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PREVALENCE OF CLINDAMYCIN RESISTANCE AMONG THE CLINICAL ISOLATES OF *STAPHYLOCOCCUS aureus* FROM A TERTIARY CARE HOSPITAL IN SOUTH INDIA

Microbiology

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ABSTRACT

The antimicrobial resistance among Staphylococcal infections is a challenge nowadays. This has led to the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat these infections. However therapy may fail either due to constitutive or inducible resistance. This study was undertaken in a tertiary care hospital to detect different phenotypes including inducible clindamycin resistance in the clinical isolates of *Staphylococcus aureus.* A total of 135 S. aureus isolates were subjected to routine antibiotic susceptibility testing, including cefoxitin disc (30 µg). Inducible clindamycin resistance was tested by 'D test' as per CLSI guidelines. Among 135 *S. aureus* isolates, 38 (28.1 %) were resistant to methicillin, whereas 97 (71.9 %) were methicillin susceptible. Out of the 51 (37.8%) erythromycin-resistant S. aureus isolates, 12 (8.9 %) showed inducible MLS_B phenotype, 9 (6.7 %) showed constitutive MLS_B phenotype while the remaining 30 (22.2 %) showed MS phenotype. Inducible and constitutive resistance was found to be higher in MRSA when compared with MSSA. Performing D test routinely guides the clinician about the inducible clindamycin resistance and helps in appropriate usage of antimicrobial agents.

KEYWORDS

Staphylococcus aureus, Clindamycin resistance, constitutive MLS B phenotype, inducible MLSB phenotype, MS phenotype

Introduction

Staphylococcus aureus, a versatile pathogen, is a leading cause of infections worldwide. It has an extraordinary ability to develop resistance on exposure to any antimicrobial agent. Nowadays, a large majority of strains are methicillin resistant and the infections caused by them have a significant impact on healthcare costs.[1] Macrolide–lincosamide–streptogramin B (MLSB) drugs are prescribed for patients with penicillin allergy.

Clindamycin, due to its excellent pharmacokinetic properties, is used in the treatment of skin and soft-tissue infections, caused by the staphylococcal species.[2] However; the possible presence of inducible clindamycin resistance causes clinical failure of therapy. This resistance is caused by ribosomal target modification that affects the activities of both macrolides and clindamycin, resulting in so-called macrolide–lincosamide–streptogramin B ($iMLS_B$) resistance, which is encoded by the erm(A) or erm(C) gene. [3] It is important to differentiate this iMLSB type of resistance from constitutive macrolide resistance that affects only macrolides and not clindamycin. [4]

The present study was designed to detect the presence of clindamycin resistance among the clinical isolates of *S. aureus* using the D test as per CLSI guidelines.[5] Also, we tried to ascertain the relationship between methicillin-resistant *S. aureus* (MRSA) and inducible clindamycin resistance.

Materials and Methods

This cross sectional study was conducted in a tertiary care hospital with the approval of the institution's ethical committee. A total of 135 isolates of *Staphylococcus aureus* isolated from various clinical specimens like pus, wound swab, urine, blood, and sterile fluids were tested. The isolates were first identified as *S. aureus* by standard techniques [6] and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15µg), penicillin (10U), ciprofloxacin (5 µg) and cefoxitin (30 µg) as per CLSI guidelines. Methicillin resistance was detected using cefoxitin as a surrogate marker.[5] Those isolates which were erythromycin resistant were further subjected to 'D test' as per CLSI guidelines. Briefly, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37 ^{0} C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance.[5]

Three different phenotypes were appreciated after testing and interpreted as follows:

1. The MS phenotype: *Staphylococcal* isolate exhibiting resistance to ER (zone size ≤13 mm) while sensitive to CL (zone size ≥21 mm) and giving circular zone of inhibition around CL.

2.The Inducible MLS_B phenotype (MLS_Bi): *Staphylococcal* isolate showing resistance to ER (zone size ≤13 mm) while being sensitive to CL (zone size ≥21 mm) and giving D-shaped zone of inhibition around clindamycin with flattening towards ER disc.

3.The Constitutive MLS_B phenotype (MLS_Bc): *Staphylococcal* isolate showing resistance to both ER (zone size ≤13 mm) and CL (zone size ≤14 mm) with circular shape of zone of inhibition if any around CL.

Results

One hundred and thirty five *Staphylococcus aureus* were tested for susceptibility to erythromycin and other antibiotics of the panel by routine disc diffusion testing; 51 (37.8%) of them were erythromycin resistant. These isolates when subjected to D test showed nine (6.7%) isolates resistant to both erythromycin and clindamycin indicating constitutive MLS_B phenotype; 42 isolates showed clindamycin sensitivity. Out of these, 12 isolates showed positive D test indicating inducible MLS_B phenotype while 30 gave negative D test indicating MS phenotype [Table 1]. Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA [Table 2].

Table 1: Susceptibility pattern of *Staphylococcus aureus* isolates to erythromycin and clindamycin

Phenotypes	No.of isolates (n=135)	(%)
E-S, CL-S	84	62.2
E-R, CL-R (constitutive MLS_B)	9	6.7
E-R, CL-S, D test positive (inducible MLS_B)	12	8.9
E-R, CL-S, D test negative (MS)	30	22.2

Table 2: Association of clindamycin resistance with methicillin resistance

Phenotypes	MRSA (%)	MSSA (%)	Total
E-S, CL-S	9 (23.7)	75 (77.3)	84 (62.2)
E-R, CL-R (constitutive MLS_B)	9 (23.7)	0	9 (6.7)
E-R, CL-S, D test positive (inducible MLS_B)	8 (21.1)	4 (4.1)	12 (8.9)
E-R, CL-S, D test negative (MS)	12 (31.6)	18 (18.6)	30 (22.2)
Total	38 (28.1)	97 (71.9)	135

Discussion

In this study majority of (62.2%) S.aureus isolates were detected from pus and wound swabs. It is in accordance with the observations of Majhi et al., 2016.[7] Prevalence of MRSA in this study was 28.1% which is comparable to the reports of Bala Murali et al., 2016.[8] In the present study, when S.aureus isolates were subjected to D-zone test, it was found that 12 (8.9%) isolates showed inducible clindamycin resistance (iMLSB phenotype) and 9 (6.7%) showed constitutive resistance (cMLSB phenotype). Gade et al reported that 13.2% strains were of iMLSB phenotype and 12.4% were of cMLSB phenotype while Shantala et al reported that 24.9% of their strains were of iMLSB phenotype and 18.3 % were of cMLSB phenotype. [9,10]

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.21.1% MRSA isolates were found to be of iMLSB phenotype which correlates well with the findings of Deotale et al who reported 27.6 % iMLSB phenotype.[2] On the contrary, Sasirekha *et al.,* and Bottega *et al.,* had shown a higher percentage of inducible resistance in MSSA compared to MRSA isolates, 0.65% in MRSA and 8.49% in MSSA; 2.1% MRSA and 5.8% MSSA respectively.[11,12]

In this study, 31.6% MRSA belonged to MS phenotype as compared to 18.6% MSSA.Similar findings were made by Deotale et al who reported 24.3% & 4.0% MS phenotype among MRSA and MSSA respectively. [2] Gadepalli et al reported 12.0% strains of the MS phenotype among the MRSA and MSSA each. [13]

Conclusion

In this study, we found that that there is a high percentage of clindamycin resistance amongst the MRSA isolates. Hence incorporation of D test in routine antimicrobial susceptibility testing by Kirby- Bauer disc diffusion method will facilitate in the judicious use of clindamycin for skin and soft tissue infections. The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible clindamycin resistance is helpful in formulating & monitoring the antibiotic policy.

Abbreviations:

iMLSB: Inducible Macrolide-Lincosamide-StreptograminB
cMLSB: Constitutive Macrolide-Lincosamide-StreptograminB
MRSA: Mehicillin Resistant *Staphylococcus aureus*
MSSA: Methicillin Susceptible *Staphylococcus aureus*
ER: Erythromycin
CL: Clindamycin

Conflict of Interest: None declared

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