ORIGINAL ARTICLE

Study of inducible and constitutive clindamycin resistance in *Staphylococcus aureus* isolated from various clinical samples in tertiary care hospital

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Abstract

Background: Macrolide, Lincosamide and Streptogramin B (MLSB) antibiotics are commonly used in treatment of *Staphylococcus aureus* infections. However, presence of inducing agents like erythromycin can trigger the expression of resistance genes making drug ineffective. The detection of this inducible clindamycin resistance is required as failure to identify it can lead to treatment failure in patients. *Aim and Objectives:* The primary objective was to identify inducible and constitutive clindamycin resistance in *Staphylococcus aureus* from various clinical specimen and to compare the results of disc diffusion (D test) and VITEK 2 for identification of inducible clindamycin resistance. The secondary objective was to compare rate of inducible clindamycin resistance between methicillin resistant S. aureus (MRSA) and Methicillin Sensitive *S. Aureus* (MSSA) isolates. *Material and Methods:* A total of 259 *Staphylococcus aureus* were isolated from various clinical specimen from 1st July 2023 to 30th June 2024. Identification and antibiotic sensitivity testing were performed by an automated VITEK 2 compact system. For detection of inducible clindamycin resistance, D test using erythromycin and clindamycin as per CLSI guidelines was performed, and two different phenotypes were interpreted as inducible MLSB (D test positive), and constitutive MLSB. *Results:* Out Of the 259 isolates, 80(30.9%) were showing inducible clindamycin resistance (D test +ve). The rates of inducible clindamycin resistance in MRSA and MSSA were 37.4% and 19.4%, respectively. *Conclusion:* Inducible and constitutive clindamycin resistance was higher among MRSA than MSSA. D test is a helpful method in routine disc diffusion testing to detect inducible clindamycin resistance. VITEK 2 and D test both have similar sensitivity to detect inducible clindamycin resistance.

Keywords: Inducible resistance, Clindamycin resistance, *Staphylococcus aureus*, D test

Introduction

Macrolide, Lincosamide, and Streptogramin B (MLSB) antibiotics are commonly used to treat staphylococcal infections. Examples of macrolides include erythromycin, roxithromycin, and clarithromycin, while lincosamides include clindamycin and lincomycin. Although they belong to different classes, they share the same mechanism of action: inhibition of bacterial protein synthesis [1-3]. Common use of MLSB antibiotics has led to an increased resistance to these agents particularly in lincosamide like clindamycin, in staphylococcal strains. Clindamycin remains an option for treating both Methicillin Susceptible *S. Aureus* (MSSA) and Methicillin Resistant *S. Aureus* (MRSA) infections. MRSA strains have been traditionally associated with Hospital Acquired Infections (HA-MRSA) and is emerging as an important cause of Community Acquired Infections (CA-MRSA) as well [4]. Expression of inducible resistance to clindamycin could reduce the effectiveness of this drug. Two different resistance

mechanisms confer macrolide (e.g., erythromycin) resistance in staphylococci [3]. The erm (erythromycin ribosome methylase) gene codes for methylation of the 23S Ribosomal Ribonucleic Acid (rRNA), which results in resistance to erythromycin and inducible or constitutive resistance to clindamycin [5-7] The msrA (macrolide streptogramin resistance) gene codes for an efflux mechanism, which results in resistance to erythromycin but susceptibility to clindamycin [5-7].

Consequently, when an erythromycin resistant and clindamycin-susceptible staphylococcal isolate is encountered, analysis for inducible clindamycin resistance must be done before clindamycin is reported to be susceptible [1]. A disc diffusion test (D zone test) for detection of inducible resistance to clindamycin in erythromycin-resistant isolates can be performed by placing a 15µg erythromycin disc and a 2µg clindamycin disc in a proximity of 15-26 mm apart adjacent positions [1]. Flattening of the clindamycin zone (formation of D) between the two disks indicates that isolate has inducible clindamycin resistance because of erm. No flattening indicates that isolate is erythromycin-resistant only (because of msrA). When an isolate demonstrates inducible resistance, clindamycin is reported as resistant [8]. The phenomenon of clindamycin-inducible resistance also exists in *S. pyogenes* and *S. pneumoniae* [4, 9]. Among MLSB drugs, macrolides are potent inducers of the erm gene, which encodes the enzyme erythromycin ribosome methylase (erm). Induction of the gene leads to cross-resistance to other MLSB antibiotics including lincosamides and streptogramin B causing them less effective [3]. *Staphylococcus aureus* isolates exhibiting constitutive resistance are resistant to both erythromycin and clindamycin on in vitro testing, while inducible resistant strains show erythromycin resistance but appear clindamycin susceptibility on disc diffusion testing. The D test detects inducible resistance and prevents false susceptibility reporting. The primary objective this study was to detect inducible and constitutive clindamycin resistance in *Staphylococcus aureus* isolates and to compare the results of D test and VITEK 2 for identification of inducible clindamycin resistance. The secondary objective was to compare rate of inducible clindamycin resistance between MRSA and MSSA isolates.

Material and Methods

Study design and period

An institutional based retrospective cross-sectional study was conducted to detect the inducible and constitutive clindamycin resistance among the *Staphylococcus aureus* isolated at a tertiary care hospital for a duration of one year from July 2023 to June 2024 after receiving approval from Institutional Review Board (NHL IRB/ 2024/ July/12/03). Inclusion criteria included all *staphylococcus aureus* isolated from clinical specimen of patient during study period. All other bacteria other than *staphylococcus aureus* were excluded especially Streptococcus species. Microsoft excel and SPSS were used for conducting data analysis.

Sample size and sampling technique

Sample size of 245 was determined using statistical formula taking a prevalence of 20% from previous studies with a degree of precision set at margin of error 0.05 and at 95% confidence interval (z score–1.96).

A total of two hundred and fifty-nine (259) *Staphylococcus aureus* isolates from various clinical specimens like pus, urine, blood, fluid, sputum, swabs, endotracheal tube, bronchoalveolar lavage and pleural fluid over a period of one year were

isolated. They were identified by using conventional [1, 9] and automated methods. Identification was done using VITEK 2 GP card (biomerieux) and antibiotic sensitivity testing was performed by AST P 628 card of automated VITEK-2 compact system. All *Staphylococcus aureus* isolates were also tested by D test for confirmation of inducible clindamycin resistance [8].

For D test 15 µg erythromycin and 2 µg clindamycin discs (HiMedia) were placed on Mueller-Hinton plate previously inoculated with *Staphylococcus aureus* isolate. The discs were positioned 15 to 26 mm edge to edge apart from each other and incubated overnight at 37°C. The D test was considered as positive when flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (D shaped zone of inhibition) and observed inducible MLSBi resistance. Strains resistant to both erythromycin and clindamycin were classified as exhibiting constitutive MLSB resistance (Figure a). Strains resistant to erythromycin but susceptible to clindamycin with a D-Zone of inhibition around clindamycin disk were classified as exhibiting inducible MLSB resistance (Figure b). Strains that were resistant to erythromycin and sensitive to clindamycin were classified as MS phenotype (Macrolide and streptogramin B resistance and lincosamide sensitive) (Figure c). Strains which were sensitive to both erythromycin and clindamycin were classified as sensitive phenotype (Figure d) (Table 1).

Results

A total of 259 *Staphylococcus aureus* isolates were included in this study. Among these isolates, clindamycin was resistant in 18 (7%) isolates by disc diffusion method (Zone size ≤ 14mm). Out of 241 isolates, 80 (30.9 %) isolates showed D test positivity by disc approximation test, 77 (29.7%) were negative in D test while 84 (32.4%) isolates

Table 1: Mechanism, type of resistance and D test interpretation for Erythromycin (E) and Clindamycin (CD)

Mechanism	Resistance Gene	Erythromycin	Clindamycin	D Test	Phenotype
Ribosome alteration	ermA or ermC	R	R (constitutive)	Negative	MLSBc
Ribosome alteration	ermA or ermC	R	S→R (inducible)	Positive	MLSBi
Efflux	mrsA	R	S	Negative	MSB/MS
No ribosome alteration or efflux	-	S	S	Negative	-

mrsA - macrolide streptogramin resistance erm- erythromycin ribosome methylase

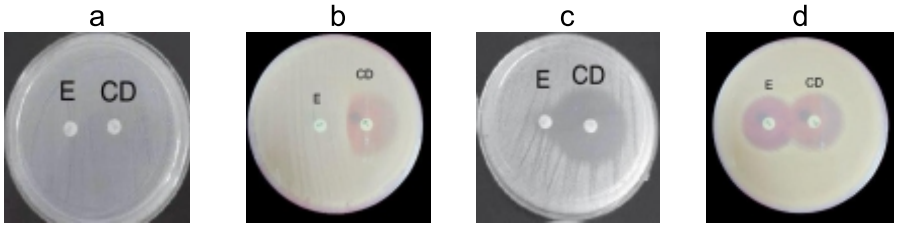

Figure 1:Showing various results of double disc test

a) Constitutive: E-R, CD-R. **b) Inducible MLSB:** E-R, CD -S/I: D Test: +ve
c) MS MLSB: E-R, CD -S/I: D Test -ve **d) No Resistance:** E-S, CD -S
E- Erythromycin, **CD-** Clindamycin

were sensitive to both erythromycin and clindamycin (Tables 2 and 3). Table 3 shows comparison between VITEK 2 result and D test. It shows that all isolates detected as inducible clindamycin resistance by VITEK 2 were also positive in D test showing 100% agreement. Out of total 259 *Staphylococcus aureus* isolates, 166 (64.1%) were MRSA and 93 (35.9%) were MSSA isolates. In phenotype-specific analysis, constitutive resistance was observed in 15 (9%) MRSA isolates and 3 (3.2%) MSSA isolates. Inducible clindamycin resistance (as detected by a positive D-test) was 62 (37.3%) in MRSA and 18 (19.4%) in MSSA isolates. Clindamycin resistance was not found in 89 (53.6%) MRSA and 72 (77.4%) MSSA isolates. Based on these findings and applying chi square 2 × 2 contingency table, *p* value for constitutive clindamycin resistance was 0.077, indicating no marked difference in between constitutive clindamycin resistance between MRSA and MSSA. Value of *p* for inducible clindamycin resistance was 0.0026, suggesting that rate of inducible clindamycin resistance was more frequent in MRSA isolates than MSSA isolate (Tables 4).

Out of 77 MRSA isolates which were clindamycin resistant, 23 were CA-MRSA and 54 were HA-MRSA. Rate of inducible clindamycin resistance was 82.6 % in CA-MRSA, while it was 79.6% in HA-MRSA suggesting there was no statistically significance in rate of inducible clindamycin resistance between CA and HA-MRSA ($p = 0.38$) (Table 5).

Table 2: Distribution of various phenotypes with constitutive and inducible clindamycin resistance in *Staphylococcus aureus* isolates (n = 259)

Erythromycin	Clindamycin	Type of resistance	Total
R	R	Constitutive	10 (3.9%)
S	R	Constitutive	8 (3.1%)
R	D+	Inducible	80 (30.9%)
R	D-	No resistance	77 (29.7%)
S	S	No resistance	84 (32.4%)
Total			259
Constitutive		**Inducible**	
18 (7%)		80 (30.9%)	

Table 3: Comparison of results of D zone test and VITEK 2 (degree of agreement)

Number of isolates positive by VITEK 2	D test positive	Degree of agreement
80	80	100

Table 4: Comparison of and Inducible Constitutive Clindamycin Resistance in MRSA and MSSA.

Type of resistance	MRSA (n-166) (64.1%)	MSSA (n-93) (35.9%)	*p*
Inducible (D+)	**62 (37.3%)**	**18 (19.4%)**	**0.0026***
D Test Negative	104 (62.7%)	75 (80.6%)	
Constitutive	15 (9%)	3 (3.2%)	0.077^{NS}
No constitutive	**151 (91%)**	**90 (96.8%)**	

**Significant at p<0.05*

Table 5: Comparison of constitutive and inducible clindamycin resistance between CA-MRSA and HA-MRSA

Type of resistance	CA-MRSA (n-23) (29.9%)	HA-MRSA (n-54) (70.1%)	*p*
Constitutive	4 (17.4%)	11 (20.4%)	0.38 ^{NS}
Inducible	**19 (82.6%)**	**43 (79.6%)**	

NS-not significant at p>0.05

Discussion

Treating staphylococcal infections in outpatient settings is challenging due to the widespread prevalence of MRSA. Clindamycin is a common choice for skin and soft tissue infections because of its favourable oral bioavailability, low cost, and excellent tissue penetration [10]. However, therapeutic failure due to MLSBi strains are now reported commonly necessitating D test testing. In our study it was observed that high prevalence of 30.9% of inducible clindamycin resistance and 7% of constitutive clindamycin resistance among all *S. aureus* isolates. Study by Manandhar *et al*. reported a similar prevalence of inducible resistance at 34.8%, while rate of constitutive resistance was 10.6% [11]. In a study by Thapa *et al*. from Nepal, a similar rate of inducible resistance at 36.5% was reported, while rate of constitutive resistance was 18.5% which was higher compared to our study [12]. In study by Shantala *et al*. reported a similar prevalence of inducible resistance at 32.5%, while rate of constitutive resistance was higher 25.4% compared to our study [13]. In our study, rate of inducible and constitutive clindamycin resistance was 37.35% and 9.04% in MRSA, while it was 19.35% and 3.22% respectively in MSSA. The difference in inducible resistance between MRSA and MSSA was statistically highly significant (p = 0.0026). In a study by Thapa *et al*. rate of inducible and constitutive resistance was 40% and 20% in MRSA, while MSSA showed 34.9% inducible resistance and 17.4% constitutive resistance [12]. Study by Lall *et al*. reported 37.1% inducible resistance and 16.6% constitutive resistance in MRSA, while MSSA showed 6% inducible resistance and 4.8% constitutive resistance [14].

In study by Nahara *et al*. in Japan, overall inducible clindamycin resistance was 31.9%. MRSA showed higher rate of inducible resistance at 58.6% compared to MSSA 23.5% ($p < 0.001$) [15]. A study by Jangla *et al*. in Mumbai, 23.5% exhibited an inducible clindamycin resistance, out of which MRSA had significantly higher rate (39%) compared to MSSA (12%) [16]. These studies have similarly found a highly significant difference in inducible clindamycin resistance between the two groups ($p < 0.001$) suggesting that inducible and clindamycin resistance is higher in MRSA compared to MSSA. In our study, high rates of inducible resistance were observed in both CA-MRSA (82.6%) and HA-MRSA (79.6%). However, there is no statistically significant difference between them ($p = 0.38$). In a study by Lall *et al*. similar findings in CA-MRSA and HA-MRSA were observed with no statistically significant difference [13].

Conclusion

The rate of inducible clindamycin resistance was significant, affecting approximately one-third (30.9%) of all *Staphylococcus aureus* isolates. Without routine D-test testing, isolates with the iMLSB phenotype would be missed, leading to clindamycin therapeutic failure. Therefore, implementing this simple, reliable, and inexpensive D-test is crucial for accurately identifying both iMLSB and cMLSB phenotypes and preventing treatment failure of clindamycin. The D-test should be a mandatory method in routine disk diffusion testing to detect inducible clindamycin resistance.

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