Original article

Inducible clindamycin resistance among *Staphylococcus aureus* isolates

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Abstract:

Introduction: Clindamycin is considered an useful alternate drug in penicillin-allergic patients in the treatment of skin & soft tissue infections caused by *Staphylococcus aureus*. *Staphylococcus spp*. can be resistant to erythromycin through either *erm* or *msr* A genes. Strains with *erm*-mediated erythromycin resistance may possess inducible clindamycin resistance but may appear susceptible to clindamycin by disc diffusion test. The objective of the present study was to know the prevalence of erythromycin-induced clindamycin resistance among clinical isolates of *S. aureus.*

Methods: A total of 250 *S. aureus* isolates from various clinical samples submitted in the Dept. of Microbiology at our tertiary care hospital were studied. Methicillin resistant *S. aureus* strains were identified by Cefoxitin disc diffusion method. Inducible clindamycin resistance was detected by erythromycin and clindamycin disc approximation test (D-zone test) as per CLSI guidelines.

Result: Among the 250 *S. aureus* isolates, 107 strains (42.8%) were detected as MRSA of which 26(24.3%) strains showed inducible clindamycin resistance (D-test positive). Only 7(5.0%) isolates of MSSA were D-test positive. 156(62.4%) isolates of *S. aureus* were sensitive to both erythromycin & clindamycin.

Conclusion: High prevalence of strains with inducible clindamycin resistance particularly among MRSA indicates that inducible clindamycin resistance testing (D-test) should be included as a part of routine antibiotic susceptibility. These isolates may be missed in routine antibiotic testing by disk diffusion method.

Keywords: Clindamycin resistance, MRSA, D test

Introduction

Staphylococcus aureus is one of the most common organisms causing nosocomial and community-acquired infections worldwide. Antibiotic resistance in this organism has become an ever-increasing problem. In *Staphylococcus*, penicillin resistance was recognized first in 1944 and methicillin resistance was recognized first in 1961.[1] Emergence of methicillin-resistant *S. aureus*- (MRSA) has left us with very few therapeutic alternatives to treat staphylococcal infections. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative with clindamycin being the preferred agent in MLS_B group for treating both methicillin-susceptible *S. aureus* (MSSA) and MRSA infections, due to its excellent pharmacokinetic properties.[2] The MLS antibiotics are structurally unrelated but are related microbiologically because of their similar modes of action. They inhibit protein synthesis by binding to the 23S r RNA2.[3]

Clindamycin resistance in *Staphy-lococcus species* can be either constitutive or inducible.[4]The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive in vitro when not placed adjacent to each other. In

such cases, in vivo therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through msrA genes i.e. efflux of antibiotic, staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro and the strain do not typically become clindamycin resistant during therapy.[5]

It is very important that the clinical microbiologists and the infectious disease experts keep a close watch on the developing patterns of drug resistance, which will help in guiding the therapy effectively. [6] The Clinical Laboratory Standards Institute (CLSI) [7] has recommended the erythromycin - clindamycin disc approximation test (D-zone test) to detect the inducible clindamycin resistance. This study was therefore aimed to find out the percentage of *S. aureus* isolates having inducible clindamycin resistance ($iMLS_B$) in our geographic area using D-test. Also, we tried to ascertain the relationship between MRSA and inducible clindamycin resistance.

Material and Methods

The study was conducted from April 2011 to February 2012 in the Department of Microbiology at our tertiary care hospital in Nagpur, Maharashtra. A total of 250 *S. aureus* strains were isolated from various clinical specimens like pus, wound swabs, aspirates, blood, and sterile fluids. Only one isolate per patient was included in the study. All the isolates were tested for their susceptibility to penicillin (10 units), gentamicin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), erythromycin (15 µg), ciprofloxacin (5 µg), pristinamycin (15 µg), vancomycin (30 µg) & linezolid (30µg) by Kirby Bauer disc diffusion method using criteria of standard zone of inhibition. Methicillin resistance was detected by cefoxitin disk diffusion method using a 30 µg disk (Hi-media laboratories Pvt. Ltd., Mumbai).

D-zone test: The erythromycin and clindamycin disc approximation test (D-test) was performed as per CLSI 2011 guidelines. The clindamycin (2µg) discs were placed at a distance of 15mm (edge to edge) from the erythromycin (15 µg) discs on the same plate and were incubated at 37°C overnight. A flattening of the zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance. S. aureus ATCC 25923 was used as control. Three different phenotypes were identified.
a) The Inducible MLS_B phenotype: Isolates which were resistant to erythromycin (zone of inhibition ≤ 13mm) and sensitive to clindamycin (zone of inhibition ≥ 21mm) with a D-shaped zone of inhibition around the clindamycin disc. [Fig. 1]
b) The Constitutive MLS_B phenotype : Isolates which were resistant to erythromycin (≤13mm) and susceptible to clindamycin (≥21mm) with circular zone of inhibition around clindamycin. [Fig. 2]

Results

Among the 250 *S. aureus* strains studied, 33 (13.2%) strains were D-test positive i.e. of the inducible MLS_B ($iMLS_B$) phenotype as compared to the 31 (12.4%) constitutive MLS_B ($cMLS_B$) phenotypic strains. (Table I) High percentage of erythromycin resistance (37.6%) was noted among *S. aureus* strains.

Out of 33 $iMLS_B$ phenotype S. *aureus* strains, 26 (78.7%) strains were isolated from pus, followed by 4 (12.1%) strains which were isolated from blood.

In our study, 107 strains (42.8%) were detected as MRSA of which 26 (24.3%) strains showed inducible clindamycin resistance. Percentage of both inducible and constitutive resistance was

found to be higher amongst MRSA isolates as compared to MSSA ($p<0.001$). (Table I)

All the *S. aureus* strains were sensitive to vancomycin and linezolid. Four *S. aureus* isolates which showed constitutive clindamycin resistance were also showed resistance to pristinamycin. All four isolates were MRSA. (Table II)

Discussion

Clindamycin, a lincosamide, is one of the most efficient antibiotics in treating staphylococcal skin and soft tissue infections, including osteomyelitis because of its excellent tissue penetration except in CNS.[8] It accumulates in abscesses and no dosage requirements are needed in the presence of renal disease. It also directly inhibits the staphylococcal toxin production and is a useful alternative for patients who are allergic to penicillin.[9] Good oral absorption makes this drug an important option in outpatient therapy or as a follow-up after intravenous therapy.

However, clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and such isolates, can undergo a rapid in vitro and in vivo conversion to a constitutive resistance phenotype.[10] Reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option. [11] Therefore accurate susceptibility data are important for appropriate therapy decisions. This is where the D-test becomes significant.

In present study, When *S. aureus* isolates were subjected to D-zone test, it was found that 33 (13.2%) isolates showed inducible clindamycin resistance ($iMLS_B$ phenotype) and 31 (12.4%) showed constitutive resistance ($cMLS_B$ phenol-type).A study from MGIMS, Sevagram reported that 14.5% strains were of iMLSB phenotype and 3.6% were of cMLSB phenotype.[11]Another study from Bangalore reported that 24.9% of their *S. aureus* strains were of iMLSB phenotype and 18.3 % were of cMLSB phenotype.[12]

There have been various reports on the pattern of the MLS_B resistance among the staphylococci; some reports indicate a high prevalence of the $iMLS_B$ phenotype, while the others indicate an increasing frequency of the $cMLS_B$ phenotype. The true incidence depends on the patient population studied, the geographical region, the hospital characteristics and methicillin susceptibility.[8]

In this study, it was found that both the inducible and constitutive clindamycin resistance were seen in significantly higher proportion among MRSA as compared to MSSA isolates ($p<0.001$). Studies from different parts of India have reported 30% to 64% of the MRSA isolates to be of the $iMLS_B$ phenotype. [12] In the present study, 26 (24.3%) of the 107 MRSA isolates were found to be of $iMLS_B$ phenotype which correlates well with the findings of Deotale et al who reported 27.6 % $iMLS_B$ resistance in the MRSA isolates.[11] On the contrary, Schreckenberger et al [13] and Levin et al [14] reported higher percentage of inducible resistance in MSSA as compared to MRSA isolates, 7-12% in MRSA and 19-20% in MSSA; 12.5% MRSA and 68% MSSA respectively.

Constitutive clindamycin resistance in our study was seen in 7.0% of MRSA isolates, which is contrary to the study from CMC, Vellore which did not find it in any of the strains.[15] 3.7% MRSA isolates which were constitutively resistant to clindamycin ($cMLS_B$ phenotype) also showed resistance to pristinamycin in our study.

In this study, 17.8% MRSA belonged to MS phenotype as compared to 7.7% MSSA. Similar findings were made by Deotale et al [11]who

reported 24.3% & 4.0% MS phenotype among MRSA and MSSA respectively. Gadepalli et al reported 12.0% strains of the MS phenotype among the MRSA and MSSA each.

In present study, 42.8% of the total isolates of the *S. aureus* were MRSA. Other studies have also shown such a high prevalence of MRSA from various parts of the country ranging from 31% to 44%. [16, 17] Lack of awareness, the indiscriminate and improper use of antibiotics before coming to the hospital might be the contributory factors for such a high prevalence of MRSA. Even though there are recent reports of the increase in emergence of vancomycin resistance of *S.aureus* worldwide.[18, 19] In our study, none of the *S.aureus* isolates were resistant to vancomycin. Linezolid also showed excellent activity against *S.aureus* isolates.

Conclusion

As clindamycin is one of the most commonly used antibiotics for MRSA isolates, the increasing clindamycin resistance in the form of $iMLS_B$ and $cMLS_B$ limits the therapeutic options for MRSA to the antibiotics like linezolid and vancomycin.

The inducible clindamycin resistance can be easily missed by routine in vitro susceptibility tests, when the erythromycin and the clindamycin discs are placed in non adjacent positions. In view of the therapeutic implications, the D test is a simple, reliable and inexpensive test to perform along with routine susceptibility testing which delineates the inducible ($iMLS_B$) and the constitutive ($cMLS_B$) resistance.

The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible clindamycin resistance is helpful in guiding anti-staphylococcal therapy. Use of D test in a routine laboratory will enable us in guiding the clinicians regarding the judicious use of clindamycin.

Table I : Comparison of different types of MLS_B resistance among *S. aureus* on D-zone test

PHENOTYPE (SUSCEPTIBILITY PATTERN)	**MRSA (%) (N=107)**	**MSSA (%) (N=143)**	**TOTAL (%) (N=250)**
Inducible Clindamycin resistance (ER-R, CL-S, D test + ve)	26 (24.3%)	07 (4.9%)	33 (13.2%)
Constitutive Clindamycin resistance (ER-R, CL-R)	21 (19.6%)	10 (7.0%)	31 (12.4%)
MS Phenotype (ER-R, CL-S, D test –ve)	19 (17.8%)	11 (7.7%)	30 (12.0%)
Susceptible to Erythromycin & Clindamycin (ER-S, CL-S)	41 (38.3%)	115 (80.4%)	156 (62.4%)

Table II : Comparison of antibiotic resistance pattern among MRSA and MSSA isolates

Antibiotics	MSSA (n=143)	MRSA (n=107)
	Resistant	Resistant
	Number (%)	Number (%)
Penicillin	116 (81.1%)	107 (100%)
Cotrimoxazole	84 (58.7%)	96 (89.7%)
Tetracycline	17 (11.9%)	45 (42.1%)
Gentamicin	05 (3.5%)	61 (57.0%)
Ciprofloxacin	45 (31.5%)	99 (92.5%)
Erythromycin	28 (19.6%)	66 (61.7%)
Clindamycin	17 (11.9%)	47 (43.9%)
Pristinamycin	0	04 (3.7%)
Vancomycin	0	0
Linezolid	0	0

Figure 1 legend: Inducible MLS_B phenotype

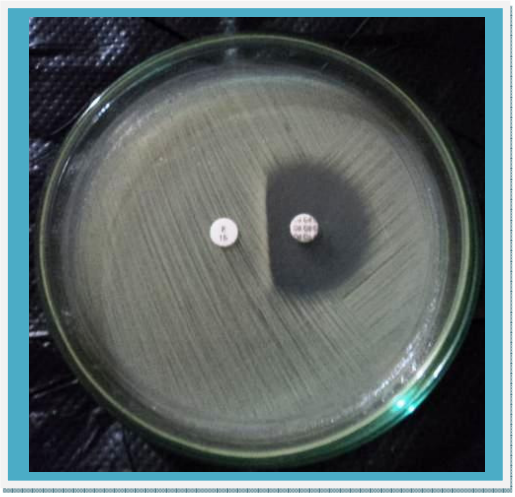
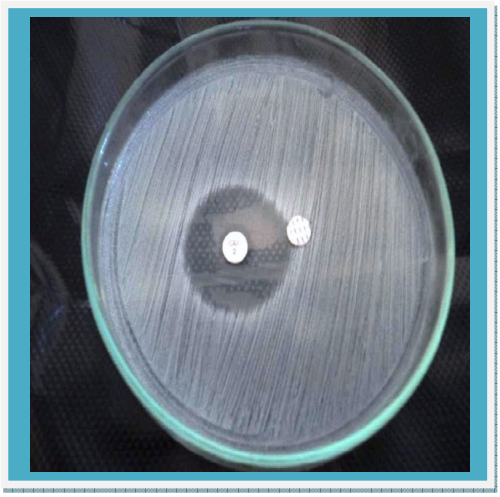


Figure 2 legend: MS phenotype



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