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INDUCIBLE CLINDAMYCIN RESISTANCE AND METHICILLIN RESISTANCE AMONGST *STAPHYLOCOCCUS AUREUS* ISOLATES: A PHENOTYPIC DETECTION

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ABSTRACT

Background: Increasing prevalence of *Methicillin resistant staphylococcus aureus* is global public health issue in both community and hospital settings. Management of *MRSA* infections is tough owing to its resistance to many antibiotics. Macrolide-lincosamide-streptogramins B (MLS_B) antibiotics are commonly used for the management of *MRSA*. Clindamycin is being the preferred agent due to its excellent pharmacokinetic properties. However, use of clindamycin in erythromycin resistant *Staphylococcus* isolates could result in treatment failure as a result of inducible clindamycin resistance in spite of showing *in vitro* sensitivity.

Aim: This study was aimed to find out the percentage of *S. aureus* having inducible clindamycin resistance ($iMLS_B$) in our geographic area using D-test and to ascertain the relationship between *methicillin-resistant S. aureus (MRSA)* and inducible clindamycin resistance.

Methods: A total of 822 *Staphylococcus aureus* isolated from different clinical samples were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. All isolates were tested for Methicillin resistance by using cefoxitin 30 µg discs. Inducible clindamycin resistance was detected by 'D' test as per CLSI guidelines.

Results: Out of the 822 *Staphylococcus aureus* isolates, 395 (48.05%) were *MRSA* and 427 (51.94%) were *MSSA*. 482 (58.63%) isolates were erythromycin resistant. These erythromycin resistant isolates when subjected to 'D' test, 89 isolates showed MS phenotype, 148 showed inducible MLS_B phenotype and 245 showed Constitutive MLS_B phenotype. Out of 395 MRSA isolates 116 (29.36%) showed Inducible MLS_B phenotype and 190 (48.10%) showed Constitutive MLS_B phenotype, while in 427 methicillin sensitive Staphylococcal isolates 32(7.49%) showed Inducible MLSB phenotype and 55 (12.88%) showed Constitutive MLS_B phenotype. The percentage of inducible and constitutive resistance was higher amongst *MRSA* isolates as compared to *MSSA* isolates.

Conclusion: Considering the higher prevalence of clindamycin resistance in *MRSA* isolates as compared *MSSA* isolates, routine D- test of *S.aureus* isolates is strongly recommended to prevent treatment failure. Therefore inducible clindamycin resistance detection should be the part of *S.aureus* sensitivity testing in all the microbiology

laboratories.

Keywords: Clindamycin, *MRSA*, D- Test, constitutive MLS_B phenotype, Erythromycin, Inducible MLS_B phenotype

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INTRODUCTION:

Staphylococcus aureus, one of the most common nosocomial and community acquired pathogens has now emerged as an ever increasing problem due to its increasing resistance to several antibiotics.[1]Emergence of methicillin resistance in *S. aureus* has left very few therapeutic alternatives. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[2] The advantages of choosing clindamycin are availability of both parenteral and oral formulations, high bioavailability, soft tissue permeability, inhibits toxin production ,no dosage adjustments are required in the presence of renal disease, can be given in penicillin allergic patients and is relatively cheap. However its increased use has resulted in widespread resistance against clindamycin.[3,4] However, resistance to this drug is again a problem. Resistance to MLS_B can occur by two mechanisms: an active efflux mechanism encoded by the *msrA* gene and target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_B Phenotype) or inducible (inducible MLS_B Phenotype).[5] It is very difficult to detect the inducible clindamycin resistance 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 *msr*A genes i.e. efflux of antibiotic, staphylococcal isolates ap- pear erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro and the strain do not typically be- come clindamycin resistant during therapy.[6] Thus to avoid clinical therapeutic failure in the resistance case me- diated by *erm* gene, it is very important to detect inducible clindamycin resistance phenotypes in vitro which can be made by erythromycin-clindamycin disc approximationtest (D-test) as its sensitivity was found 100% in different studies when compared with *erm* and *msr* gene detection by polymerase chain reaction. There is a wide variation in the rate of inducible clindamycin resistance in different places.[7] This study was conducted to determine the prevalence of inducible clindamycin resistance among clinical *S. aureus* isolates and also tostudy their association with *MRSA* in our set up.

MATERIALS AND METHODS

This observational study was conducted in the Department of Microbiology, Swami Ramanand Teerth Rural Medical College, Ambajogai, Beed, Maharashtra for a period of 1 year and 6 months from January 2022 to June 2023 .A total of 822 isolates of *Staphylococcus aureus* were isolated from various clinical samples e.g. pus, blood, urine, sputum, body fluids, throat swabs, swabs from surgical and non-surgical wounds sent for bacteriological cultures from patients of all age groups and both sexes from various departments. Repeated samples and samples showing the possible signs of contaminations were excluded. Isolates were identified on the basis of colony characteristics, Gram staining, catalase test, slide coagulase test, tube coagulase test, growth on mannitol salt agar and DNase test.[8] Antibiotic susceptibility pattern of *S. aureus* was carried out by modified Kirby Bauer disc diffusion method on Mueller Hinton agar. Methicillin-resistance was detected using a 30 mg cefoxitin disc and inducible resistance to clindamycin was tested by D-test as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] A lawn culture of the isolate which was adjusted to 0.5 McFarland's concentration was made on a Mueller-Hinton agar plate and discs of clindamycin (2 mg) and erythromycin (15mg) (Hi-Media, Mumbai, India) were placed at a distance of 15 mm (edge to edge) as per the CLSI recommendations, along with routine antibiotic susceptibility testing. Three different phenotypes were appreciated and interpreted. This interpretation was done only for erythromycin resistant *S.aureus* strains.

MS phenotypes: MS phenotypes were the staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and giving a circular zone of inhibition around clindamycin.

Inducible MLS_B ($iMLS_B$) phenotype: Inducible MLS_B ($iMLS_B$) phenotypes were the staphylococcal isolates showing resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D- shaped zone of inhibition around clindamycin withflattening toward erythromycin disc.

Constitutive MLS_B ($cMLS_B$) phenotype: Constitutive MLSB ($cMLS_B$) phenotypes were labeled for erythromycin zone size ≤13 mm and clindamycin zone size ≤14 mm with the circular shape of the zone of inhibition (if any) around clindamycin or with *Staphylococcus* isolates showingno zones of inhibition around both erythromycin and clindamycin. Quality control of the erythromycin and clindamycin discs was performed with *S. aureus* ATCC25923, *S. aureus.*

RESULTS

Among the 822 clinical isolates of *S. aureus*, 395 (48.02%)were *methicillin sensitive S.aureus* (*MRSA)* and 427 (51.94%) were *methicillin sensitive S.aureus* (MSSA) [Table 1]

Of the 822 *S. aureus* isolates, 340(41.36%) had the erythromycin-sensitive and clindamycin-sensitive phenotype, 245 (29.8%) had a constitutive resistance phenotype ($cMLS_B$), 148 (18 %) had the inducible resistance ($iMLS_B$) phenotype, and 89 (10.83%) had an MS_Bphenotype [Table 3].

Among the 395 *MRSA* isolates, 190 (48.10%) had the constitutive, 116 (29.36%) had the $iMLS_B$ resistance and 25 (6.32%) had the MS phenotype. Among the 427 *MSSA* isolates, 55 (12.88%) and 32 (7.49%) isolates were found to have the $cMLS_B$ and $iMLS_B$ resistance phenotypes respectively whereas 64 (14.98%) exhibited the MS phenotype [Table 3].

Among 482(58.63%) erythromycin resistant *S. aureus* isolates, 245(50.82%) had a constitutive resistance phenotype($cMLS_B$),148(30.83%)had the inducible resistance ($iMLS_B$) phenotype, and 89 (18.46%) had an MS_B phenotype [Table 4 & Figure 1].

DISCUSSION

The proportion of *MRSA* has increased worldwide since last decades.Its prevalence varies markedly across different countries and among hospitals of the same country.[7]Improper infection preventions practices in the hospital set up, discriminate use of antibiotics ,intravascular catheterization ,hospitalization in intensive care unit etc. contribute in the emergence of *MRSA*. [10] Our study showed prevalence rate of 48.05% which is similar to study done by More et.al(45.92%)[11] and Nikam et al.,(43.5%)[5].However higher rates of MRSA were also observed by Bala et al.,(80.5%)[3], Phukan et al.,(74.42%) [4]and lower rate of MRSA noted by Ghosh et al.,(23.9%)[12] ,Sharma et al., (25.25%)[13].Thses variations could be due to the differences in the circulating clones or due to the variations in the infection prevention practices and trends of antibiotics prescription in different hospital set up. Emergence of *MRSA* has left us with very few therapeutic options available to treat staphylococcal infections. The Macrolide – Lincosamide-Streptogramin B(MLS_B)family of antibiotics is commonly used to treat these infections Among all thses drugs Clindamycin is the drug of choice by most of the clinicians because of its excellent pharmacokinetic properties in particularly skin and soft tissue infections and as an alternative in penicillin allergic patients. [5,14]

In this study, of 822 *S. aureus* studied over a period of 1 year and 6 months, Erythromycin resistance was seen in 482(58.63%) isolates. Similar high prevalence of Erythromycin resistance has reported by Adhikari et al (54.4%)[7] and Shaikh et al(52.48%)[15].Among the erythromycin-resistant *S. aureus*, 148 (30.7%) isolates tested positive for inducible clindamycin resistance by D-test. Our findings are consistent with the results of singh et al.,(29.4%)[16],Banik et al.,(27.36%)[2], Nikam et al.,(25.74%) [5]where as panwala et al.,[17]prabhu et al.,[6]Fatima et al.,[18]have reported 37.5% ,37.5%& 39.3% respectively. These observations suggest that if the D-test would not have been performed, nearly one-third of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in the therapeutic failure. The incidence of constitutive clindamycin resistance is high (51.2%) in our hospital setting, similar to those observed by Pal et al.,(46.97%)[19]. singh et al.,(53.8%)[16], Fatima et al.(48%)[18],Banik et al.,(43.15%)[2], Nikam et al.,(47.52%)[5].Lower percentage was observed in panwala et al.,(15%)[17], prabhu et al.(16.6%)[6]. These all studies shows that there is a wide variation in incidence of clindamycin resistance among clinical isolates of staphylococcus aureus in different geographical areas.In the present study, 18.46% of erythromycin resistant staphylococcal isolates showed true clindamycin susceptibility. Patients with infections caused by such isolates can be treated with clindamycin without emergence of resistance during therapy

In our study the incidence of inducible clindamycin resistance and constitutive clindamycin resistance is higher among *MRSA* (29.36% and 48.10%) as compared to *MSSA* (7.49%and 12.88%), respectively. This was in concordance with Nikam et al.,[5] who have reported inducible clindamycin resistance and constitutive clindamycin resistance (30% and 43.42%) in *MRSA* and (3% and 15.84 %) in *MSSA* respectively. However singh et al.,[16] reported inducible clindamycin resistance and constitutive clindamycin resistance (25% and 64.8%) in *MRSA* and (8.7% and 4.6%) in *MSSA* respectively. On the contrary, Levin et al.,[20] showed a higher percentage of inducible resistance in *MSSA* (68%) as compared to *MRSA* (12.5%). Uzunović et al.,[21] also showed higher inducible resistances in *MSSA* as compared to *MRSA.*

Overall prevalence of inducible clindamycin resistance among isolates was found to be somewhat high in our set up, this study showed higher percentage of resistance to erythromycin and clindamycin among *MRSA*. This indicates that wide use of erythromycin and clindamycin for treatment of staphylococcal infection in our set up, as wide consumption of macrolides results emergence of macrolide resistant *staphylococcus species* due to selective pressure.As this resistant patterns can be decreased by reduction in the use of macrolides.This study emphasizes the

need to do likewise in our set up to reserve its as alternative drug of choice for treating infection caused by *MRSA*.This study showed only 6.32% of *MRSA* among erythromycin resistant isolates as MS phenotype which means clindamycin can be used as treatment option only for less number of *MRSA* which are erythromycin resistant .So there is a least chance of clinical efficacy of clindamycin while treating erythromycin resistant *MRSA* infections as an alternative to vancomycin .These findings further emphasize the need of D- test to be performed routinely in our set up to avoid clinical failure while using clindamycin as an alternative to anti-MRSA antibiotics like vancomycin and linezolid. *MRSA* is now growing public health problem. The relationship between *MRSA* and iCR appears to be clinically insignificant even though a highly positive correlation coefficient is in present study observed. This is alarming sign that clindamycin therapy failure may occur without prior testing for inducible resistant phenotypes. It should be necessary to prepare local sensitivity data which help in guiding empiric therapy and for preparing antibiotic policy.

Globally, AMR is on the rise, particularly in developing countries, like India. Over the counter sale of antibiotics, lack of effective regulations on antibiotics use, incom- plete dosing, excessive use of wide-spectrum antibiotics for common infections, and empiric therapy without lab- oratory diagnosis are all common. These practices usually cure infections, so most health settings opt for and retain these practices, but in return, these settings act as a factory of resistant mutants. This, in part, is because of a lack of sufficient resources to set up standard laboratory facilities covering all geography, particularly in remote skirts of India. AMR is a public health threat that demands urgent attention. Surveillance of this type reports the updated AMR profile of the circulating pathogens in the region, which in turn can be used for formulasting policies with strong strategies to check AMR.

Production of erm gene and its subtypes detected by molecular methods like DNA probing, Polymerase chain reaction, RFLP etc. These tests have not done in present study. These tests are available at research institute only. This is limitation of present study.

CONCLUSIONS

High prevalence of *MRSA* and clindamycin resistance ($cMLS_B$ and $iMLS_B$) warrants the need of development, adoption and enforcement of Antimicrobial stewardship programme and infection prevention and control practices in our hospital settings. Clindamycin resistance in the form of $_iMLS_B$ and $_cMLS_B$ limits the therapeutic options for *MRSA* to the antibiotics like linezolid and vancomycin. Therefore to identify these resistance mechanisms phenotypically, D-test should be routinely performed that will help us in guiding the clinicians regarding the judicious use of clindamycin.

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TABLES

Total samples	***MRSA*, *n* (%)**	***MSSA*, *n* (%)**
822	395(48.05%)	427(51.94%)

Table1: Occurrence of *methicillin-resistant Staphylococcus aureus* and *methicillin-sensitive Staphylococcus aureus* isolates in ***all Staphylococcus aureus isolates***

Total samples	Erythromycin sensitive, ***n* (%)**	Erythromycin resistant, ***n* (%)**
822	340(41.36%)	482(58.63%)

Table2: Erythromycin Susceptibility in *Staphylococcus aureus* isolates

Phenotypes	MRSA (%)	MSSA (%)	Total (%)
Erythromycin-sensitive, Clindamycin-sensitive	64 (16.2%)	276 (64.64%)	340 (41.36%)
Erythromycin-resistant,Clindamycin-resistant (constitutive MLS_B)	190 (48.10%)	55 (12.88%)	245 (29.80%)
Erythromycin-resistant, Clindamycin-sensitive, D-testpositive (inducible MLS_B)	116 (29.36%)	32 (7.49%)	148 (18%)
Erythromycin-resistant, Clindamycin-sensitive, D-testnegative (MS)	25 (6.32%)	64 (14.98%)	89 (10.83)
Total	395 (48.05%)	427 (51.94%)	822

Table 3: *Susceptibility to erythromycin and clindamycin* *among* *Staphylococcus aureus* *isolates and association with Methicillin resistance*

Phenotypes	Total (%)
Erythromycin-resistant,Clindamycin-resistant (constitutive MLS_B)	245 (50.82%)
Erythromycin-resistant, Clindamycin-sensitive, D-testpositive (inducible MLS_B)	148 (30.83%)
Erythromycin-resistant, Clindamycin-sensitive, D-testnegative (MS)	89 (18.46%)
Total	482

Table 4: Susceptibility to clindamycin and Clindamycin among erythromycin Resistant *Staphylococcus aureus isolates*

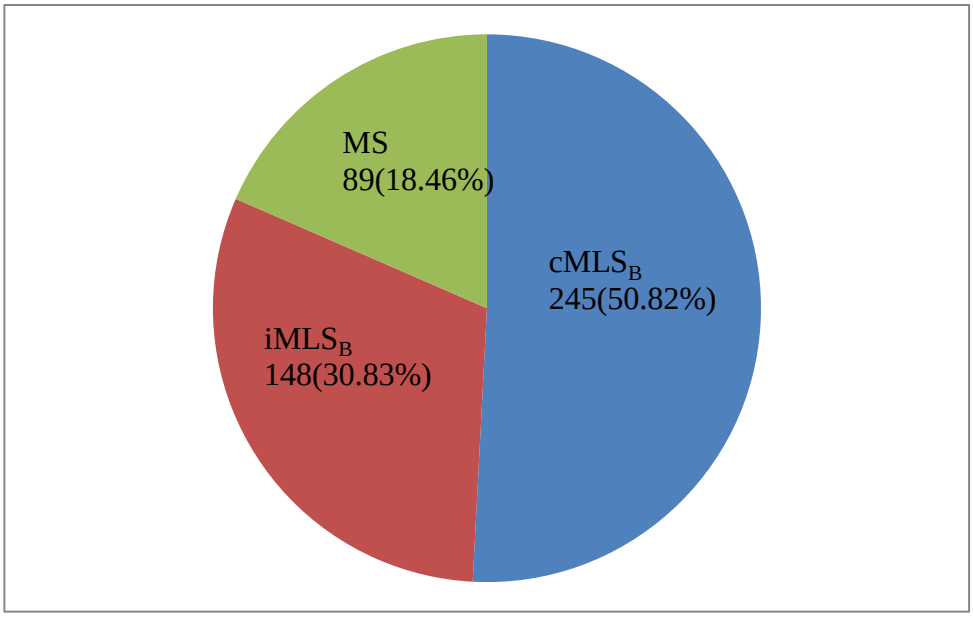

Figure:1 Pie chart showing proportion of Susceptibility to clindamycin and Clindamycin among erythromycin Resistant *Staphylococcus aureus isolates*