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ORIGINAL ARTICLE

Inducible Clindamycin Resistance in Staphylococcus Aureus-A Therapeutic Challenge

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ABSTRACT

In modern medical practice, multidrug resistant Staphylococcus aureus isolates have limited therapeutic options. Clindamycin is a useful drug in treating Staphylococcal infection. This study was undertaken to determine the prevalence of inducible clindamycin resistance in clinical isolates of Staphylococcus aureus.

Inducible clindamycin resistance was tested by the clindamycin disc induction test D-zone test) as recommended by the Clinical and Laboratory Standards Institute (CLSI) previously known as NCCLS), 2004 guidelines.

18.6% of Staphylococcus aureus isolates were positive for inducible clindamycin resistance and belonged to the $iMLS_B$ phenotype. All $iMLS_B$ phenotypes (100%) were sensitive to vancomycin and linezolid, Moreover, all $iMLS_B$ phenotypes were methicillin resistant Staphylococcus aureus (MRSA).

We conclude that the clindamycin disc induction test (D-zone test) is easy, which should be performed routinely by all clinical microbiology laboratories to guide the clinicians about the $iMLS_B$ phenotype of Staphylococcus aureus to prevent misuse of antibiotics.

Key Words: Staphylococcus aureus, Inducible clindamycin resistance, D-zone test.

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Introduction

Antibiotic resistance in Staphylococcus aureus has become an ever-increasing problem. In Staphylococcus, penicillin resistance was recognized first in 1944 and methicillin resistance was recognized first in 1961 [1]. In 1997, the intermediate susceptibility to vancomycin (VISA), heteroVISA and vancomycin resistant Staphylococcus aureus (VRSA) had been reported [2].

The increasing frequency of Methicillin resistant Staphylococcus aureus (MRSA) infections and the changing patterns of antibiotic resistance have led to renewed interest in macrolides, lincosamides and streptogramin$_B$ (MLS_B) antibiotics, especially in patients who are allergic to penicillin.

Clindamycin, a semisynthetic derivative of lincomycin has excellent tissue penetration (except for the central nervous system), rapid oral absorption, no requirement of dosage adjustment in the presence of renal disease and it is one of the most efficient antibiotics in treating Staphylococcal skin and soft tissue infections, including osteomyelitis.

The chemical structures of macrolides, lincosamides and streptogramin $_B$ are very different, but their mechanism of action is identical [3]. All 3 antibiotics block protein synthesis by inhibiting peptidyltransferase. Bacteria develop cross resistance quite often to MLS_B due to overlapping binding sites in 23SrRNA. Three types of MLS_B resistance can be observed- i) Constitutive (c MLS_B), ii) Inducible ($iMLS_B$) and iii) MS_B Phenotype.

Most commonly, the resistance to MLS_B antibiotics occurs from the acquisition of the *erm* genes which encode enzymes that methylate 23SrRNA [3].

In constitutive MLS_B resistance, active methylase mRNA is produced in absence of an inducer and the strains show a high level of cross-resistance to MLS_B drugs. In inducible MLS_B resistance, the bacteria produce inactive mRNA which is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. Bacterial strains having an inducible *erm* gene are resistant to the inducer but appear to be susceptible to clindamycin by the disc diffusion method, thereby confusing Microbiologists. On getting the report as 'Clindamycin sensitive', clinicians have only two options, either to avoid prescribing a useful drug such as clindamycin or to lead to a therapeutic failure by using it. Inducible clindamycin resistance is not detected by standard broth microdilution testing, automated susceptibility testing devices, the standard disc diffusion test or the E-test (AB Biodisk) [4].

The issues of the detection and the reporting of inducible MLS_B in Staphylococci have been addressed in 2004 at the Clinical and Laboratory Standards Institute (CLSI) guidelines, formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for antibiotic susceptibility testing [5]. The CLSI has described the test methods that can routinely detect inducible clindamycin resistance.

In the MS_B phenotype, a fully operational efflux pump that has specificity for 14- and 15-membered macrolides and streptogramin $_B$, is responsible for MS_B resistance. The efflux system involves the *msr*(A) and chromosomal genes to constitute the operational efflux pump[6]. Clindamycin is neither an inducer nor a substrate for the pump and the strains are fully sensitive to clindamycin.

Aims and Objectives

The present study was undertaken

- To determine the prevalence of inducible clindamycin resistance in the clinical isolates of Staphylococcus aureus.
- To study the antibiotic sensitivity pattern of Staphylococcus aureus strains having the $iMLS_B$ phenotype.

Material and Methods

This study was conducted for a period of 10 months from September 2007 to June 2008. A total number of 366 Staphylococcus aureus strains were isolated and identified from clinical specimens such as pus, blood, body fluids, drain fluids, sputum, throat swab etc. following standard procedures [7] in the department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (M), Wardha.

Antibiotic susceptibility tests were performed by the Kirby-Bauer disc diffusion

method [8]. Methicillin resistance was detected, based on CLSI recommendations, using a 1µg oxacillin disc[9] Staphylococcus aureus ATCC 25923 was used as control strain for the disc diffusion method.

D-zone test: The Erythromycin and Clindamycin double disc susceptibility test (D-zone test) was performed as per NCCLS guideline 2004 [5]. A disc containing erythromycin (15 µg) was placed 15mm from centre to centre of a clindamycin (2 µg) disc. Inducible resistance to clindamycin is manifested by flattening or blunting of the clindamycin zone of inhibition adjacent to the erythromycin disc, giving a D-shape to the zone of inhibited growth. [Table/Fig 1]

(Table/Fig 1) D-Zone test: D-shaped blunting of a circular zone of inhibition around Clindamycin (cd) disc on the side facing Erythromycin (E) disc.

Strains resistant to both erythromycin and clindamycin were defined as showing a constitutive MLS_B ($cMLS_B$) phenotype. Strains that were resistant to erythromycin and sensitive to clindamycin (no induction) were defined as showing the MS_B phenotype [10].

Results

Among the 366 Staphylococcus aureus strains studied, 68 (18.6%) strains were D zone positive i.e. of the inducible MLS_B ($iMLS_B$) phenotype, which were resistant to erythromycin and sensitive to clindamycin by routine antibiotic sensitivity tests.

Out of 68 $iMLS_B$ phenotype Staphylococcus aureus strains, 48 (70.6%) strains were isolated from pus, followed by 9 (13.2%) strains which were isolated from blood culture [Table/Fig 2]. All 68 (100%) $iMLS_B$ phenotype Staphylococcus aureus strains were sensitive to vancomycin and linezolid and 23 (33%) strains were sensitive to gatifloxacin [Table/Fig 3].

Specimens	Inducible MLS_B S. aureus strains	
	Numbers	Percentages
Pus	**48**	**70.6**
Blood	**9**	**13.2**
Tip of I.V. canula	**5**	**7.3**
Others (tissue materials, body fluids, drain fluids)	**6**	**8.8**

(Table / Fig 2) Isolation of inducible MLS_B ($i MLS_B$) Staphylococcus aureus strains from different clinical specimens (n= 68)

Antibiotics	Susceptible strains	
	number	percentage
Linezolid	68	100
Vancomycin	68	100
Penicillin	0	0
Ciprofloxacin	15	22
Amikacin	21	30
Gattifloxacin	23	33

(Table / Fig 3) Antibiotic susceptibility profile of inducible MLS_B Staphylococcus aureus strains (n= 68)

In our study, the inducible MLS_B ($iMSL_B$) phenotype of Staphylococcus aureus strains were 68 (18.6%) as compared to the 14 (3.8%) constitutive MLS_B ($cMLS_B$) phenotypic strains and the 3 (0.8%) MS_B phenotypic strains[Table/Fig 4]. Out of 366 Staphylococcus aureus strains, 189 (51.6%) strains were Methicillin resistant Staphylococcus aureus (MRSA) and 177 (48.3%) strains were Methicillin sensitive Staphylococcus aureus. (MSSA) [Table/Fig 5]. Out of 177 MSSA strains, 2(1.1%) were of the $cMLS_B$ phenotype. In the present study, out of 189 MRSA strains, 80 (42.3%) were clindamycin resistant and 109 (57.7%) were clindamycin sensitive [Table/Fig 6].

	Inducible MLS_B Phenotype	**Constitutive MLS_B Phenotype**	**MS_B Phenotype**
Number of strains	**68**	**14**	**3**
Percentage	**18.6**	**3.8**	**0.8**

(Table/Fig 4) Comparison of different types of MLS_B resistance in Staphylococcus aureus isolates (n=366)

Total S. aureus strains	MRSA		MSSA	
	Numbers	Percentage	Numbers	Percentage
366	**189**	**51.6**	**177**	**48.3**

(Table / Fig 5)Prevalence of Methecillin resistant Staphylococcus aureus (MRSA) and Methecillin sensitive Staphylococcus aureus (MSSA) strains

Total MRSA strains	Clindamycin Resistant			Clindamycin Sensitive	
	$iMLS_B$	$cMLS_B$	Total	MS_B	Total
189	68 (36%)	12 (6.3%)	80 (42.3%)	3 (1.58%)	109 (57.7%)

(Table / Fig 6) Prevalence of Clindamycin resistant and Clindamycin sensitive strains amongst MRSA

Discussion

In our study, 68 (18.6%) Staphylococcus aureus strains were of the $iMLS_B$ phenotype, whereas Angel et al from CMC, Vellore, reported that 23.2% strains were of the $iMLS_B$ phenotype [11] and Fiebelkorn et al reported that 28% [10] and Dizbay et al reported that 90% [12] of their Staphylococcus aureus strains were of the $iMLS_B$ phenotype. No MSSA strain was of the $iMLS_B$ phenotype in the present study. But other workers have found that 4% to 15% of their MSSA strains were of the $iMLS_B$ phenotype. [13], [14], [15] Out of 189 MRSA strains, 68 (36%) were of the $iMLS_B$ phenotype, though several studies from different parts of India have reported that 30% to 64% of their MRSA strains were of the $iMLS_B$ phenotype [11], [13], [15], [16], [17].

Though the incidence of the $cMLS_B$ phenotype is quite high outside India, [10], [13] Angel et al have not found any $cMLS_B$ resistance in Staphylococcus aureus strains.[11] We found 14 (3.8%) Staphylococcus aureus strains with the cMLSB phenotype, out of which 12 (6.3%) were MRSA strains and 2 (1.1%) were MSSA strains. Gadepalli et al had reported 12% strains of the MS_B phenotype among the Staphylococcus aureus strains [17] but in our study, only 3 (0.8%) strains were of the MS_B phenotype.

In the present study, the prevalence of the MRSA strains were 51.6%, which is somewhat similar to the results obtained by S. Anuprabha et al (MRSA strains were 54.8%) [19]. Our hospital is a tertiary care hospital situated in a rural set up and caters to patients from villages of Vidarbha and the adjoining areas of Madhya Pradesh and Andhra Pradesh. Lack of awareness and the indiscriminate and the improper use of antibiotics before coming to the hospital might be the contributory factors for the high prevalence of MRSA in our study.

The true incidence of the $iMLS_B$ phenotype of Staphylococcus aureus depends on the patient population studied, the geographical region, the hospital characteristics and methicillin susceptibility (MRSA or MSSA) [13]. The data from India is scanty [11], [16], [17].This is the first report from central India for MLS_B resistance.

Though the confirmation of the $iMLS_B$ phenotype can be done by detecting the *erm* gene, the D-test is an easy test to perform for the detection of the $iMLS_B$ phenotype. All of our 68 $iMLS_B$ phenotype Staphylococcus aureus strains showed a false sensitivity zone by the routine Kirby-Bauer disc diffusion method. There are a few reports of clindamycin treatment failure in infections with Staphylococcus aureus strains with inducible clindamycin resistance [20], [21].

Methicillin resistant Staphylococcus aureus (MRSA) has been identified as one of the clinically important multidrug resistant organisms (MDRO) and therapeutic options for it are limited significantly. In a previous study conducted in our laboratory, we have already reported that 16.8% MRSA strains were resistant to 10 commonly used antibiotics including gentamicin, ciprofloxacin, erythromycin etc. in 1997. [(22)] But in that study, linezolid and vancomycin were not included.

In the present study, 47(24.9%) strains were resistant to all commonly used antibiotics [Table/Fig 3]. But all MRSA strains were sensitive to linezolid and vancomycin. For MRSA strains, clindamycin is commonly used to treat skin and bone infections because of its tolerability and excellent tissue penetration and also, no renal

adjustments are needed. Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy [15]. But without the D-zone test, our 68 Staphylococcus aureus isolates with inducible clindamycin resistance would have been misclassified as Clindamycin sensitive, resulting in therapeutic failure. This is where the D-zone test becomes significant and important.

As clindamycin is one of the most commonly used antibiotics for MRSA strains, 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.

Conclusion

We hereby conclude that without the D-zone test, all Staphylococcus aureus isolates with inducible clindamycin resistance would have been misidentified as clindamycin susceptible by routine antibiotic susceptibility testing methods, resulting in the misuse of clindamycin and treatment failure.

Hence, all clinical microbiology laboratories should perform the D-zone test as per the CLSI guidelines 2004, which is simple and inexpensive, when Staphylococci appear to be erythromycin resistant and clindamycin susceptible by routine tests.

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