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Inducible clindamycin resistance in *staphylococcus aureus* isolated from clinical samples at tertiary care hospital Ahmedabad

Mehul Patel[1,*], Vicky Gandhi[2]

[1]Tutor, [2]Assistant Professor, Dept. of Microbiology, Veer Narmad South Gujarat University, Surat, Gujarat

***Corresponding Author:**
Email: mrpatel.jsb@gmail.com

Abstract

Introduction: Clindamycin is most commonly used drug in treatment of erythromycin resistant *Staphylococcus aureus* causing skin and soft tissue infection. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes leading to treatment failure. Thus it is necessary to detect such resistance by simple D test on regular basis.

Aim: To study the induction of clindamycin resistance and characterize the *Staphylococcus aureus* phenotypes based on their susceptibility pattern and induction testing.

Material & Methods: Total 400 *Staphylococcus aureus* strains were isolated from different clinical samples. All isolates were tested for MRSA using Cefoxitin (30µg) by Kirby Bauer disc diffusion method. All 400 *S. aureus* strains were tested to see different induction phenotypes by D test as per CLSI guidelines.

Result: Out of 400 isolates of *S. aureus*, 75 (18.7%) isolates showed inducible clindamycin resistance, 117 (29.3%) isolates showed constitutive resistance, 79(19.7%) isolates showed MS phenotype of resistance and 129 (32.3%) isolates were sensitive to both erythromycin and clindamycin drugs. Inducible resistance was more in MRSA strain (32.3%) as compared to the MSSA strain (9.9%).

Conclusion: This study showed that D test should be done as routine disc diffusion test to detect inducible clindamycin resistance in *Staphylococcus aureus* strains so clindamycin therapeutic failure can be reduced.

Keywords: Inducible clindamycin resistance, Constitutive resistance, MS phenotype, MRSA

Introduction

Staphylococcus aureus is responsible for both nosocomial and community acquired infections that range from minor skin and soft infection to life threatening systemic infection.[1,2] Emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) has kept very few treatment options for us. The Macrolides – Lincosamide – Streptogamin B (MLSB) used as good alternative with Clindamycin as preferred drug.[3] Though, the widespread use of MLSB antibiotics has lead to rise in number of MLSB resistant *S. aureus* strains.[4]

Resistance to MLSB antibiotics occurs by two different mechanisms. The most common mechanism is target side modification by *erm* genes. Other mechanism is active efflux mechanism encoded by the *mrs*A gene.[5,6] *erm* gene mediated clindamycin resistance expressed either constitutively (constitutive MLSB phenotype) or inducibly (inducible MLSB phenotype).[7]

Strains with inducible resistance are hard to detect routinely because they appear as erythromycin resistant and clindamycin sensitive in vitro when they are kept separately. Strains having in vitro inducible MLSB resistance (iMLSB), because of presence of erythromycin ribosomal methylase (*erm*) gene, also have a increased rate of mutation to constitutive MLSB resistance (cMLSB), which could be used during clindamycin therapy and leads to its clinical failure.[8]

Material and Method

The study was conducted at Microbiology department, B.J. Medical College, Ahmedabad during the period from October 2011 to May 2013. Total 400 *Staphylococcus aureus* strains were isolated from different clinical samples by standard microbiology techniques.[9,10]

All *S. aureus* strains were tested for methicillin resistance by using Cefoxitin (30µg) by disc diffusion method. An inhibition zone of 19mm or less indicated MRSA. All *S. aureus* strains were tested for D test as per CLSI guidelines to detect inducible clindamycin resistance.[11] For that erythromycin (15µg) disc was placed at a distance of 15mm to 25 mm measured center to center from clindamycin (2µg) disc on a Muller Hinton agar inoculated with 0.5% McFarland standard bacterial suspension. After overnight incubation at 37°C, flatting of zone (D shaped) around clindamycin between two discs, indicate inducible clindamycin resistance. Different phenotypes were seen after testing and then interpreted as following.[12] (Table 1)

Quality control was done by Negative and positive control strains-ATCC BAA-976(MS phenotype, *msrA* gene positive) and BAA-977(iMLSB phenotype, *ermA* gene positive) were inoculated onto each plate.

Table 1: Characteristics of clindamycin induction test phenotypes as tested by disc diffusion

Induction test phenotype	Resistance phenotype	Induction test description
D	iMLSB	Blunted D-shaped clear zone around CLI disc proximal to ERY disc.
D+	iMLSB	Blunted D-shaped zone around CLI disc proximal to ERY disc and small colonies growing to CLI disc in otherwise clear zone.
Neg	MS	Clear zone around CLI disc; no induction.
HD	cMLSB	Two zones of growth appear around CLI disc. One zone is a light hazy growth extending from the CLI disc to the second zone where the growth is much heavier.
R	cMLSB	No hazy zone. Growth up to CLI and ERY discs.
S	No resistance	Clear susceptible zone diameters.

Result

Total 400 strains of *Staphylococcus aureus* were isolated from different clinical samples. Table 2 shows different clinical samples from which *S. aureus* isolated. *S. aureus* strains most commonly isolated from swab (53.1%) and pus (23.8%) samples.

Table 2: Sample distribution by sample type

Specimen	No. of isolates	Percentage (%)
Swab	212	53.1
Pus	96	23.8
Blood	46	11.6
Tracheal aspirate	29	7.3
CSF	7	1.8
Fluid	7	1.8
Drain	3	0.6
Total	400	100

All *S. aureus* strains were tested for cefoxitin sensitivity. Table 3 shows sensitivity pattern of *S. aureus* for cefoxitin drug.

Table 3: Cefoxitin susceptibility pattern observed

Susceptibility	Number of isolates	Percentage (%)	Interpretation
Resistant	158	39.5	MRSA
Sensitive	242	60.5	MSSA

Among 400 *S. aureus* strains tested for erythromycin and clindamycin in combination (D test), 117 (29.3%) strains were found resistance to both the drugs; 129 (32.2%) were sensitive to both drugs and 154 (38.5%) were resistance to erythromycin and sensitive to clindamycin. There was no isolate which showed sensitive to erythromycin and resistance to clindamycin.

Table 4: Different phenotypes by Induction test

Resistance Phenotype	Induction test phenotype	ERY susceptibility	CLI susceptibility	Number of isolates	Percentage (%)
iMLSB	D	R	S	51	12.8
	D+	R	S	24	5.9
MS	Neg	R	S	79	19.7
cMLSB	HD	R	R	29	7.3
	R	R	R	88	22
No resistance	S	S	S	129	32.3

Table 4 shows the characteristics and distribution of the induction test phenotypes and resistance phenotype observed in the present study. The induction tests revealed D, D+, Neg, HD, R and S phenotypes in 51 (12.8%), 24 (5.9%), 79 (19.7%), 29 (7.3%), 88 (22%) and 129 (32.3%) isolates respectively. The resistant phenotype observed that among the 400 isolates, 117 (29.3%) showed constitutive MLSB resistance while 75 (18.7%) were observed to show inducible MLSB resistance. MS phenotype was observed for 79 (19.7%) isolates while the remaining 129 (32.3%) were susceptible to both erythromycin and clindamycin. Figure 1-6 shows different induction phenotypes observed during study.

Fig. 1: D Phenotype Fig 2: D + Phenotype

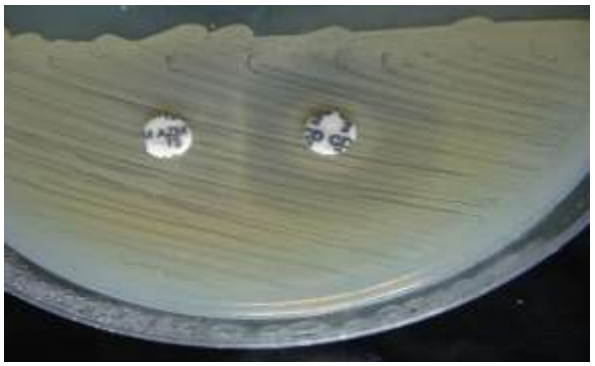

Fig. 3: HD Phenotype

Fig. 4: Neg Phenotype

Fig. 5: R Phenotype

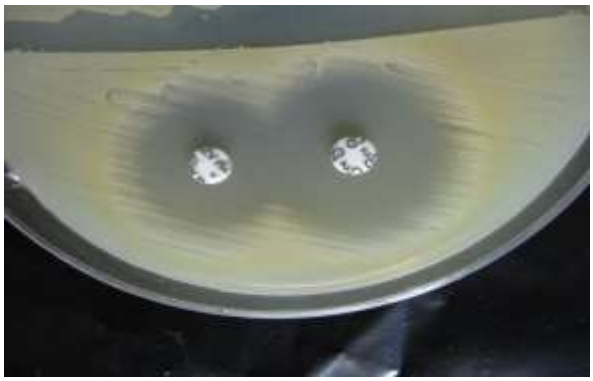

Fig. 6: S Phenotype

This shows the six phenotypes observed during CLI induction testing of S.aureus by disk diffusion. E 15 µg, ERY disc; Cd 2 µg, CLI disc.

Table 5: Resistant phenotypes observed in MRSA isolates

Resistant Phenotype	Induction Phenotype	Number of isolates with Induction phenotypes	Total of resistant phenotype	Percentage (%)
iMLSB	D	35	51	32.3
	D+	16		
MS	Neg	42	42	26.6
cMLSB	HD	16	45	28.5
	R	29		
No resistance	S	20	20	12.6

Table 5 showed the resistance phenotypes observed among the MRSA isolates. Of the total of 158 MRSA isolates in the present study, 51 (32.3%) showed iMLSB phenotype and cMLSB was observed in 45 (28.5%) isolates. Of the remaining isolates 42 (26.6%) showed MS phenotype while 20 (12.6%) isolates showed no resistance.

Table 6: Resistant phenotypes observed in MSSA isolates

Resistant Phenotype	Induction Phenotype	Number of isolates with Induction phenotypes	Total of resistant phenotype	Percentage (%)
iMLSB	D	16	24	9.9
	D+	8		
MS	Neg	37	37	15.3
cMLSB	HD	13	72	29.8
	R	59		
No resistance	S	109	109	45

Table 6 showed the resistance phenotypes seen in the 242 MSSA isolates of the present study. iMLSB phenotype was observed in 24 (9.9%) isolates while 72(29.8%) and 37 (15.3%) isolates showed cMLSB and MS phenotypes respectively. No resistance was observed in 109 (45%) of the isolates.

Discussion

The increase frequency of *Staphylococcus* infections among patients and changing pattern in antimicrobial susceptibility and increase number of MRSA isolates leave us very few therapeutic options such like clindamycin.[13] Because of its good oral bioavailability, excellent tissue penetration and cost makes clindamycin as good option for outpatient therapy or intravenous therapy.[6,14] Since the iMLSB resistance mechanism is not detected by routine standard susceptibility testing, D test become a crucial part of routine antimicrobial susceptibility test for all clinical isolates of *S. aureus*.[15]

In our study we found two distinct induction phenotypes (D, D+) and four non-inducible phenotypes (HD, R, S, Neg) by using erythromycin and clindamycin disc diffusion method. The induction phenotype identify by D test constituted 75 (18.7%) of the total isolates. These included D and D+, which were observed in 51 (12.8%) and 24 (5.9%) isolates respectively. Non-inducible phenotypes were include Neg, HD, R or S phenotypes, which were observed in 79 (19.7%), 29 (7.3%), 88(22%) and 129(32.3%) isolates respectively.

Table 7: Comparison of resistance phenotype in various studies

Study	Fiebelkorn et. al.[3]	Lim Jung-A et. al.[16]	Jorgensen et. al.[17]	Gadepalli et. al.[12]	Present study
Place of study	Texas	Seoul	Texas	Delhi	Ahmedabad
Total isolates	130	493	75	200	400
iMLSB	34(26.2%)	72(14.6%)	22(29.3%)	42(21%)	75(18.7%)
cMLSB	39(30%)	--	25(33.3%)	53(26.5%)	117(29.3%
MS	--	--	--	24(12%)	79(19.7%)
S	16(12.3%)	--	28(37.4%)	81(40.5%)	129(32.3%)

Table 7 compares the results of resistance phenotypes observed in various studies. The results obtained in the present study were comparable to those reported by Fiebelkorn et.al, Lim Jung-A et. al, Jorgensen et.al and Gadepalli et.al for the iMLSB and cMLSB isolates. The results for MS phenotype were comparable to that observed by Gadepalli et.al. The results obtained for S phenotype were similar to those of Gadepalli et.al, but were significantly different from those of the Fiebelkorn et.al.

In our study MRSA isolates included 51 (32.3%) isolates of iMLSB phenotype, 45 (28.5%) of cMLSB, 42 (26.6%) of MS phenotype while 20 (12.6%) isolates showed no resistance. The MSSA strains included 24 (9.9%) isolates of $iMLS_B$ phenotype, 72 (29.8%) isolates showed cMLSB and 37 (15.3%) had MS phenotypes. No resistance was observed in 109 (45%) of the isolates.

Table 8: Comparison of the resistance phenotypes in relation to MRSA/MSSA for various studies

Comparison		Marr et. al.[18]	Goyal R. et. al.[19]	Patel M. et. al.[20]	Gadepalli et. al.[12]	Present study
MRSA	Total	36	150	272	104	158
	iMLSB	4(11.1%)	76(50.7%)	138(50.7%)	31(30%)	51(32.3%)
	cMLSB	--	25(16.7%)	--	39(38%)	45(28.5%)
	MS	--	24(16%)	--	11(12%	42(26.6%)

MSSA	Total	64	--	122	94	242
	iMLSB	22(34.4%)	--	73(59.8%)	09(10%)	24(9.9%)
	cMLSB	--	--	--	15(14%)	72(29.8%)
	MS	--	--	--	11(12%)	37(15.3%)

Table 8 shows the comparison of the resistance phenotypes in relation to MRSA/MSSA strains. Among MRSA isolates, results of iMLSB and cMLSB were comparable to the one reported by Gadepalli et. al, while variable from study by Goyal et. al. The iMLSB resistance phenotypes reported in the MSSA strains were comparable to the results of Gadepalli et. al. In the present study, both constitutive and inducible resistance phenotypes were higher in MRSA isolates as compared to MSSA strains. Similar results were reported by Gadepalli et. al.[12]

The goal of routine detection of inducible clindamycin resistance among *S. aureus* is twofold. First, potential for clinical failure in patients infected with iMLSB strains have been documented.[14,20,21] However, to declare all macrolide-resistant *S.aureus* as clindamycin resistant would deny possibly safe and effective treatment for patients infected with isolates that carry only the macrolide efflux mechanism. So, the other benefit of regular testing for inducible clindamycin resistance is to detect those strains that remain sensitive to clindamycin in spite of macrolide resistance. That's why, regular testing of *S.aureus* for inducible clindamycin resistance is perceived.

Conclusion

Our study concludes that prevalence of D test positive was perceived more in *S. aureus* isolates and also in MRSA isolates as compare to MSSA isolates. So D test is must in laboratory as routine laboratory test to guide the clinicians for wise use of clindamycin in skin and soft tissue infection and to prevent clindamycin therapeutic failure in D test positive isolates.

References

1. Lowy D. Franklin. *Staphylococcal* infections. In Kasper et.al. *Harrison's Principles of Internal Medicine*. 16th edition; 2005;814-823.
2. Washington Winn Jr et.al. *Staphylococci* and related gram-positive cocci. In Washington Winn Jr et.al (eds) *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th Edition. 2006. 623-671.
3. Fiebelkorn KR, Crawford SA, McElmeel ML. Practical disc diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase negative *Staphylococci*. Journal of Clinical Microbiology 2003;41:4740-44.
4. Ajantha GS, Kulkarni RD, Shetty J et al. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates by using the lower limit of recommended inter disc distance. Indian J Pathology and Microbiology 51:376-378.
5. Steward CD, Raney PM, Morell AK et al. Testing for induction of clindamycin resistance in erythromycin resistant isolates of *Staphylococcus aureus*. J Clin Microbiology 2005;43:1716-21.
6. Leclercq R (2002) Mechanism of resistance to macrolide and lincodamides: nature of the resistance elements and their clinical implication. Clin Infect Diseases 34: 482-492.
7. Lim HS, Lee H et al. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* at Korean tertiary care hospital. Yonsei Med J 2006;47:480-484.
8. Siberry K, Carroll Karen et al. Brief report: Failure of clindamycin treatment of methicillin resistance *staphylococcus aureus* expressing inducible clindamycin resistance in vitro. Clinical Infectious Diseases 2003;37:1257-1260.
9. Kloos WE, Banerman TL. *Staphylococcus* and *Micrococcus*, Chapter 22. Manual of clinical microbiology. 7th edition. Murray PR, Baron EJ. ASM Press; 1999. Page 264-82.
10. Brocks GF, Butel JS, Morse SA. The *Staphylococci*. In Brooks GF et al. Jawets, Melnick, Adelberg's Medical Microbiology. 23rd edition. McGraw-Hill 2004:223-230.
11. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing. CLSI 2014.Approved Standard.
12. Gadapalli R et al. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. Indian journal of Medical Research. 2006;123:571-573.
13. Frank Al, Marcinak JF et al. Clindamycin treatment of methicillin resistance *Staphylococcus aureus* infection in children. Paediatric Infectious Diseases Journal 21:530-534.
14. Drinkovic D, Fuller ER et al. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrobial Chemotherapy. 2001;48:315-316.
15. Gupta V, Datta P, Rani H et al. Inducible clindamycin resistance in *Staphylococcus aureus*. A study from North India. J Postgrad Med 2009;55:176-179.
16. Lim Jung-A et al. Prevalence of resistance to macrolide, lincosamides and streptogramin antibiotics in gram positive cocci isolated in Korean hospital. Journal of Antimicrobial Chemotherapy. 2002;49;489-495.
17. Jorgensen J.H et.al. Detection of inducible clindamycin resistance in *Staphylococci* in conjunction with performance of automated broth susceptibility testing. Journal of Clinical Microbiology. 2004;12(4);1800-1802.
18. Marr J.K, Lim A.T, Yamamoto L.G et.al. Erythromycin-induced resistance to clindamycin in *Staphylococcus aureus*. 2005;64(1),6-8.
19. Goyal R., Singh N.P, Manchanda V., Mathur M. Detection of clindamycin susceptibility in macrolide resistant phenotypes of *Staphylococcus aureus*. Indian Journal of Medical Microbiology. 2004;22(4);251-254.
20. Patel Mukesh et.al. Prevalence of inducible clindamycin among community and hospital associated *Staphylococcus aureus* isolates. Journal of Clinical Microbiology.2006;44(7);2481-2484.
21. Vester Brite, Douthwaite Stephen. Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrobial Agents and Chemotherapy. 2001;45(1);1-12.