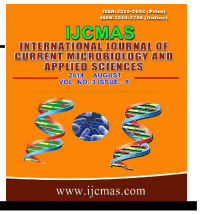
Int.J.Curr.Microbiol.App.Sci (2014) 3(9) 689-694

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 689-694**
http://www.ijcmas.com



Original Research Article

Detection of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care hospital

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Keywords

Staphylococcus, Constitutive clindamycin resistance, Inducible clindamycin resistance, MS Phenotype, D-test.

A B S T R A C T

The resistance to different antimicrobial agents among staphylococci is an increasing problem. Clindamycin (CL) is considered to be one of the alternative drug in these infections. Inducible clindamycin resistance cannot be detected by the routine conventional antimicrobial susceptibility test. This study demonstrates a simple, reliable method (double-disc diffusion test) for detecting inducible resistance to clindamycin in erythromycin-resistance (ER-R) isolates of *S. aureus*. Therefore, this study was undertaken to detect inducible clindamycin resistance among isolates of *Staphylococcus aureus.* Aims: To detect the prevalence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in our hospital by Disk approximation test (D-test). Materials and Methods: - A total 450 isolates of *Staphylococci aureus* from various clinical samples were used in this study. Results: - A total 135 (30%) isolates were resistant to Erythromycin. Out of these 84 isolates were MRSA and 51 were MSSA. Constitutive clindamycin resistance (cMLSB) was 03.55%, Inducible clindamycin resistance (iMLSB) was 11.11% and MS Phenotype was 15.33% in all isolates of *Staphylococci aureus*. Conclusion: - In conclusion the D – test is an easy, simple, test to perform along with routine susceptibility testing therefore D-test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

Introduction

Staphylococci aureus is causes variety of pyogenic infections in man, ranging from skin and soft tissue infections to life threatening endocardities. The emergence of resistance to antimicrobial agent among staphylococci is an increasing problem.

Clindamycin is commonly used for the treatment of serious soft tissue infection produced by *Staphylococci aureus* due to its excellent pharmakinetic properties(2).

Increasing frequency of MRSA infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide lincosamides – streptogramin B (MLSB) antibiotics to treat staphylococcal infection. Clindamycin is

also used as an alternative for patients who are allergic to penicillin. Clindamycin is an alternative choice for MRSA infections especially in penicillin-allergic patients. However, subinhibitory concentration of erythromycin is a common inducer of inducible clindamycin resistance (ICR) (6). Wide spread use of MLSB antibiotics in serious Staphylococcal infections results in emergence of increased number of strains acquiring resistance to MLSB antibiotics (13).

The expression of MLSB phenotype can be inducible (iMLSB) or constitutive (cMLSB). Inducible resistance is seen when the inactive mRNA produced by the production of enzyme methylase becomes active in the presence of an inducer, while active methylase mRNA is produced in strains where constitutive expression is seen (9).

Materials and Methods

A total 450 isolates of *Staphylococci aureus* from various clinical samples were used in this study at our institute Bharati Vidyapeeth university medical college and hospital, Sangli. The isolates were identified using conventional methods like Colony morphology, Gram staining, Catalase test, tube coagulate and slide coagulase test, mannitol fermentation and DNase test. (3) Isolates were initially screened for routine antibiotics sensitivity test by Kirby-Bauer disc diffusion method for various antibiotics.(8) MRSA was detected by using Cefoxitine (30ug) and Oxacillin(1ug) antibiotics disc as per CLSI guidelines on Muller Hinton agar supplemented with 2% Nacl followed by incubation at 35^{o}c. (4). Initially isolates were screened for Erythromycin resistance. The isolates that were found to be Erythromycin resistant were further studied for inducible and constitutive Clindamycin resistance by D – test. (6) To detect inducible clindamycin resistance, there is a specific disk diffusion method that shows resistance is induced by erythromycin. In this method an erythromycin disk is placed next to clindamycin disk. When erythromycin diffuses, induces resistance to clindamycin and results in flattening of the clindamycin zone of inhibition just next to the erythromycin disk, making a D shape, so this method is called D-test. The ideal inter-disk distance between the antibiotics is yet not clear and Clinical and Laboratory Standards Institute (CLSI) recommends a range of 15 to 26 mm disk separation.
Three different phenotypes were used for interpretation like as following manner.

a] iMLSB phenotype – Demonstration of flattened Clindamycin zone (D – shaped) between Erythromycin and Clindamycin disc showed inducible Clindamycine resistance, (iMLSB Phenotype).

b] cMLSB phenotype – Growth up to Clindamycin and Erythromycin discs indicates resistance to both Erythromycin and Clindamycin (cMLSB phenotype).

c] MS phenotype – Isolates sensitive to Clindamycin and resistant to Erythromycin but circular zone of inhibition around Clindamycin (D – test negative) was labeled as MS phenotype. Figure No. 1, 2, and 3 show cMLSB, iMLSB and MS phenotype

Results and Discussion

Total 450 isolates of *Staphylococcus aureus* were obtained from different clinical samples like, pus, wound swab, urine, blood, body fluids. MRSA was documented in 166 (36.88%) and MSSA in 284 (63.11%). A total 135 (30%) isolates were resistant to Erythromycin. Out of these 84 isolates were MRSA and 51 were MSSA. (Table No 1)

In MRSA isolates, 41 (24.69%) isolated showed iMLSB, 16 (9.63%) isolates resistant to both Erythromycin & Clindamycin, and 27 (16.245) isolates were showed MS phenotype.

In the case of MSSA, out of these 51 isolates, 9 (3.16%) were showed D – test positive (iMLSB) and 42 (14.78%) showed MS phenotype. The overall percentage of resistance to all three phenotype is shown in Table no – 2. This study was in concordance with a few of studies reported by Deotales, (5) Jadhav savita (7). Various studies from different regions show different prevalence of inducible Clindamycin resistance among MRSA and MSSA. (tableNo.3)

Accurate drug susceptibility data of the infecting microbe is an essential step in making appropriate therapeutic decisions. The initial step before starting the antimicrobial therapy of infected individuals is performing the antimicrobial susceptibility testing for clinical isolates to avoid indiscriminate usage of antibiotics on trial and error basis. Determination of resistance to MLSB antibiotics will be beneficial in selecting the appropriate treatment for Staphylococcal infections. Commonest antibiotic being preferred while treatment of these staphylococcal infections in case of failure to beta-lactam antibiotics is clindamycin. The clinical failure of Clindamycin therapy has been reported before. (15)

Resistance to macrolide, lincosamides, streptogramin B (MLSB) antibiotics, most commonly results from acquisition of Erythromycin resistant methylase genes (erm gene) which encode enzymes that methylate the 23 sr RNA.

Resistance to MLSB can be inducible or constitutive. Macrolide – induced Clindamycin resistance was observed among the clinical isolates of *Staphylococci aureus* since 1968 which could not be detected by the routine disc diffusion method from such isolates constitutive resistant mutants are emerged and results in treatment failure with Clindamycin in vivo which would be demonstrated on D – test cross ref of (11). However Clindamycin resistance can develop in Staphylococcal isolates with inducible phenotype and from such isolates, spontaneous constitutively resistant mutants have arisen during Clindamycin therapy. (16)

In our study we found 30% Erythromycin resistant isolates correlate with Indian study. (5) Amongst these 57 (42%) D test positive, 16 (11.85%) were constitutive and 45.92% were showed MS phenotype.

It was also observed that percentage of inducible resistance and MS phenotypes were higher amongst MRSA 28.91% and 16.26% respectively as compared to MSSA it is 3.16 and 14.78 respectively. Other studies also reported 22.6% in MRSA and 4% in MSSA (5) (1). On the contrary, Schreckenberger et al (14) and Levin et al (10) showed a higher percentage of inducible resistance in MSSA compared to MRSA, 7-12% in MRSA and 19-20% in MSSA; 12.5% MRSA and 68% MSSA, respectively.

In our observation constitutive resistance was 3.55% in all isolates. This is contrary to the one Indian study by Angel et al (2) which did not observe this in any of the isolates.

Table No.1 Distribution of Erythromycin resistant *S. aureus*

Staphylococcus aureus	Number of isolates(n=450)	Erythromycin resistant (n= 135)
MRSA	166	84 (50.60%)
MSSA	284	51 (17.95%)

Table No.2 Phenotypic pattern of inducible Clindamycin resistance among MRSA and MSSA

Phenotype	**MRSA (n=84)**	**MSSA (n=51)**	**Total (n = 135)**
Ery- R, CL - R (cMLSB).	16 (9.63%)	0%	03.55%
Ery- R, CL - S D test +ve (iMLSB)	41 (24.69%)	09 (03.16%)	11.11%
Ery- R, CL - S D test negative (MS Phenotype)	27 (16.26%)	42 (14.76%)	15.33%

Ery- Erythromycin, CL Clindamycin, R- resistant, S- Sensitive

Table No.3 Various reports of the prevalence of inducible Clindamycin resistance among MRSA and MSSA

Name of investigator	Year	MRSA	MSSA
Yiilmaz G et al [16]	2007	24.40%	14.80%
Rahabar M et al [12]	2007	22.60%	04.00%
Ajantha GS et al[1]	2008	74.00%	45.00%
Ciraj AM et al[3]	2009	38.00%	12.90%
Deotale V et al[5]	2010	27.60%	01.60%
Jadhav Savita et al [7]	2011	24.82%	01.66%
In our study	2012	24.69%	03.16%

Figure.1 Constitutive clindamycin resistance

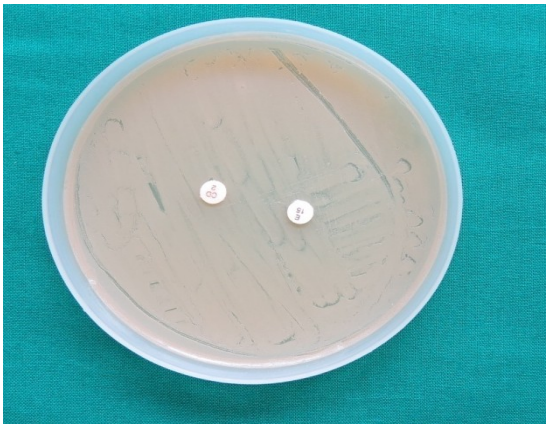


Figure.2 Inducible clindamycin resistance

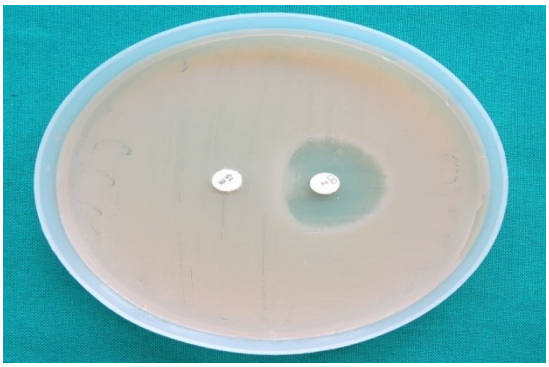
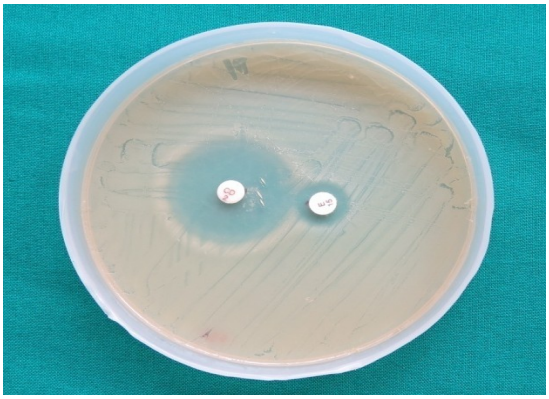


Figure.3 MS Phenotype



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