The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 7, 2025

FREQUENCY OF INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATES AT ABBAS INSTITUTE OF MEDICAL SCIENCES

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DOI: https://doi.org/10.5281/zenodo.16671958

Keywords
Clindamycin, D-test, inducible resistance, MRSA, MSSA, Staphylococcus aureus

Article History
Received on 28 April 2025
Accepted on 09 June 2025
Published on 08 July 2025

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Abstract

Background: *Staphylococcus aureus is a leading cause of hospital- and community-acquired infections. Clindamycin is commonly used for treating these infections; however, the emergence of inducible clindamycin resistance (iMLSB phenotype) may lead to therapeutic failure if not properly detected. The D-test is a reliable method for identifying such resistance patterns in routine diagnostics.*

Objectives: *To determine the frequency of inducible clindamycin resistance in Staphylococcus aureus isolates at Abbas Institute of Medical Sciences, Muzaffarabad.*

Study Design & Setting: *A cross-sectional study conducted in the Department of Microbiology, Abbas Institute of Medical Sciences, over a six-month period following ethical approval from August 2024 to April 2025.*

Methodology: *A total of 178 non-repetitive S. aureus isolates were obtained from clinical specimens including pus, urine, blood, and CSF. Standard microbiological procedures were used for identification. Methicillin resistance was determined using cefoxitin (30 µg) disc diffusion. Inducible clindamycin resistance was detected via the D-test, placing clindamycin and erythromycin discs 15 mm apart on Mueller-Hinton agar. Data were analyzed using SPSS version 23, with $p \leq 0.05$ considered significant.*

Results: *Of 178 S. aureus isolates, 108 (60.7%) were MSSA and 70 (39.3%) were MRSA. Inducible clindamycin resistance was observed in 41 (23.0%) isolates overall–significantly more prevalent in MRSA (41.4%) than MSSA (11.1%) ($p < 0.001$). Resistance was higher in adults and inpatients, especially in pus samples.*

Conclusion: *A considerable frequency of inducible clindamycin resistance was noted, especially among MRSA strains. Routine D-testing is essential for guiding*

appropriate antibiotic therapy

INTRODUCTION

As the prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) rises and antibiotic resistance patterns shift, less effective treatments for these infections are readily accessible.[1] As a result, medical professionals need to reconsider their antibiotic prescription practices and turn to macrolides, lignosamides, and streptogramin group B (MLSB) antibiotics.[2] The MLSB antibiotic clindamycin has superior pharmacokinetics, making it the drug of choice for treating MRSA infections.[3] Antibiotics belonging to the MLSB class bind to the 23 rRNA of the 50S ribosomal subunit, which inhibits bacterial protein synthesis. Despite their structural differences, these drugs suppress bacterial protein synthesis.[4] Resistance to MLSB antibiotics, particularly clindamycin, has grown due to their extensive use.[5] Resistance to clindamycin can be either constitutive or inducible, depending on whether a macrolide inducer is present or not.[6] It is possible to induce clindamycin resistance by using erythromycin. Because these infections are difficult to detect using standard laboratory procedures, clinical isolates having inducible clindamycin resistance pose a significant threat.[7] The Clinical and Laboratory Standardisation Institute (CLSI) suggests the disc diffusion induction test, also known as the erythromycin-clindamycin disc approximation test (D test), as a means to identify S. aureus isolates with the clindamycin resistance inducible phenotype.[8]

A descriptive cross-sectional investigation was carried out by Paradhan S et al. using clinical samples collected from a tertiary care center's in-patient and out-patient departments. The inducible clindamycin resistant genotype was found in 41 out of 141 (or 29.1%) of the Staphylococcus aureus bacteria. The prevalence of inducible clindamycin resistance was greater among MRSA (47.5%).[9] Out of 161 S. aureus isolates found in different clinical samples, Gupta DK et al. found that 73% of them were methicillin-resistant Staphylococcus aureus. Of the isolates tested, 61.4% were resistant to erythromycin. A total of 21.1% of the S. aureus bacteria tested positive for erythromycin also showed evidence of inducible (iMLSB) resistance.[10]

Clinical treatment failure and inappropriate use of clindamycin could result from a failure to identify inducible clindamycin resistance in S. aureus. Different regions have different patterns of clindamycin resistance in S. aureus samples. In order to assess the extent of resistant clindamycin induction in S. aureus isolates derived from our local environment, this study has been organised. In order to enhance antibiotic utilisation and direct empirical treatment in our clinical context, local data will be crucial. In the long run, this will assist doctors keep their patients from getting the wrong dose of clindamycin, which is good for their health.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at Abbas Institute of Medical Sciences, Muzaffarabad from August 2024 to April 2025. The sample size was determined with the WHO sample size calculator according to the formula for a single proportion. A clindamycin resistance frequency of 21.1%, with a 95% confidence interval and an absolute precision of 6%, necessitated a sample size of 178. Non-probabilistic Consecutive sampling was utilised for sample selection. The study included clinical specimens that tested positive for Staphylococcus aureus. These specimens were obtained from various inpatient units, including Neonatal Intensive Care Units (NICUs), hospital wards, and outpatient departments. The specimens included pus, urine, blood, and cerebrospinal fluid (CSF). Specimens were excluded if they were redundant, gathered in a compromised container, or sourced from patients already undergoing anti-staphylococcal antibiotic treatment.

Data including patient age, gender, type of specimen (blood, urine, CSF, pus), origin of specimen submission (outpatient or inpatient), and the location of the inpatient (NICU, PICU, or general ward) were recorded. All S. aureus isolates were categorized as methicillin-sensitive Staphylococcus aureus (MSSA) or methicillin-resistant Staphylococcus aureus (MRSA) according to the operational definitions. Inducible clindamycin

resistance testing (D-test) was performed on all S. aureus isolates as per standard protocol and each isolate was labeled as either inducible clindamycin resistance positive or negative. All relevant data were documented on a structured proforma.

A phenotypic approach utilising a 30 µg cefoxitin disc was used to ascertain methicillin sensitivity and resistance in Staphylococcus aureus isolates. Isolates were classified as Methicillin Sensitive Staphylococcus aureus (MSSA) if they produced an inhibitory zone of 22 mm or more, and as Methicillin Resistant Staphylococcus aureus (MRSA) if their zone was 21 mm or less. The D-test, or double disc diffusion, was used to identify inducible clindamycin resistance. The Mueller-Hinton agar plate was inoculated with a lawn culture of S. aureus isolates that were susceptible to clindamycin but resistant to erythromycin. Then, a 2 µg clindamycin disc and a 15 µg erythromycin disc were placed on the plate, with a distance of 15 mm between them. We checked the plates for flattening of the clindamycin zone close to the erythromycin disc after they had been incubated at 37°C for one night. Inducible clindamycin resistance was considered present when a blunted or D-shaped zone was observed.

Data analysis was performed using SPSS version 23. The normality of numerical variables was assessed using the Shapiro-Wilk test. Age was presented as mean and standard deviation, or as median and range in case of non-normal distribution. Categorical variables such as gender, type of specimen, origin of specimen, inpatient location, type of S. aureus (MSSA/MRSA), and presence or absence of inducible clindamycin resistance were summarized in frequencies and percentages. The data were stratified by age group (children/adults), gender, type of specimen, origin of specimen, inpatient location, and type of S. aureus to assess their effect on the distribution of inducible clindamycin resistance. A p-value less than or equal to 0.05 was deemed statistically significant when the post-stratification chi-square test for significance was employed.

RESULTS

A total of 178 Staphylococcus aureus isolates were included in the study. The demographic and clinical characteristics of patients are summarized in Table 1. Out of 178 patients, 58 (32.6%) were children (<18 years), and 120 (67.4%) were adults (≥18 years). The majority of patients were male (57.3%), while females comprised 42.7% of the study population. Regarding specimen types, pus was the most frequently submitted specimen (53.9%), followed by urine (20.2%), blood (16.9%), and CSF (9.0%). Most specimens were submitted from inpatients (69.7%), and among them, the most common inpatient location was the general ward (33.7%), followed by NICU (13.5%), PICU (10.7%), and adult ICU (11.8%). For 30.3% of patients, location was not applicable as specimens were from outpatient settings.

The distribution of S. aureus strains into MSSA and MRSA is shown in Table 2. MSSA accounted for 108 (60.7%) of isolates, while MRSA was identified in 70 (39.3%) of the total isolates. The frequency of inducible clindamycin resistance among all S. aureus isolates is presented in Table 3. A total of 41 isolates (23.0%) showed inducible clindamycin resistance, while 137 (77.0%) were negative for inducible resistance.

When stratified by type of S. aureus, inducible clindamycin resistance was observed in 29 (41.4%) of MRSA isolates compared to 12 (11.1%) of MSSA isolates (Table 4). This indicates that inducible resistance was more prevalent in MRSA isolates. Stratification of inducible clindamycin resistance with respect to demographic and clinical variables is presented in Table 5. Inducible resistance was more commonly observed in adults (26.7%) compared to children (15.5%) with a statistically significant p-value of 0.046. No significant difference was noted with respect to gender (p = 0.872), though slightly higher resistance was noted among females (23.7%) than males (22.5%). Among different specimen types, the highest rate of inducible resistance was observed in pus specimens (28.1%), followed by blood (20.0%), CSF (18.8%), and urine (13.9%), though this association was not statistically significant (p = 0.073). Inpatients showed a higher rate of inducible resistance (26.6%) compared to outpatients (14.8%), with a p-value of 0.094. Regarding location of inpatients, the highest rate of inducible resistance was found in general ward patients (30.0%), followed by adult ICU (28.6%), NICU (20.8%), and PICU (21.1%), though no

statistically significant association was found (p = 0.421).

Table 1: Demographic and Clinical Characteristics of Patients (n = 178)

Variable	Category	Frequency n (%)
Age Group	<18 years	58 (32.6%)
	≥18 years	120 (67.4%)
Gender	Male	102 (57.3%)
	Female	76 (42.7%)
Type of Specimen	Blood	30 (16.9%)
	Urine	36 (20.2%)
	CSF	16 (9.0%)
	Pus	96 (53.9%)
Origin of Specimen	Outpatient	54 (30.3%)
	Inpatient	124 (69.7%)
Location of Inpatient	NICU	24 (13.5%)
	PICU	19 (10.7%)
	Adult ICU	21 (11.8%)
	General Ward	60 (33.7%)
	Not Applicable	54 (30.3%)

Table 2: Distribution of MSSA and MRSA Among Isolates (n = 178)

Type of *Staphylococcus aureus*	Frequency n (%)
MSSA	108 (60.7%)
MRSA	70 (39.3%)

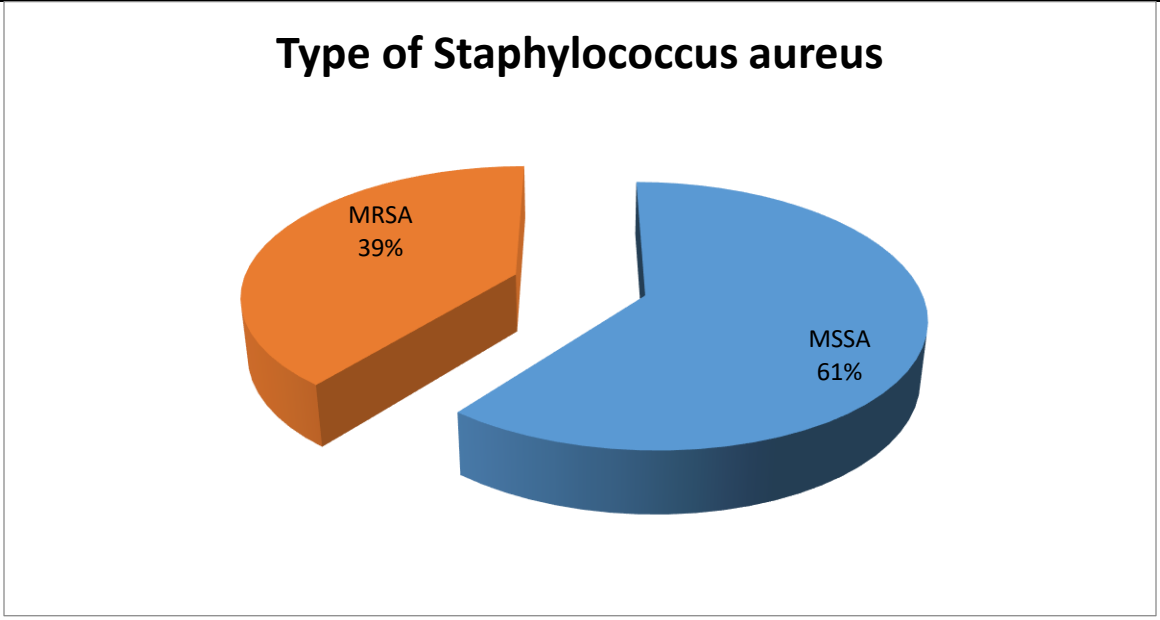

Figure: Distribution of MSSA and MRSA Among Isolates

Table 3: Frequency of Inducible Clindamycin Resistance Among Isolates (n = 178)

Inducible Clindamycin Resistance	Frequency n (%)
Positive	41 (23.0%)
Negative	137 (77.0%)

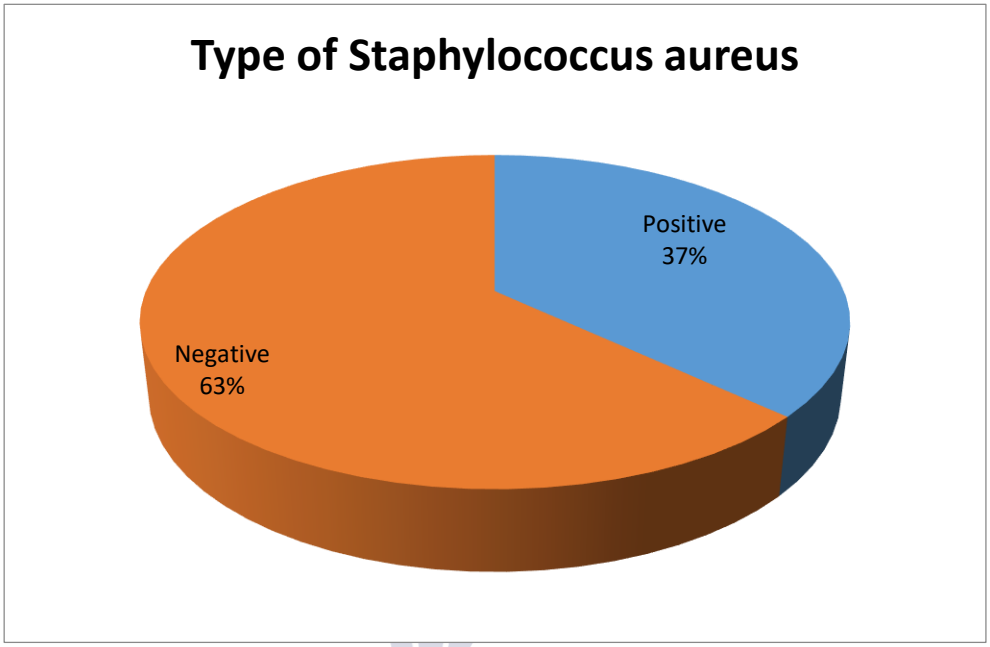

Figure: Inducible Clindamycin Resistance Among Isolates

Table 4: Association Between Type of *S. aureus* and Inducible Clindamycin Resistance (n = 178)

Type of *S. aureus*	iMLSB Positive n (%)	iMLSB Negative n (%)	Total n (%)
MSSA	12 (11.1%)	96 (88.9%)	108 (100%)
MRSA	29 (41.4%)	41 (58.6%)	70 (100%)

Table 5: Inducible Clindamycin Resistance Stratified by Demographic and Clinical Variables (n = 178)

Variable	Category	iMLSB Positive	iMLSB Negative	p-value
Age Group	<18 years	9 (15.5%)	49 (84.5%)	0.046
	≥18 years	32 (26.7%)	88 (73.3%)	
Gender	Male	23 (22.5%)	79 (77.5%)	0.872
	Female	18 (23.7%)	58 (76.3%)	
Type of Specimen	Blood	6 (20.0%)	24 (80.0%)	0.073
	Urine	5 (13.9%)	31 (86.1%)	
	CSF	3 (18.8%)	13 (81.2%)	
	Pus	27 (28.1%)	69 (71.9%)	
Origin of Specimen	Outpatient	8 (14.8%)	46 (85.2%)	0.094
	Inpatient	33 (26.6%)	91 (73.4%)	
Location (Inpatients)	NICU	5 (20.8%)	19 (79.2%)	0.421
	PICU	4 (21.1%)	15 (78.9%)	
	Adult ICU	6 (28.6%)	15 (71.4%)	
	General Ward	18 (30.0%)	42 (70.0%)	

DISCUSSION

Staphylococcus aureus is a major cause of skin, soft tissue, and invasive infections, with rising concern due to antibiotic resistance. Clindamycin is often preferred for its efficacy and good tissue penetration, especially in community-acquired MRSA cases.[12] However, inducible resistance to clindamycin (iMLSB phenotype), not detectable by routine susceptibility testing, can lead to treatment failure. The D-test helps identify this resistance pattern by detecting inducible clindamycin resistance in erythromycin-resistant strains.[13] This study was conducted to determine the frequency of inducible clindamycin resistance in S. aureus isolates at a tertiary care hospital.

The present study demonstrated that 23.0% of Staphylococcus aureus isolates exhibited inducible clindamycin resistance (iMLSB), with a significantly higher prevalence among MRSA strains (41.4%) compared to MSSA strains (11.1%). These findings are closely related to those reported by Greesh et al. (2023), who found iMLSB in 37.8% of MRSA and 9.4% of MSSA isolates, supporting our observation that inducible resistance is notably higher in methicillin-resistant strains.[20] Our results are also in line with Seifi et al. (2012), who reported inducible resistance in 20.5% of MRSA isolates, and a lower rate (10.6%) among MSSA, comparable to our MSSA group (11.1%).[14] Similarly, Gangurde et al. (2014) documented iMLSB in 27.8% of MRSA and 6.8% of MSSA, again highlighting a pattern consistent with our findings.[22]

The overall frequency of inducible resistance in our study was higher than that reported in a systematic review by Memariani et al. (2021), which estimated the pooled prevalence of iMLSB in Iran as 10.4%.[19] This discrepancy may be attributed to geographic variation, differences in study populations, and local antibiotic prescribing practices. However, their observation that iMLSB strains are frequently isolated from wound and blood samples resonates with our results, where pus (53.9%) and blood (16.9%) were the most common specimen types. Our MRSA proportion (39.3%) falls within the range reported by Thapa et al. (2021)[17] (39.5%) and Jahanbakhshi et al. (2024) (65.9%).[18] The iMLSB frequency among S. aureus isolates in our study (23.0%) is comparable to the 36.5% reported by Thapa et al., though they observed a higher burden, potentially due to higher multidrug resistance (67%) in their sample.[17] In contrast, Fasih et al. (2010) reported discordant clindamycin-erythromycin resistance in 6% of isolates, among which 72% were D-test positive, again indicating that routine susceptibility testing may underestimate iMLSB prevalence unless D-testing is specifically performed.[16]

The current study also supports findings from Zafar et al. (2024), who found that the D-test had good concordance with automated systems like Vitek2 for detecting iMLSB in MRSA isolates. This underlines the reliability of the D-test as a routine phenotypic method, especially in resource-limited settings.[15] Additionally, our adherence to a 15 mm disc spacing for the D-test aligns with the findings of Nikam et al. (2017), who demonstrated that incorrect disc spacing may significantly underestimate inducible resistance.[21] This supports the methodological rigor in our study and affirms the accuracy of our iMLSB detection rate.

The study provides valuable local data on the frequency of inducible clindamycin resistance, especially in MRSA strains. It used a standardized phenotypic method (D-test) for reliable detection. Inclusion of both inpatient and outpatient samples enhances generalizability. However, the study was limited to a single-center, which may not reflect broader population trends. Molecular confirmation of resistance genes was not performed. Also, clinical outcomes of patients with iMLSB resistance were not assessed.

CONCLUSION

Inducible clindamycin resistance was observed in a significant proportion of S. aureus isolates, particularly among MRSA strains. Routine D-testing is essential to prevent therapeutic failure. Local antibiogram updates should incorporate inducible resistance patterns for better antibiotic stewardship.

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