

PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATES RESISTANT TO ERYTHROMYCIN

Syed Asim M¹, Prajna Sharma², Anita K. B³, Swathi V⁴, Narendra Nayak⁵

¹Senior Resident, Department of Microbiology, University College of Medical Sciences, New Delhi.

²Assistant Professor, Department of Microbiology, A. J. Institute of Medical Sciences, Kuntikan, Mangalore.

³Professor, Department of Microbiology, A. J. Institute of Medical Sciences, Kuntikan, Mangalore.

⁴Specialist, Department of Microbiology, Sulaiman Al Habib Hospital, Buraydah, Al Qassim, Saudi Arabia.

⁵Professor and HOD, Department of Microbiology, A. J. Institute of Medical Sciences, Kuntikan, Mangalore.

ABSTRACT

BACKGROUND

Clindamycin is an effective drug to treat Methicillin-Resistant Staphylococcus Aureus (MRSA). Reporting S. aureus as susceptible to clindamycin without checking for inducible clindamycin resistance may lead to therapeutic failure. Therefore, D-test is used to screen inducible clindamycin resistance.

MATERIALS AND METHODS

All the S. aureus isolates resistant to erythromycin were taken. Erythromycin (15 µg) disc and clindamycin (2 µg) disc were placed 15 mm apart on Mueller-Hinton agar plates as per CLSI guidelines and incubated at 37°C for 18-24 hours. Flattening of zone (D shape) around clindamycin was taken as D-test positive.

RESULTS

Out of 270 S. aureus isolates, 80 were resistant to erythromycin. D-test was positive in 29 isolates, out of which 23 were MRSA. These MRSA isolates were also resistant to most of the other routinely used antibiotics. This study showed that inducible clindamycin resistance is as high as 36.2% in erythromycin resistant S. aureus and 10.7% among S. aureus as such.

CONCLUSION

We conclude that this simple and effective method can be implemented for accurate identification of inducible clindamycin resistance in S. aureus to prevent treatment failure. Clinical laboratories should guide the clinicians about the inducible clindamycin resistance by performing D-test routinely and prevent misuse of antibiotics.

KEYWORDS

D-test, Methicillin-Resistant Staphylococcus Aureus (MRSA), Clindamycin, Erythromycin, Inducible MLSB Phenotype.

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BACKGROUND

Staphylococcus aureus (S. aureus) is one of the most common pyogenic bacteria infecting man. It is the most important bacteria causing nosocomial infections, abscesses and other pyogenic infection, endocarditis, pneumonia and various other infections. S. aureus promptly acquires antimicrobial resistance after the introduction of new antibiotics. Erythromycin (a macrolide) and clindamycin (a lincosamide) represent two distinct classes of antimicrobial agents that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells. In staphylococci, resistance to both of these antimicrobial agents can occur

through methylation of their ribosomal target site.¹ Such resistance is typically mediated by erm genes. Resistance to macrolides also can occur by efflux, typically mediated by the msrA gene.² Another resistance mechanism, inactivation of lincosamides by chemical modification (such as mediated by the inuA gene) appears to be rare.³ Macrolide-Lincosamide-Streptogramin B (MSLB) resistance, a target site modification resistance results in resistance to erythromycin, clindamycin and streptogramin B. This mechanism can be constitutive where the rRNA methylase is always produced or can be inducible where methylase is produced only in the presence of an inducing agent. Clindamycin is a weak inducer, but erythromycin is an effective inducer. In vitro, Staphylococcus aureus isolates with constitutive resistance are resistant to erythromycin and clindamycin and isolates with inducible resistance are resistant to erythromycin, but appear susceptible to clindamycin. In vivo, therapy with clindamycin may select for constitutive erm mutants, which may lead to clinical failure.⁴ Isolates with msrA-mediated efflux also appear erythromycin resistant and clindamycin susceptible by in

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Corresponding Author:
Dr. Syed Asim M,
University College of Medical Sciences,
Dilshad Garden, New Delhi.
E-mail: drprajnaasharma@gmail.com
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vitro tests; however, such isolates do not typically become clindamycin resistant during therapy. An in vitro induction test can distinguish staphylococci that have inducible erm-mediated resistance from those with msrA mediated resistance (D-test). For erythromycin-resistant isolates, induction tests can help laboratories determine whether results for clindamycin should be reported as sensitive (if D test is negative) or as resistant (if D test is positive). In this study, we reassessed the reliability of simply placing erythromycin and clindamycin disks in adjacent positions in a standard disk diffusion method and access the inducible clindamycin resistance.

The iMLSB (inducible clindamycin) type of resistance is not recognised using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests, the VITEK system, etc.^{5,6} Further reports on inducible clindamycin resistance are scanty in India. Therefore, this study was undertaken to determine the incidence of MLSB resistance in the clinical isolates of *S. aureus* and to study the antibiotic sensitivity pattern of *S. aureus* isolates having the iMLSB phenotype in our hospital.

MATERIALS AND METHODS

This was a prospective study conducted at A.J. Institute of Medical Sciences, Mangalore, for a period of 6 months from January 2015 to June 2015. A total of 302 staphylococcus isolates were collected from various clinical samples (pus, wound swab, blood, e.t.c.). Staphylococci were identified upto species level by conventional methods like Gram stain, characteristic growth on nutrient agar, slide and tube coagulase test, mannitol fermentation test, DNase test as per CLSI guidelines.⁷

Among 302, staphylococcus isolates, 270 were *S. aureus* and 32 were Coagulase-Negative Staphylococcus (CoNS). Erythromycin resistance was seen among 91 isolates, out of which 80 were *S. aureus* and 11 were CoNS. All erythromycin-resistant isolates were further subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method and also for inducible clindamycin resistance as described below.

Antibiotic Susceptibility Testing

The erythromycin resistant isolates were subjected to susceptibility testing by Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates using erythromycin (15 µg) and clindamycin (2 µg). The test is performed by disk diffusion placing a 15 µg erythromycin disk in proximity to a 2 µg clindamycin disk (around 15 mm) on an agar plate that has been inoculated with a staphylococcal 0.5 McFarland bacterial suspension isolate and then incubated overnight at 37°C for 18-24 hours.^{7,8} Sensitivity was also tested with other routinely used antibiotics like linezolid (30 µg), chloramphenicol (30 µg), amikacin (30 µg), vancomycin (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), gentamicin (30 µg) and tetracycline (30 µg). The results were interpreted as per Clinical Laboratory Standards Institute guidelines.⁷

D-Test

Isolates that were erythromycin resistant were tested for inducible resistance by the 'D test' as per CLSI guidelines as described above.⁷ A flattening of the zone of inhibition around the clindamycin disk proximal to the erythromycin disk (zone of inhibition shaped like the letter D) is considered a positive result and indicates that the erythromycin has induced clindamycin resistance (a positive "D-zone test").

- Isolates, which were clindamycin susceptible and erythromycin resistant with a D-shaped inhibition zone around the clindamycin disc were considered to be inducible MLSB phenotypes.
- Isolates showing both erythromycin and clindamycin resistance was considered constitutive MLSB phenotypes (D test negative).

Tests showing erythromycin resistance and clindamycin sensitive were considered as MS phenotypes.

	Erythromycin	Clindamycin	D test
MS phenotype	R	S	-
Inducible MLSB phenotype	R	S	+
Constitutive MLSB phenotype	R	R	-

Table 1. Erythromycin Resistance Types

RESULTS

Among 302 staphylococcus isolates, 270 were *S. aureus* and 32 were CoNS. Erythromycin resistance was seen among 91 isolates out of which 80 were *S. aureus* and 11 were coagulase-negative staphylococcus species (Figure 1).

Among the 80 erythromycin resistant, *S. aureus* 68 were MRSA and 12 were MSSA (Figure 2). Inducible clindamycin resistance (D-test +ve) was seen in 23 isolates of MRSA and 6 isolates of MSSA, therefore, a total of 29 isolates were D-test positive in *S. aureus*. These MRSA isolates also showed resistance to most other routinely used antibiotics. Our study showed the highest percentage of MRSA occurrence in patients with the age group of 20-30 years.

The percentage of inducible clindamycin resistance (D-test) was 10.24% (n=29) among all Staphylococcus aureus isolates (n=270), but D-test was much more prevalent in erythromycin-resistant Staphylococcus aureus, i.e. 36.2% (n=80) isolates. The susceptibility of iMLSB phenotypes isolated were amikacin 90.2%, gentamicin 70%, ciprofloxacin 50%, chloramphenicol (30 µg) 55.2%, co-trimoxazole 33.3% and tetracycline 56.2%, but all were 100% sensitive to vancomycin and linezolid.

Among the 32 CoNS, 5 of them showed inducible clindamycin resistance, which again signified their importance in testing for inducible clindamycin resistance. It can also be noted that maximum number of inducible clindamycin resistance (D-test +ve) among *S. aureus* isolates was seen with MRSA isolates (23 out of 34) compared to MSSA isolates (6 out of 34) Figure 3.

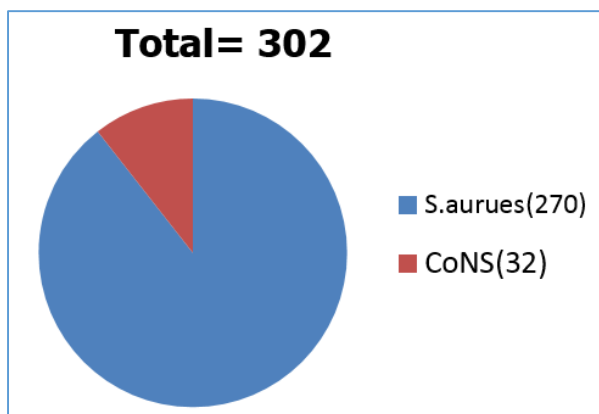


Figure 1. Isolates in the Study Group (n=302)

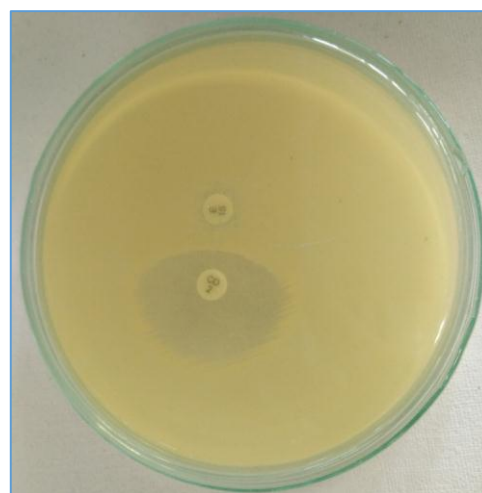


Figure 4. D-Test Positive Pattern

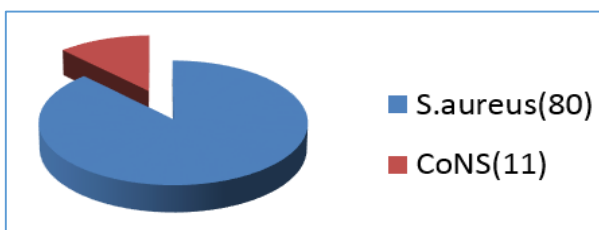


Figure 2. Distribution of Erythromycin Resistance in the Study Group (n=91)

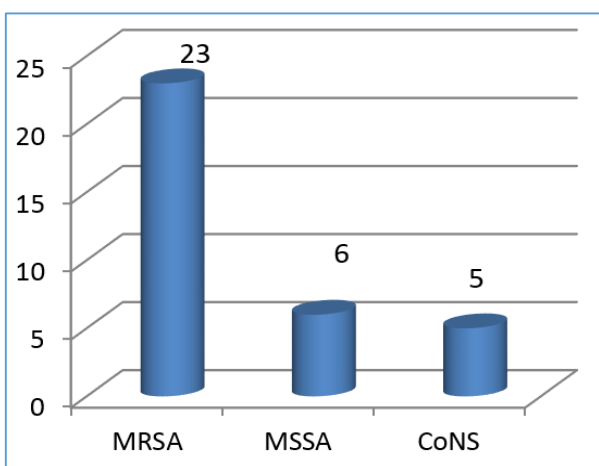


Figure 3. Inducible Clindamycin Resistance among MRSA, MSSA, CoNS (n=34)

	Total	MRSA	MSSA	CoNS
MS phenotype	56.1%	44%	5.5%	6.6%
Inducible MLSB phenotype	37.3%	25.5%	6.6%	5.5%
Constitutive MLSB phenotype	6.6%	5.5%	1.2%	Nil

Table 2. Erythromycin Resistant Isolates (n=91)

Sl. No.	Antibiotics	Percentage of Susceptibility
1.	Amikacin (30 µg)	90.2%
2.	Chloramphenicol (30 µg)	55.2%
3.	Co-trimoxazole (25 µg)	33%
4.	Ciprofloxacin (5 µg)	50%
5.	Gentamicin (30 µg)	70%
6.	Linezolid (30 µg)	100%
7.	Tetracycline (30 µg)	50%
8.	Vancomycin (30 µg)	100%

Table 3. Antibiotic Susceptibility Pattern of S. Aureus

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 frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration and the fact that it accumulates in abscesses and no renal dosing adjustments are needed.¹⁰ Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. Clindamycin is a good alternative for the treatment of both methicillin-susceptible and methicillin-resistant Staphylococcal infections.

In 1969, McGehee and colleagues demonstrated the development of clindamycin resistance in vivo and in vitro in erythromycin-resistant staphylococci.¹¹ Other investigators have confirmed the rapid in vitro conversion of inducible to constitutive MLSB resistance in staphylococci.¹² There have also been a number of reported clindamycin or lincomycin therapy failures in serious infections due to staphylococci with inducible MLSB resistance indicating that it is not uncommon. This has led to questioning the safety of clindamycin use against any erythromycin-resistant staphylococci. Because of the high reported incidence of inducible MLSB resistance, particularly in S. aureus, it has been suggested that in vitro erythromycin resistance could serve as a surrogate for all MLS agents regardless of susceptibility test results and that disk induction testing be performed on isolates from serious CNS infections.¹³

The incidence of iMLSB resistance varies significantly by geographical region. In our study, the percentage of inducible clindamycin resistance among erythromycin-resistant strains was more in MSSA strains (50%) compared to MRSA (33.8%). In a study done by Sasirekha B et al in Bangalore showed inducible-clindamycin resistance in 9.15% isolates of S. aureus and 22.4% in erythromycin-resistant S. aureus.¹⁴ A similar study done by Schreckenberger et al⁶ and Levin et al¹⁵ showed higher percentage of inducible resistance in MSSA (20%) as compared to MRSA (12%), 12.5% MRSA and 68% MSSA, respectively. The very high rates of methicillin resistance

among *S. aureus* isolates have been noted in developed countries especially in Western Pacific regions both in community acquired and nosocomial infections. In West Asia, MRSA prevalence ranged from 12 to 49.4% in six different hospitals of Saudi Arabia.¹⁶ In European countries, MRSA rates varied from 0.6% in Sweden to 40.2-45% in Belgium, Greece, Ireland, Italy, UK and Israel.¹⁷ In our study, methicillin-resistance *S. aureus* was found to be 24.45% (n=302). Similar prevalence rate of MRSA was obtained from other workers in India-26.9% by Shittu and Lin (2006)¹⁸ and 26.6% Mehta et al (2007),¹⁹ although lesser and higher percentage have been published. The differences in the prevalence of MRSA among different countries and between different regions in a country could be due to difference in the study design, population and geographical distribution and the variation is probably due to differential clonal expansion and drug pressure in community. Further, it emphasises the importance of local surveillance in generating relevant local resistance data that can guide empiric therapy. In our study, there was no isolate with reduced susceptibility to glycopeptides and all isolates were found susceptible to vancomycin and linezolid.

For appropriate therapy decisions, accurate susceptibility data are important. In staphylococci, in vitro susceptibility testing for clindamycin may indicate false susceptibility by the disk diffusion testing with erythromycin and clindamycin disks in nonadjacent positions and broth microdilution method. However, if inducible resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in those patients with true clindamycin-susceptible isolates. In this study, we have described a simple, reliable and effective method to detect inducible resistance to clindamycin in erythromycin-resistant isolates of *S. aureus* and CoNS.

REFERENCES

- [1] Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother* 1995;39(3):577-585.
- [2] Ross JI, Eady EA, Cove JH, et al. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport supergene family. *Mol Microbiol* 1990;4(7):1207-1214.
- [3] Brisson-Noël A, Delrieu P, Samain D, et al. Inactivation of lincosaminide antibiotics in staphylococcus. Identification of lincosaminide O-nucleotidyltransferases and comparison of the corresponding resistance genes. *J Biol Chem* 1988;263(31):15880-15887.
- [4] Drinkovic D, Fuller ER, Shore KP, et al. Clindamycin treatment of staphylococcus aureus expressing inducible clindamycin resistance. *J Antimicrob Chemother* 2001;48(2):315-316.
- [5] Fiebelkorn KR, Crawford SA, McElmeel ML, et al. Practical disc diffusion method for detection of inducible clindamycin resistance in staphylococcus aureus and coagulase-negative staphylococci. *J Clin Microbiol* 2003;41(10):4740-4744.
- [6] Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible resistance in staphylococcus aureus and coagulase-negative staphylococci in a community and a tertiary care hospital. *J Clin Microbiol* 2004;42(6):2777-2779.
- [7] Performance standards for antimicrobial disk susceptibility tests: approves standard M2-A8. 8th edn. Wayne, PA: CLSI 2003.
- [8] Gadepalli R, Dhawan B, Mohanty S, et al. Inducible clindamycin resistance in clinical isolates of staphylococcus aureus. *Indian J Med Res* 2006;123(4):571-573.
- [9] Frank AL, Marcinak JF, Mangat PD, et al. Clindamycin treatment of methicillin resistant staphylococcus aureus infections in children. *Pediatr Infect Dis J* 2002;21(6):530-534.
- [10] Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. *Mayo Clin Proc* 1999;74(8):825-833.
- [11] McGehee RF, Barrett FF, Finland M. Resistance of staphylococcus aureus to lincomycin, clindamycin and erythromycin. *Antimicrob Agents Chemother* 1968;8:392-397.
- [12] Panagea S, Perry JD, Gould FK. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother* 1999;44(4):581-582.
- [13] Sanchez ML, Flint KK, Jones RN. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. *Diagn Microbiol Infect Dis* 1993;16(3):205-213.
- [14] Sasirekha B, Usha MS, Amruta JA, et al. Incidence of constitutive and inducible clindamycin resistance among hospital-associated staphylococcus aureus, Springerlink.com .1007/s13205-013-0133-5.
- [15] Levin TP, Suh B, Axelrod P, et al. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant staphylococcus aureus: report of a clinical failure. *Antimicrob Agents Chemother* 2005;49(3):1222-1224.
- [16] Hussain W, Ikram A, Butt T, et al. In vitro susceptibility pattern of staphylococcus aureus clinical isolates at Rawalpindi. *Pak J Pathol* 2008;19(1):12-15.
- [17] Blandino G, Marchese A, Ardito F, et al. Antimicrobial susceptibility profiles of pseudomonas aeruginosa and staphylococcus aureus isolated in Italy from patients with hospital-acquired infections. *Int J Antimicrob Agents* 2004;24(5):515-518.
- [18] Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of staphylococcus aureus in KwaZulu-Natal province, south Africa. *BMC Infect Dis* 2006;6:125.
- [19] Mehta M, Dutta P, Gupta V. Bacterial isolates from burn wound infections and their antibiograms: a eight-year study. *Indian J Plast Surg* 2007;40(1):25-28.