

**PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATES IN A TERTIARY CARE HOSPITAL: AN ALARM BEFORE “NO ANTIBIOTIC ERA”**Nita Gangurde<sup>1</sup>, Preeti Bajaj<sup>2</sup>, Sunita Phatale<sup>3</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: INTRODUCTION:** Among Macrolide-Lincosamide-Streptogramin (MLS) antibiotics Clindamycin has been used most commonly because of its excellent pharmacokinetics. Because of increasing use, resistance to this drug is problem. The inducible resistance can only be detected if the erythromycin and Clindamycin discs are placed adjacent to each other. It is very important for each microbiology lab to check for inducible Clindamycin resistance. **MATERIALS & METHODS:** Total 325 Staphylococcus aureus isolates were studied. Isolates showing resistance to Erythromycin were tested for inducible Clindamycin resistance by disc approximation test, performed as per CLSI 2011 guideline S. **RESULTS:** 32% isolates were Methicillin Resistant Staphylococcus aureus (MRSA) and 68% were Methicillin Sensitive Staphylococcus aureus (MSSA). Inducible Clindamycin resistance was observed in 13.53% isolates, constitutive resistance was found in 12.61% isolates & MS phenotype was observed in 16.61% isolates. Inducible resistance, constitutive resistance and MS phenotype were higher in MRSA (27.80 %, 18.26% & 20.20% respectively) as compared to MSSA (6.78%, 9.95% & 14.93% respectively). **CONCLUSION:** Inducible Clindamycin resistance testing should be done as routine practice. IF not done it can lead to treatment failure and ultimately irrational use of other higher antibiotics. So there is need to guide the clinicians by delivering appropriate reports to prevent the stage of “NO ANTIBIOTIC ERA”

**KEYWORDS:** Inducible, Constitutive Clindamycin resistance, D test, MS phenotype.

**INTRODUCTION:** Staphylococcus aureus is ubiquitous organism. Also is one of the most common bacteria infecting man.<sup>1,2</sup> Nowadays it is commonly known as cause of Hospital acquired and community acquired infection.<sup>3</sup> Initially Penicillin was drug of choice to treat S.aureus infections. But indiscriminate use of Penicillin led to production of resistant strains which are now known as Methicillin Resistant Staphylococcus aureus (MRSA). Methicillin resistance was first reported in 1961 and has now become a global problem.<sup>4</sup> Since then Macrolide-Lincosamide-Streptogramin (MLS) antibiotics were used to treat the MRSA infections.<sup>3,5-7</sup> Indiscriminate use of MLS antibiotics has led to high degree of resistance to these drugs leading to emergence of MLS resistant strains.<sup>8-11</sup>

Among all these antibiotics Clindamycin has been used most commonly because of its excellent pharmacokinetics.<sup>1</sup> Also can be used as an alternative for patients who are allergic to penicillin.<sup>12,13</sup> But because of increasing use, resistance to this drug is again a problem. MLS antibiotics are structurally unrelated but are related microbiologically because of their similar mode of action.<sup>12</sup> Macrolide resistance is mediated by two mechanisms- MLS type B (MLSb) or efflux mechanism phenotypes. MLSb resistance can be of two types – constitutive or inducible.<sup>3,15</sup> Strains with inducible resistance to Clindamycin are difficult to detect in routine laboratory practices, because in routine testing the strains show resistance to erythromycin but sensitive to Clindamycin.

## ORIGINAL ARTICLE

The inducible resistance can only be detected if the erythromycin and Clindamycin discs are placed adjacent to each other.<sup>1,16-18</sup> If the inducible Clindamycin resistance is not detected it leads to false sensitive report to Clindamycin and leads to treatment failure. Therefore it is very important for each microbiology lab to check for inducible Clindamycin resistance and make clinician aware about the same.

**AIMS AND OBJECTIVES:** To find out the prevalence of Staphylococcus aureus isolates from various clinical samples showing inducible Clindamycin resistance in our institute.

**MATERIALS AND METHODS:** The present study was conducted in the Department of Microbiology, Dr Vasant Rao Pawar Medical College, Hospital & Research Centre, Nasik, India. The study was conducted during February 2013 to January 2014. Total 325 Staphylococcus aureus isolates from various clinical samples were included in the study. Identification of Staphylococcus aureus was done by conventional methods with catalase and tube coagulase tests.

After identification the isolates were tested for antibiotic susceptibility to following drugs with Kirby Bauer disc diffusion method. Methicillin resistance was detected by using Cefoxitin disc (30 µg) from Hi-Media Laboratories, Mumbai. All the isolates showing resistance to Erythromycin were further tested for inducible Clindamycin resistance by disc approximation test. The test was performed as per CLSI 2011 guideline.<sup>19</sup> 0.5 McFarland standard of Erythromycin resistant Staphylococcus aureus isolates were prepared.

Lawn culture from the broth was done on Muller Hinton agar plates. Clindamycin (2µg) and Erythromycin (15µg) discs were placed at distance of 15mm (edge to edge) on same plate of Mueller Hinton agar. Plates were incubated at 37°C overnight. Flattening of zone (D shaped zone) around Clindamycin disc in the area between the two discs was taken as positive D-zone test and interpreted as inducible Clindamycin resistance. The control used here was Staphylococcus aureus ATCC 25923.

Three types of phenotypes were observed as follows:

- Isolates showing resistance to erythromycin and sensitivity to Clindamycin with D shaped inhibition zone around Clindamycin disc- inducible (iMLSb) Clindamycin resistance.
- Isolates showing resistance to erythromycin and sensitivity to Clindamycin with circular inhibition zone around Clindamycin disc- MS phenotype.
- Isolates showing resistance to erythromycin and resistance to Clindamycin – were considered as constitutive (cMLSb) Clindamycin resistance.

**RESULTS:** Total 325 Staphylococcus aureus isolates were studied. Out of these 104 isolates showed resistance to Cefoxitin. So 104 isolates were Methicillin Resistant Staphylococcus aureus (MRSA). And 221 isolates were sensitive to Cefoxitin. Thus 221 isolates were Methicillin Sensitive Staphylococcus aureus (MSSA).

TOTAL NUMBER	MRSA n (%)	MSSA n (%)
325	104 (32%)	221(68%)

Table 1: Percentage of MRSA and MSSA among Total isolates of Staphylococcus aureus

## ORIGINAL ARTICLE

Out of total 325 isolates inducible Clindamycin resistance was observed in 44(13.53%) isolates. Out of these 44 isolates 29 (27.80%) were MRSA and 15(6.78%) were MSSA. Also out of total 325 isolates constitutive resistance was found in 41 (12.61%) isolates. Out of which 19 (18.26%) were MRSA and 22 (9.95%) were MSSA. MS phenotype was observed in 54 (16.61%) isolates.

Out of 104 MRSA isolates 29 (27.80%) showed inducible Clindamycin resistance, 19 (18.26%) showed constitutive resistance and 21(20.20%) isolates were identified as MS phenotypes. And remaining 35 (33.74%) were sensitive to both erythromycin and Clindamycin.

Also out of 221 MSSA isolates 15 (6.78%) showed inducible Clindamycin resistance, 22 (9.95%) showed constitutive resistance and 33 (14.93%) isolates were identified as MS phenotypes. And remaining 151 (68.34%) were sensitive to both erythromycin and Clindamycin.

PHENOTYPE	MRSA % (n=104)	MSSA % (n=221)	TOTAL % (n=325)
Inducible Clindamycin Resistance (iMLSb)	27.80 (29)	6.78 (15)	13.53 (44)
Constitutive Clindamycin Resistance (cMLSb)	18.26 (19)	9.95 (22)	12.61 (41)
MS phenotype	20.20 (21)	14.93 (33)	16.61 (54)
Erythromycin- Sensitive Clindamycin – Sensitive	33.74(35)	68.34 (151)	57.23 (186)

**Table 2: Percentage of three different phenotypes observed among total isolates of Staphylococcus aureus**

**DISCUSSION:** Emergence of Methicillin resistance in Staphylococcus aureus has left us with very few therapeutic options available to treat Staphylococcal infections.<sup>12, 13</sup> The Macrolide-Lincosamide-Streptogramin B (MLSb) family of antibiotics is commonly used to treat these infections. Among all these drugs Clindamycin is the drug of choice by most of the clinicians because of its excellent pharmacokinetic properties.<sup>1, 3, 12</sup> But nowadays because of injudicious use of Clindamycin, strains with Clindamycin resistance have been found.

Clindamycin resistance can develop in Staphylococcal isolates with inducible phenotype and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro testing and in vivo during Clindamycin therapy.<sup>17,20</sup> In constitutive resistance methylase is always produced but in case of inducible resistance methylase is produced only in presence of inducer.<sup>3</sup>

The 'erm' genes encode enzymes responsible for inducible and constitutive resistance and 'msrA' gene is responsible for MS phenotype.<sup>1,16-18</sup> Organisms with inducible Clindamycin resistance will pose a diagnostic challenge, because unless the two relevant discs are placed adjacent to and at a proper distance from one another, the isolate will appear resistant to Erythromycin but sensitive to Clindamycin.<sup>4</sup>

Reporting Staphylococcus aureus as susceptible to Clindamycin without checking for inducible resistance may result in inappropriate Clindamycin therapy. On the other hand negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility and provides a very good therapeutic option.<sup>17,21-23</sup> Inducible MLSb resistance is not recognized by using standard susceptibility test methods including standard broth based or agar dilution susceptibility test.<sup>12,13</sup> Inducible Clindamycin resistance can be detected by disc diffusion test, 'D' shaped zone of inhibition around Clindamycin disc if an Erythromycin disc is placed nearby (15 mm). This D test is a simple,

reliable and inexpensive test to perform along with routine susceptibility testing which delineates the inducible (iMLSb) resistance.<sup>16</sup>

Since accurate drug susceptibility data of the infective agents is the most important factor in making appropriate therapeutic decisions [12, 24, 25], if inducible resistance can be reliably detected, Clindamycin can be safely and effectively used in patients with true Clindamycin susceptible strains.<sup>26</sup>

In our study among total 325 *Staphylococcus aureus* we found 32% (n=104) isolates resistant to Methicillin (MRSA) and 68% (n=221) were sensitive to Methicillin (MSSA). Our findings are similar to study by B. Sasirekha et al, who have reported MRSA prevalence as 27.45% and MSSA prevalence as 72.54%. Similar prevalence rate of MRSA was obtained by other workers in India- 22.8% BY Pal and Ayyagari (1991),<sup>27</sup> 26.9% by Shittu % Lin (2006)<sup>28</sup> and 26.6% by Mehta et al (2007).<sup>29</sup> Also varied percentage was obtained by few workers -2.4% by Pulimood et al (1996) <sup>30</sup>, 54.85 by Dar et al (2006)<sup>31</sup> and 65% by Borg et al (2006).<sup>32</sup>

This difference in prevalence of MRSA among different countries and between different regions in a country could be due to varied population and geographical distribution and drug pressure in community.

In our study, *Staphylococcus aureus* isolates when tested for inducible Clindamycin resistance, it was found that 44(13.53%) isolates showed inducible Clindamycin resistance (iMLSb) and 41 (12.61%) showed constitutive resistance (cMLSb). Our these findings are similar with the study by Gade N.D. et al<sup>16</sup> who have reported it as 13.2% and 12.4% respectively. Also a study from Bangalore reported that as 24.9% and 18.3% respectively.<sup>16,33</sup>

Also we found in our study that among MRSA isolates 27.8% showed inducible Clindamycin resistance, 18.26% showed constitutive resistance and 20.20% were MS phenotypes. Our these findings correlate well with findings of study by Gade et al, V. Deotale et al. Gade et al reported inducible Clindamycin in MRSA as 24.3%, constitutive as 19.6% & MS phenotype as 17.8% and Deotale et al reported them as 27.3%, 7.3% and 24.3% respectively. But studies from Veena M et al, Smita Sood and Vandana K E et al have reported quite high prevalence of resistance among MRSA.

In our study we found 6.78% of isolates from MSSA positive for inducible Clindamycin resistance, 9.95% positive for constitutive resistance and 14.93% were MS phenotypes. Our these findings are in concordance with other similar studies from Gade et al, B.Sasirekha et al,<sup>32</sup> Kalpana Date et al.<sup>14</sup> But on the contrary studies by Veena M et al,<sup>1</sup> SmitaSood<sup>34</sup> have reported the percentage quite high. Veena M et al reported inducible resistance in MSSA as 16.22%, constitutive as 21.62% and MS phenotype as 62.16%. Similarly Smita Sood reported 60% as inducible resistance among MSSA.

Also we found higher percentage of inducible Clindamycin resistance in MRSA (27.8%) as compared to MSSA (18.26%) isolates, which matches well with similar studies from Gade et al, Veena et al, Mahima Lall et al,<sup>3</sup> Deotale et al, Kalpana Date et al, Amrutkrishan et al. On the contrary, Schreckenberger et al<sup>35</sup> and Levin et al<sup>36</sup> reported higher percentage of inducible resistance in MSSA as compared to MRSA isolates, 12.5% MRSA and 68% MSSA respectively. Constitutive resistance in our study was seen 18.26% of MRSA isolates which in concordance with study by Gade et al who reports it as 19.6% and study by Mahima Lall who reports it as 16.6%. but on the contrary other similar studies report the same upto high percentage. Studies by Veena et al, Kalpana Date et al, Smita Sood have reported it as 23.73%, 52.63% and 38% respectively.

## ORIGINAL ARTICLE

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In our study we found 54 (16.61%) isolates as MS phenotypes. We found the MS phenotype in 20.20% of MRSA isolates and P.Sreeniwasa, Shantala G.B. et al report it as 22.22% and 15.07% respectively. On the contrary Debmita D et al has reported it upto 48% and Zorgani et al reported the same as 7.7% only. In MSSA the MS phenotype in our study was 14.93%. Gadepalli et al, Shantala et al and Debmita D. report the same as 12%, 16.34% and 16% respectively.

So in our study we found the difference regarding inducible Clindamycin resistance in between MRSA and MSSA isolates was highly significant ( $\chi^2 = 26.89$ ,  $df=1$ ,  $p<0.001$ ). Also difference regarding constitutive resistance in between MRSA and MSSA isolates was significant ( $\chi^2 = 4.43$ ,  $df=1$ ,  $p<0.05$ ). But difference regarding MS phenotype in between MRSA and MSSA isolates was not significant ( $\chi^2 = 1.41$ ,  $df=1$ ,  $p= 0.2347$ ).

It is now clear that had D test not been performed, nearly half of the erythromycin resistant isolates would have been misidentified as Clindamycin sensitive resulting in therapeutic failure. True incidence of MLSb phenotype of Staphylococcus aureus depends on the patient population studied and geographical region, the hospital characteristics and Methicillin susceptibility (MRSA or MSSA).<sup>33</sup>

**CONCLUSION:** Since Clindamycin resistance is on higher side among MRSA isolates, it indicates that inducible Clindamycin resistance testing should be done as routine practice in antibiotic susceptibility testing. If not done there is threat of these isolates getting missed and falsely reported sensitive to Clindamycin. Which can lead to treatment failure and ultimately irrational use of other higher antibiotics like Vancomycin, Teicoplanin etc. so there is need to guide the clinicians by delivering appropriate reports to prevent the stage of "NO ANTIBIOTIC ERA"

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## ORIGINAL ARTICLE

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## ORIGINAL ARTICLE

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