PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATES IN A TERTIARY CARE HOSPITAL: AN ALARM BEFORE "NO ANTIBIOTIC ERA"

Nita Gangurde¹, Preeti Bajaj², Sunita Phatale³

HOW TO CITE THIS ARTICLE:
Nita Gangurde, Preeti Bajaj, Sunita Phatale. “Prevalence of Inducible Clindamycin Resistance among Staphylococcus Aureus Isolates in a Tertiary Care Hospital: an Alarm Before "No Antibiotic Era". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 18, May 05; Page: 4839-4846, DOI: 10.14260/jemds/2014/2521

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 a 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.¹² Nowadays it is commonly known as cause of Hospital acquired and community acquired infection.³ 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.⁴ Since then Macrolide-Lincosamide-Streptogramin (MLS) antibiotics were used to treat the MRSA infections.³⁵⁻⁷ Indiscriminate use of MLS antibiotics has led to high degree of resistance to these drugs leading to emergence of MLS resistant strains.⁸⁻¹¹ Among all these antibiotics Clindamycin has been used most commonly because of its excellent pharmacokinetics.¹ Also can be used as an alternative for patients who are allergic to penicillin.¹²¹³ 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.¹² 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.³¹⁵ 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.
The inducible resistance can only be detected if the erythromycin and Clindamycin discs are placed adjacent to each other. 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 Vasantrao 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. 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:
- a) Isolates showing resistance to erythromycin and sensitivity to Clindamycin with D shaped inhibition zone around Clindamycin disc - inducible (iMLSb) Clindamycin resistance.
- b) Isolates showing resistance to erythromycin and sensitivity to Clindamycin with circular inhibition zone around Clindamycin disc - MS phenotype.
- c) 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
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 | 27.80 (29)     | 6.78 (15)      | 13.53 (44)      |
| Constitutive Clindamycin Resistance | 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.\(^{12,13}\) 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.\(^{1,3,12}\) 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.\(^{17,20}\) In constitutive resistance methylase is always produced but in case of inducible resistance methylease is produced only in presence of inducer.\(^{3}\)

The ‘erm’ genes encode enzymes responsible for inducible and constitutive resistance and ‘msrA’ gene is responsible for MS phenotype.\(^{1,16-18}\) 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.\(^{4}\)

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.\(^{17,21-23}\) Inducible MLSb resistance is not recognized by using standard susceptibility test methods including standard broth based or agar dilution susceptibility test.\(^{12,13}\) 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. 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.

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), 26.9% by Shittu % Lin (2006) and 26.6% by Mehta et al (2007). Also varied percentage was obtained by few workers - 2.4% by Pulimood et al (1996), 54.85 by Dar et al (2006) and 65% by Borg et al (2006).

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 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.

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, Kalpana Date et al. But on the contrary studies by Veena M et al, Smita Sood 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, Deotale et al, Kalpana Date et al, Amrutkrishan et al. On the contrary, Schreckenberger et al and Levin et al 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.
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.Sreeniwas, 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%. Gadeppalli 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 ($x^2 = 26.89$, df=1, $p<0.001$). Also difference regarding constitutive resistance in between MRSA and MSSA isolates was significant ($x^2 = 4.43$, df=1, $p<0.05$). But difference regarding MS phenotype in between MRSA and MSSA isolates was not significant ($x^2 = 1.41$, 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).33

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”

REFERENCES:
1. Veena Manjunath, Eshwar Singh, Ramya. T. G, Mrudula Raj Prakash, Archana Sharma. D-Test – Its role in detection of inducible resistance to Clindamycin in Staphylococcus aureus with special reference to MRSA. Int J Biol Med Res. 2012; 3(1): 1430-1432.
2. Ryan KJ. Staphylococci .In: Ryan KJ, Ray CG, editors. Sherris medical microbiology.4th ed. New York: McGraw Hill; 2004.p. 261-71.
3. Lt Col Mahima Lall, Brig A. K. Sahni. Prevalence of inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Medical Journal Armed Forces India (2013)1-5.
4. Amruthkishan Upadhya, Sunil kumar Biradar. Prevalence of inducible clindamycin resistance in Staphylococcus aureus in a tertiary care hospital in north-east Karnataka, India. Health sciences: An International Journal 2011; 1(3): 21-24
5. Lim JA, Kwon AE, Kim SK, Chong Lee K, Choi EC. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in Korean hospital. J Antimicrob Chemother. 2002; 49: 489-495.
6. Lina G, Quaglia A, Reverdy, Leclercq R, Vandenesch, Etienne. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother. 1999;43:1062-1066
7. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis Pegler. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. J Antimicrob Chemother. 2001; 48:315-316.
8. Ravisekhar Gadepalli, Benu Dhawan Srujana Mohanty, Arti Kapil Bimal K. Das, Rama Chaudhry. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res 123, April 2006, pp 571-573
9. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis 2005; 58: 104-6.
10. Fokas S, Fokas S, Tsironi M, Kalkani M, Dionysopoulou M. Prevalence of inducible Clindamycin resistance in macrolide-resistant Staphylococcus spp. Clin Microbiol Infect 2005: 337-40.
11. Azap ÖK, Arslan H, Timurkaynak F, Yarar G, Oruc E, Gagir U. Incidence of inducible Clindamycin resistance in staphylococci: first results from Turkey. Clin Microbiol Infect 2005; 11:582-4.
12. A. M. Ciraj, P. Vinod, G. Sreejith, K. Rajani Inducible clindamycin resistance among clinical isolates of staphylococci Indian Journal Of Pathology and Microbiology - 52(1), January - March 2009
13. Fiebelkorn KR, Crawford SA, Mc Elmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J Clin Microbiol 2003;41: 4740-4.
14. Kalpana Date, Mamta Choudhary, Vilas Thombare. Inducible clindamycin resistance in clinical isolates of staphylococci in a rural hospital. Int J Biol Med Res. 2012; 3(3): 1922-1925
15. Delialioglu Nuran, Aslan Gonul, Ozturk Candan, Baki Vildan, Sen Sebahat. Inducible clindamycin resistance in staphylococci isolated from clinical samples. J Infect Dis. 2005;58:104-106.
16. Gade N, Qazi MS. Inducible clindamycin resistance among Staphylococcus aureus isolates. Indian Journal of Basic & Applied Medical Research; September 2013: Issue-8, Vol.-2, P. 961-967
17. VDeotale, DK Mendiratta, U Raut, P Narang. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical Samples. Indian Journal of Medical Microbiology, (2010) 28(2) :124-6
18. G. S. Ajantha, Raghavendra D. Kulkarni, Jeevan Shetty, C. Shubhada, Pavithra Jain. Phenotypic detection of inducible Clindamycin resistance among Staphylococcus aureus isolates by using the lower limit of recommended inter-disk distance. Indian Journal Of Pathology and Microbiology- 51(3), July - September 2008
19. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth informational supplement. CLSI document. M100-S20. Pennsylvania: Clinical and Laboratory Standards Institute; 2011.
20. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol 2007;56:342-5.
21. Rodrigues Perez LR, Caierao J, Souza Antunes AL, Alves’Azevedo P. Use of D test method to detect inducible clindamycin resistance in coagulase negative staphylococci(CoNS) Braz J Infect Dis 2007;11:186-8.
22. Vidhya R, Parimala S, Beena P M. Inducible clindamycin resistance in Staphylococcus aureus isolates from a rural tertiary care hospital, Kolar. J Clin Biomed Sci 2013; 3(3):125-28
23. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians 2011;3:25-27
24. Watanakunakorn C. Clindamycin therapy of Staphylococcus aureus endocarditis: Clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin. Am J Med 1976; 60: 419-25.

25. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clin Infect Dis 2003; 37: 1257-60.

26. N Pal, B Sharma, R Sharma, L Vyas. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. J Postgrad Med 2010; 56: 182-5.

27. Pal N, Ayyagari A (1991). Drug resistance pattern of Methicillin resistant Staphylococcus aureus. Ind Paediatrics 28: 731-733.

28. Shittu AO, Lin J (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in Kwa Zulu-Natal province, South Africa. BMC Infect Dis 6: 125.

29. Mehta M, Dutta P, Gupta V (2007). Bacterial isolates from burn wound infections and their antibiograms: an eight-year study. Indian J Plast Surg 40: 25-28.

30. Pulimood TB, Lalitha MK, Jesudson MV, Pandian R, Selwyn JJ (1996). The spectrum of antimicrobial resistance among Methicillin resistant Staphylococcus aureus in a tertiary care centre in India. Ind J Med Res 103: 212-215.

31. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M et al (2006). Molecular epidemiology of clinical and carrier strains of methicillin resistant Staphylococcus aureus in the hospital settings of north India. Ann Clin Microbiol Antimicrobial 5(1): 22.

32. Borg M, Scicluna E, De Kraker M, Van de Sande-Bruinsma N, Tiemersma E, Gur D et al (2006). Antibiotic resistance in the south eastern Mediterranean—preliminary results from the AR medical project. European surveillance 11(7): 639.

33. B. Sasirekha, M. S. Usha, J. A. Amruta, S. Ankit, N. Brinda, R. Divya. Incidence of constitutive and inducible clindamycin resistance among hospital-associated Staphylococcus aureus.3 Biotech: February 2014, Volume 4, Issue 1, pp 85-89.

34. Smita Sood. Inducible Clindamycin Resistance amongst Clinical Staphylococcal Isolates from an Urban Hospital in North-West, India. Research and Reviews: Journal of Medical and Health Sciences | Volume 2 | Issue 4 | October-December, 2013

35. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible Clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol 2004; 42: 2777-9.

36. Levin TP, Suh B, Axelrod P, Truant A, Fekete T. Potential clindamycin Resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: Report of a clinical failure. Antimicrob Agents Chemother 2005; 49: 1222-4.
| AUTHORS:                                      | NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR: |
|----------------------------------------------|---------------------------------------------------|
| 1. Nita Gangurde                             | Dr. Nita Gangurde, #2/2, Sai Dwar Appartments,    |
| 2. Preeti Bajaj                              | Sambhaji Chawk, Untwadi Road, Nashik- 422002.     |
| 3. Sunita Phatale                            | E-mail: nitagangurde@gmail.com                    |

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Dr. Vasantrao Pawar Medical College & Hospital, Nashik.
2. Professor, Department of Pathology, Dr. Vasantrao Pawar Medical College & Hospital, Nashik.
3. Tutor, Department of Microbiology, Dr. Vasantrao Pawar Medical College & Hospital, Nashik.

Date of Submission: 08/04/2014.
Date of Peer Review: 09/04/2014.
Date of Acceptance: 18/04/2014.
Date of Publishing: 01/05/2014.