

Research Article**Inducible Clindamycin Resistance: A Potential Threat in Treating
Staphylococcus aureus Infection****Rajdeep Saha^{1*}, Paramita Das², Papiya Biswas³, Jaya Das⁴**¹Assistant Professor, ²⁻⁴Post graduate trainee, Department of Microbiology, Calcutta National Medical College, 32, Gorachand Road, Kolkata-14, West Bengal, India***Corresponding author**

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Abstract: Clindamycin is an aminoglycoside used in treatment of skin & soft tissue infections caused by both Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin sensitive *Staphylococcus aureus* (MSSA). *Staphylococcus aureus* becomes resistant to erythromycin through either erm or msr A genes. Strains with erm-mediated erythromycin resistance may possess inducible clindamycin resistance but appear susceptible to clindamycin by disc diffusion test. The objective was to determine the prevalence of erythromycin induced clindamycin resistance among clinical isolates of *S. aureus* in our tertiary care hospital. A total of 243 *Staphylococcus aureus* isolates from various clinical samples submitted in the dept. of Microbiology at our tertiary care hospital were studied. Inducible clindamycin resistance was detected by erythromycin and clindamycin disc approximation test (D-zone test) as per CLSI guidelines. Among the 243 *S. aureus* isolates, 73.1% and 28.7% of MRSA and MSSA respectively showed erythromycin resistance. 31.2% and 14.8% of MRSA and MSSA were found to be positive for D test. Along with conventional antibiogram routine D-zone testing for detection of clindamycin resistance can reduce hospital acquired Staphylococcal infection.

Keywords: *Staphylococcus aureus*, Clindamycin resistance, D test

INTRODUCTION

Staphylococcus aureus can produce a wide variety of diseases, from relatively benign skin infections such as folliculitis and furunculosis to deep-seated and life-threatening conditions, including cellulitis, deep abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis.

Staphylococcus aureus is a frequent cause of bacterial infections in both developed and developing countries. [1-3]. It is a highly versatile, virulent, multidrug resistant adaptable pathogen, causing wide variety of life-threatening infections like infections of skin, soft tissue, respiratory system, bone, joints and endovascular tissues [4, 5].

Clindamycin, an aminoglycoside, is used to treat localised as well as severe systemic infections caused by drug resistant *Staphylococcus aureus*. However, emergence of resistance to this drug during the course of treatment is now of major concern. This may be due to the widespread use of macrolides (eg. erythromycin, clarithromycin), lincosamides (eg. Clindamycin) and group B streptogramins (quinupristin), that have a common binding site (23S rRNA component of 50S ribosomal subunit) and thereby

leading to the development of cross resistance to these drugs.

The mechanism of resistance to MLS_B by staphylococcal strains is of three types: (a) Target site modification by erm gene resulting in rRNA methylase production that can be either constitutive (constitutive MLS_B) or inducible (iMLS_B phenotypes) where methylase is produced only in the presence of an inducer like erythromycin; (b) Resistance is by efflux of antibiotic by mrs A gene (MS phenotype) and iii) by inactivation of lincosamides by chemical alteration mediated by the inu A gene [6, 7].

When tested in vitro, constitutively expressed MLS_B phenotypes are found to be resistant to both erythromycin and clindamycin. Inducible phenotypes (iMLS_B) are resistant to erythromycin and sensitive to clindamycin in the absence of an inducer. These iMLS_B phenotypes, when tested in the presence of an inducer (erythromycin), show D shape zone of inhibition indicating clindamycin resistance. In contrast, MS phenotypes are resistant to erythromycin and sensitive to clindamycin without D zone, indicating efflux of macrolide antibiotic.

Thus, it is becoming increasingly important to identify and assess the prevalence of iMLS_B strains that may develop resistance to lincosamides during the course of treatment in our tertiary care hospital.

EXPERIMENTAL SECTION

This prospective study was conducted for a period of 9 months from July 2013 to April 2014. A total of 243 non duplicate Staphylococcal isolates were recovered from various clinical samples at the Microbiology Laboratory of our tertiary care hospital. Duplicate isolates from the same patient were not included in the study. Of 243 *S. aureus* isolates, 161 (66.2 %) were recovered from pus, 45 (18.5 %) from sputum, 18 (7.4 %) from ear swab, 9 (3.7%) from blood, 5 (2.1 %) from urine and 5 (2.1%) from synovial fluid.

Isolates were identified up to species level by conventional methods. Antimicrobial susceptibility of all isolates were performed by modified Kirby Bauer disc diffusion method on Mueller–Hinton agar plates according to Clinical and Laboratory Standards institute (CLSI) guidelines. Antibiotics tested for *Staphylococcus aureus* by disc diffusion technique were erythromycin (15 µg), clindamycin (2µg), mupirocin (5 µg), linezolid (30 µg), vancomycin (30 µg), teicoplanin (30 µg), rifampicin (5 µg), chloramphenicol (30 µg), co-trimoxazole (30 µg), ciprofloxacin (5 µg), gentamicin(30 µg), amikacin (30 µg), and coamoxyclav (20/10). The results were interpreted as per Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute 2014).

Isolates of Erythromycin resistant *Staphylococcus aureus* were further tested for inducible resistance by the ‘D test’ as per CLSI guidelines. Erythromycin (15µg) disc was placed at a distance of 12 mm (edge to edge) from clindamycin (2µg) on Mueller–Hinton agar plates previously inoculated with 0.5 McFarland bacterial suspensions. Plates were checked after 18 h of incubation at 37⁰C. Interpretation of the inhibition zone diameters was as follows:

If an isolate was erythromycin resistant and clindamycin susceptible, with a D-shaped inhibition zone around the clindamycin disc, it was considered to be positive for inducible resistance (D test positive, iMLS_B phenotype) (Fig. 1).

If the isolate was erythromycin resistant and clindamycin susceptible, with both zones of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D test negative, MS phenotype), but to have an active efflux pump (Fig. 2).

If the isolate was erythromycin resistant and clindamycin resistant, the isolate was considered to have the macrolide – lincosamide – streptogramin B constitutive (cMLS_B phenotype) (Fig. 3).

If the isolate was susceptible to both erythromycin and clindamycin, showing clear zones around both discs, the isolate was considered to be susceptible (S phenotype).

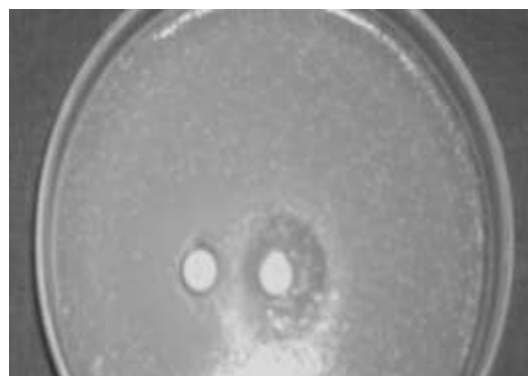


Fig. 1: Showing positive D-zone test

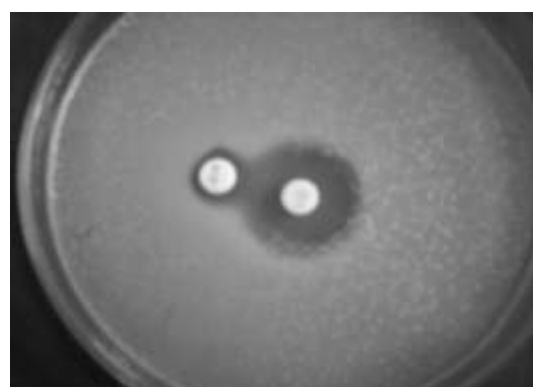


Fig. 3: Showing negative D-zone test



Fig. 3: Showing constitutive MLS_B Phenotype

The quality control of the erythromycin and clindamycin disc was performed with *S. aureus* ATCC 25923.

RESULTS AND DISCUSSION

Out of 243 Staphylococcal isolates 149 (61.3%) were found to be methicillin resistant (MRSA) and the remaining 94 (38.7%) isolates were found to be susceptible to methicillin (MSSA) (Fig. 4).

Amongst the total MRSA and MSSA isolates, 73.1% (i.e. 109 isolates) and 28.7% (i.e. 27 isolates) respectively showed resistance to erythromycin (Fig. 4).

These isolates when subjected to D zone test 45 (41.3%, n=109) isolates in case of MRSA and 9 (33.3%, n=27) isolates in case of MSSA were found to be resistant to both erythromycin and clindamycin indicating constitutive MLS_B Phenotype [MLS_B (c)] (Fig. 3). 34 (31.2%, n=109) isolates of MRSA and 4 (14.8%, n=27) isolates of MSSA showed positive D test indicating inducible MLS_B phenotype [MLS_B (i)] (Fig. 1) ; whereas 30 (27.5%, n=109) isolates of MRSA and 14 (51.9%) isolates of MSSA gave negative D test indicating MS phenotype (Fig. 2, 6). The results of this study fairly coincided with several studies from different parts of India that have reported that 30% to 64% of their MRSA strains were of the iMLSB phenotype.

Though the confirmation of the iMLSB phenotype can be done by detecting the erm gene, the D-test is an easy test to perform for the detection of the iMLSB phenotype.

CONCLUSION

The prevalence of inducible clindamycin resistance may vary from hospital to hospital. Although we did not study the prevalence of inducible clindamycin resistance in our area, from the current study, we can conclude that there is a fairly high percentage of inducible clindamycin resistance amongst the staphylococcal isolates which shows erythromycin resistance.

Use of D test in a routine laboratory will enable us in guiding the clinicians regarding judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not a suitable drug for D test positive isolates while it can definitely prove to be a drug of choice in case of D test negative isolates.

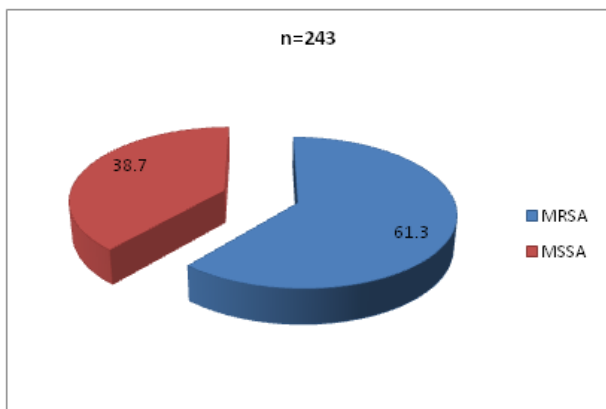


Fig. 4: Distribution of *Staphylococcus aureus* according to their methicillin resistance (n= total no. of isolates)

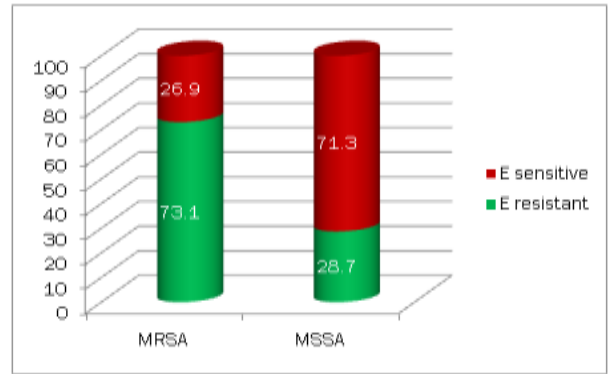


Fig. 5: Distribution of MRSA & MSSA according to erythromycin sensitivity (in percentage)

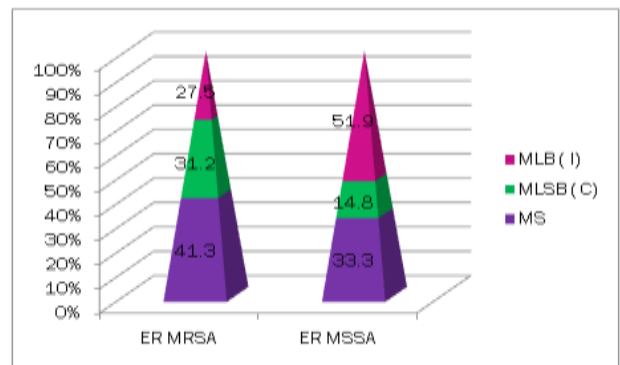


Fig. 6: Percentage of different phenotypes of Erythromycin resistant MRSA & MSSA

REFERENCES

1. Deleo FR, Otto M, Kreiswirth BN, Chambers HF; Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet, 152010; 375 (9725): 1557-68.
2. Kennedy AD, Deleo FR; Epidemiology and virulence of community-associated MRSA. Clinical Microbiology Newsletter, 2009; 31(20): 153-160.
3. Rehm SJ; *Staphylococcus aureus*: the new adventures of a legendary pathogen. Cleve Clin J Med., 2008; 75(3): 177-180, 183-186, 190-192.
4. Cohen, ML; Epidemiology of drug resistance: implications for a post-antimicrobial era. Science, 1992; 257(5073): 1050-1055
5. Tomasz A; Multiple-antibiotic-resistant pathogenic bacteria. A report on the Rockefeller University workshop. N Engl J Med., 1994; 330:1247-1251.
6. Martínez-Aguilar G1, Hammerman WA, Mason EO Jr., Kaplan SL; Clindamycin treatment of invasive infections caused by community-acquired, methicillin resistant and methicillin-susceptible *Staphylococcus aureus* in children. Pediatr Infect Dis J., 2003; 22(7): 593-598.
7. Frank AL1, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC *et al.*; Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J., 2002; 21(6): 530-534.