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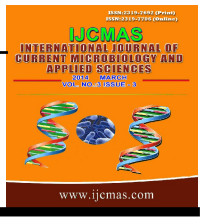

Original Research Article

Prevalence of inducible Clindamycin resistance in *Staphylococcus aureus* from clinical samples: A study from a teaching hospital in Andhra Pradesh, India

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A B S T R A C T

Methicillin Resistant *Staphylococcus aureus* (MRSA), known to cause nosocomial infections has lately been associated with community acquired infections[7]. It is more important to treat Staphylococcal infections with safe and effective drugs[10] like Clindamycin. However, resistance to Macrolide, Lincosamine and Streptogramin antibiotics has been reported to be mediated by the *msr*A gene coding for efflux mechanism and *erm* gene which encoding the enzymes conferring resistance to MLS_B antibiotics. Routine antibiotic sensitivity tests for Clindamycin may fail to detect inducible Clindamycin resistance due to the presence of the *erm* gene thereby resulting in failure of treatment. D test, if performed on a routine basis can overcome this. A total of 108 Staphylococcus aureus isolates from urine, pus, sputum, throat swab, suction tips and other body fluids were subjected to Clindamycin resistance by D Test using Erythromycin and Clindamycin disks. Inducible Clindamycin resistance was detected in 46.34% of methicillin-resistant *Staphylococcus aureus* isolates and in 23.8% of methicillin-sensitive *Staphylococcus aureus* isolates. Although Clindamycin is considered a safe and effective agent for treatment of Staphylococcal infections, all especially the MRSA strains must be subjected to D test to avoid treatment failure.

K e y w o r d s

Methicillin Resistant Staphylococci; Erythromycin; Clindamycin; Inducible Resistance; Constitutive Resistance; D test.

Introduction

Since 1960s, Methicillin Resistant Staphylococcus aureus (MRSA) has emerged as one of the most notorious pathogens. Conventionally, it was known to cause a variety of nosocomial infections (Gosbell Iain, 2001) but since 1990s, this scenario has radically changed and MRSA has now become one of the major cause of community acquired infections accounting for >50% of Staphylococcal infections in the USA (Maria Adriana Cataldo et al., 2010). The clinical manifestations may range from simple abscesses to life threatening infections like necrotizing fasciitis, pneumonia (Maria Adriana Cataldo et al., 2010) skin and soft tissue infections (Participating Physicians and Microbiologists, 2002-2003). Due to the high resistance to most of the antibiotics by MRSA, Vancomycin is normally the

drug of choice. As Vancomycin has many side effects, it has led to interest in the alternatives for Vancomycin especially in Macrolide, Lincosamine Streptogramin- B (MLS_B) family of antibiotics (Kavitha Prabha et al., 2011).

Erythromycin (*ERY*) a macrolide and Clindamycin (*CLI*) a lincosamide represent two distinct classes of antimicrobial agents of the MLS_B family. Their mechanism of action and resistance is very similar. Both of them bind to the 50s ribosomal subunit thereby inhibiting protein synthesis (Eun- Jeong Yoon et al., 2008). The resistance to these two drugs can be mediated by *msrA* gene conferring the efflux mechanism or via the *erm* gene which encodes for the enzyme producing inducible or constitutive resistance to MLS_B (Eun- Jeong Yoon et al., 2008**;** Laclercq, 2002). The resistance is constitutive ($cMLS_B$) when R-methylase is produced and inducible ($iMLS_B$) when methylase is produced only in the presence of an inducing agent. *ERY* is a very effective inducer and *CLI* is a weak inducer (Gupta et al.,).

In vitro, *Staphylococcus aureus* isolates with constitutive resistance are resistant to both *ERY* and *CLI* whereas those with inducible resistance are resistant to *ERY* but appear to be sensitive to *CLI* ($iMLS_B$). These isolates, when used along with Clindamycin, *erm* mutants for constitutive resistance emerge, which lead to failure in treatment (Mukesh Patel et al., 2006). This resistance goes undetected by Kirby Bauer method however, it is detected by a simple D test. The result is observed as a flattening zone in the area between *ERY* and *CLI* disc, in the shape of a 'D' which indicates inducible Clindamycin resistance.

Antimicrobial sensitivity testing is important for treating infections, but inducible Clindamycin resistance test if not done, may lead to improper treatments (Gerard Lina Alain Quaglia et al., 1999).
The incidence of inducible resistance to Clindamycin may vary in different geographical regions. Since there is no substantial evidence of the Clindamycin resistance pattern in our geographical region, this study was done to know the prevalence of inducible Clindamycin resistance ($iMLS_B$) among MRSA and MSSA.

Materials and Methods

The present study was carried out in the Department of Microbiology, Mallareddy Institute of Medical Sciences, Hyderabad, Andhra Pradesh. A total 108 isolates of *Staphylococcus aureus* from pus/wound swab, sputum, throat swab, suction tip, pleural fluid and urine obtained from both out and in patients of this hospital over a period of eight months (January 2013-August 2013) were included in the study. *Staphylococcus aureus* were identified using standard microbiological culture and biochemical reactions and then subjected to antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method (Kloos WE., Banerman TL., 1999) on Mueller Hinton agar plates using Erythromycin (15 μg), Norfloxacin (5 μg), Fusidic acid (10 μg), Vancomycin (30 μg), Clindamycin (2 μg), Oxacillin (1 μg), and Cefoxitin (30 μg) as per CLSI guidelines (Gerard Lina Alain Quaglia et al., 1999). An inhibition zone of 10 mm or less around Oxacillin disc and 19 mm or less around Cefoxitin disc indicates MRSA. All Erythromycin-resistant and Clindamycin-sensitive *Staphylococcus* strains were subsequently tested by D-test for identifying inducible Clindamycin

resistance. On Mueller Hinton agar, Standard recommendations for inoculum preparation and inoculation were followed using McFarland's Indictor (Kloos and Banerman TL., 1999). *ERY* disc was placed at a distance of 15 mm (edge to edge) from *CLI* disc. Following overnight incubation at 37° C, appearance of *CLI* inhibition zone close to *ERY* disc was noted (Fiebelkorn et al., 2003). A flattening zone in the area between *ERY* and *CLI* disc, in the shape of a 'D' which indicates inducible Clindamycin resistance[2].

The results based on phenotypic variations are shown in Table 1 (Christine D. Steward et al., 2005).

Controls used

*Staphylococcus aureus (*ATCC 25923) strains.

In house strains of *Staphylococcus aureus* showing D-test positive.

Results and Discussion

Of the 108 *Staph aureus* isolates, 41 were MRSA (Fig 1) and 67 of them were MSSA(Fig 2) (Chart: 1). They were all subjected to susceptibility testing to Erythromycin (15 μg), Norfloxacin (5 μg), Fusidic acid (10 μg), Vancomycin (30 μg), Clindamycin (2 μg), Oxacillin (1 μg), and Cefoxitin (30 μg) by routine disc diffusion testing on Meuller Hinton agar. 76 (70.37%) of 108 were erythromycin resistant and the rest 32 (29.63%) were sensitive to both Erythromycin and Clindamycin (Fig 5). Of the MRSA strains, 46.34% were D test positive (Fig 3) while 26.83% were D test negative (Fig 4). 16 (35.82%) of MSSA samples were D test positive and 27 (40.3%) were negative for D test (Chart 2). The rate of both inducible and constitutive resistance was higher in MRSA samples than MSSA (Chart 3).

MRSA and MSSA among various clinical samples is shown in Table: 2. Highest number of 49(45.37%) of *Staphylococcus* isolates was from pus sample, showing inducible resistance of 14.29% in MRSA & 12.24% in MSSA (Table: 2)

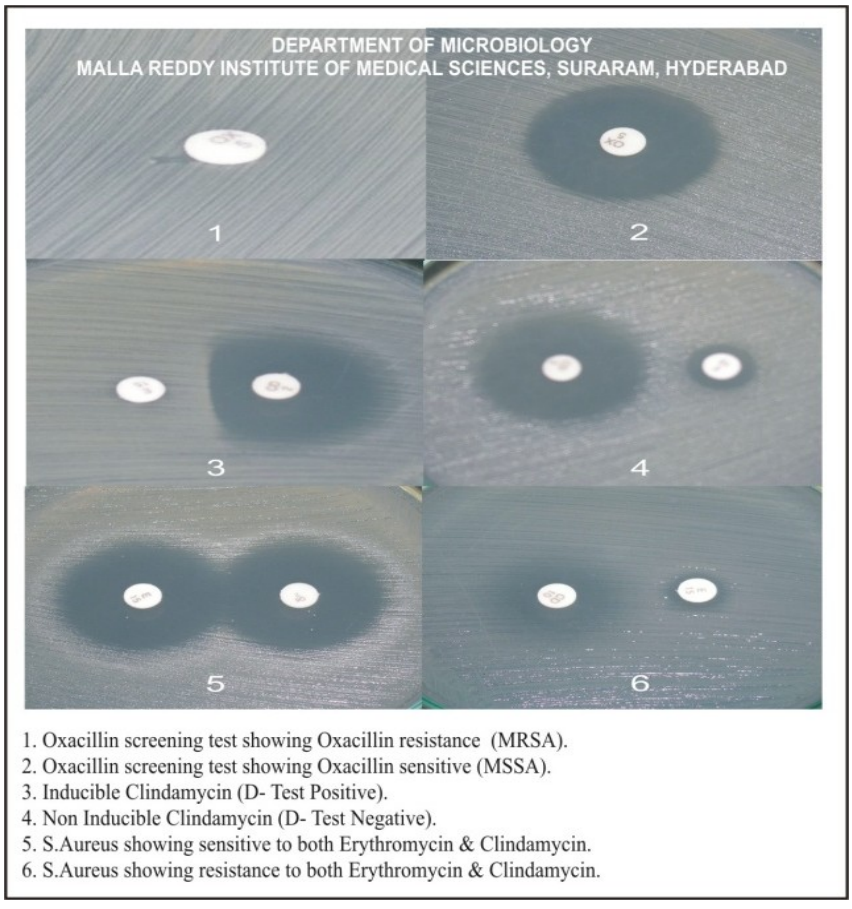
The Antibiotic Sensitivity test for any clinical isolate is often very crucial in determining the course of treatment, especially so in the multidrug resistance pathogens. Emergence of Methicillin Resistant Staphylococcus aureus has left us with very little therapeutic options to treat Staphylococcal infections. Clindamycin, which is a Lincosamine has excellent oral bioactivity making it a very good alternative to intravenous drugs. It distributes evenly throughout the body and penetrates easily into the tissues. It is orally administered and is easily metabolized and subsequently excreted in urine and bile (Anouk et al., 2010; Martinez Aguilar et al., 2003).

However, the emerging resistance to inducible Clindamycin is a concern, thereby discouraging the use of this drug. Reporting *Staphylococcus aureus* as susceptible to Clindamycin based on MS Phenotype (resistant to Erythromycin and Sensitive to Clindamycin with D test Negative) without checking for the inducible resistance (resistant to Erythromycin and sensitive to Clindamycin with D test Positive) may result in inappropriate therapy. On the other hand, negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility, thereby giving a good therapeutic option.

Table.1 Interpretation of Phenotypic Variations of *Staphylococcus aureus*

Type of phenotype	Erythromycin	Clindamycin	D test result	Character of the phenotype
Sensitive	Sensitive	Sensitive		
MS Phenotype	Resistant zone size ≤13 mm	Sensitive zone size ≥21 mm	Negative	Circular zone of inhibition around Clindamycin
Inducible Phenotype	Resistant zone size ≤13 mm	Sensitive zone size ≥21 mm	Positive	Have D Shaped zone of inhibition around clindamycin with flattening towards Erythromycin disc
Constitutive phenotype	Resistant zone size ≤13 mm	Resistant zone size ≤14 mm		

Fig 1-6: Phenotypic variations of Staphylococcus aureus



1. Oxacillin screening test showing Oxacillin resistance (MRSA).
2. Oxacillin screening test showing Oxacillin sensitive (MSSA).
3. Inducible Clindamycin (D- Test Positive).
4. Non Inducible Clindamycin (D- Test Negative).
5. S.Aureus showing sensitive to both Erythromycin & Clindamycin.
6. S.Aureus showing resistance to both Erythromycin & Clindamycin.

Chart.1 Number of MRSA and MSSA *Staphylococcus aureus*

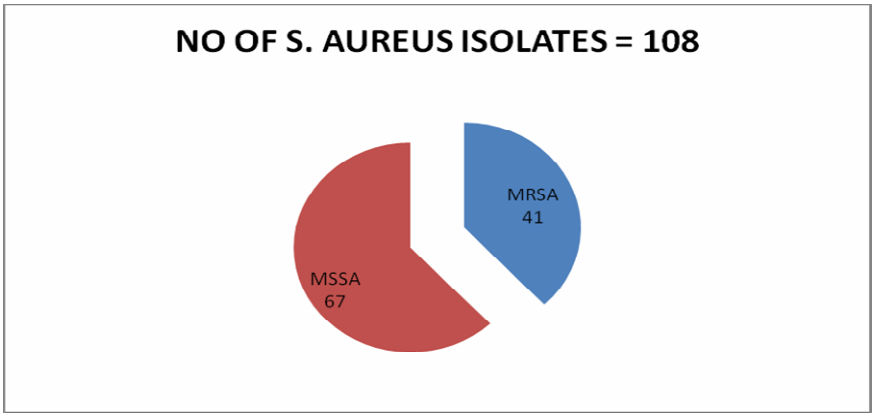


Chart.2 Phenotypic Variations among Isolates

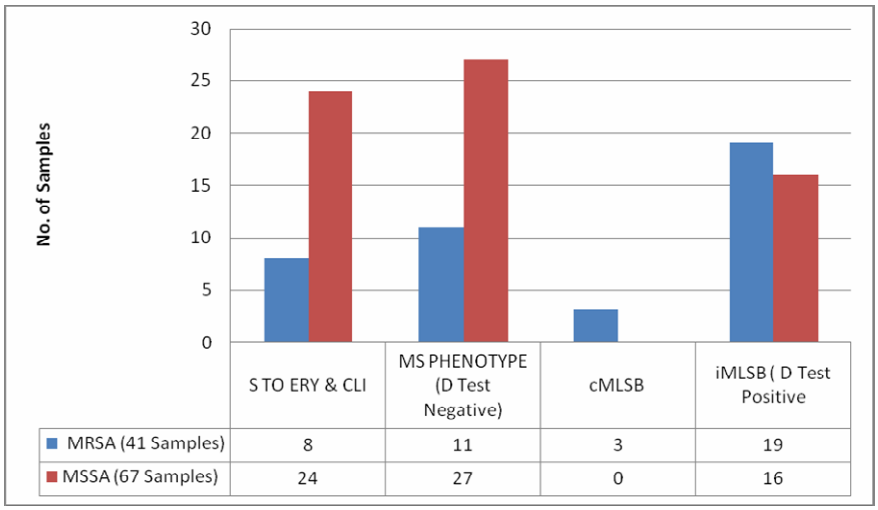


Chart.3 Inducible and Constitutive Resistance among MRSA and MSSA

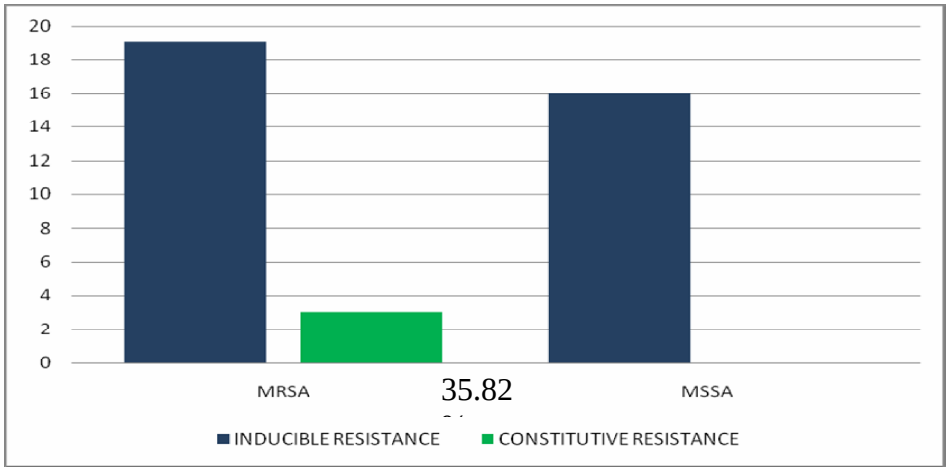


Table2 Clinical sample wise distribution of Inducible Clindamycin Resistance

Sample	Total No of Isolates	MRSA					MSSA				
		D-Test +ve (E-R Cd-S) with D-shape	D-test –ve (E-R Cd-S)	Sensitive to (E-S Cd-S)	Resistant to (E-R Cd-R)	Total MRSA isolates	D-Test +ve (E-R Cd-S) with D-shape	D-test -ve (E-R Cd-S)	Sensitive to (E-S Cd-S)	Resistant to (E-R Cd-R)	Total MSSA isolates
Pus	49 (45.37%)	7 (14.29%)	4 (8.16%)	6 (12.24%)	3 (6.12%)	**20**	6 (12.24%)	10 (20.41%)	13 (26.53%)	–	**29**
Urine	26 (24.07%)	4 (15.38%)	2 (7.69%)	1 (3.84%)	–	**7**	3 (11.53%)	8 (30.7%)	8 (30.7%)	–	**19**
Sputum	15 (13.89%)	3 (20%)	1 (6.67%)	1 (6.67%)	–	**5**	3 (20%)	5 (33.3%)	2 (3.3%)	–	**10**
Throat swab	9 (8.33%)	2 (22.22%)	2 (22.22%)	–	–	**4**	2 (22.22%)	3 (33.3%)	–	–	**5**
Suction tip	6 (5.55%)	2 (33.33%)	1 (16.67%)	–	–	**3**	1 (16.67%)	1 (16.67%)	1 (16.67%)	–	**3**
Pleural fluid	3 (2.78%)	1 (33.33%)	1 (33.33%)	–	–	**2**	1 (33.33%)		–	–	**1**
Total	***108 (100%)***	***19 (17.59%)***	***11 (10.19%)***	***8 (7.40%)***	***3 (2.78%)***	***41***	***16 (14.81%)***	***27 (25%)***	***24 (22.22%)***	**–**	***67***

Table.3 Results from other studies on Inducible Clindamycin Resistance in MRSA and MSSA

Author	Inducible Clindamycin rates in MRSA	Inducible Clindamycin rates in MSSA
Present Study	46.34%	35.8%
Manjunath et al ()	47.2%	21.67%
Ajantha et al	74%	45%
Levin TP et al	12.31%	68%
Delialiodlu et al	5.4%	10.7%
Matthew VN et al	12%	9.5%
Kavitha Prabhu et al[2]	20%	6%
Deotale et al	27.6%	1.6%
Gurdal Yilmaz et al	24.4%	14.8%

Since iMLSB is not detected by standard antibiotic sensitivity testing, it becomes imperative to perform a D test as a routine technique to confirm the sensitivity to Clindamycin.

This study observed an Inducible Clindamycin rate of 17.59% among MRSA and 14.58% among MSSA . Similar results from other studies are shown in table: (3)

It is observed in our study that the occurrence of Inducible Clindamycin resistance is more in MRSA than in MSSA in our area. This is in concurrence with studies of Manjunath et al, Ajantha et al, Matthew VN et al, Kavitha Prabhu (Table 2). In studies by Levin et al, Delialiodlu et al, the Inducible Clindamycin rate is more in MSSA.

In cases of Methicillin Resistant Staphylococcus aureus infections, where the range of drugs is limited, Clindamycin can be used as an alternative to Vancomycin, as Vancomycin has many limitations. But the Macrolide resistance by *Staph. aureus* varies with different regions. This study is the only study done in this area, it can be concluded that there is fairly a high rate of inducible Clindamycin resistance here. A simple D test can overcome the ambiguity regarding the inducible Clindamycin resistance and confirm its sensitivity, which would help clinicians for appropriate treatment.

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References

Anouk E., Muller, Johan W. Mouton, Paul M. Oostvogel, P. Joep Dörr, Rob A. Voskuyl, Joost DeJongh, Eric A. P. Steegers and Meindert Danhof, 2010. Pharmacokinetics of Clindamycin in Pregnant Women in the Peripartum Period. Antimicrob. Agents Chemother. 54(5), 2175-2181.

Christine D. Steward**,** Patti M. Raney**,** Allison K. Morrell**,** Portia P. Williams**,** Linda K. McDougal**,** Laura Jevitt**,** John E. McGowan Jr. and Fred C. Tenover**.,** 2005. Testing for Induction of Clindamycin Resistance in Erythromycin-Resistant Isolates of *Staphylococcus aureus. J Clin* Microbiol. 43(4), 1716-1721

Doolal V, Mendinatta DK., Raut U. and Narang P., 2010. Inducible Clindamycin Resistance in Staphylococcus aureus isolated from clinical samples. IJMM. 28, 124 – 126.

Eun- Jeong Yoon, Ae-Ran Kwon, Yu-Hong Min and Eung- Chil Choi, 2008. Foggy D-shaped zone of inhibition in *Staphylococcus aureus* owing to a dual character of both inducible and constitutive resistance to macrolide–lincosamide–streptogramin B. J Antimicrob Therapy. 61(3), 533-540.

Fiebelkorn KR., Crawford SA., McElmeel ML. and Jorgensen JH., 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase negative staphylococci. J Clin Microbiol. 41,4740-4744.

Gerard Lina Alain Quaglia, Marie-Elisabeth Reverdy, Roland Leclercq, Francois Vandenesch and Jerome Etienne, 1999. Distribution of genes encoding resistance to Macrolides, Lincosamines and Streptogramins

among Staphylococci Antimicrob Agents Chemother. 43(5), 1062-66.

Gosbell Iain G., 2001. Methicillin Resistant Staphylococcus aureus – Impact on dermatology practice. Am J Clin Dermatol. 45 (4), 219 – 259.

Gupta V., Datta P., Rani H. and Chander J., 2009.Inducible clindamycin resistance in *Staphylococcus aureus*: A study from North. J PG Med, 55(3), 176-179.

Gurdal Yilmaz, Kemalettin Aydin, Serap Iskender, Rahmet Caylan and Iftihar Koksal, 2007. Detection and prevalence of Inducible Clindamycin resistance in Staphylococci. J Med Microbiol. 56(3), 342-345.

Kavitha Prabha, Sunil Rao and Venkayakrishna Rao, 2011. Inducible Clindamycin Resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians. 3(1), 25-27.

Kloos WE., Banerman TL., 1999. Staphylococcus and Micrococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenoverrr FC, Yolken RH, editors. Chapter 22. Manual of Clinical Microbiology. Washington DC. ASM Press; 264-82

Laclercq R., Mechanisms of resistance to macrolides and lincosamides, 2002. Nature of resistance elements and their clinical implications. Clin Infect Dis, 34,482-92.

Maria Adriana Cataldo, Fabrizio Tagrietti and Nicola Petrosillo, 2010. Methicillin Resistant Staphylococcus aureus – A community health Threat. PG Med. 12.

Martinez Aguilar G, Hammermann WA, Manson EO and Kaplan SL, 2003. Clindamycin treatment of invasive infections caused by community acquired Methicillin resistant and methicillin susceptible Staphylococcus aureus in children. Pediatr Infect Dis J. 22, 593-598.

Mukesh Patel, Ken B. Waites, Stephen A. Moser, Gretchen A. Cloud and Craig J. Hoesley, 2006. Prevalence of Inducible Clindamycin Resistance among Community- and Hospital-Associated *Staphylococcus aureus* isolates: J Clin Microbiol. 44(7), 2481-2484.

Participating Physicians and Microbiologists., Outbreaks of community associated methicillin resistant Staphylococcus aureus skin infections : Los Angeles County, California, 2002-2003. MMWR Morb Mortal Wkly Rep. 52 (5), 88.