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RESEARCH ARTICLE OPEN ACCESS

Phenotypic Characterization of Macrolide-Lincosamide-Streptogramin B Resistance in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (S.aureus) **is a prevalent organism causing infections in the community and hospital. A variety of antibiotics are used, including the Macrolide-Lincosamide-StreptograminB (MLS_B) family of antibiotics in which clindamycin is the preferred agent. Widespread use of these antibiotics leads to resistance to these MLS_B antibiotics; a D-test can characterize the different MLS_B phenotypes. This study was taken up with an objective to perform a double disc diffusion test for detecting different phenotypes in *S.aureus* with particular reference to inducible clindamycin resistance. Out of a total of 174(100%) strains of *S.aureus,* 98(56.32%) were MRSA, and 76(43.68%) were MSSA. All isolates were tested by D-test. A total of 47(27.01%) were of $cMLS_B$ phenotype, 31(17.82%) were of $iMLS_B$ phenotype, and 96(55.17%) were of MS phenotype. The majority of MRSA strains were $cMLS_B$ phenotype(76.60%) and $iMLS_B$ phenotype (64.52%) in comparison to MSSA isolates. Although $iMLS_B$ phenotypes are present in both MRSA and MSSA, iMLSB was more in MRSA isolates. Appropriate susceptibility data is essential for a clinician to start clindamycin therapy to prevent therapeutic failures with inducible MLS_B resistance in *S.aureus* isolates. It will be appropriate for all the clinical laboratories to report inducible Clindamycin resistance in *S.aureus* strains (both MRSA and MSSA), for which D-test is a reliable testing method.**

Keywords: *Staphylococcus aureus,* MRSA, MSSA, D-test, inducible clindamycin resistance, MLS_B phenotypes

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INTRODUCTION

Staphylococcus aureus (S.aureus) is a commonly encountered organism causing infections both in hospital and community settings[1].The genus *Staphylococcus* contains 32 species, of which 16 species are found in humans. *S.aureus* is one of the most virulent species having many virulence factors like surface proteins, capsular polysaccharides, cytotoxins, superantigens, enzymes responsible for producing an array of ailments from superficial infections to deep-seated and life-threatening infections[2].

Treatment of *S.aureus* infections is usually with antibiotics like β lactams, glycopeptides, quinolones, oxazolidinone, etc. *S.aureus* has developed resistance to multiple antibiotics by various mechanisms like efflux of the drug, drug inactivation, target alteration, production of β lactamase, etc.[3] Emergence of Methicillin-Resistant *S.aureus*(MRSA) strains which is a typical hospital acquired organism and acquiring multidrug resistance has still complicated the treatment . The Macrolide-Lincosamide-StreptograminB(MLS_B) family of antibiotics are the agents used against such strains. MLS_B includes Macrolide (Eg: Erythromycin, Azithromycin, Spiramycin), Lincosamides (Eg: Clindamycin, Lincomycin), and StreptograminB (Eg: Quinupristin, Dalfopristin). These agents are different chemically, but all of them act by inhibition of protein synthesis, among which clindamycin is the recommended agent due to its pharmacokinetics, and its ability to reach various tissues, including bones[4].

Widespread use of the MLS_B group of antibiotics leads to an increase in *S.aureus* strains becoming resistant to these drugs, which can be due to any of the following mechanisms:

1. *erm*, a gene of *S.aureus* produces rRNA methylase, which brings about changes in the antibiotic binding site. The production of *the erm* gene can be either constitutive or inducible, leading to $cMLS_B$ or $iMLS_B$ phenotypes, respectively.
2. Efflux of antibiotics by *msrA* gene, which is called MS phenotype.
3. Inactivation of lincosamide by chemical alteration by *the inuA* gene[5].

Organisms develop resistance to these groups of antibiotics by acquiring genes called *erm* genes responsible for producing methylases. *S.aureus* strains harbor the genes like *erm* A, B, C & Y in their plasmids, conferring resistance to MLS_B antibiotics. The resistance can be inducible resistance where the strains exhibiting this type of resistance don't encode for methylases but become active only in the presence of antimicrobial agents like erythromycin, which is an inducer of *erm* genes. Another type of resistance exhibited by organisms is called constitutive resistance, in which methylases are produced even in the absence of inducer like erythromycin[6].

The isolates having the inducible *erm* gene exhibit resistance to agents like erythromycin, which are the inducer but will appear to be susceptible to the lincosamide and the non-inducer macrolides. Hence, using antibiotics like clindamycin will lead to the selection of constitutive mutants leading to treatment failures[7].

So while testing in vitro, interpretation of different phenotypes has to be done. $cMLS_B$ phenotypes are resistant to macrolides like erythromycin and lincosamides like clindamycin. $iMLS_B$ phenotypes are resistant to erythromycin and appear sensitive to clindamycin when tested without an inducer. But, in the presence of inducer of *erm* gene like erythromycin, they are resistant to clindamycin with a D-shaped zone of inhibition. MS phenotypes are sensitive to clindamycin without a D zone and resistant to erythromycin due to drug efflux mechanisms[4].

Determination of inducible clindamycin resistance by double disc diffusion test is advisable to avoid false sensitive reporting of clindamycin. The use of clindamycin in $iMLS_B$ phenotypes can lead to treatment failure because of the selection of $cMLS_B$ phenotypic strains. D-test which is an induction test useful in distinguishing *S.aureus* isolates which have inducible *erm* mediated resistance, i.e., $iMLS_B$ phenotypes from those with resistance due to drug efflux mechanism, i.e., MS phenotypes, and it is essential to test in vitro to differentiate $iMLS_B$ and MS phenotype strains to avoid clinical therapeutic failure[8,9].

Inducible Clindamycin resistance can be tested phenotypically by double disc diffusion test (D-test) or genotypically by molecular methods like Polymerase Chain Reaction(PCR) for detecting *erm* gene.[10] Though molecular techniques like PCR are more sensitive, its cost, requirement for technical expertise, and non-availability at all testing

facilities make it less preferable than simple, easy to perform D-test.

MATERIALS AND METHODS

This is a descriptive cross-sectional study conducted in the microbiology department at Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Andhra Pradesh, India, after the Institutional Ethics Committee approval for a period of two years, i.e., from January 2018 to December 2019.

A total of 339 *Staphylococcus aureus* isolates obtained from various clinical samples were incorporated in the study and were characterized by conventional tests, including Gram's staining, culture, and standard biochemical tests. Antibiotic sensitivity testing of all the isolates was done by Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) by using antibiotic discs (obtained from HIMEDIA lab Mumbai)of Penicillin (10units), Cefoxitin (30mcg), Ciprofloxacin (5mcg), Linezolid (30mcg), Erythromycin (15mcg) and Clindamycin (2mcg); interpreted as sensitive, intermediate and resistant as per CLSI guidelines. Vancomycin was reported by performing E-test. Identification of methicillin sensitive *S.aureus* (MSSA) and MRSA strains were according to CLSI guidelines[10]. Double disc diffusion test was done for all the isolates by placing Clindamycin(2mcg) and Erythromycin(15mcg) discs 15mm apart.

Flattening of the zone of inhibition around the clindamycin disc facing the Erythromycin disc was considered D-test positive, indicating inducible clindamycin resistance (Fig. 1). All such isolates were reported as clindamycin resistant.

The strains were interpreted as constitutive MLS_B phenotype if resistant to erythromycin with zone size ≤13mm and clindamycin with zone size ≤14mm, and those strains that were resistant to erythromycin with zone size ≤13mm and sensitive to clindamycin with zone size ≥21mm without D-zone was interpreted as MS phenotype[11]. (Table 1)

S.aureus ATCC 25923 was used as a control strain. Results tabulated and analyzed statistically.

RESULTS

Of the total 339 *S.aureus* isolates,165 were sensitive to both erythromycin and clindamycin. D-test further characterized the remaining 174 isolates resistant to either erythromycin or clindamycin, or both.

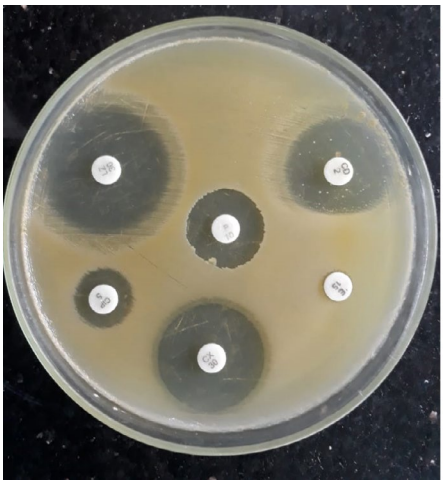

Fig. 1. Showing Positive D-test

Table 1. Showing the interpretation of the D-test

D-test phenotype	Erythromycin(E) Zone size	Clindamycin(CD)	D-test interpretation
Constitutive MLSB(cMLSB)	R(≤13mm)	R(≤14mm)	Growth up to CD and E discs
Inducible MLSB (iMLSB)	R(≤13mm)	S(≥21mm)	Flattened D shaped zone of inhibition around CD adjacent to E disc
MS phenotype	R(≤13mm)	S(≥21mm)	No D-zone, clear zone around Clindamycin disc

S: Sensitive R: Resistant CD: Clindamycin E: Erythromycin

Fig. 2. Showing MRSA & MSSA isolates

Table 2. Showing different *S.aureus* phenotypes

Phenotype	No. of isolates
$cMLS_B$	47(27.01%)
$iMLS_B$	31(17.82%)
MS phenotype	96(55.17%)
Total	174(100%)

Table 3. Showing phenotypes among MRSA and MSSA isolates

Phenotype	Methicillin Resistance		Total
	MRSA	MSSA	
$cMLS_B$	36(76.60%)	11(23.40%)	47
$iMLS_B$	20(64.52%)	11(35.48%)	31
MS phenotype	42(43.75%)	54(56.25%)	96
Total	98	76	174

Out of the 174 isolates, 98(56.32%) were MRSA, and 76 isolates were MSSA(43.68%), as shown in Fig. 2.

All the 174 isolates of *S.aureus* were subjected to D-test to characterize as $cMLS_B$, $iMLS_B$, or MS phenotype. Among the 174 isolates tested, 47(27.01%) strains were of $cMLS_B$ phenotype, 31(17.82%) strains were of $iMLS_B$ phenotype, and 96(55.17%) strains were of MS phenotype, as shown in Table 2.

Out of the total 98 isolates of MRSA 36(76.60%) were $cMLS_B$,20 (64.52%) $iMLS_B$ and 42(43.75%) MS phenotype.Out of total 76 MSSA isolates 11(23.40%) were $cMLS_B$, 11(35.48%) $iMLS_B$ and 54(56.25%)MS phenotype as shown in Table 3.

In the present study, the $iMLS_B$ phenotype was more in MRSA isolates (64.52%) than in MSSA isolates(35.48%).

DISCUSSION

Clindamycin is an excellent and preferred agent to treat superficial infections with *S.aureus* and a preferred antibiotic in patients allergic to penicillin[12]. Resistance to clindamycin in *S.aureus* strains with inducible phenotype may be reported as sensitive if not tested by D-test giving a false sensitive report which could result in treatment failure and also the emergence of constitutive *erm* mutants[13].

The incidence of $iMLS_B$ in our study was 17.82% which was comparable with Toleti et al.[14] (18%), Lall et al.[9] (20.3%), and Adaleti et al.[15](22%). Bingo et al.[16] had reported an incidence of $iMLS_B$ to be 28.5% which is higher than in our study. Prabhu K et al.[17] had reported 10.52% of $iMLS_B$, which was less compared to our study. 31(17.82%) isolates of Staphylococci would have been reported as sensitive if not tested with a D-test, conveying a false report to the treating clinician.

In the present study, among MRSA isolates, $cMLS_B$ phenotypes were 36(76.60%), $iMLS_B$ phenotypes 20(64.52%), and MS phenotypes were 42(43.75%), and in MSSA isolates, $cMLS_B$ phenotypes are 11(23.40%) $iMLS_B$ are 11(35.48%), and MS phenotype are 54(56.25%). In our study, both $cMLS_B$ and $iMLS_B$ phenotypes are more in MRSA isolates compared to MSSA isolates.

According to Toleti et al.[14], the prevalence of $iMLS_B$ phenotype was 22.72% in MRSA isolates and 11.11% in MSSA isolates, and Bingo S et al. [16] found that $iMLS_B$ phenotypes in MRSA were 91.9%. In MSSA, it was 8.1%, and according to Lall M et al. [9] MRSA isolates showing $iMLS_B$ phenotype are 37.1%, and MSSA was 6%. According to Prabhu K et al.[17] $iMLS_B$ phenotypes in MRSA was 20% and MSSA was 6.5%, and these results were similar to our study showing $iMLS_B$ phenotypes more in MRSA isolates than in MSSA isolates.

In a study done by Adaleti R et al. 15, The $iMLS_B$ phenotypes in MRSA were 18.2%, and MSSA was 40% which can be considered because the frequency of $iMLS_B$ phenotypes varies widely, ranging from 7 to 94%[18] and there are also few other studies showing higher percentage in MSSA isolates than MRSA isolates[19].

CONCLUSION

Clindamycin is a preferred antibiotic in superficial Staphylococcal infections and an alternative in penicillin-allergic patients.

False sensitive reports can lead to Clindamycin therapy failures and the selection of a constitutive resistant mutant in an $iMLS_B$ strain. So it will be appropriate that all clinical laboratories test and report inducible clindamycin resistance in both MRSA & MSSA by double disc diffusion test, which is a straight forward method to identify $iMLS_B$ phenotypes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All listed author(s) have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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