RESEARCH ARTICLE

The Prevalence of Inducible and Constitutive Macrolide-lincosamide-streptogramin B-Resistant ($iMLS_B$ and $cMLS_B$) Phenotypes among Clinical Isolates of *Staphylococcus aureus* at a Tertiary Care Hospital in South Mumbai

ABSTRACT

Introduction: Antimicrobial resistance in *Staphylococcus aureus* has led to renewed interest in macrolide-lincosamide-streptogramin B (MLS_B) group of antibiotics. Clindamycin is preferred alternative for the treatment of both Methicillin-resistant *S. aureus* (MRSA) and Methicillin-sensitive *S. aureus* (MSSA) infections. Multiple resistance mechanisms among MLS_B antibiotics lead to treatment failure. The resistance to clindamycin can be inducible or constitutive. Routine susceptibility testing may fail to detect inducible clindamycin resistance among staphylococci due to *erm* genes which may lead to treatment failure, thereby necessitating simple D test for the detection of inducible clindamycin resistance. **Materials and Methods:** A total of 331 *S. aureus* isolates were evaluated and subjected to routine antibiotic susceptibility testing by Kirby–Bauer disc diffusion method, including cefoxitin 30 mcg for the detection of MRSA. As per Clinical and Laboratory Standards Institute (CLSI) guidelines, D test was performed on erythromycin-resistant isolates to detect inducible clindamycin resistance. **Results:** Sixty-two (18.7%) isolates had inducible clindamycin resistance, 90 (27.1%) had constitutive resistance. Inducible and constitutive resistance was found to be higher in MRSA (23.7% and 36%) as compared to MSSA (11.6% and 14.5%). **Conclusion:** Study indicates that it should mandatory to perform D test in routine disc diffusion testing for the detection of inducible clindamycin resistance.

Key words: Antibiotic resistance, Infectious diseases, Inducible and constitutive clindamycin resistance, Staphylococci

INTRODUCTION

Staphylococcus aureus is one of the most common causative organisms of health care as well as community-acquired infections in every region of the world. Moreover, increase in the prevalence of methicillin resistance among *Staphylococci* is a matter of concern.[1] Due to this, there has been a renewed interest in the usage of macrolide-lincosamide-streptogramin B (MLS_B) antibiotics for the treatment of *S. aureus* infections. Moreover, clindamycin is the preferred agent because of its exceptional pharmacokinetic properties.[2]

Clindamycin can be used as an alternative antibiotic in penicillin-allergic patients for the treatment of skin and soft-tissue infections caused by *S. aureus*. It achieves high intracellular levels in phagocytic cells, high levels in bone, and appears to be able to reduce toxin production in toxin-producing strains of staphylococci. Except central nervous system, it has very good tissue penetration.[3] It is a good option for outpatient prescription or as a follow-up drug after intravenous therapy because of its good oral absorption.[4] However, a possibility of inducible clindamycin resistance among *Staphylococcal* isolates is a major concern in use of clindamycin.[5]

There are three mechanisms of resistance to the MLS_B class of antibiotics: Modification of target site, enzymatic inactivation, and impermeability or macrolide efflux pumps.[6]

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Resistance due to modification of ribosomal target is mediated by erythromycin ribosomal methylases encoded by *ermA*/*ermC* genes and it affects the activities of macrolides as well as clindamycin. This type of resistance may be inducible or constitutive.[7,8] The constitutive resistance MLS_B ($cMLS_B$) strains can easily be detected by standard susceptibility testing methods because they are resistant to both macrolides and lincosamides alike. However, the inducible resistant MLS_B ($iMLS_B$) strains appear erythromycin resistant and clindamycin sensitive in routine laboratory tests, unless the tests include measures that result in induction of clindamycin resistance.[4] In such cases, therapy with clindamycin may

select *erm* mutants, resulting in treatment failure.[4] D test can be performed to detect inducible clindamycin resistance[9] in which erythromycin disc is placed in close proximity to clindamycin disc. As erythromycin diffuses through the agar, there is a flattening or blunting of the zone of inhibition of clindamycin adjacent to the erythromycin disc, this gives a "D" shape to the zone (D-zone effect). Inducible clindamycin resistance should be detected in all the isolates of *S. aureus* because the different resistance phenotypes vary widely between geographic regions and even between different hospitals.[4]

The present study was conducted in the Department of Microbiology Bombay Hospital and Medical Research Center to know the prevalence of inducible and constitutive clindamycin resistance among *S. aureus* in our tertiary care hospital.

MATERIALS AND METHODS

A total of 331 *S. aureus* isolates from various clinical specimen such as pus, blood, sputum, bronchoalveolar lavage, tracheal secretion, urine, wound swab, and fluids from patients attending Bombay Hospital and Medical Research Center between January 2018 and December 2018 were included in the study.

We identified isolates as per standard microbiological methods and susceptibility testing was done by Kirby–Bauer disc diffusion method using Mueller-Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) recommendations.[9] Methicillin resistance was detected using cefoxitin (30 µg) disc. Erythromycin-resistant isolates were subjected to D test to determine inducible clindamycin resistance as per CLSI guidelines[9] by placing erythromycin (15 µg) and clindamycin (2 µg) disc at adjacent position, 15 mm apart on Mueller-Hinton agar. We observed different phenotypes when clindamycin and erythromycin discs were placed near each other and following are the interpretations:

MS phenotype

Resistant to erythromycin but sensitive to clindamycin with a circular zone of inhibition around clindamycin.

iMLS$_B$ phenotype

Resistant to erythromycin and showing D shaped zone of inhibition around clindamycin towards erythromycin disc. This indicates a resistance phenotype due to expression of *erm*-gene coded methylases.[4] It is positive D test for the detection of inducible clindamycin resistance [Figure 1].

cMLS$_B$ phenotype

Isolates resistant to both erythromycin and clindamycin known as constitutive clindamycin resistance.

RESULTS

Out of total 331 *S. aureus* isolates, 194 (58.6%) were Methicillin-resistant *S. aureus* (MRSA) and 137 (41.3%) were Methicillin-sensitive *S. aureus* (MSSA). Erythromycin resistance was seen in 239 (72.2%) isolates. Inducible clindamycin resistance was 18.7%. It was high in MRSA isolates (23.7%) as compared to MSSA isolates (11.6%). Constitutive clindamycin resistance was 27.1%. It was high in MRSA isolates (36%) as compared to MSSA isolates (14.5%). Among all 331 strains isolated, 87 (26.2%) were sensitive to both erythromycin and clindamycin. MS phenotype, that is, erythromycin resistant and clindamycin sensitive were also 87 (26.2%). An unusual phenotype showing erythromycin sensitive and clindamycin resistant was seen in five isolates, of which three were MRSA and two isolates were MSSA. The details of various susceptibility patterns are shown in Table 1.

DISCUSSION

Clindamycin is a desirable option for the treatment of *Staphylococcal* infections as it is effective, safe, and convenient parenteral as well as oral antibiotic.[10] However, the possibility of inducible resistance is a matter of concern. If inducible clindamycin resistance is not checked, it may give a wrong report of sensitivity to clindamycin, thereby giving rise to treatment failure. On the other hand, negative result for inducible clindamycin resistance confirms sensitivity to clindamycin and provides a good therapeutic choice to the clinician.[11]

We found high rate of erythromycin resistance 239 (72.2%) in our study. Among those, 62 (18.7%) were having inducible clindamycin resistance (iMLS$_B$) which was detected by D test and 90 (27.1%) were having constitutive clindamycin resistance (cMLS$_B$). Remaining 87 (26.2%) were D test negative and were sensitive to clindamycin

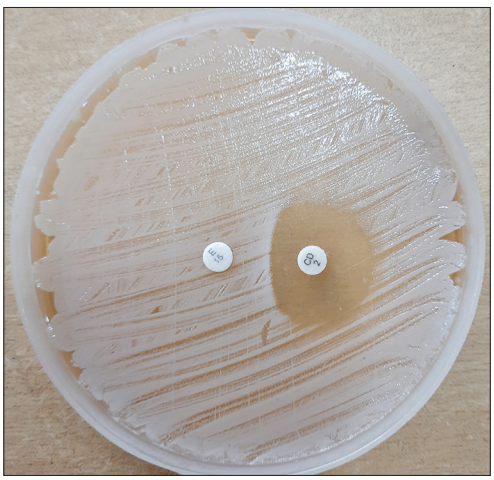

Figure 1: Positive D test

Table 1: Susceptibility patterns of *Staphylococcus aureus*

Phenotype	MRSA (%)	MSSA (%)	Total (%)
ERY-S, CL-S	28 (14.4)	59 (43)	87 (26.2)
ERY-R, CL-R ($cMLS_B$)	70 (36)	20 (14.5)	90 (27.1)
ERY-R, CL-S, D Test +($iMLS_B$)	46 (23.7)	16 (11.6)	62 (18.7)
ERY-R, CL-S, D Test -(MS phenotype)	47 (24.2)	40 (29.1)	87 (26.2)
Other -ERY-S, CL-R	3 (1.5)	2 (1.4)	5 (1.5)
Total	194 (58.6)	137 (41.3)	331

ERY: Erythromycin, CL: Clindamycin, S: Sensitive, R: Resistant, cMLSB: Constitutive resistance to clindamycin, iMLSB: Inducible resistance to clindamycin

(MS phenotype). Our observations suggest that if we had not performed D test, there was a chance to misidentify approximately 25% of the erythromycin-resistant isolates as clindamycin sensitive leading to treatment failure. We also observed that percentages of inducible resistance and constitutive resistance were higher amongst MRSA isolates (23.7% and 36%, respectively) as compared to MSSA isolates (11.6% and 14.5%, respectively) which is consistent with few other studies.[1,2,4]

In erythromycin-resistant *S. aureus* infections, some clinicians may avoid using clindamycin on account of uncertainty about the reliability of susceptibility reports for clindamycin when D-test results are not available, as well as confusion over the clinical importance of this inducible resistance.[12] Hence, clinical microbiology laboratories should consider performing routine testing and reporting for inducible clindamycin resistance in *Staphylococcal* isolates so that use of clindamycin is judiciously undertaken especially for the treatment of MRSA infections before switching over to vancomycin.

CONCLUSION

Performing routine D test on the erythromycin-resistant *S. aureus* isolates will determine the true susceptibility to clindamycin. The prevalence of inducible clindamycin resistance may vary in different hospitals. Therefore, clinical microbiology laboratories should consider performing routine testing and reporting of inducible clindamycin resistance in *S. aureus* isolates. This can help clinicians in appropriate treatment choice for the patients with *S. aureus* infections. Furthermore, this will ensure that clindamycin remains a viable and excellent alternative for the treatment of staphylococcal infections.

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