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Original Article

Evaluation of the D-Test for Detecting Macrolide Lincosamide Streptogramin B Resistance (MLSB) In Staphylococcus Aureus Isolates: A Diagnostic Efficacy Study in Tertiary Care Hospital in Central India

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ABSTRACT

Background: Inducible clindamycin resistance among *Staphylococcus aureus* isolates has been increasingly reported from Indian tertiary-care hospitals, with D-test identified as a critical phenotypic tool for its detection.[1]
Recent Indian studies have shown that routine disc diffusion testing alone may falsely categorize inducible MLSB-resistant isolates as clindamycin susceptible.[2]
Failure to detect inducible MLSB resistance has been associated with inappropriate antimicrobial therapy and potential treatment failure.[3]
Objectives: To evaluate the diagnostic utility of the D-test in detecting inducible clindamycin resistance among *Staphylococcus aureus* isolates.
Methods: A laboratory-based observational study was conducted on clinical isolates of *Staphylococcus aureus*. Identification was performed using standard microbiological techniques. Antimicrobial susceptibility testing was carried out by the Kirby–Bauer disc diffusion method, and the D-test was applied to erythromycin-resistant, clindamycin-susceptible isolates.
Results: A total of 51 unique *Staphylococcus aureus* clinical isolates were analyzed. After antimicrobial susceptibility and D-testing, 7 isolates (13.7%) showed inducible MLSB resistance despite appearing clindamycin-susceptible in routine tests, while 44 isolates (86.3%) were D-test negative.
All D-test positive isolates came from male patients, but gender was not statistically linked to D-test positivity (p = 0.18). Patients with D-test positive isolates had a higher median age, with a significant association between increasing age and D-test positivity (p = 0.033).
The D-test enabled identification of inducible clindamycin resistance in isolates that would have otherwise been misclassified as clindamycin susceptible on routine testing. This highlights the diagnostic importance of incorporating the D-test into routine antimicrobial susceptibility workflows.
Conclusion: Routine implementation of the D-test is essential for accurate detection of inducible clindamycin resistance and rational antimicrobial selection in *Staphylococcus aureus* infections.

***Keywords**: Staphylococcus aureus; D-test; MLSB resistance; Clindamycin; Antimicrobial susceptibility.*

INTRODUCTION

Recent Indian studies have established inducible macrolide-Lincosamide-streptogramin B (MLSB) resistance as a clinically relevant resistance mechanism in *Staphylococcus aureus*, particularly in tertiary-care hospital settings. Majhi et al. demonstrated both inducible and constitutive MLSB resistance among clinical isolates in Eastern India, highlighting the inadequacy of routine susceptibility testing without D-test confirmation.[4]

Earlier investigations from South India reported that erythromycin-resistant, clindamycin-susceptible isolates frequently harbour inducible resistance detectable only by the D-test, emphasizing the risk of false clindamycin susceptibility reporting.[5] Similar findings were subsequently documented from Maharashtra, where inducible MLSB resistance was identified in isolates that would otherwise be misclassified using routine disc diffusion alone.[6]

Independent studies from Karnataka, North India, Kashmir, and Eastern India have consistently confirmed the presence of inducible[8] MLSB resistance across diverse geographic regions, underscoring the need for routine D-test implementation in clinical microbiology laboratories.[7]

The present study was undertaken to evaluate the diagnostic utility of the D-test for detecting MLSB resistance among *Staphylococcus aureus* isolates in a tertiary care hospital in Central India.

AIM

To assess the diagnostic efficacy of the D-test in identifying MLSB resistance patterns among *Staphylococcus aureus* isolates.

OBJECTIVES

1. To isolate and identify *Staphylococcus aureus* from various clinical specimens using standard microbiological techniques.
2. To determine the prevalence of MLSB resistance phenotypes among *Staphylococcus aureus* isolates.
3. To detect inducible clindamycin resistance using the D-test in erythromycin-resistant isolates.

MATERIALS AND METHODS

This prospective laboratory-based observational study was conducted in the Department of Microbiology of a tertiary care hospital in Central India.

Study Duration:

This prospective laboratory-based observational study was conducted over a period of **3 months**, in the Department of Microbiology of a tertiary care hospital in Central India.

Sample Size Calculation:

The sample size was calculated based on the expected prevalence of **inducible clindamycin resistance (iMLSB phenotype)** among *Staphylococcus aureus* isolates, as reported in Indian studies.

Previous Indian studies have reported the prevalence of inducible MLSB resistance ranging from **10% to 20%**. Considering an expected prevalence (**p**) of **15%**, a confidence level of **95%**, and an absolute allowable error (**d**) of **10%**, the sample size was calculated using the standard formula for estimating a single proportion:

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where:

- n= required sample size
- Z= standard normal variate at 95% confidence level (= **1.96**)
- p= expected prevalence = **0.15**
- q= $1 - p$= **0.85**
- d= allowable error = **0.10**

Substituting the values:

$$n = \frac{(1.96)^2 \times 0.15 \times 0.85}{(0.10)^2}$$

$$n = \frac{3.84 \times 0.1275}{0.01}$$

$$n = \frac{0.4896}{0.01}$$

$$n = 48.96$$

Thus, the **minimum required sample size was calculated to be 49 isolates**.

In the present study, a total of **51 non-duplicate *Staphylococcus aureus* isolates** were included over the **18-month study period**, which satisfies the calculated sample size requirement.

Clinical specimens including pus, wound swabs, blood, ear discharge, and other sterile body fluids were processed according to standard microbiological protocols. Identification of *Staphylococcus aureus* was performed using colony morphology, Gram staining, catalase test, and slide and tube coagulase tests.

Antimicrobial susceptibility testing was carried out on Mueller–Hinton agar using the Kirby–Bauer disc diffusion method. Erythromycin and clindamycin discs were placed 15–20 mm apart for performance of the D-test. Flattening of the clindamycin inhibition zone adjacent to the erythromycin disc was interpreted as inducible MLSB resistance.

RESULTS

Distribution of Isolates

A total of **51 non-duplicate clinical isolates of *Staphylococcus aureus*** were included in the study. All isolates fulfilled the inclusion criteria and were subjected to antimicrobial susceptibility testing followed by D-test where indicated.

Detection of Inducible Clindamycin Resistance

Out of 51 isolates, **7 (13.7%)** demonstrated a **positive D-test**, indicating inducible MLSB resistance. The remaining **44 isolates (86.3%)** were D-test negative. All D-test positive isolates appeared clindamycin susceptible on routine disc diffusion testing but showed characteristic flattening of the clindamycin inhibition zone adjacent to the erythromycin disc on D-test.

Gender-wise Distribution

All **7 D-test positive isolates (100%)** were recovered from male patients. No inducible MLSB resistance was detected among female patients. Statistical analysis did not demonstrate a significant association between gender and D-test positivity (Chi-square test, $p = 0.18$).

Age Distribution

Patients yielding D-test positive isolates had a higher median age compared to those with D-test negative isolates. Non-parametric analysis using the Mann–Whitney U test demonstrated a statistically significant association between increasing age and D-test positivity ($p = 0.033$).

Summary of Observations

The D-test enabled identification of inducible clindamycin resistance in isolates that would have been misclassified as clindamycin susceptible on routine testing, thereby improving the accuracy of suscept

DISCUSSION

The prevalence of inducible MLSB resistance observed in the present study (13.7%) is comparable with findings reported from several Indian tertiary-care centres.

Majhi et al. reported inducible clindamycin resistance among *Staphylococcus aureus* isolates in Eastern India, emphasizing the diagnostic gap associated with routine susceptibility testing without D-test confirmation.[4] Gade and Qazi similarly documented inducible resistance among erythromycin-resistant isolates in South India.[5]

Studies from Maharashtra and Karnataka have reported comparable prevalence of inducible MLSB resistance, reinforcing the reproducibility and reliability of the D-test across laboratories.[4,5,8] . Reports from North India, Kashmir, and Eastern India have further confirmed the widespread distribution and clinical relevance of inducible MLSB resistance.[6, 9–11]

The findings of the present study are consistent with these reports and support the routine incorporation of the D-test into antimicrobial susceptibility testing. Accurate detection of inducible clindamycin resistance is essential to prevent inappropriate clindamycin therapy and potential treatment failure, particularly in settings with a high burden of MRSA infections.

CONCLUSION

The D-test is a simple, reliable, and essential phenotypic method for detecting inducible clindamycin resistance in *Staphylococcus aureus*. Routine implementation of the D-test is recommended in clinical microbiology laboratories to ensure accurate susceptibility reporting and rational antimicrobial therapy ability reporting.

Table 1. Distribution of D-test Results among *Staphylococcus aureus* Isolates (n = 51)

D-test result	Number (n)	Percentage (%)
Positive (Inducible MLSB)	7	13.7
Negative	44	86.3
Total	**51**	**100**

Table 2. Association of D-test Positivity with Gender

Gender	D-test Positive	D-test Negative	Total
Male	7	30	37
Female	0	14	14
Total	**7**	**44**	**51**

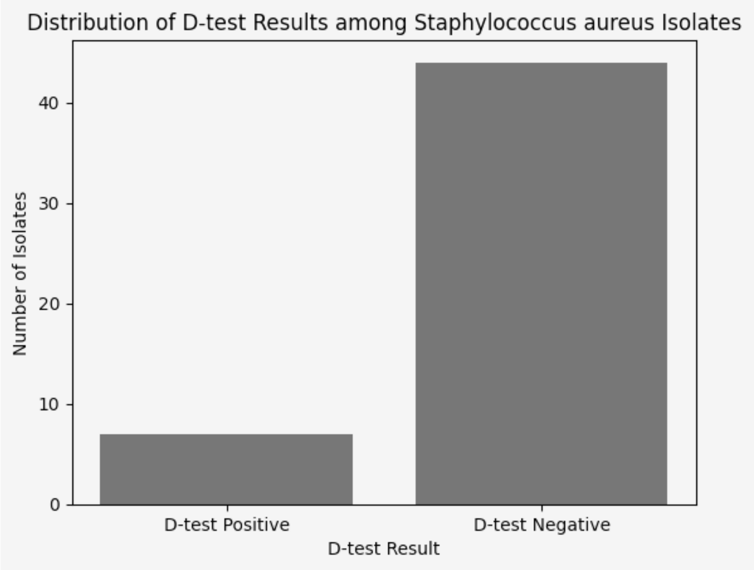

Figure 1. Distribution of D-test results among *Staphylococcus aureus* isolates

Bar diagram showing the proportion of D-test positive and D-test negative *Staphylococcus aureus* isolates.

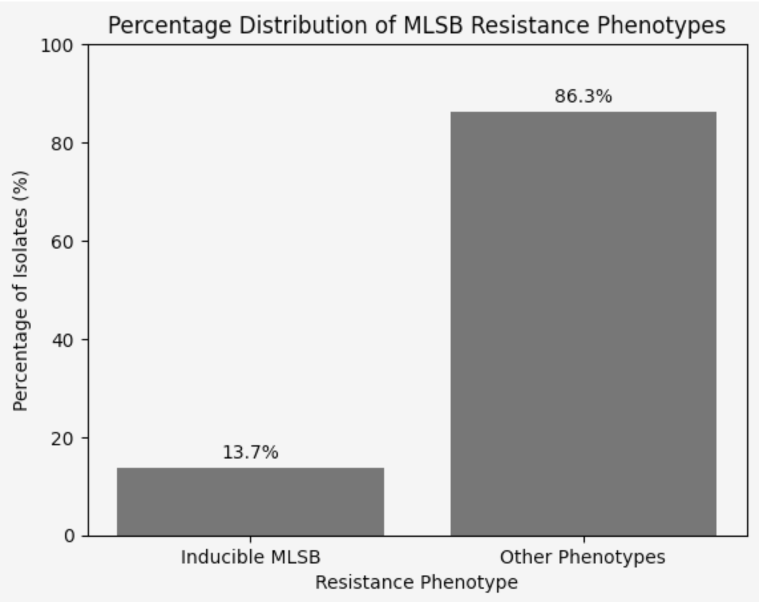

Figure 2. Percentage Distribution of MLSB resistance phenotypes among *Staphylococcus aureus* isolates

Bar diagram showing the percentage distribution of inducible MLSB resistance and other resistance phenotypes among *Staphylococcus aureus* isolates studied (n = 51).

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