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# A STUDY ON INDUCIBLE CLINDAMYCIN RESISTANCE AMONG MRSA ISOLATES FROM THE CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL , WARANGAL.

Clinical Microbiology

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## ABSTRACT

**Introduction:** Staphylococcus aureus is one of the most common human pathogens capable of causing a wide range of infections, which are hospital acquired as well as community acquired. Increasing incidence of methicillin resistance among staphylococci has led to usage of macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat S.aureus infections, with clindamycin being the preferable agent owing to its excellent pharmacokinetic properties. Inducible clindamycin resistance may lead to therapeutic failure. **Aim And Objectives:** To detect the prevalence of constitutive and inducible clindamycin resistance in clinical isolates of methicillin resistance staphylococcus aureus (MRSA) by D-test method to improve the clinical outcomes in patients. **Materials And Methods:** This is a prospective study done in a total of 120 MRSA isolates obtained from different clinical samples. Methicillin resistance was detected using cefoxitin disc-diffusion method. D-test will be performed by Kirby-bauer disc diffusion method using Mueller-Hinton agar (MHA) according to standard CLSI guidelines. **Results:** Out of 120 MRSA isolates, 108 shows erythromycin resistance and 96 shows clindamycin sensitive. Among erythromycin resistant isolates 11% showed constitutive resistance ($MLSB_C$), 26% showed inducible clindamycin resistance ($MLSB_I$) 63% showed MS phenotype. **Conclusion:** The frequency of constitutive and inducible clindamycin resistance in MRSA isolates emphasizes that D-test is very important and it must be included as routine antimicrobial sensitivity test in diagnostic laboratories.

## KEYWORDS

MRSA, Inducible clindamycin resistance, D-test.

## INTRODUCTION:

*Staphylococcus aureus* is one of the most common organisms causing nosocomial and community-acquired infections worldwide[1,2,3] About 30% of the general population is colonized with *S. aureus* and up to 3% carry methicillin-resistant *Staphylococcus aureus* (MRSA) in their nose[4]. These bacteria can cause a wide range of infections from mild folliculitis to potentially fatal systemic illnesses such as bacteremia or endocarditis[5].

Increasing incidence of methicillin resistance among staphylococci has led to usage of macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat S.aureus infections, with clindamycin being the preferable agent owing to its excellent pharmacokinetic properties.

Clindamycin is a lincosamide antibiotic, developed in 1966 by chemically modifying the naturally occurring lincomycin. Clindamycin belongs to the macrolide -lincosamide- streptogramin B (MLSB) family. Its spectrum of activity includes staphylococci, streptococci, most anaerobic bacteria, *Chlamydia* and certain protozoa. The wide spread use of the MLSB family of antimicrobials has led to the emergence of resistance.[6] Clindamycin represents a useful option for therapy of various staphylococcal infections.

However, clinical failure of Clindamycin therapy was documented for some of the strains. These failures were due to that routine antibiotic susceptibility test cannot identify inducible resistance to Clindamycin. Mainly, there are two types of macrolide–lincosamide–streptogramin (MLS) resistance, one is constitutive and the other one is inducible. Constitutive resistance can be seen by standard antibiotic susceptibility testing that is done routinely; however, the inducible resistance to Clindamycin can be missed out by routine antibacterial sensitivity testing as it requires an inducer of methylase synthesis such as erythromycin (ER).[7]

Hence that D-test is employed to detect these strains and to improve the clinical outcomes in patients. In this study, we have performed *D*-test on the strains of MRSA isolates obtained from various clinical samples like pus, swab, blood, urine and other body fluids at a tertiary care hospital.

## MATERIALS AND METHODS:

This is a prospective study of 6 months duration from January 2024 to June 2024 done on 120 isolates obtained from various clinical samples sent for culture sensitivity to Microbiology department, MGM Hospital.

***Inclusion Criteria*** **:** Among MRSA isolates, erythromycin resistance and clindamycin either sensitive/ resistant isolates were included in this study.

***Exclusion Criteria*** **:** MSSA isolates and erythromycin sensitive in MRSA isolates were excluded.

### Identification of *Staphylococus aureus*

#### *Gram staining*

Gram staining was performed on all the isolates using ATCC Staphylococcus aureus (25923) as the positive control and ATCC Escherichia coli (25922) as the negative control. All the Gram-positive cocci isolates were included for further testing.

#### *Catalase test*

Catalase test was performed on all the isolates using ATCC Staphylococcus aureus (25923) and ATCC Enterococcus faecalis (29212) as positive and negative controls, respectively. Catalase-positive strains were subjected for further identification.

#### *Coagulase test*

Slide and tube coagulase test was performed using the standard protocols and only coagulase-positive strains were included and identified as Staphylococcus aureus.

#### *Culture*

The samples were inoculated on nutrient agar, blood agar and Mac Conkey agar. The inoculated plates were incubated at 37 ºC for 16 to 24 hours. Colonies grown on agar plates were identified based on their morphology, microscopic appearance, culture characteristics and other biochemical reactions according to standard operative procedures.[8]

### MRSA Detection

Methicillin resistance detected using cefoxitin disc diffusion method. Interpretation was done according to CLSI guidelines[9]. Zone of inhibition > 22mm was considered as MSSA and < 21mm was considered as MRSA.

### D-test

D-test will be performed by Kirby-bauer disc diffusion method using mueller-hinton agar (MHA) according to standard CLSI guidelines.[9]

For the antibiotics 15mcg for erythromycin (ER) disc and 2mcg of clindamycin (CL) disc is used. The MHA plate is streaked with an inoculum of the test organisms that is standardized using 0.5 McFarland standard.

Antibiotic discs of ER and CL are kept 15-20mm apart. The plates are incubated for 16 to 18h at 37$^0$C. After the incubation, the test will be interpreted, and appearance of growth of organisms near the edges of the disc will be considered as resistant.

Three different phenotypes were appreciated after testing and interpreted as follows:

**(I) MLSB$_i$ phenotype** (Inducible clindamycin-resistant phenotype): Staphylococcal isolates showing resistance to erythromycin while being sensitive to CL giving D-shaped zone of inhibition around CL with flattening toward the erythromycin disc (D-test positive) as shown in Figure-1.

**(ii) MLSB$_C$ phenotype** (Constitutive clindamycin-resistant phenotype): This phenotype showed resistance to both erythromycin and CL.(Figure-2).

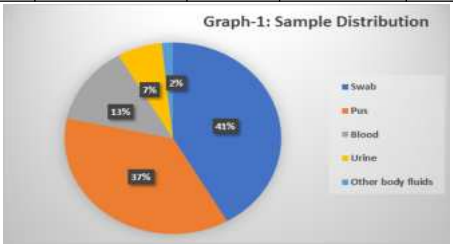
**(iii) MS phenotype**: Staphylococcal isolates exhibiting resistance to erythromycin (zone size <13 mm) while sensitive to CL (zone size >21 mm) and giving circular zone of inhibition around CL disc was MS phenotype (Figure-3).

## RESULTS:

A total of 120 MRSA strains were isolated from various clinical samples like Swab (41%), Pus (37%) blood (13%), Urine (7%) and other body fluids (2%) shown in Graph-1. All these 120 samples were confirmed as MRSA by the Cefoxitin disc diffusion method. Among 120 isolates 12 (10%) were erythromycin sensitive, 108 (90%) were erythromycin resistant and 96 (80%) were clindamycin sensitive and 24 (20%) were clindamycin resistant. D-test was performed on all erythromycin resistant samples. In that 12 (11%) were constitutive MLSB$_C$phenotypes, 28 (26%) strains were MLSB$_i$ and 68 (63%) were MS Phenotypes. (Table-1)

**Table – 1: Phenotypic distribution among MRSA isolates.**

| Isolates | Erythromycin Resistant | D-test Phenotype | | |
|---|---|---|---|---|
| MRSA | 108 | MLSB$_C$ No(%) | MLSB$_i$ No (%) | MS No (%) |
| | | 12 (11%) | 28 (26%) | 68 (63%) |



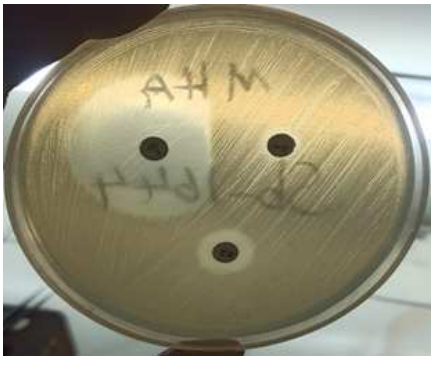



**Figure:1** D-test Positive (MLSB$_i$phenotype)

**Figure:2** MLSB$_c$phenotype

**Figure:3** MS phenotype

## DISCUSSION:

*Staphylococcus aureus* is one of the most common organisms causing both hospital and community acquired infections[10]. Resistance to many antimicrobial agents signifies the problem and limits the treatment options. Last years, developing of new effective agents to treat such infections is considered as an important new challenge in health care settings to overlap the changing pattern of resistance[11]. Clindamycin, which belongs to the Lincosamide family is an excellent antibiotic for treating MSSA as well as MRSA infections. Its versatility lies in the fact that it is well absorbed even if administered orally or intravenously and it is an alternative to cases that are allergic to penicillin.[12,13,14] Clindamycin cannot be considered as a alternative drug unless without finding out the inducible clindamycin resistance , which can lead to failed or inappropriate treatment. So the D-test is employed to detect these strains. The good thing about *D*-test is that even if it is negative, it assures that CL therapy is going to be successful.[15]

In our study out of 120 MRSA isolates 10% was MLSB$_C$ phenotype, 23% was MLSB$_i$ phenotype and 57% of MS phenotype. The present study was compared with various other studies as shown in (Table-2).[16-19] Among them Seifi et al.[19] showed similar findings with our study which reported 15.9% of MLSB$_C$ phenotype, 20.45% of MLSBi phenotype and 52.3% of MS phenotype. Our study shows higher prevalence of inducible phenotype than the constitutive phenotype. This was also supported by almost other studies done elsewhere as shown in (Table-2)

**Table – 2: Comparison of erythromycin resistance pattern and different phenotypes in the present study with other studies.**

| Study | Erythromycin Resistant | MLSB$_C$ phenotype | MLSB$_i$ Phenotype | MS phenotype |
|---|---|---|---|---|
| **Vasanthi *et al.*[16]** | 50% | 10% | 10% | 30% |
| **Mallamgunta *et al.*[17]** | 54% | 15% | 14% | 25% |
| **Mallikarjun *et al.*[18]** | 70% | 3% | 32% | 35% |
| **Seifi *et al.*[19]** | 88.6% | 15.9% | 20.45% | 52.3% |
| **Present study** | **90%** | **10%** | **23%** | **57%** |

## CONCLUSION:

MRSA has became a major global health problem worldwide, both in community and hospitals.

The frequency of constitutive and inducible clindamycin resistance among MRSA isolates emphasizes that D-test is very important and should be performed to facilitate the appropriate treatment of patients. Hence, it must be included as a routine antimicrobial sensitivity test in diagnostic laboratories.

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