e-ISSN: 2319-9865
p-ISSN: 2322-0104
Research and Reviews: Journal of Medical and Health Sciences

Inducible Clindamycin Resistance amongst Clinical *Staphylococcal* Isolates from an Urban Hospital in North-West, India.

Smita Sood*

Department of Laboratory Medicine (SRL Ltd.), Fortis Escorts Hospital, Jaipur, India.

Research Article

Received: 24/08/2013
Revised: 09/09/2013
Accepted: 26/09/2013

***For Correspondence**

Department of Laboratory Medicine (SRL Ltd.), Fortis Escorts Hospital, Jaipur, India.

Keywords: Inducible clindamycin resistant, *staphylococcus*, Rajasthan

ABSTRACT

A major concern regarding the use of Clindamycin for *Staphylococcal* infections is the possible presence of inducible resistance to clindamycin. *In vitro* routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance thus necessitating the need to detect such resistance by a simple D test on routine basis .The present study was conducted to determine the incidence of inducible clindamycin resistance in *Staphylococcal* isolates in an urban hospital in North west India. A total 129 *Staphylococcal* strains (*Staphylococcus aureus* and Coagulase Negative *staphylococci*) isolated from various clinical specimens were subjected to disc-approximation method (D-test) to detect inducible clindamycin resistance with standard erythromycin and clindamycin discs.15.50% inducible Clindamycin resistance phenotype, 20.93% constitutive Clindamycin resistance phenotype and 3.10% MS phenotype was detected among 129 clinical isolates of *staphylococci*. In case of a *Staphylococcal* infection, inducible MLS_Bi resistance in *Staphylococcus aureus* strains (40%) was found to be lesser than in CONS strains (60%). This study indicates the importance of D test to differentiate inducible clindamycin resistant isolates of *Staphylococci* so as to facilitate the optimal treatment of patients.

INTRODUCTION

Staphylococcus aureus and *Coagulase Negative Staphylococci* are recognized as causing nosocomial and community acquired infections in every region of the world. The resistance to antimicrobial agents among *Staphylococci* is an increasing problem [1].

The Macrolide-Lincosamide-Streptogramin B (MLS_B) family of antibiotics is commonly used in the treatment of *Staphylococcal* infections. Clindamycin a protein synthesis inhibitor is an attractive option for use in the scenario of increasing drug resistance among the *Staphylococci* especially for skin and soft tissue infections and as an alternative in Penicillin allergic patients. This drug has excellent tissue penetration, requires no renal dosing adjustments and has a good oral absorption. All these factors make it convenient for outpatient prescription or as follow-up after intravenous therapy [2]. However, one important issue with Clindamycin treatment is the risk of clinical failure during therapy.

The MLS family of antibiotics has three different mechanisms of resistance: target site modification, enzyme antibiotic inactivation and macrolide efflux pumps [3]. Macrolide antibiotic resistance in *Staphylococcus aureus* and *Coagulase negative Staphylococci* (CONS) may be due to an active efflux mechanism encoded by *msr A* (macrolides streptogamin resistance) genes , conferring resistance to macrolides and type B streptogamin only [4] or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B streptogamin (MLS_B resistance). *Erm (*erythromycin resistant methylase*)* genes are responsible for encoding enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA and reducing binding by MLS agents to the ribosome [5].

Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if isolates are not tested for inducible Clindamycin resistance. This resistance missed by using standard susceptibility test methods such as standard broth-based or agar dilution susceptibility tests. The

e-ISSN: 2319-9865
p-ISSN: 2322-0104

inducible MLS_B resistance can be detected by a simple test known as Disk approximation test or D test. Low level of Erythromycin is an inducer of MLS_{Bi} phenotype and this is the basis of performing D test [6]. Failure to identify inducible MLS_B resistance may lead to clinical failure of Clindamycin therapy. Conversely, labeling all erythromycin-resistant *staphylococci* as Clindamycin resistant prevents the use of Clindamycin in infections caused by truly Clindamycin-susceptible *Staphylococcal* isolates.

The present study was thus designed to investigate the prevalence of Erythromycin – induced Clindamycin resistance in *Staphylococci* isolated from various clinical specimens at an urban hospital in North West India.

MATERIALS AND METHODS

129 isolates of *Staphylococci* comprising of *Staphylococcus aureus* and *Coagulase negative Staphylococci* were obtained from various clinical samples received in the Microbiology lab of an urban hospital in Northwest India over a period of 8 months (October 2008-May 2009). Duplicate isolates from the same patient were not included. Isolated bacteria were identified by the conventional microbiological methods including colony morphology, Gram's stain, Slide and Tube Coagulase test [7].

Methicillin resistance was determined by Cefoxitin (30 mcg) disc diffusion test according to the recommendations of CLSI (Clinical Laboratory Standards Institute) [8]. Isolates were initially screened for resistance to Erythromycin using disc diffusion testing. The isolates found resistant to Erythromycin were further screened for inducible Clindamycin resistance. A 0.5 Mc Farland equivalent suspension of organism was inoculated onto a Mueller Hinton Agar plate as described in the CLSI recommendations. Clindamycin (2 mcg) and Erythromycin (15 mcg) discs were placed 15 – 20 mm apart from the center on Mueller Hinton Agar. Plates were analyzed after 18 hours of incubation at 37 degree C.

Interpretations of zone of diameters were as follows:

- Erythromycin sensitive ER-S>=23mm
- Erythromycin intermediate sensitive 14-22mm
- Erythromycin resistant ER-R<=13mm
- Clindamycin sensitive CL-S>=21 mm
- Clindamycin intermediate sensitive 15-20mm
- Clindamycin resistant CL-R<=14mm

If Erythromycin zone is <=13mm and Clindamycin zone is >=21mm and both in inhibition zones have a circular shape the organism was considered as negative for inducible Clindamycin resistance on D-testing and were defined as showing MS phenotype.

If Erythromycin zone is <=13mm and the Clindamycin zone is >=21mm with a D shaped inhibition zone around Clindamycin the organism was considered as positive for inducible Clindamycin resistance on D testing and were defined as showing inducible Clindamycin resistance (MLS_{Bi}).

Strains where resistance to both Erythromycin and Clindamycin were observed were defined as showing constitutive MLS_B resistance (MLS_{BC}).

Quality control of the Erythromycin and Clindamycin discs were performed with *Staphylococcus aureus* ATCC 25923. In house strains of *Staphylococcus aureus* showing D-test positive repeatedly was used as a positive control for inducible Clindamycin resistance.

OBSERVATIONS AND RESULTS

Among the 129 *Staphylococcal* isolates studied, 43 (33.33%) were *Staphylococcus aureus* and 86 (66.66%) were *Coagulase negative Staphylococci* (CONS).

Table- 1 shows the categorization of the *Staphylococcal* isolates along with their specimen source. Among the 129 *Staphylococcal* isolates, 10 (7.75%) were Methicillin resistant *Staphylococcus aureus (*MRSA) and 33 (25.58%) were Methicillin sensitive *Staphylococcus aureus*, 45 (34.88%) were Methicillin sensitive *Coagulase negative Staphylococci* (MSCONS) and 41 (31.78%) were Methicillin resistant *Coagulase negative Staphylococci* (MRCONS).

e-ISSN: 2319-9865
p-ISSN: 2322-0104

Table 1: Frequency distribution of *Staphylococci* isolated from various clinical samples

SAMPLE	TOTAL ISOLATES	MSSA		MRSA		MSCONS		MRCONS	
		N	%	N	%	N	%	N	%
PUS	33	17	51.51	4	12	5	15	7	21
BLOOD	43	3	6.9	1	2.3	22	51	17	40
RESPIRATORY SPECIMENS	11	2	18.18	1	9	4	36	4	36
SWABS	10	3	30	1	10	4	40	2	20
SEMEN	5	2	40	0	0	1	0	2	40
C.S.F	8	1	12.5	0	0	6	75	1	13
BODY FLUIDS	6	2	33.33	0	0	3	50	1	17
OTHERS	13	3	23.07	3	23	0	0	7	54
TOTAL	129	33	25.58	10	7.8	45	35	41	32
		Staphylococcus aureus=43 33.33%				CONS=86 66.66%			

Of the 129 clinical *Staphylococcal* isolates 51 (39.53%) showed Erythromycin resistance. Of the Erythromycin resistant *Staphylococcal* isolates 20 (15.50% of total isolates) belonged to MLS_{Bi} phenotype and showed inducible Clindamycin resistance, 27 *Staphylococcal* isolates (20.93% of total isolates) showed constitutive resistance to Clindamycin. Resistance to Erythromycin and susceptibility to Clindamycin with no flattening of Clindamycin inhibition zone i.e. MS phenotype was observed in 4(3.10% of total) isolates. Resistance phenotypes of the isolates are provided in Table 2.

Table 2: Distribution of various resistance phenotypes in *Staphylococcal* species

ORGANISM ISOLATED		ERYTHROMYCIN RESISTANT (n=51)	MLS_{Bi}		MLS_{BC}		MS PHENOTYPE	
			n	%	n	%	n	%
S.aureus (n= 43)	MSSA (n=33)	5	3	60	0	0	2	50
	MRSA (n=10)	8	5	62.5	3	38	0	0
Coagulase negative *Staphylococci* (n=86)	MSCONS (n=45)	14	5	35.71	7	26	2	50
	MRCONS (n=41)	24	7	29.16	17	63	0	0
TOTAL		51	20	39.21	27	53	4	7.84

The isolation rates of MLS_{Bi} phenotypes from clinical samples are provided in Table 3. Among the MLSBi isolates 60% were CONS and 40% *Staphyloccocus aureus*.

Table 3: Distribution of MLS_{Bi} isolates in various clinical specimens

	MLS_{Bi} ISOLATES(n=20)			
SAMPLE	MSSA	MRSA	MSCONS	MRCONS
PUS	2	2	0	1
BLOOD	0	0	3	4
RESPIRATORY SECRETIONS	0	0	0	1
SWABS	1	0	1	0
SEMEN	0	0	0	0
C.S.F	0	0	1	1
BODY FLUIDS	0	0	0	0
OTHERS	0	3	0	1
TOTAL	3 (15%)	5 (25%)	5 (25%)	7 (35%)

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 [9]. Clindamycin is a good alternative for treatment of both Methicillin – resistant and susceptible *Staphylococci* [10].

Clindamycin resistance can develop in *Staphylococcal* isolates with the inducible phenotype, and spontaneous constitutively resistant mutants have been selected from such isolates both in *vitro and in vivo* during Clindamycin therapy [11].

In this study we found a total of 8 (18.60%) *Staphylococcus aureus* and 12 (13.95%) CONS isolates with Erythromycin resistance and Clindamycin sensitive phenotype which demonstrated inducible resistance. Overall the results indicate a high incidence 20 (15.50%) of inducible Clindamycin resistance. Inducible clindamycin rates of 23.6% in *Staphylococci* isolated from various clinical samples in Nasik, Maharashtra has been reported [12]. Pal *et al*. in their previous study conducted in the same region have also demonstrated inducible clindamycin resistance to be 23.48% among the *staphylococci* isolated from various clinical samples [13]. Another recent study from Mumbai reports 34.66% of Erythromycin resistant *Staphylococci* to exhibit inducible clindamycin resistance [14]. Similar to our findings a study from an urban hospital in Dhaka also reported MLS_{Bi} to be 20 % [15].

Studies have reported that the incidence of constitutive and inducible MLS_B phenotype is higher in MRSA [16, 17]. Our results demonstrate that in case of a *Staphylococcal* infection inducible MLS_{Bi} resistance in *Staphylococcus aureus* strains (40%) is lesser than in CONS strains (60%). Lim *et al* (2006) also reported that inducible Clindamycin resistance strains more prevalent in CONS [18].

We found 15.50% *Staphylococcal* isolates with inducible Clindamycin resistance phenotype, 20.93% with constitutive Clindamycin resistance phenotype and 3.10% with MS phenotype. 60% of Methicillin sensitive *Staphylococcus aureus* (MSSA) and 62.5% of Methicillin resistant *Staphylococcus aureus* (MRSA) of Erythromycin resistant and clindamycin susceptible strains exhibited inducible Clindamycin resistance. 35.71% Methicillin sensitive Coagulase negative staphylococcus (MSCONS) and 29.16% Methicillin resistant Coagulase negative staphylococcus (MRCONS) with Erythromycin resistant and Clindamycin susceptibility exhibited inducible clindamycin resistance. Gadepalli *et al* (2006) have reported 26.5% of the *S.aureus* isolates to exhibit constitutive MLS_{Bc} phenotype, 21% to exhibit inducible MLS_{Bi} phenotype and 12% to exhibit MS phenotype [19]. No constitutive resistant phenotype was reported by Angel *et al* (2008) in their study of prevalence of inducible Clindamycin resistance in Gram positive organisms from South India [20].

CONCLUSION

This study demonstrates that the D shape of Clindamycin zone adjacent to Erythromycin disc in a conventional disc diffusion test can serve to detect *S. aureus* or *CONS* strains with inducible resistance to Clindamycin. As the D test is simple, inexpensive and easy to perform it can be included as a part of routine antibiotic susceptibility testing. Additionally it can be used to survey the MLS_B resistance of *Staphylococci* strains from specific geographical regions/hospitals. However the confirmation of *erm* gene in *Staphylococcal* isolates with a positive D test would asset in standardization of the test.

REFERENCES

1. Fokas S, Fokas S, Tsironi M, Kalkani M, Dionysopouly M. Prevalence of inducible Clindamycin resistance in macrolide resistant Staphylococcus spp. Clin Microbial Infect. 2005; 11: 337-340.
2. Drinkovic D, Fuller E R, Shore KP, Holland DJ, Ellis –Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible Clindamycin resistance. J Antimicrob Chemother. 2001; 48: 315-316.
3. Yalmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible Clindamycin resistance in *Staphylococci*. J Med Microbiol. 2007; 56 (Pt 3): 342-5.
4. Ross J I, Eady E A, Cove J H, Cunliffe W J, Baumberg S, Wootton J C. Inducible erythromycin resistance in *staphylococci* is encoded by a member of the ATP-binding transport super-gene family. Mol Microbiol 1990; 4: 1207-1214.
5. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide-lincosamide-streptogramin resistance determinants. Antimicrob Agents Chemother. 1999; 43:2823-2830.
6. Ciraj A M, Vinod P, Sreejith G, Rajani K. Inducible Clindamycin resistance among clinical isolates of *Staphylococci*. Ind J Pathol Microbiol. 2009; 52(1):49-51.
7. Kloos W E and Bannerman TL: Staphylococcus and Micrococcus In PR Murray, EJ Baron, MA Pfaller, FC Tenover and RH Yolken (eds.), Manual of Clinical Microbiology. 6th ed 1995;pg 282-298 ASM Press, Washington.
8. Clinical and laboratory Standards Institute. (2009) Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement (M100-S19) Wayne, PA.
9. Frank AL, Marcinak JF, Mangal PD, Tjhie JT, Kelkar S ,Schreckenberger PC, Quinn JP. Clindamycin treatment of Methicillin-resistant *Staphylococcus aureus* in children. Pediatrics Infect Dis J. 2002; 21: 530-534.
10. Fiebelkorn KR, Crawford SA, Mc Elmeel ML, Jorgensen JH. Practical disc diffusion method for detection of inducible Clindamycin resistance in *Staphylococcal* aureus and Coagulase negative *staphylococci*. J Clin Microbiol. 2003; 41:4740-4744.

11. Drinkovic D, Fuller E R, Shore KP, Holland DJ, Ellis –Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible Clindamycin resistance. J Antimicrob Chemother. 2001; 48: 315-316.
12. Mane M , Kadu A, Gangurde N. Inducible Clindamycin Resistance among *Staphylococcal* Isolates from Different Clinical Samples International Journal of Health Sciences and Research 2012; 2(2):1-7.
13. Pal N, Sharma B, Sharma R, Vyas L. Detection of inducible clindamycin resistance among *Staphylococcal* isolates from different clinical specimens in western India. J Postgrad Med 2010; 56(3):182-5.
14. Samant SA, Pai CG. Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococci*. International Journal of Research in Pharmaceutical and Biomedical Sciences 2013; 4 (2):521-525.
15. Akhter S, Asna SMZH , Rahman MM .Inducible Clindamycin resistance amongst *Staphylococci* isolated from clinical samples in urban hospital of Dhaka City. Ibrahim Med Coll J. 2011; 5(1): 6-8.
16. Ajanta GS, Kulkarni RD, Shetty J, Shukhada C, Jain P .Phenotypic detection of Inducible clindamycin resistance among S. aureus isolates by using the lower limit of recommended inter disk distance. Ind J Path Microbiol 2008; 51(3): 376-378.
17. Mittal V, Kishore S, Siddique ME. Prevalence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* detected by phenotypic method: A preliminary report. Journal of Infectious Diseases and Immunity. 2013; 5(1): 10-12.
18. Lim H S, Lee H, Roh K H, Yum J H, Yong D, Lee K, Chong Y. Prevalence of inducible Clindamycin resistance in *Staphylococcal* isolates at a Korean tertiary care hospital. Yonsei Med J. 2006; 47(4): 480-484.
19. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das B K, Chaudhary R. Inducible Clindamycin resistance in clinical isolates of *Staphylococcus aureus*. Ind J Med Res 2006; 123: 571-573.
20. Angel MR, Balaji V, Prakash J, Brahmadathan KN, Mathews MS. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J Med Microbiol 2008; 26: 262-4.