staphylococcus aureus* remains a major cause of both hospital-acquired and community-acquired infections. Resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics compromises clindamycin therapy, an important alternative for treating methicillin-resistant *S. aureus* (MRSA). Accurate phenotypic detection of inducible resistance prevents treatment failures and contributes to antimicrobial stewardship.

Objectives: To identify constitutive (cMLSB), inducible (iMLSB), and MS phenotypes among *S. aureus* isolates using the D-test and compare the results with automated VITEK-2 system outcomes.

Methods: This cross-sectional study included 250 *S. aureus* isolates collected from patients attending various departments of Index Medical College Hospital & Research Centre (IMCHRC), Indore, between November 2021 and May 2024. Isolates were identified using standard microbiological procedures, and antimicrobial susceptibility testing was performed according to CLSI guidelines. The D-test was conducted for all erythromycin-resistant isolates, and the results were correlated with VITEK-2 findings. Statistical analysis was performed using SPSS v25.

Results: Among the 250 isolates, 148 (59%) were MRSA and 102 (41%) were MSSA. D-test identified 26% of MRSA and 11% of MSSA as iMLSB, and 40% of MRSA and 11% of MSSA as cMLSB. The D-test showed 57.7% sensitivity and 100% specificity compared with VITEK-2. A statistically significant association ($p < 0.05$) was observed between MRSA and inducible resistance.

Conclusion: Routine implementation of the D-test is essential in diagnostic microbiology to identify inducible clindamycin resistance, especially in MRSA isolates. Early detection prevents therapeutic failure and helps formulate targeted antibiotic policies.

Keywords: *Staphylococcus aureus*, MRSA, clindamycin resistance, D-test, iMLSB, cMLSB, VITEK-2.

INTRODUCTION

Staphylococcus aureus is a Gram-positive pathogen responsible for a wide spectrum of infections ranging from minor skin and soft tissue infections to endocarditis, osteomyelitis, and sepsis[1]. The widespread use of macrolide and lincosamide antibiotics has contributed to the emergence of resistance mechanisms that complicate treatment [1-3]. Clindamycin remains an important alternative to beta-lactams in the management of MRSA infections due to its excellent tissue penetration and ability to inhibit toxin production[4-5]. However, resistance mediated by methylation of the 23S rRNA binding site can render clindamycin ineffective, leading to clinical failures if not properly detected [6]. The *erm* (erythromycin ribosomal methylase) genes—particularly *ermA*, *ermB*, and *ermC*—mediate resistance through methylation of the 23S rRNA[7-8]. This modification blocks antibiotic binding to the 50S ribosomal subunit and results in either constitutive (cMLSB) or inducible (iMLSB) resistance phenotypes [3-5]. The D-test, recommended by the Clinical and Laboratory Standards Institute (CLSI), remains the gold standard phenotypic method for detecting inducible clindamycin resistance[11]. Despite the availability of automated systems like VITEK-2, phenotypic confirmation remains indispensable due to possible misclassification of inducible resistance[9-10].

This study aims to determine the distribution of MLSB resistance phenotypes among clinical isolates of *S. aureus*, compare D-test results with automated VITEK-2 detection, and evaluate the correlation between MRSA and MLSB resistance patterns in central India.

MATERIALS AND METHODS

Study Design and Setting:

A prospective cross-sectional study was conducted in the Department of Microbiology, Index Medical College Hospital & RC, Indore, from November 2021 to May 2024. Ethical clearance was obtained from the institutional ethics committee.

Sample Collection and Processing:

Two hundred and fifty non-duplicate *S. aureus* isolates were collected from pus, wound swabs, sputum, urine, and blood. The isolates were identified using colony morphology, Gram staining, catalase, coagulase, and mannitol fermentation tests[1415].

Antimicrobial Susceptibility Testing:

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar as per CLSI (2024) guidelines[1]. The antibiotics tested included erythromycin (15 µg), clindamycin (2 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and trimethoprim-sulfamethoxazole (25 µg). Methicillin resistance was determined using cefoxitin discs (≤21 mm = MRSA; ≥22 mm = MSSA).

D-Test

Procedure:

Erythromycin and clindamycin discs were placed 15 mm apart on an inoculated MHA plate and incubated at 37°C for 18–24 hours. Flattening of the clindamycin inhibition zone adjacent to the erythromycin disc was recorded as a positive D-test, indicating iMLSB phenotype[10-11]. Resistance to both antibiotics indicated cMLSB phenotype, and resistance to erythromycin alone with clindamycin sensitivity indicated MS phenotype.

Automated Detection:

The VITEK-2 Compact system (bioMérieux, France) was used for confirmatory susceptibility testing and compared with D-test results[12].

Statistical Analysis:

All data were analysed using SPSS v25 software. Sensitivity, specificity, and correlation coefficients were calculated. A p-value < 0.05 was considered statistically significant.

RESULTS

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

Table 1. Distribution of MLSB phenotypes among MRSA and MSSA isolates

Phenotype	MRSA (n=148)	MSSA (n=102)	Total (%)
iMLSB	39 (26%)	11 (11%)	50 (20%)
cMLSB	59 (40%)	11 (11%)	70 (28%)
MSB	10 (7%)	6 (6%)	16 (6%)
Sensitive	40 (27%)	74 (72%)	114 (46%)

Table 2. Comparison between D-test and VITEK-2 results

Method	Positive (MLSB)	Negative	Total	Percentage of positivity
D-test	120	130	250	48
VITEK-2	208	42	250	83

Discussion

The present study highlights a substantial prevalence of MLSB resistance among *Staphylococcus aureus* isolates, emphasizing the continued relevance of phenotypic testing in routine diagnostics. The proportion of MRSA (59%) was consistent with national surveillance data reported across tertiary-care hospitals in India[15-16]. The D-test revealed that nearly one in five isolates demonstrated inducible clindamycin resistance, underscoring the importance of performing this simple yet crucial assay in all erythromycin-resistant cases[18].

Several Indian studies have reported iMLSB rates ranging from 12–35% and cMLSB from 25–50%, aligning closely with the results of the present investigation. The predominance of cMLSB over iMLSB observed in this study supports the global trend that constitutive resistance remains more widespread due to chromosomal activation of *ermA* and *ermC* genes[14]. Similar patterns have been observed in Nepal, Sri Lanka, and Iran, indicating that geographical differences in antibiotic use and infection-control policies contribute significantly to resistance variability[11-15].

The observed concordance between the D-test and VITEK-2 in detecting MLSB resistance was moderate (sensitivity 57.7%, specificity 100%). Although automated systems provide rapid results, they may misinterpret inducible resistance as clindamycin susceptibility when using only erythromycin resistance as a marker.[18] This underlines the need for D-test confirmation to prevent false-negative interpretations that can lead to clindamycin treatment failure [17]. Previous reports from Europe and North America also highlight similar discrepancies between automated systems and phenotypic tests. Therefore, incorporating D-testing into laboratory workflows remains cost-effective and essential, especially in low-resource settings. [18]

The correlation between MRSA and iMLSB phenotypes suggests a strong linkage between methicillin resistance and macrolide resistance determinants. This association has been attributed to co-location of resistance genes on mobile genetic elements, facilitating horizontal gene transfer among staphylococcal populations[15-17]. The integration of plasmid-borne *ermC* and transposon-associated *ermA* genes likely enhances the adaptive capacity of MRSA strains to withstand multiple antibiotic pressures[18]. These findings are consistent with molecular studies confirming co-expression of *erm* genes in MRSA isolates[20].

Phenotypic characterization offers critical clinical implications. Clindamycin, when used without prior D-testing, may fail in cases of undetected inducible resistance. Routine implementation of the D-test is thus essential not only to ensure accurate antimicrobial susceptibility reporting but also to prevent therapeutic failure in MRSA infections[11-14]. Moreover, identifying iMLSB and cMLSB phenotypes helps epidemiologists monitor resistance trends, guiding empirical therapy protocols and infection-control interventions.[12-17].

In light of these findings, strengthening hospital-based antimicrobial stewardship programs is

imperative. Educational initiatives for clinicians and laboratory professionals on the interpretation and implications of D-test results can substantially reduce inappropriate clindamycin prescriptions[14-16]. Additionally, national-level surveillance integrating both phenotypic and molecular approaches will improve early detection of emerging resistance phenotypes[17-19]. Further studies employing next-generation sequencing and molecular epidemiology could elucidate genetic linkages and transmission pathways of *erm* gene variants [20].

Conclusion

The study demonstrates a high prevalence of MLSB resistance, particularly among MRSA isolates, emphasizing the need for D-test inclusion in routine antimicrobial susceptibility testing. Despite automation, phenotypic confirmation remains the cornerstone for reliable detection of inducible clindamycin resistance. Continuous surveillance and integration of phenotypic data with molecular findings are vital for guiding therapy and curbing antibiotic resistance spread in hospital environments.

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