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*RESEARCH ARTICLE*

# PHENOTYPIC DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS* SPECIES ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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## *Abstract*

**Introduction:** Clindamycin is an effective drug used as an alternative to Vancomycin to treat skin and soft tissue infections caused by *Staphylococcus* spp. Reporting *Staphylococcus* as susceptible to Clindamycin without checking for inducible Clindamycin resistance may lead to therapeutic failure.

**Aim:** To detect inducible Clindamycin resistance (MLSBi) among clinical isolates of Staphylococci by using a simple double-disc diffusion test (D-test). Methods: It was a prospective study conducted for 6 months from June 2022 to November 2022. All the Staphylococcal isolates from various samples were taken . Inducible clindamycin resistance (MLSBi) was detected by Erythromycin and Clindamycin disc approximation test (D-test) following CLSI guidelines.

**Results:** A total of 216 *Staphylococcus* species were isolated from different samples. Out of the total samples *Staphylococcus aureus* were 42% and Coagulase negative *Staphylococcus* (CoNS) were 58%. Sensitivity to vancomycin, linezolid and teicoplanin were 100%. Methicillin resistance is seen in 21% of *Staphylococcus aureus* and 23% of CoNS. The different susceptibility patterns to clindamycin in both *Staphylococcus aureus* and CoNS were noted. Constitutive (MLSBc) phenotype was most prevalent (48%) followed by MS phenotype (43%) and inducible (MLSBi) phenotype (9%) among Erythromycin resistant Staphylococcus isolates. Sensitive phenotype (S) is detected in 27%. MLSBi was more frequent in Methicillin Resistant *Staphylococcus aureus* (MRSA) (28%) and MRCoNS (10%) than in MSSA (4%) and MSCoNS (2%).

**Conclusion:** D-test should be included as a mandatory method in routine disc diffusion antimicrobial testing to detect inducible Clindamycin resistance in Staphylococci for the optimum treatment of patients.



## Introduction:-

*Staphylococcus aureus (S. aureus*) and CoNS are causative agents for widespectrum of diseases . Emergence of Methicillin-Resistant Staphylococcus strains which is a typical hospital acquired organism and acquiring multidrug resistance has still complicated the treatment. This has led to renewed interest in the usage of Macrolide-Lincosamide- StreptograminB ($MLS_B$) antibiotics to treat Staphylococcal infections[1]. Macrolides such as



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erythromycin, roxithromycin, clarithromycin and lincosamides such as clindamycin and lincomycin belong to different classes of antimicrobials but act through the same mechanism that is by inhibition of protein synthesis[2]. Clindamycin has long been an option for treating both methicillin susceptible S. aureus (MSSA) and methicillin resistant *S. Aureus* (MRSA) infections. Widespread use of MLSB antibiotics has led to an increase in resistance to these antibiotics especially clindamycin, among staphylococcal strains[3].

Macrolide resistance may be constitutive or inducible in the presence of a macrolide inducer[4].

This mechanism can be constitutive, where methylase is always produced, or can be inducible, where methylase is produced only in presence of a macrolide inducer. Among MLSB drugs, only macrolides are good inducers of the enzyme erythromycin ribosome methylase (erm). Once induced, the gene product confers cross-resistance to other membersof the group including lincosamides and streptogramin B. *Staphylococcus aureus* isolates with constitutive resistance show resistance to erythromycin and clindamycin on in vitro testing, whereas isolates with inducible resistance show resistance to erythromycin but appearsusceptible to clindamycin on disc diffusion testing[5].

A double disc diffusion test (D test) for detecting inducible resistance to clindamycin in erythromycin-resistant isolates can be performed by placing a 15 mg erythromycin disc in proximity to a 2 mg clindamycin disc in adjacent positions. This test helps to distinguish staphylococci that have inducible resistance from those with constitutive resistance. For erythromycin-resistant isolates, D test can help to determine whether clindamycin could be used as a therapeutic option (reported as susceptible when the D test is negative or reported as resistant when the D test is positive)[6].

Therefore, it is very crucial to identify actual MLSB resistance for prescribing appropriate therapy in infected patients. If the patient is prescribed with clindamycin without the proper identification of MLSB resistance, it can lead to treatment failure.

The present study was designed to detect the presence of inducible clindamycin resistance among clinical isolates of staphylococci.

## Materials and Methods:-

**Study design:**
Prospective study from June 2022 to November 2022

**Inclusion criteria:**
All staphylococcal isolates from clinical specimens like pus, woundswab, aspirates, blood, urine and sterile fluids.

**Exclusion criteria:**
All bacterial isolates other than staphylococcus were excluded.

**Procedure:** All samples received were cultured on Blood and MacConkey agar and incubated at 37°C for 18-24 hours. Staphylococcal isolates were identified by a battery of standard biochemical tests. Antimicrobial susceptibility testing was performed by Kirby-Bauer's disc-diffusion method on Muller Hinton Agar. Cefoxitin (30 µg) disc was used todetect Methicillin resistance. The isolates that were erythromycin resistant and clindamycin susceptible were further subjected to D test . The erythromycin disc (15 µg) was placed 15 mm apart, edge to edge from the clindamycin disc (2 µg) on the inoculated Muller Hinton Agar plate and was incubated at 37°C for 18-24 hours. After incubation, the plates wereexamined to detect any flattening or blunting of the shape of the clindamycin zone. CLSI2022 guidelines were followed for performing the tests and its interpretation. All the mediaand antibiotic discs used were of Hi-media.

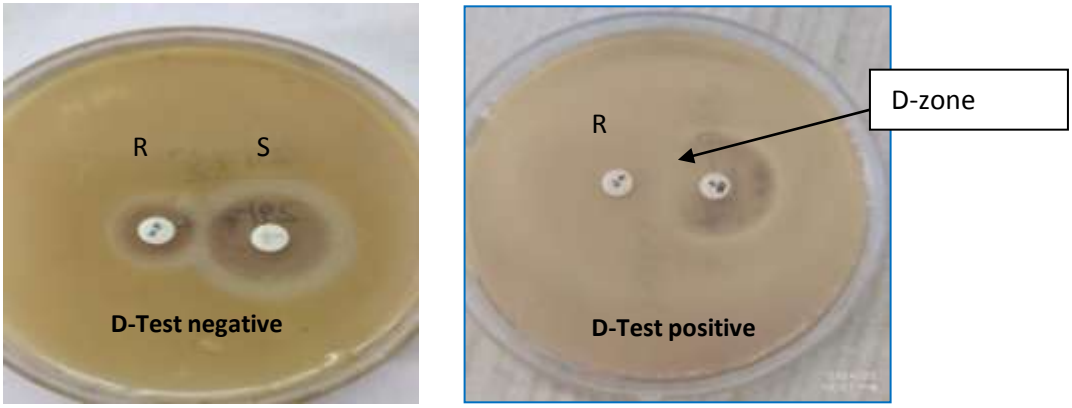



**Figure 1:-** (a) D-test negative, showing Erythromycin resistant, Clindamycin susceptible, clear circular zone. (b) D-test positive, showing Erythromycin resistant and blunting of zone of inhibition around theClindamycin disc. (R-Resistance, S- Susceptible).

**Table 1:-** Interpretation of erythromycin and clindamycin zone sizes in Staphylococcus*

| | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin (E) | ≥23 mm | 14-22 mm | ≤13 mm |
| Clindamycin (CD) | ≥21 mm | 15-20 mm | ≤14 mm |

* CLSI guidelines 2022: Performance std for Antimicrobial Disk Susceptibility Tests.

Different phenotypes observed and interpreted were:
**Sensitive (S) phenotype**: Inhibition of growth around Erythromycin (zone size ≥23 mm) and Clindamycin (zone size ≥21 mm). Sensitive to both E and CD.

**Constitutive MLSB phenotype (MLSBc)**: Presence of growth around Erythromycin (zone size ≤13 mm) and Clindamycin (zone size ≤14 mm). Resistant to both E and CD.

**Inducible MLSB phenotype (MLSBi)**: Presence of growth around Erythromycin (zone size≤13 mm) and clearance around clindamycin (zone size ≥21 mm), giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

**MS phenotype**: Presence of growth around erythromycin (zone size ≤13 mm) and inhibition of growth around clindamycin (zone size ≥21 mm) and giving circular zone of clearance around clindamycin (D test negative).

**Table 2:-** Different phenotypes based on susceptibility to Erythromycin and Clindamycin.

| Phenotype | Erythromycin (E) | Clindamycin (CD) |
|---|---|---|
| S | Sensitive | Sensitive |
| MLSBc | Resistant | Resistant |
| MLSBi (D-testpositive) | Resistant | Sensitive |
| MS (D-test negative) | Resistant | Sensitive |

## Results:-

Among the 216 isolates studied 90 (42%) were *S.aureus* and 126 (58%) were coagulase- negative staphylococci (CoNS). Pus was the most common specimen from which 124 (57%) Staphylococci were isolated, followed by blood 75 (35%), body fluids 8 (4%), urine 6 (3%) and catheter tips 3 (1%). 19 (21%) out of 90 Staphylococcus aureus and 29 (23%) out of 126 CoNS were Methicillin resistant (Table-3)

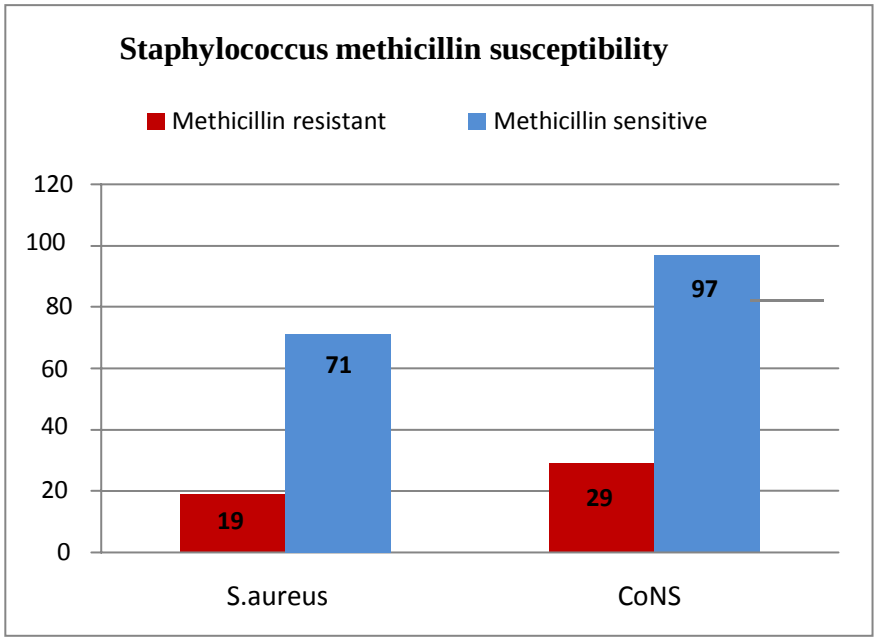



**Figure 2:-** Methicillin susceptibility in Staph aureus & CoNS.

**Table 3:-** Staphylococcus isolated from specimen and their methicillin susceptibility.

| Sample | Total no. Of samples (n=216) | Staph aureus (n=90) | | CoNS (n=126) | |
|---|---|---|---|---|---|
| | | MRSA(n=19) (21%) | MSSA(n=71) (79%) | MRCoNS (n=29) (23%) | MSCoNS(n=97) (77%) |
| Pus | 124 (59%) | 16 | 56 | 14 | 38 |
| Blood | 75 (36%) | 1 | 8 | 14 | 52 |
| Body fluids | 8 (4%) | 1 | 6 | 0 | 1 |
| Urine | 6 (3%) | 1 | 0 | 1 | 4 |
| Catheter tips | 3 (1%) | 0 | 1 | 0 | 2 |

*S.aureus* were most commonly isolated in the age group 21-30 years (22, 24%), followed by 31-40 years (18, 20%), 0-10 years (13, 14%), 41-50 years (13, 14%), 11-20 years (8, 9%), 51-60 years (8, 9%), >60 years(8, 9%). CoNS were most commonly isolated in the age group 0-10 years (35,28%), followed by 21-30 years (20,16%), 31-40 years (18,14%), 51-60 years (17,13%), 41-50 years (14, 11%), >60 years (11, 9%) (Table-4)

**Table 4:-** Staphylococcus isolates - Age wise distribution.

| Age group | Staph aureus (n=90) | CoNS (n=126) |
|---|---|---|
| 0-10 years | 13 (14%) | 35 (28%) |
| 11-20 years | 8 (9%) | 11 (9%) |
| 21-30 years | 22(24%) | 20 (16%) |
| 31-40 years | 18 (20%) | 18 (14%) |
| 41-50 years | 13 (14%) | 14 (11%) |
| 51-60 years | 8 (9%) | 17 (13%) |
| 61-70 years | 8 (9%) | 8 (6%) |
| >70 years | 0 | 3 (2%) |

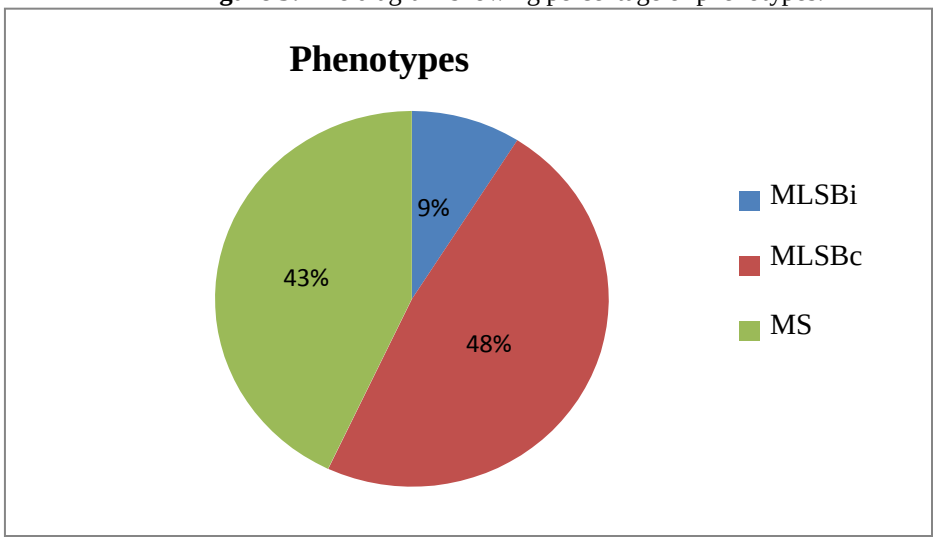
The frequency of susceptibility pattern to Erythromycin as well as different patterns of susceptibility to Clindamycin in both *Staphylococcus aureus* and CoNS was noted (Table-5)

**Table 5:-** Phenotypes of staphylococcal isolates.

| | MLSBi (D-test positive) E-R, CD-S | MLSBc E-R, CD-S | MS (D-test negative) E-R, CD-S |
|---|---|---|---|
| **MRSA** | 6 (43%) | 4 (5%) | 5 |
| **MSSA** | 3 (21%) | 6 (8%) | 25 |
| **MRCoNS** | 3 (21%) | 15 (20%) | 8 |
| **MSCoNS** | 2(15%) | 51 (67%) | 29 |
| **Total** | **14(9%)** | **76 (48%)** | 67 |

E- Erythromycin, CD- Clindamycin

**Figure 3:-** Pie diagram showing percentage of phenotypes.




The inducible clindamycin resistance (D-test positive) was more commonly seen in methicillin resistant *Staphylococcus aureus* (43%) followed by CoNS (21%) as compared to methicillin susceptible *Staphylococcus aureus* (21%) followed by CoNS (15%)- (Table-5)

**Table 6:-** Pattern of inducible clindamycin resistance in staphylococci with respect to methicillinsusceptibility.

| Methicillin susceptibility | D - Test | | Total | Chi-square | p-value(<0.05) |
|---|---|---|---|---|---|
| | Positive | Negative | | | |
| MRSA | 6 (32%) | 13 (68%) | 19 | | |
| MSSA | 3 (4%) | 68 (96%) | 71 | 12.4611 | 0.000416 |
| MRCoNS | 3 (10%) | 26 (90%) | 29 | | |
| MSCoNS | 2 (2%) | 95 (98%) | 97 | 4.0194 | 0.044981 |

## Discussion:-

The increasing frequency of Staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin therapy to treat such infections. Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration. Good oral absorption makes it an important option in an outpatient therapy or follow-up after intravenous therapy. Clindamycin is a good alternative for the treatment of both methicillin- resistant and susceptible Staphylococcal infections.

In the present study, Staphylococcus was isolated most commonly from pus samples 124 (57%) which is higher compared to previous studies Prashant singh et al (43%), Juyal Det et al (46.3%), Lyall et al (31.1%). While Staphylococcus was isolated most commonly in urine samples in the study of Ciraj et al.
Methicillin resistant Staphylococcus was isolated more in studies done by Lyall et al (91.5%) followed by Modukuru et al (56.32%), Panwala et al (53%), Mahima lal et al (45.9%). The present study shows 21% isolates of methicillin resistant Staphylococcus which correlates with Adhikari et al (25.1%).

In the study done by Prashant singh et al, Methicillin resistant CoNS isolated were 33%, while in study of Juyal Det et al MRCoNS was 28%. The present study shows 23% of MRCoNS. In Ciraj et al only 4% of MRCoNS were isolated.

Erythromycin resistant Staphylococcus isolated in the present study was 73%, which correlates with Panwala et al (70.25%). Lyall et al shows 51.7%, while Pal et al shows 50.52%, and Prabhu et al shows 28.4%.

The present study done in kakinada shows 9% inducible clindamycin resistance (MLSBi) which is less compared to Modukuru GK et al done at vijayawada (18%). The present study correlates with Sasirekha B et al done at Bangalore (9.15%). While Ciraj et al done at Manipal shows 13.1%, Prabhu et al done at Mangalore shows 10.52% of MLSBi. Based on these studies the inducible clindamycin resistance varies highly with regard to geographic areas.

## Conclusion:-

Inducible clindamycin resistance cannot be recognised in routine antimicrobial susceptibility testing. So, the D-test is an easy test to perform along with routine susceptibility testing. The incidence of resistance is highly variable with regard to geographic locality. Hence the local data regarding inducible clindamycin resistance is helpful in guiding antibiotic policy.

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