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INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* FROM A TERTIARY CARE TEACHING HOSPITAL IN SOLAPUR, MAHARASHTRA

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ABSTRACT

Background- Clindamycin is commonly used to treat skin and soft tissue infections caused by *Staphylococcus aureus* especially methicillin resistant *S. aureus* (MRSA) infections. The resistance to antimicrobial agents among *Staphylococci* is an increasing problem globally. Inducible clindamycin resistance detection is important as it guides physician for better patient outcome. The study has been undertaken with the aim to detect inducible clindamycin resistance both inducible as well as constitutive MLS_B (Macrolide-Lincosamide-Streptogramin-B) phenotype among Staphylococcus aureus isolates.

Material and method- A total of 269 non duplicate *Staphylococcus aureus* isolates from various clinical samples were obtained from patients attending OPD or admitted in the hospital (IPD). These isolates were included in the study. Identification of *Staphylococcus aureus* was based on culture characteristics and other standard biochemical tests. Antimicrobial susceptibility testing was done on Mueller Hinton agar by Kirby baeur disc diffusion method. MRSA were detected using disc of Cefoxitin 30 µg, zone of inhibition less than 22 mm in *Staphylococcus* isolates were considered as MRSA. Inducible clindamycin resistance was detected by D-zone test as per CLSI guidelines.

Result-Among 269 *Staphylococcus aureus* isolates MRSA were 66 (24.53%) while MSSA were 203 (75.5%). Majority of MRSA (n=66) & MSSA (n=203) isolates were from patients admitted in the hospital (IPD) which were 61 (92.42%) and 155 (76.4%) respectively. *Staphylococcus* isolates were predominantly isolated from pus/wound swab 135 (50.2%) followed by blood 62 (23.04%), urine 58 (22%), tracheal aspirate 09 (3.34%) and pleural fluid 03 (1.11%). Among *Staphylococcus aureus* isolates MS phenotype accounted for 13.01% of isolates, iMLSB phenotype were 15% while cMLSB phenotype seen in 20.44% of the isolates. On comparison between MRSA and MSSA isolates cMLSB phenotype and iMLSB phenotype is common among MRSA. Among MRSA $cMLS_B$ seen in 39.4% isolates and $iMLS_B$ in 30.33% isolates respectively as compared to MSSA where $cMLS_B$ was seen in 16.74% isolates while $iMLS_B$ phenotype was seen in 10% of isolates. MS phenotype in MRSA was seen in 15.2% of isolates while in MSSA MS phenotype seen in 10% of isolates.

Conclusion- Present study will help in assessment of the magnitude of clindamycin resistance among clinical isolates of *S. aureus*. Routine testing of such resistance will guide physicians for optimum therapy, to avoid treatment failure and better patient outcome.

KEYWORDS : MRSA, MSSA, inducible clindamycin resistance, iMLSB (inducible macrolide-streptogramin B phenotype), cMLSB (constitutive macrolide-streptogramin B phenotype), D-zone test.

INTRODUCTION

Staphylococcus aureus is one of the most common pyogenic bacteria infecting humans[1].These organism's cause variety of infections ranging from furuncle, carbuncle to serious life threatening infections[2]. These organisms are implicated in both health care associated infections (HAI) as well as community acquired infections (CAI). Over the years there has been rapid increase in strains which are resistant to Methicillin (MRSA), infection with such strains make treatment difficult as drugs like vancomycin, teicoplanin, daptomycin, quinopristin, dalfopristine are used for treatment of such infections[3].These drugs are highly toxic and also increases hospital stay and financial burden on the patients.

Due to injudicious use of these drugs, decrease susceptibility or resistance is commonly encountered now-a-days. To combat this condition alternative therapeutic options such as Macrolide-Lincosamide- Streptogramin-B (MLS) are explored as treatment options. Macrolide- Erythromycin, Lincosamide-Clindamycin, Streptogramin- Quinopristin and Dalfopristine antimicrobial agents are collectively known as MLS_B agents and are currently used to treat Staphylococcal infections[4].

MLS_B agents are structurally unrelated but act through common mechanism of protein synthesis inhibition[5]. Erythromycin and Clindamycin represents two different classes of antibiotics that act by binding at 50-s ribosomal subunit of bacteria and causes inhibition of protein synthesis. Macrolide resistance in *Staphylococcus aureus* occur by more than one mechanism. The resistance to macrolide may occur by efflux pump mechanism which is mediated by msr gene. Inducible and constitutive resistance to Macrolide, Lincosamide and Streptogramin –B resistance is mediated by erm gene[6].

The resistance mechanism can be constitutive where r-RNA methylase is always produced (C MLS_B) or the resistance mechanism can be inducible where methylase is produced only in the presence of an inducing agent ($iMLS_B$). Erythromycin is a strong inducer whereas Clindamycin is a weak inducer. *Staphylococcus aureus* isolates when tested in vitro may show constitutive resistance i.e. resistance to both Erythromycin and Clindamycin ($cMLS_B$), however isolates with inducible resistance are resistant to Erythromycin but appear sensitive to Clindamycin ($iMLS_B$). In such conditions if Clindamycin is used for treatment it may result in clinical failure. Inducible Clindamycin resistance can be easily detected by in vitro testing by keeping disc of Erythromycin 15µg and Clindamycin 2µg 15-20 mm on lawn culture of *Staphylococcus aureus* suspension adjusted at 0.5 Mac Farland. The plate is incubated at 37°C for 18-24 hours. A Flattening zone in area between the disc indicates that organism has inducible Clindamycin resistance, which is commonly known as D-test[7,8].

The present study aims to detect inducible clindamycin resistance in *Staphylococcus aureus* isolates, as no such study has been done previously at our institute, it will help physicians in selection of drugs for appropriate treatment and better clinical outcome.

MATERIALAND METHODS-

This study was conducted over a period of 6 months from Dec 2018 to May 2019 in Microbiology department at a tertiary care hospital in Solapur, Maharashtra. The study was approved by institutional ethical committee. A total of 269 non duplicate isolates of *Staphylococcus aureus* were obtained from various clinical samples like Pus/wound swab, blood, urine, tracheal aspirate and pleural fluid. The isolates were identified as *Staphylococcus aureus* using standard biochemical test[9]. Antibiotic susceptibility testing was done by Kirby baeur disk diffusion test. Methicillin resistance was detected using 30µg Cefoxitin disc and interpretation were made as per CLSI guidelines[10] 2018. Along with routine antibiotic susceptibility testing, three different phenotypes were also interpreted only for those strains which were resistant to Erythromycin. A Lawn culture of the test isolate was

adjusted to 0.5 Mc Farland concentration on Mueller Hinton agar. Disc of Erythromycin (15µg) and Clindamycin (2µg) were placed 15-20 mm apart if after overnight incubation flattening of zone of inhibition was observed towards Clindamycin, it was observed towards clindamycin, it is known as D test positive[10].

Test Interpretation:

a. The isolate is sensitive to Erythromycin and Clindamycin was considered susceptible phenotype.
b. Erythromycin resistant and Clindamycin sensitive isolate with no D- zone known as MS phenotype.
c. Erythromycin resistant and Clindamycin sensitive isolates (D-zone present), I MLS_B phenotype.
d. Erythromycin and Clindamycin resistant isolate $cMLS_B$ phenotype.

RESULTS-

Among 269 *Staphylococcus aureus* isolates MRSA were 66 (24.53%) while MSSA were 203 (75.5%). Majority of MRSA (n=66) & MSSA (n=203) isolates were from patients admitted in the hospital (IPD) which were 61 (92.42%) and 155 (76.4%) respectively. *Staphylococcus* isolates were predominantly isolated from pus/wound swab 135 (50.2%) followed by blood 62 (23.04%), urine 58 (22%), tracheal aspirate 09 (3.34%) and pleural fluid 03 (1.11%). Among *Staphylococcus aureus* isolates MS phenotype accounted for 13.01% of the isolates, iMLSB phenotype were 15% while cMLSB phenotype seen in 20.44% of the isolates. On comparison between MRSA and MSSA isolates cMLSB phenotype and iMLSB phenotype is common among MRSA. Among MRSA $cMLS_B$ seen in 39.4% isolates and $iMLS_B$ in 30.33% isolates respectively as compared to MSSA where $cMLS_B$ was seen in 16.74% isolates while $iMLS_B$ phenotype was seen in 10% of the isolates. MS phenotype in MRSA was seen in 15.2% of the isolates while in MSSA MS phenotype seen in 10% of the isolates.

DISCUSSION

It is important to accurately identify and report *Staphylococcus aureus* isolates and their antibiotic susceptibility pattern as this may change the course of treatment and clinical outcome in patient. Prevalence of *S. aureus* isolates and its antibiotic sensitivity pattern may vary depending upon geographical region, patient's age, sample origin, source of strain. The prevalence of inducible Clindamycin resistance may be variable even between different hospitals due to population under study and local prescribing trends of the hospitals.

In the present study overall incidence of inducible Clindamycin resistance. In *Staphylococcus aureus* is 15%. Studies done by various workers have shown variation in $iMLS_B$ phenotype prevalence rate. Study done by Rahbor et al[11] showed prevalence of $iMLS_B$phenotype to be 10.8% while study done by Majtaba Moosavian et al[12] showed quite high prevalence of $iMLS_B$ phenotype of 32.3%. Jethwani et al[13] showed 43% of *S. aureus* isolates of $iMLS_B$ phenotype which is quite high as compared to our study.

In the present study $iMLS_B$ was predominantly seen in 30.33% of MRSA isolates while MSSA isolates where $iMLS_B$was seen were 10% of isolates.

In the present study majority of patients with MRSA infection were seen in indoor patient both $iMLS_B$ and $cMLS_B$ phenotypes were commonly seen among MRSA isolates. In the present study sensitive "s" phenotype was seen in 51.55% of *Staphylococcus aureus* isolates while MS phenotype was seen in 13.01% of *Staphylococcus aureus* isolates. "S" phenotype was more common among MSSA isolates. In the present study $cMLS_B$ in MRSA isolates accounted to 39.4% as compared to MSSA where $cMLS_B$ was seen in 16.74% of *Staphylococcus aureus* isolates.

CONCLUSION

High prevalence of Clindamycin resistance, constitutive or inducible resistance is commonly encountered now-a days, which may be due to misuse or overuse of drugs. Antibiotic susceptibility testing is essential and simple method for detection of inducible and constitutive Clindamycin resistance and should be routinely reported by the laboratories in order to streamline the treatment options for the clinicians for better patient outcome.

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