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Testing for Induction of Clindamycin Resistance in Erythromycin-Resistant Isolates of *Staphylococcus aureus in* a Tertiary Care Hospital

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A B S T R A C T

The increasing resistance to macrolide, lincosamide, streptogramin B (MLSB) agents among *Staphylococcus aureus* is becoming a challenge to microbiologist. Clindamycin has been a useful drug for treatment of infection caused by the staphylococcus aureus, but change in clindamycin sensitivity pattern due to various mechanisms is leading to therapeutic failure. One of the important mechanisms is mediation of resistance by erm genes. Staphylococcus strains which have erm genes show inducible clindamycin resistance that cannot be determined by routine disk diffusion test resulting in treatment failure. Resistance may be constitutive (cMLSB phenotype) or inducible (iMLSB phenotype).The iMLSB phenotypes are distinguished by erythromycin-clindamycin disk approximation test. A total of 142 clinically significant *Staphylococcus aureus* isolated from pus, urine, blood, fluid, sputum, ear swabs, endotrachealtube, ophthalmic , and umbilical discharge.These isolates were initially identified by colony morphology, Gram staining, catalase test, slide coagulase test, tube coagulase test and mannitol fermentation. The isolates were subjected to routine antibiotic sensitivity testing including cefoxitin by Kirby Bauer disk diffusion test. Inducible clindamycin resistance was detected by double disk approximation test (D-test)as per CLSI guidelines on erythromycin resistant isolates.For detection of inducible clindamycin resistance, D test using erythromycin and clindamycin as per CLSI guidelines was performed, and three different phenotypes were interpreted as methicillin-sensitive (MS) phenotype (D test negative), inducible MLSB (iMLSB) phenotype (D test positive), and constitutive MLSB phenotype. Of the 142 isolates, 50 were identified as methicillin resistant *S. aureus*, while 92 were methicillin sensitive *S. aureus*. The rates of inducible clindamycin resistance in methicillin resistant *S. aureus* (MRSA), methicillin sensitive*S. aureus* (MSSA) were 36% and 2.2%, respectively. The inducible clindamycin resistance was significantly more among MRSA compared to methicillin sensitive *S. aureus* (MSSA) (P value < 0.0001). Overall the rate of inducible clindamycin resistance is 14.1%,constitutive clindamycin resistance 2.8% and MS phenotype is 6.3%. Majority of the MRSA isolates were susceptible to clindamycin, vancomycin and linezolid, while most of them were resistant to erythromycin,gentamicin,ciprofloxacin,tetracycline and sulfamethoxazole-trimethoprim. Clindamycin is the drug of choice in many staphylococcal, streptococcal and anaerobic infections. The D-test is easy to perform and inexpensive to know clindamycin sensitivity. We feel that this test should be made mandatory as a routine work in clinical microbiology laboratories. Therapeutic failures can be prevented if we don't use clindamycin for treatment of patients with infections caused by staphylococci with inducible clindamycin resistance.

Introduction

Staphylococcus aureus and *coagulase-negative Staphylococci* (CoNS) are important causes of nosocomial and community-acquired infections. Treatment of these infections is a growing problem because of the increasing methicillin resistance among *Staphylococci* spp. Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives available to treat Staphylococcal infections. The macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics serves as an alternative, with clindamycin being the preferred agents due to its excellent pharmacokinetic properties. However widespread use of MLSB antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLSB antibiotics.

Although erythromycin and clindamycin are in separate antimicrobial agent classes, macrolides and lincosamides, respectively, their mechanisms of action (inhibition of protein synthesis) and mechanisms of resistance are similar. The cross-resistance for 3 antibiotic families (macrolides e.g., erythromycin, clarithromycin, azithromycin; lincosamides e.g., clindamycin; and group B streptogrammins e.g., quinupristin) that share a common binding site is called as the MLS_B phenotype. The two main mechanisms of resistance are production of methylase enzyme encoded by a multiallele plasmid-borne gene erm that alters the ribosomal binding site of the antimicrobial agents and efflux pumps. In Staphylococci, the MLS_B resistance can be either constitutive ($cMLS_B$) or inducible ($iMLS_B$). If it is constitutive, in vitro susceptibility tests will show resistance to all 3 antibiotic classes, while if it is inducible, in vitro tests will show resistance to macrolides, but susceptibility to clindamycin will be retained, unless induced by a macrolide (i.e. erythromycin). Isolates that are erythromycin resistant but clindamycin susceptible may either possess inducible clindamycin resistance ($iMLS_B$) or have efflux pumps that remove macrolides but not clindamycin from the microbe.

It is important to determine if resistance (whether inducible or constitutive) to clindamycin exists when it is being considered for therapy. Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if Staphylococci are not tested for inducible CL resistance by the disk diffusion induction test (D-test).This study demonstrates a very simple method of detecting inducible resistance to clindamycin in erythromycin resistant Staphylococcal isolates i.e D test .

The main objectives of this study to speciate *Staphylococcus aureus* isolates from various clinical samples and also to detect inducible resistance to clindamycin in erythromycin resistant staphylococcal isolates.

Materials and Methods

The study was carried out in the Department of Microbiology, Dr. B.R.A.M.C, K.G.Halli, Bengaluru for a period of 9 months from October 2014 to June 2015.A total of 142 clinically significant *Staphylococcus aureus* isolated from pus, urine, blood, fluid, sputum, ear swabs, endotracheal tube ophthalmic and umbilical discharge. Identification of staphylococcal isolates was done based on colony morphology on 5% sheep blood agar, Gram stain and catalase test. Coagulase test by the plasma tube method and sugar fermentation tests were done to distinguish between *S. aureus* and *coagulase negative Staphylococci*. The isolates were subjected to susceptibility

testing by Kirby Bauer disc diffusion method on Mueller Hinton agar plates using erythromycin, (15 µg), clindamycin (2 µg), penicillin (10 IU), ciprofloxacin (5 µg), gentamicin (10 µg), cefoxitin (30 µg), vancomycin (30 µg) and linezolid (30 µg) as per Clinical Laboratory Standards Institute (CLSI) guidelines (7). Methicillin resistance was detected by using a 30 µgcefoxitin disc. Staphylococcus ATCC 25923 was used as the control strain for the disc diffusion method.

D-test

Those isolates which were erythromycin resistant were subjected to 'D test' as per CLSI guidelines. A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate. The test was performed with erythromycin (15 µg) disc placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc, followed by overnight incubation at 37^{o}C. Three different phenotypes were interpreted as follows.

1. cMLS$_B$ phenotype – isolates showing resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin.

2. iMLS$_B$ phenotype – isolates showing resistance to erythromycin (zone size≤ 13mm), while being sensitive to clindamycin (zone size ≥21mm) with a D shaped zone of inhibition around clindamycin with flattening towards it.

3. MS phenotype-isolates showing resistance to erythromycin (zone size≤ 13mm)while being sensitive to clindamycin(zone size ≥21mm)with a circular zone of inhibition around clindamycin.

Results and Discussion

A total of 142 staphylococcus aureus isolates were included, out of which 50 were MRSA and 92 were MSSA.

Categorisation of the isolates along with sources is depicted in table 1.

These isolates when subjected to D test showed 4 isolates resistant to both erythromycin and clindamycin indicating constitutive MLSB phenotype, 138 isolates showed clindamycin sensitivity. Out of these, 20 isolates showed positive D test indicating inducible MLSB phenotype while 9 gave negative D test indicating MS phenotype.

The overall percentage resistance for all three phenotypes was as follows

Inducible clindamycin resistance-14.1% (figure 1)

Constitutive clindamycin resistance-2.8% (figure 2)

MS Phenotype-6.3%

Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA as shown in table 2 below.

The treatment options for the isolates which were iMLS $_B$ showed all these 20 isolates to be 100% sensitive to vancomycin, teicoplanin, tigecycline and linezolid, moderately sensitive to gentamicin and amoxycillin/clavulanic acid whereas sensitivity was least to cotrimoxazole and ciprofloxacin as shown in table below.

Table.1. Sources and Categorization of *Staphylococcus aureus* Isolates

SPECIMEN	MRSA	MSSA	TOTAL
PUS	40(80)	63(68.5)	103(72.5)
EAR SWAB	4(8)	3(3.3)	7(4.9)
ET TUBE	2(4)	1(1.1)	3(2.1)
SPUTUM	2(4)	2(2.2)	4(2.8)
CORNEAL SCRAPING	0(0)	1(1.1)	1(0.7)
WOUND SWAB	1(2)	15(16.3)	16(11.3)
THROAT SWAB	1(2)	4(4.3)	5(3.5)
URINE	0(0)	1(1.1)	1(0.7)
HIGH VAGINAL SWAB	0(0)	1(1.1)	1(0.7)
UMBILICAL DISCHARGE	0(0)	1(1.1)	1(0.7)
TOTAL	50(35.2)	92(64.78)	142

Table.2 Distribution of Isolates

PHENOTYPE	MRSA(%)	MSSA(%)	TOTAL
ERY-S,CL-S	22(44)	87(94.5)	109(76.8)
ERY-R,CL-R	4(8)	-	4(2.8)
ERY-R,CL-S,D TEST POSITIVE	18(36)	2(2.2)	20(14.1)
ERY-R,CL-S,D TEST NEGATIVE	6(12)	3 (3.2)	9(6.3)
TOTAL	50(35.2)	92(64.8)	142

Table.3 Percentage Antimicrobial Resistancein D Test +ve and –ve Isolates

Antibiotics	D test negative	D test positive
Penicillin/ampicillin	92	96
Doxycycline	62	43
Gentamicin	60	40
Amikacin	40	50
Amoxicillin clavulanic acid	49	55
Ciprofloxacin/levofloxacin	83	89
Cotrimoxazole	77	85
Piperaciilin/tazobactam	40%	60%
Tigecycline	0	0
Vancomycin	0	0
Teiocoplanin	0	0
Linezolid	0	0

Figure.1 D-Test Showing Inducible Clindamycin Resistance

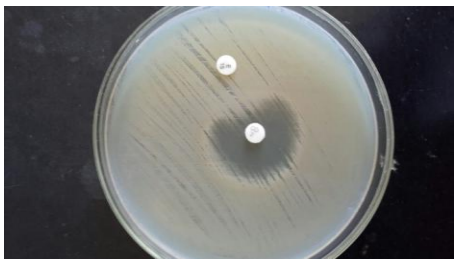
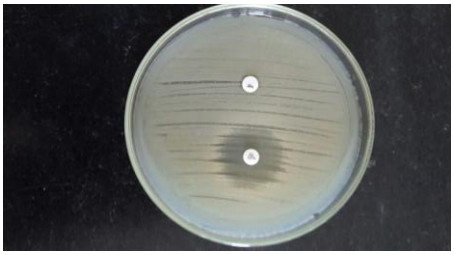


Figure.2 Test Showing Constitutive Clindamycin Resistance



For any clinical microbiology laboratory, the differentiation of *erm*-mediated inducible MLS $_B$ (iMLS $_B$ phenotype) isolates from isolates with *msrA*-mediated (MS phenotype) resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant *Staphylococcus aureus* isolate. In recent times, clindamycin has become an excellent drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients. Also, clindamycin has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics. Since the iMLS $_B$ resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of *Staphylococcus aureus.* Failure to identify iMLS $_B$ resistance may lead to clinical failure of clindamycin therapy. Conversely, labeling all erythromycin-resistant *Staphylococci* as clindamycin-resistant prevents the use of clindamycin in infections caused by truly clindamycin-sensitive Staphylococcal isolates. Hence, Clinical and Laboratory Standards Institute (CLSI) recommends routine testing of all Staphylococcal isolates for iMLS$_B$. In our study ,142 isolates were obtained over a period of nine months(2014-2015) majority were obtained from pus(72.5%)followed bywound swab (11.3%),ear swab(4.9) , throat swab (3.5), sputum(2.8), urine (0.7), corneal scraping(0.7)etc. This is similar to study conducted by Kanwal Deep Singh Lyall *et al*., in which out 0f the 593 *S. aureus* isolates, majority were obtained from pus (31.1%) followed by blood and body fluids (27.3%); central line/neck line/ umbilical catheter, etc. (20.2%); urine (12.6%); and respiratory samples(8.7%). In our study we found high percentage of erythromycin-resistant *S.aureus* isolates (23.2) which is similar to study conducted by Kavithaprabhu *et al*., who showed (28.42%) isolates erythromycin resistant. Among the 142 *Staphylococcus aureus* strains, we found 50 (35.2%) to be MRSA and 92(64.8) to be MSSA, which is lower than that reported by Vineeta Mittal *et al*., in

which out of 260 isolates 105 were MRSA and 155(59.61) were MSSA. In our study, 18(36%)of MRSA strains showed iMLSB phenotype 2(2.2%) of MSSA strains showed iMLSB phenotype, which is similar to study conducted by Gadepalli *et al*., in which 30%MRSA strains were of iMLSB phenotype and 10% MSSA phenotype of iMLSB phenotype. Study conducted by Yilmaz *et al*., also showed inducible resistance of 24.4% in MRSA and 14.8% in MSSA. Similar study conducted by Mohd Rahabar *et al*., showed inducible resistance of 22.6% in MRSA and 10% in MSSA. On contrary studies, conducted by Schrecken berger *et al*., showed inducible resistance higher in MRSA than MSSA(7-12% AND 19-20% respectively). In the present study the constitutive clindamycin resistance was present in(8%) of MRSA and no strains of MSSA isolates, which is similar to studies conducted by Deotale *et al*., who got7.3%of MRSA isolates of constitutive resistance, which is contrary to the only study from India, by ANGEL *et al*., which did not find it any of the strains The low constitutive clindamycin resistance in our study may also be attributed to the fact that drug is not commonly used and hence there is less selection of resistant strains. The drugs which are recommended for treatment of MRSA-associated infections are vancomycin, linezolid, co-trimoxazole, tetracycline, rifampicin in combination with co-trimoxazole or tetracycline and clindamycin. Resistance against co-trimoxazole, tetracycline and rifampicin has also increased these days.In our study, majority of the MRSA isolates were susceptible to vancomycin and linezolid, while most of them were resistant to erythromycin, gentamicin, ciprofloxacin, tetracycline and sulfamethoxazole-trimethoprim, similar to the study by Gupta V *et al.*, in north India.

Clindamycin is the drug of choice in many staphylococcal, streptococcal and anaerobic infections. The D-test is easy to perform and inexpensive to know clindamycin sensitivity. We feel that this test should be made mandatory as a routine work in clinical microbiology laboratories. Therapeutic failures can be prevented if we don't use clindamycin for treatment of patients with infections caused by staphylococci with inducible clindamycin resistance.

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