International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

*Original Research Article*

# Occurrence of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus* in a Tertiary Care Hospital

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## ABSTRACT

**Background:** *Staphylococcus aureus* causes wide range of infections, ranging from minor skin infections, chronic bone infection to devastating septicemia and endocarditis. In vitro, *S. Aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (iMLSB). There are limited reports on prevalence of inducible clindamycin resistance among *S.aureus* from this geographical area.
**Aim:** To study the occurrence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*
**Method:** Isolates of *S. aureus* obtained from various clinical samples were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. The clinical isolates were tested for Methicillin resistance using cefoxitin 30 µg discs. Inducible clindamycin resistance was detected by 'D' test as per CLSI guidelines.
**Result:** A total 161, *S. aureus* were isolated and identified from various clinical samples out of which 118 (73%) were MRSA and 43 (27%) were MSSA. Erythromycin resistance was seen in 99 (61.4%) isolates. Among the erythromycin resistant *S.aureus*, iMLSB resistance was observed in 34 (21.1%) isolates and constitutional resistant types cMLSB in 51 (31.67%) and MS phenotype in 76 (47.20%).
**Conclusion:** Occurrence of Inducible Clindamycin resistance was observed in isolates of *S.aureus*. D test is a simple and comparatively easy method which can be used in a routine laboratory and will enable in guiding the clinicians regarding judicious use of clindamycin.
***Keywords:*** Constitutive clindamycin resistance, D test, Inducible clindamycin resistance, MRSA, *Staphylococcus aureus*

## INTRODUCTION

*Staphylococcus aureus* cause of a wide range of infections ranging from minor skin lesions to septicemia and endocarditis. [1] Penicillin and methicillin resistance was first recognized way back in1944 and 1961 A.D. respectively in *Staphylococcus spp*. [2] Multidrug resistant *S. aureus* are increasingly being reported nowadays with high resistance to macrolides (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin), leaving very few therapeutic options. [3] Newer antibiotics like vancomycin, linezolid, and quinupristin dalfopristin have been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concerns over how long these uniform susceptibilities will hold good. [4,5] Macrolides have been used as an alternative

to penicillin and cephalosporin in the treatment by gram positive bacteria, but the development of resistance to macrolide has limited its use.

In vitro, *S. aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (iMLSB). [6] In such cases, patients harbouring iMLSB Staphylococci with in vivo therapy with clindamycin may select constitutive *erm*mutants and leads to the development of constitutive resistance and therapeutic failure. [7] Different studies have shown a wide variation in the rate of inducible clindamycin resistance in different places. [8-12] Lack of data from this geographical area prompted us to carry out a study to determine the occurrence of inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus* from a tertiary care hospital using D test, [13,14] a very simple method which can be used in routine microbiological practice and may help in guiding the clinicians regarding judicious use of clindamycin.

## MATERIALS AND METHODS

This prospective study was carried out in Clinical Microbiology Laboratory, Post Graduate Department of Microbiology, Subharti Medical College, and associated Chhatrapati Shivaji Subharti Hospital Meerut for a period of 1 year (June 2016 to May 2017).

The clinical samples (including pus, urine, blood, ICD fluids, CSF &others) received during the study period from various inpatient units such as Intensive Care Units (ICUs), Neonatal Intensive Care Units (NICUs), wards and outpatient departments were processed for isolation and identification of bacterial pathogen as per standard bacteriological techniques. [15] The demographic detail of the patient such as name, age, gender, date of admission, clinical diagnosis and previous antibiotic history if any was documented in asset Proforma. Approval from the institutional ethical and research committee was obtained before starting the study.

**Antibiotic susceptibility testing (AST):**

The clinical isolates of *S. aureus* was subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method on Mueller Hinton agar (Hi-Media Labs, Mumbai) plate according to CLSI guidelines 2017. [16,17]

**Disks tested for Gram positive cocci includes:** Penicillin G (10 units), cefoxitin (30µg), erythromycin (15µg), clindamycin (2µg), cotrimoxazole (1.25/23.75µg), ampicillin (10µg), tetracycline (30µg), doxycycline (30µg), ciprofloxacin (5µg), moxifloxacin (5µg), gentamicin (10µg), linezolid (30µg), vancomycin (30µg).

**Inducible clindamycin resistance (D-Test):**

The inducible clindamycin resistance was detected by D-test, as per CLSI recommendations. [16,17] Briefly, for detection of inducible clindamycin resistance, a disk approximation test was performed. A 2 2µgclindamycin disc was placed, 21 mm away from the edge of a 15µg erythromycin disc. The plates were incubated. Following overnight incubation at 37°C, three different phenotypes were appreciated and interpreted as follows:

**a) Inducible macrolide-lincosamide-streptogramin B (iMSLB) phenotype: D Test Positive:** iMLSB*S. aureus* isolates which showed resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

**b) Constitutive MSLB ($cMSL_B$) phenotype:** *S. aureus* isolates which showing resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape zone of inhibition around clindamycin.

**c) Methicillin – sensitivity (MS) phenotype:** *S.aureus* isolates exhibiting resistance to erythromycin (zone size ≤13 mm) but sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of

inhibition around clindamycin that is D test negative.

## RESULT

A total of 161 *S. aureus* were isolated and identified from various clinical samples during the study period. *S.aureus* was predominantly isolated from pus 82 (50.93%), followed by blood 43 (26.70%), urine 13 (8.07%), tracheal aspirate 5 (3.01%) and ICD fluid 4 (2.48%).

Out of these, 118 (73%) were MRSA (Methicillin Screen positive) as compared to MSSA 43 (27%)[Fig.1]. The MRSA isolated in our clinical lab were predominantly from IPD samples (n=105) as compared from OPD samples (n=36)[Fig.2].

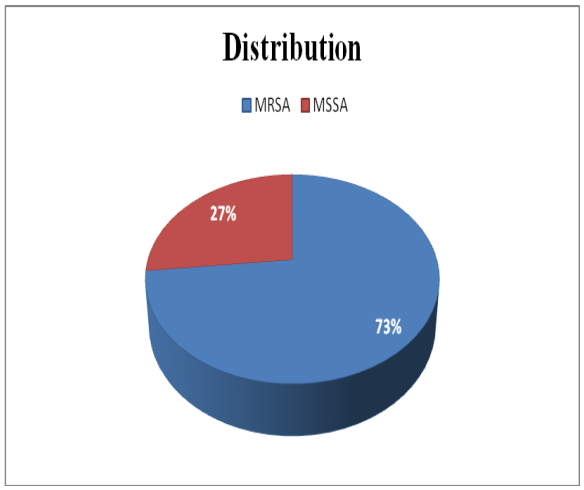


**Fig.1: Distribution of Methicillin Resistant and Methicillin Sensitive isolates of *Staphylococcus aureus* (n=161)**

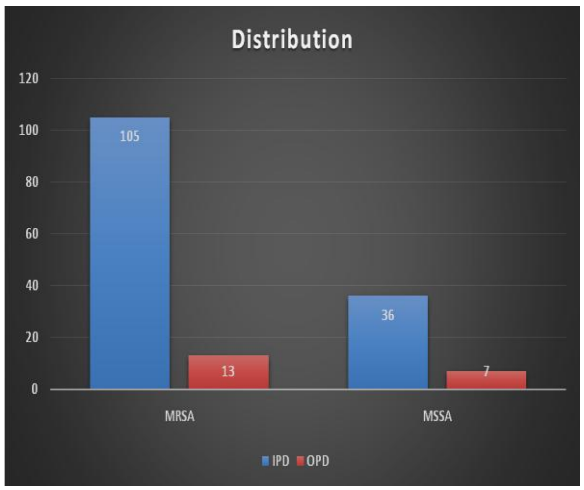


**Fig 2: Distribution of MRSA and MSSA among IPD and OPD patients**

The clinical isolates of *Staphylococcus aureus* showed high level of resistance to various antibiotics like penicillin (93.78), ampicillin (93.16%), erythromycin (61.49) etc., including resistance to linezolid 1.2%, which is a matter of therapeutic concern. However, all our isolates were sensitive to Vancomycin (MIC <2ugm/ml).[Table 1]

**Table 1: Antibiotic Susceptibility pattern of *Staphylococcus aureus* to other antimicrobial agents (n=161)**

| Antimicrobial agents | Resistant isolates (%) |
|---|---|
| Penicillin | 151(93.78) |
| Ampicillin | 150 (93.16) |
| Erythromycin | 99 (61.49) |
| Cotrimoxazole | 124 (77.01) |
| Clindamycin | 85 (52.79) |
| Ciprofloxacin | 113 (70.18) |
| Gentamicin | 42 (26.08) |
| Vancomycin | 0 (0) |
| Linezolid | 02 (1.2) |

**Table2: Distribution of various Phenotypes in isolates of *S.aureus* (n=161)**

| Phenotype | No. | % |
|---|---|---|
| iMLSB | 34 | 21.11 |
| cMLSB | 51 | 31.67 |
| MS-Phenotype | 76 | 47.20 |
| Total | 161 | 100 |

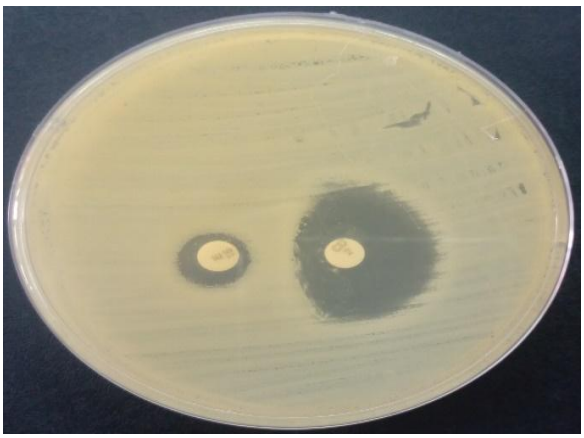

**Fig 3: Inducible MLSB isolate (iMLSB): D test positive, clindamycin therapy failure**

**Fig 4: Constitutive MLSB(cMLSB) phenotype**

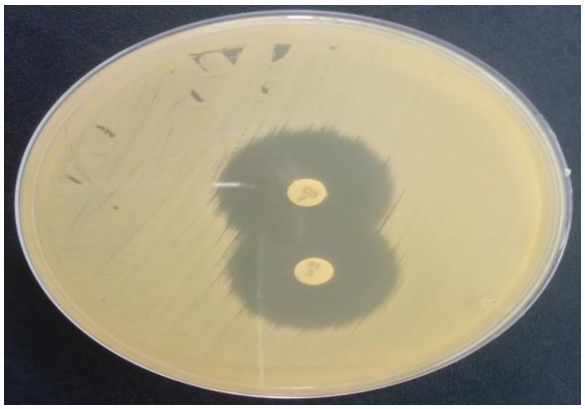

**Fig 5: MS phenotype – Successfultreatment with clindamycin**

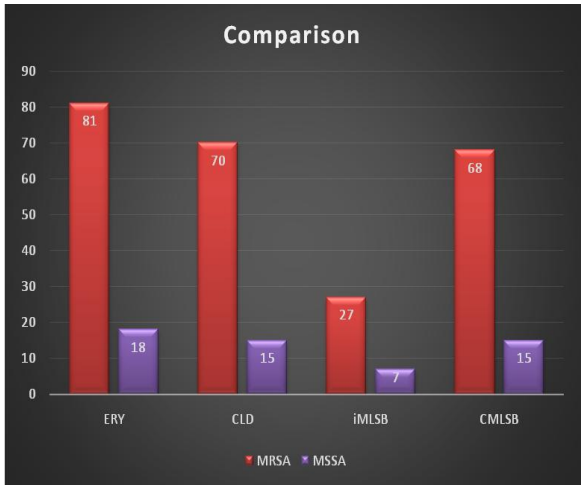


**Fig.6: Comparison of Erythromycin, Clindamycin, iMLSB&cMLSB resistance among MRSA and MSSA**

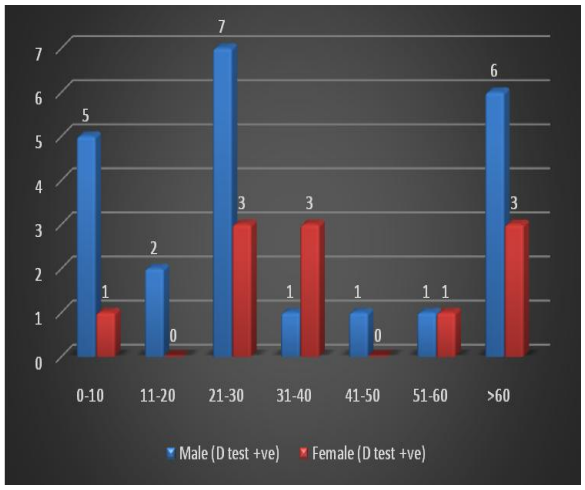


**Fig. 7: Age and gender wise distribution of D test positive cases**

**Table 3: Comparison of type of Erythromycin Resistant *S.aureus* in Different Studies**

| STUDY | YEAR | IMLSb | CMLSb | MS-Phenotype |
|---|---|---|---|---|
| Steward *et al.* [28] | 2005 | 16.4% | 12.5% | 7.8% |
| Deotale *et al.* [30] | 2010 | 14.5% | 3.6% | 14.17% |
| Debasmita *et al.* [31] | 2013 | 50.35% | 15.1% | 34.55% |
| Reghae*t al* [29] | 2016 | 12.7% | 8.1% | 41.8% |
| Present study | 2018 | 21.1% | 31.37% | 47.20% |

Out of the isolates of *S.aureus*,34 (21.11 %) were inducible macrolide-lincosamide-streptogramin B (iMSLB) phenotype i.e. D test positive, 51(31.67%) were constitutive MSLB (cMSLB) phenotypeand76 (47.20%) were Methicillin – sensitivity (MS) phenotype.[Table 2,Fig. 3,4,5]

**Table 4: Comparison of ERSA and ESSA in different studies**

| STUDY | YEAR | ERSA* | ESSA** |
|---|---|---|---|
| Deotale *et al.* [30] | 2010 | 32.39% | 67.61% |
| Prabhu*et al.* [34] | 2011 | 28.42% | 71.57% |
| Present Study | 2018 | 61.4% | 38.6% |

*ERSA: Erythromycin resistant *S.aureus* **ESSA: Erythromycin sensitive *S.aureus*

Comparing Erythromycin, Clindamycin, iMLSB&cMLSB resistance among MRSA and MSSA clinical isolates it was observed that resistance to both erythromycin and clindamycin and both the phenotypes were more commonly seen in MRSA isolates as compared to MSSA isolates [Fig.6]

Looking at the age and gender wise distribution of patients with D test positive isolates of *S. aureus*, maximum isolates were recovered from patients in the age group of 21-30 years and it was predominant in males .The male: female ratio was 1.2:1[Fig 7]

## DISCUSSION

*S. aureus* may cause severe morbidity and fatal infections and the rapid evolution of antibiotic resistance in this pathogen is of considerable concern. Methicillin was indicated for treatment of Staphylococcal infections due to penicillinase producing staphylococci. Methicillin resistant strains gradually evolved during last three decades which accounted for less than 0.1% of *S.aureus* in 1960s.

A total of 161 clinical isolates of *S. aureus* was obtained during the study period, predominantly from pus (50.93%), followed by blood (26.70%) and urine (8.07%). Similarly, studies carried out by Adhikari *et al*, [18] and Lyall *et al*, [19] also reported maximum rate of isolation of

*S.aureus* from pus followed by blood and urine with a mild variation in percentage.

Out of the *S.aureus* isolated (73%) were MRSA. The MRSA were isolated predominantly from IPD samples as compared to OPD samples and the predominant clinical samples being pus followed by blood and urine. Various studies across different geographical area have reported different prevalence rate of MRSA; Toleti *et al*, [20] have reported a prevalence rate of 64.70%, Jarajreh *et al*. [21] in their study conducted in Saudi Arabia have also reported a higher prevalence rate of 77.5% . Much higher rate of MRSA (91.5%) have been reported by Lyall, *et al*. [19] On the contrary Singh *et. al.* [22] and Adhikari *et. al*., [18] reported a much lower rate of 37.8 % and 25.1% respectively in their studies. MRSA have become well established as hospital acquired pathogens. [23] Currently, measures to control *S.aureus* infection are challenged by a large and continuing increase in the prevalence of MRSA worldwide. [24,25]

Knowledge about the susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the emergence of multidrug resistant organisms. There are many options available for treatment of MRSA and MSSA infections, with clindamycin being one of the good alternatives. [13] Good oral absorption makes it an important option in outpatient therapy as a follow-up after intravenous therapy. Clindamycin is also a good alternative antibiotic for the penicillin – allergic patients. [26] However, tremendous use of clindamycin in infections may develop therapeutic failure in inducible resistant phenotype (iMLSB) and from such isolates, spontaneous constitutively resistant mutants have arisen both in vivo and in vitro testing and during clindamycin therapy. [27] Clindamycin is a drug which is useful for treating both methicillin- susceptible and resistant staphylococcal infections.

Since the iMLSB resistance mechanism is not recognized using standard susceptibility test methods and its prevalence varies from hospital to hospital and geographic location. D- test is a simple & cost effective test which can be done in routine antimicrobial susceptibility test for all clinical isolates of *S.aureus*. [19]

In the present study, erythromycin resistance was seen in 61.4% isolates. Among the erythromycin- resistant *S.aureus*, iMLSB resistance was observed in 21.1% isolates and cMLSB in 31.67% and MS phenotype in 47.20%. A study carried out by Steward *et al*, reported maximum iMLSB phenotype (16.4%) followed by cMLSB (12.5%) and MS phenotype 7.8%. [28] Similarly studies carried out by Regha *et al*., [29] and Deotale *et al*., [30] also reported iMLSB as the predominant phenotype followed by cMLSB and then MS phenotype. On the contrary, Dubey *et al*., in 2013 reported iMLSB maximum followed by MS phenotype and cMLSB, [31] showing that studies carried out by different workers showed different rates. Comparison of type of Erythromycin resistant *S. aureus* by different workers is shown in Table 3

Macrolide resistance is by diverse mechanisms. The resistance to macrolide can be mediated by *msr*(*A*) gene coding for efflux mechanism or via *erm* gene encoding for enzymes that confer inducible or constitutive resistance to MLSB antibiotics. In constitutive resistance, r-RNA methylase is always produced (cMLSB); where as in inducible, methylase is produced only in the presence of an inducing agent (iMLSB). [32] Clindamycin is a good alternative for the management of serious soft tissue infections due to limited options of antibiotics available for the treatment of methicillin - resistant staphylococcal infections because of limitation of vancomycin which is a last resort of drug. [33]

In the present study, a comparatively high level (61.4%) of resistance to erythromycin (ERSA; Erythromycin resistant *S.aureus*) was seen as compared to ESSA; Erythromycin sensitive *S. aureus* 38.6%. The rate of resistance to erythromycin was more in MRSA isolates.

Lower rate of ERSA was seen in other studies. [34] [Table 4].

## CONCLUSION

A total of 21.1% isolates showed iMLSB resistance (D test positive)indicating that if D test is not performed routinely, nearly half of the Erythromycin resistant isolates would have been misidentified as Clindamycin sensitive resulting in therapeutic failure. D test is a simple and cost effective test that can be used in routine Clinical Microbiology laboratory and will help in guiding the clinicians regarding judicious use of clindamycin.

**Limitation**

Though our study demonstrates the use of D test in a routine laboratory which will enable in guiding the clinicians regarding judicious use of clindamycin. The study has following limitation:-

The molecular test or detection of MRSA (mecA) gene and erythromycin (erm) gene could not be carried out due to limited resources.

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How to cite this article: Gupta DK, Pandey A, Thakuria B et.al. Occurrence of inducible clindamycin resistance in clinical isolates of *staphylococcus aureus* in a tertiary care hospital. Int J Health Sci Res. 2019; 9(8):71-77.

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