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Inducible and constitutive clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care Hospital of Muzaffarnagar Medical College and Hospital, Muzaffarnagar

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Abstract: *Staphylococcus aureus (S. aureus) has been continuously acquiring resistance to many antibiotics at an alarming speed. Penicillin resistance was first noticed in 1944 and methicillin resistance was first observed in 1961 [1]. Recent emergence of inducible clindamycin resistant S. aureus, has further limited our choice of antibiotics.*

This study was undertaken to find out the prevalence of inducible ($iMLS_B$) and constitutive clindamycin resistance ($cMLS_B$) among the clinical isolates of S. aureus.

A total of 100 non-duplicate clinical isolates of S. aureus were collected from June 2014 to March 2015. D-test was performed in routine by placing clindamycin (CLI) disc 2μg and erythromycin (ERY) disc 15μg approximately 15-26 mm apart measured edge to edge on a Muller-Hinton agar plate that has been inoculated with a Staphylococcus isolate incubated at 35±2°C in ambient air.

In this study, 92 (92%) of S. aureus isolates were found to be methicillin resistant (MRSA) and 8 (8%) tested sensitive to cefoxitin, i.e., methicillin sensitive S. aureus (MSSA). Among 92 strains of MRSA, a total of 36(39.1%) exhibited $iMLS_B$ resistance, 16 (17.40%) were positive for constitutive macrolide, lincosamide and streptogramin B resistance ($cMLS_B$) phenotype and 8 (8.70%) belonged to macrolide and streptogramin (MS) phenotype. Among 8 isolates of MSSA, only 2 (25%) strains were found positive for $iMLS_B$ resistance and rest 6 strains were sensitive to clindamycin.

D-test should be performed routinely on all isolates of S. aureus in order to check $iMLS_B$ resistance.

Keywords: *Staphylococcus aureus, D-zone test, inducible clindamycin resistance*

I. Introduction

The Macrolide, lincosamide, and streptogramin (MLS) antibiotics have similar inhibitory effects on bacterial protein synthesis, but they are chemically distinct. In the treatment of Gram-positive infections, MLS antibiotics are used widely. However, this widespread use has led to an increase in the number of staphylococcocci strains resistant to MLS antibiotics [2, 3].

Clindamycin is an alternative drug for infections due to *Staphylococcus aureus* in case of intolerance to penicillin or resistance to methicillin. Furthermore, clindamycin represents an attractive option for several reasons. First, clindamycin is available in both intravenous and oral formulations. Second, the drug has a remarkable distribution into the skin and skin structures. Third, community-acquired methicillin-resistant *S. aureus* (CA-MRSA), which has rapidly emerged in recent years as a cause of skin and soft-tissue infections, is frequently susceptible to several antibiotics, including clindamycin [4, 5]. Finally, it has been shown that clindamycin inhibits the production of toxins and virulence factors in gram-positive organisms through inhibition of protein synthesis (6). Clindamycin has excellent tissue penetration (except for the central nervous system) and accumulates in abscesses, and no renal adjustments are needed [7].

Macrolide antibiotic resistance in *Staphylococcal aureus* and coagulase-negative *staphylococci* (CNS) may be due to an active efflux mechanism encoded by msrA (conferring resistance to macrolide and type B streptogramins only) or may be due to ribosomal target modification affecting macrolides, lincosamide, and type B streptogramins (MLS_B resistance) [8].

erm genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, thereby reducing binding by MLS agents to ribosome [9-11]. The msrA gene confers the so-called MS phenotype (resistance to erythromycin, inducible resistance to streptogramin B, and susceptibility to clindamycin) by efflux [3,11,12]. Strains with inducible MLS_B resistance demonstrate in vitro resistance to 14- and 15- member macrolides (e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins; strains with constitutive MLS_B resistance show in vitro resistance to all agents [3,9,10]. Inducible MLS_B resistance cannot be determined using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests [9].

Reporting *Staphylococcus spp.* as susceptible to clindamycin without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy.

II. Material and Methods

One hundred non-duplicate clinical isolates of *S. aureus* from June 2014 to March 2015 were subjected to D test. Out of one hundred isolates, 92 (92%) were found to be methicilin resistant *S. aureus* (MRSA) strains and 8 (8%) methicillin sensitive *S. aureus* (MSSA) strains. Testing of methicillin resistance was done with 30 µg disc of cefoxitin as per Clinical Laboratory and Standard Institute (CLSI), 2014 guidelines [13].

D-test was performed by placing clindamycin CLI disc 2µg and erythromycin ERY disc 15µg approximately 15-26 mm apart measured edge to edge on a Muller-Hinton agar plate that has been inoculated with a *Staphylococcus* isolate (0.5 McFarland standard) incubated at 35±2°C in ambient air. Flattening of the zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) = inducible clindamycin resistance (Figure1). D-test was performed as per Clinical Laboratory and Standard Institute (CLSI), 2014 guidelines [13].

Staphylococcus aureus ATCC 25923 strains, was used to check the quality control of ERY and CLI discs. In house positive and negative controls were also used.

Interpretation of erythromycin and clindamycin zones was done according to the description given below in the table 1.

Table 1. Interpretation of erythromycin and clindamycin zones in S. aureus			
	Sensitive	Intermediate	Resistant
Erythromycin Clindamycin	≥ 23 mm ≥ 21 mm	14-22 mm 15-20 mm	≤ 13 mm ≤ 14mm
CLSI Guidelines 2014: Performance standards for Antimicrobial disc Susceptibility Tests			

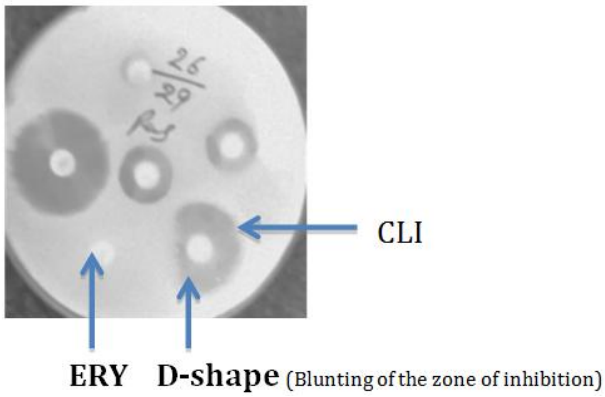

Figure 1 Positive D-test. (Erythromycin and Clindamycin discs were placed in adjacent positions)

D-test phenotypes and their characteristics description is given below in table 2. The results were read according to the details mentioned in the table.

Table 2. D-test phenotype categories and their characteristics				
D test phenotype	Resistance phenotype	CLI result	ERY result	Double disc test description
D+	Inducible MLS_B	S	R	Blunted, D shaped clear zone around CLI disc Proximal to ERY disc
D -	MS	S	R	Clear zone around CLI disc
R	Constitutive MLS_B	R	R	Growth up to CLI and ERY discs
S	No resistance	S	S	Clear zone around discs
S- Sensitive, R- Resistance, CLI- Clindamycin, ERY- Erythromycin.				

III. Results

In our study, 92 (92%) of *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA) and 8 (8%) tested sensitive to cefoxitin (MSSA) [Table 3]. A total of 38(38%) *S. aureus* isolates belonged to iMLSB phenotype. Among 92 MRSA, a total of 36 (39.1%) exhibited $iMLS_B$ resistance, 16 (17.40%) were of $cMLS_B$ phenotype and 8 (8.70%) belonged to MS phenotype. Among 8 isolates of MSSA, only 2 (25%) strains exhibited $iMLS_B$ resistance and rest 6(75%) strains were sensitive to clindamycin (Table 3).

Table 3. Distribution of isolates			
Susceptibility pattern (Phenotype)	MRSA (%)	MSSA (%)	Total (%)
ERY-S, CLI-S	32 (34.8)	6(75%)	38
ERY-R, CLI-R (constitutive MLS_B)	16 (17.4)	0 (0%)	16
ERY-R, CLI-S, D-test positive (Inducible MLS_B)	36 (39.1)	2 (25%)	38
ERY-R, CLI-S, D-test negative (MS)	08 (8.7)	0 (0%)	08
Total	92	8	100

IV. Discussion

Our study revealed an extremely high percentage of MRSA 92(92%). A recent study carried out by the Indian Council of Medical Research (ICMR) in the fifteen selected centres of the country during the year 2008-2009, has reported prevalence of MRSA varying from 21% at Apollo Health Centre (AHC), Hyderabad to 84% at Regional Institute of Medical Sciences, Imphal [14].

In Korea, the prevalence of MRSA has been estimated to be more than 70% among all clinical isolates in early 2010s [15].

Prevalence of clindamycin resistance from different centres in India is given in table 4.

Table 4.	**MRSA**			**MSSA**		
Author's name	$iMLS_B$ Phenotype%	$cMLS_B$ Phenotype%	MS Phenotype%	$iMLS_B$ Phenotype%	$cMLS_B$ Phenotype%	MS Phenotype%
Gadepalli *et a*l (2006) [16]	30	38	12	10	15	12
Angel *et a*l (2008) [17]	64	0	12	5	0	25
Ciraj *et al* (2009) [18]	38	15.3	0	12.9	0	9.7
Vandana *et a*l (2009) [19]	48.7	0.05	30.7	9.5	1.4	56.1
Shrestha *et al* (2009) [20]	39.7	44.4	11.1	0	2.7	13.7
Deotale *et al* (2010) [21]	34	9	30	2	0	5
Pal *et al* (2010) [22]	43.6	38.8	18.7	6.93	7.3	10.9
Prabhu *et al* (2011) [23]	20	16.7	13.3	6.2	6.2	6.2
Mittal *et al* (2013) [24]	47	9	14	13	7	25

According to reports from different regions of India, the prevalence of inducible clindamycin resistance varies from 20% to 64%. In our centre, it is 38%, similar to reported by Ciraj et al. However, the incidence of constitutive and inducible MLS_B resistance varies by geographic region and even from hospital to hospital, with some studies showing higher local incidence of either constitutive or inducible MLS_B resistance in staphylococcal isolates [10, 11,25,26, 27].

V. Conclusion

D-test should be a routine test in order to guide the clinician about the susceptibility of S. aureus to clindamycin. Clindamycin is a preferred drug of treatment in skin and soft tissue infections, especially in MRSA and in patients allergic to penicillin. Also, among the paediatrician, this is a preferred antibiotic in children due to the limited choice of the antibiotics. Appropriate use of this drug can avoid the therapeutic failure during therapy. Moreover, during this study, we found a very high percentage of MRSA (92%) isolates as compared to other studies in the country. Stringent hospital antibiotic policy and effective infection control measures need to be advocated immediately.

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