

TITLE

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I. INTRODUCTION

The antibiotics Macrolide, Lincosamide, and Streptogramin (MLS) are chemically different, but they have similar inhibitory effects on bacterial protein synthesis. MLS antibiotics are widely used in the treatment of Gram-positive infections. However, due to their widespread use, there has been an increase in the number of MLS antibiotic-resistant staphylococci strains¹. Clindamycin is an alternative drug for Staphylococcus aureus infections 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 forms. Second, the medication has a remarkable penetration into skin and skin structures.

Finally, methicillin-resistant S.aureus (CA-MRSA), which has recently emerged as a cause of skin and soft-tissue infections is frequently susceptible to several antibiotics, including clindamycin².

Finally, clindamycin has been shown to inhibit the production of toxins and virulence factors in gram-positive organisms by inhibiting protein synthesis. Clindamycin has excellent tissue penetration (except for the central nervous system), accumulates in abscesses, and requires no renal adjustments. Macrolide antibiotic resistance in Staphylococcus aureus and coagulase-negative staphylococci (CNS) can be attributed to an active efflux mechanism encoded by *msrA* (which confers resistance to macrolides and type B streptogramins only) or to ribosomal target modification affecting macrolides, lincosamide, and type B streptogramins (MLSB resistance)³. *erm* genes encode enzymes that provide inducible or constitutive resistance to MLS agents by methylating the 23S rRNA, reducing MLS agent binding to the ribosome⁴. The MS phenotype (resistance to erythromycin, inducible resistance to streptogramin B, and susceptibility to clindamycin) is conferred by the *msrA* gene via efflux.

Inducible MLSB resistance strains show in vitro resistance to 14- and 15-member macrolides (e.g., erythromycin), but appear susceptible to 16-member macrolides, lincosamides, and type B streptogramins; constitutive MLB resistance strains show in vitro resistance to all agents. Standard susceptibility test methods, such as broth-based or agar dilution susceptibility tests, cannot detect inducible MLB resistance⁵. This study will be undertaken to find out the prevalence of inducible (iMLSB) and constitutive clindamycin resistance (MISB) among the clinical isolates of S. aureus.

AIM AND OBJECTIVE:

To find out the prevalence of inducible (MI SB) and constitutis clindamycin resistance (eMLSB)among the clinical isolates of S. aureus.

II. MATERIAL AND METHODS

STUDY DESIGN: The prospective study was conducted in the Department of Microbiology, MMC. Muzaffarnagar,

STUDY PLACE :- Muzaffarnagar Medical College and hospital

STUDY DURATION: 15 days

SAMPLE SIZE:-A total of 30 non-duplicate clinical isolates of S. aureus were collected

SAMPLING TECHNIQUE: Simple random technique

STUDY PROCEDURE: thirty non-duplicate clinical isolates of S. aureus were subjected to D test. Testing of methicillin resistance were done with 30 ug disc of cefoxitin as per Cimical Laboratory and Standard Institute (CLSI), 2014 guidelines⁶. D-test was performed by placing clindamycin CLI disc 2ug and erythromycin ERY disc 15 approximately 15-26 mm apart measured edge to edge on a Muller Hinton agar plate that has feet inoculated with a Staphylococcus isolate (0.5 McFarland standard) and incubated at 35°C in ambient air.

Flattening of the zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) inducible clindamycin resistance.

D-test was performed as per Clinical Laboratory and Standard Institute (CLSI), 2014 guidelines⁶. Staphylococcus aureus ATCC 25923 strains were used to check the quality control of ERY and CLI disc. Interpretation of erythromycin and clindamycin zones was done according to the description given below in the table 1.

Table 1. Interpretation of erythromycin, clindamycin zones in s.aureus

	SENSITIVE	INTERMEDIATE	RESISTANT
ERYTHROMYCIN	≥23mm	14-22mm	≤13mm
CLINDAMYCIN	≥21mm	15-20mm	≤14mm

CLSI Guideline 2014: performance standards for antimicrobial disc susceptibility test Table 2. D-test phenotype categories and their characteristics

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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	ConstitutiveMLS _B	R	R	Growth upto CLI and ERY discs
S	No resistance	S	S	Clear zone around discs

STATISTICAL ANALYSIS: The data was compiled in the form of table's and percentages.**ETHICAL CLEARANCE:** Ethical clearance was taken from ethical committee of Muzaffarnagar Medical College, Muzaffarnagar.

III. REVIEW OF LITERATURE -

Staph bacteria are one of the most common causes of skin infections and sometimes produce relatively minor skin infections such as pimples and boils. However, they can cause more serious illnesses such as surgical wound infections, bloodstream infections, bone infections, and pneumonia. In the past few decades, a more dangerous form of staph has emerged. This form is known as methicillin-resistant Staphylococcus aureus and is usually referred to by the acronym MRSA. What sets MRSA apart is that it is resistant to an entire class of antibiotics called beta- lactams. This group of antibiotics includes methicillin, and the more commonly prescribed penicillin, amoxicillin, and oxacillin among others.

Inducible clindamycin resistance in staphylococci and streptococci can be detected by the **disk diffusion method** using clindamycin and erythromycin disks or broth microdilution methods. **D- zone** test is performed by disk diffusion, placing a 15-µg erythromycin disk in proximity to a 2-µg clindamycin disk on an agar plate that has been inoculated with a staphylococcal or streptococcal isolate; the plate is then incubated overnight.

A study conducted by **Prabhu et al.** in India evaluated the prevalence of inducible and constitutive clindamycin resistance among 100 clinical isolates of Staphylococcus aureus. The study found that 17% of the isolates were inducibly resistant to clindamycin, while 4% were constitutively resistant. The study also found that the resistance to erythromycin was significantly higher among the inducibly resistant isolates compared to the constitutively resistant isolates.⁷ One such study by V Gupta study was aimed to find out the percentage of Staphylococcus aureus having inducible clindamycin resistance (iMLS B) in our geographic area using D-test. Also, we tried to ascertain the relationship between Methicillin-resistant Staphylococcus aureus (MRSA) and

inducible clindamycin resistance, association of these iMLS B isolates with community or nosocomial setting and treatment options for these iMLS B isolates. Among 200 *Staphylococcus aureus* strains, 50 (25%) were found to be MRSA and 36 were D-test positive. Also, MRSA isolates showed both higher inducible resistance and constitutive resistance to clindamycin as compared to Methicillin-sensitive *Staphylococcus aureus* (MSSA). Out of 36 isolates of *Staphylococcus aureus* showing inducible clindamycin resistance, 24 were from the outpatient department and 12 were recovered from indoor patients. All isolates of *Staphylococcus aureus* showed 100% sensitivity to vancomycin and linezolid.⁸

Overall, these studies suggest that inducible resistance to clindamycin is more common among clinical isolates of *S. aureus* than constitutive resistance. Additionally, the prevalence of inducible resistance is higher among MRSA isolates than MSSA isolates. These findings highlight the importance of identifying the resistance mechanisms of *S. aureus* isolates to guide the selection of appropriate antibiotics for the treatment of infections caused by this pathogen.

IV. RESULTS

In our study, 28 (92%) of *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA) and 3 (8%) tested sensitive to cefoxitin (MSSA) (Table 3). A total of 11 (38%) *S. aureus* isolates belonged to iMLSB phenotype. Among 28 MRSA, a total of 11 (39.1%) exhibited iMLS resistance. 5 (17.40%) were of cMLSB phenotype and 3 (8.70%) belonged to MS phenotype. Among 3 isolates of MSSA only 1 (25%) strains exhibited iMLS resistance and rest 2 (75%) strains were sensitive to clindamycin (Table 3).

Table 3. Distribution of isolates

Susceptibility pattern(phenotype)	MRSA(%)	MSSA(%)	Total (%)
ERY-S, CLI-S	9 (34%)	2(75%)	11
ERY-R, CLI-R (ConstitutiveMLSB)	5 (17%)	0(0%)	5
ERY-R, CLI-S,D-test positive (inducibleMLSB)	11 (39%)	1(25%)	11
ERY-R ,CLI-S,D-Test negative (MS)	3 (8%)	0 (0%)	3
Total	28	3	30

V. 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, Imphal⁹.

In Korea, the prevalence of MRSA has been estimated to be more than 70% among all clinical isolates in early 2010.¹⁰

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

Author's name	iMLSB Phenotype %	cMLSB Phenotype %	MS Phenotype %	iMLSB Phenotype %	cMLSB Phenotype %	MS phenotype %
Grapelli et al (2006) ¹¹	30	38	12	10	15	12
Angel et al(2008) ¹²	64	0	12	5	0	25
Ciraj et al(2009) ¹³	38	15.3	0	12.9	0	9.7
Vandana et al (2009) ¹⁴	48.7	.05	37.7	9.5	1.4	56.1
Shrestha et al (2009) ¹⁵	39.7	44.4	11.1	0	2.7	13.7
Deotale et al(2010) ¹⁶	34	9	30	2	0	5
Pat et al(2010) ¹⁷	43.6	38.8	18.7	6.93	7.3	10.9
Prabhu et al (2011) ⁷	20	16.7	13.3	6.2	6.2	6.2
Mittal et al(2013) ¹⁸	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 resistance varies by geographic region and even from hospital to hospital, with 7 some studies showing higher local incidence of either constitutive or inducible MLS, resistance in staphylococcal isolates^{19,20,21}

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SUMMARY

The antibiotics Macrolide, Lincosamide, and Streptogramin (MLS) are chemically different, but they have similar inhibitory effects on bacterial protein synthesis. MLS antibiotics are widely used in the treatment of Gram-positive infections. However, due to their widespread use, there has been an increase in the number of MLS antibiotic-resistant staphylococci strains. Clindamycin is an alternative drug for *Staphylococcus aureus* infections 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 forms. Second, the medication has a remarkable penetration into skin and skin structures.

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Finally, clindamycin has been shown to inhibit the production of toxins and virulence factors in gram-positive organisms by inhibiting protein synthesis. Clindamycin has excellent tissue penetration (except for the central nervous system), accumulates in abscesses, and requires no renal adjustments. Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) can be attributed to an active efflux mechanism encoded by *msrA* (which confers resistance to macrolides and type B streptogramins only) or to ribosomal target modification affecting macrolides, lincosamide, and type B streptogramins (MLS^B resistance). *erm* genes encode enzymes that provide inducible or constitutive resistance to MLS agents by

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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.