Prevalence of Different Resistance Pattern in Staphylococcal Aureus Isolates From Tertiary Care Hospital

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Abstract

Introduction: Staphylococcus aureus is common isolated pathogen from clinical specimen with increasing trend of antimicrobial resistance. Staphylococcus aureus and Coagulase-negative Staphylococci (CoNS) infections have become common among both hospitalized and non-hospitalized patients.

Aim: The aim of this study was to determine the inducible clindamycin resistance among the MRSA and MSSA.

Material and Method: This study was prospective study which includes the entire sample obtained in the Department of Microbiology laboratory from Tertiary Care Hospital with bacterial infection and confirmed by positive culture. The standard microbiological protocol for detection of inducible clindamycin, Disc approximation method (D–test) was performed.

Result: Out of 124 Staphylococcus aureus isolates, 17 (13.7%) were inducible clindamycin resistant; 6 (35.29%) from (cefoxitin susceptible) MSSA and 11 (64.71%) from (cefoxitin resistance) MRSA.

Conclusion: The incidence of inducible clindamycin resistance among the Staphylococcus aureus was high. This high rate screen among the MRSA and MSSA provokes the necessity of performing the D- test before starting the antibiotic therapy.

Keywords: *Inducible Clindamycin Resistance (ICR), Staphylococcus aureus, MRSA, MSSA, D-test.*

Introduction

Staphylococcus aureus and Coagulase-negative Staphylococci (CoNS) infections have become common among both hospitalized and non-hospitalized patients. Staphylococcus aureus is one of the pyogenic bacteria most often infecting humans[1]. The development of Staphylococcoi resistance to antimicrobial agents is a growing concern.Staphylococcus aureus is known for acquiring antimicrobial resistance promptly after the introduction of new antibiotics[2].Reports of methicillin resistance in Staphylococcus aureus followed rapidly upon the introduction of methicillin in 1961. MRSA are present worldwide today, and most are resistance to multidrug[3]. Study of early isolates of MRSA showed that a key genetic component responsible for resistance, *mecA,* is not native to the *Staphylococcus aureus* genome. The cassette mec (SCCmec) of Stapylococcal chromosome has been characterized as a novel, mobile

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resistance factor which differs from both transposons and bacteriophages[4].

MRSA usually spreads by clones; however, the mec gene is known to have been transmitted between Staphylococcus aureus strains, and probably between other staphylococcal species. Nevertheless,it is noteworthy that some forms of SCCmec bear various additional genetic elements (Tn554, which encodes resistance to macrolides, clindamycine and streptogramine B; and pT181, which encodes resistance to tetraclines) which may confer resistance to additional groups of antibiotics; these genetic elements are especially common in HA-MRSA[5]. Additionally, strains that are resistance to erythromycin will easily become Clindamycin resistance due to inducibility of resistance. Clindamycin, a protein synthesis inhibitor, is a frequent therapeutic choice for staphylococcal infections, especially infections of the skin and soft tissue, and as an alternative in patients with penicillin allergies[6].

As for the central nervous system, it does have exceptional tissue penetration[7]. The drug accumulates in abscesses, and no changes to renal dosage are needed. Good oral absorption makes it convenient for outpatient prescription or as follow-up drug after intravenous therapy [6]. Resistance to this drug, however, is yet another problem. Staphylococcal resistance to clindamycin can be either inducible or constitutive (iMLS $_B$ - inducible Macrolide-Lincosamide-Streptogramin B resistance). It is known that treatment with clindamycin in patients harboring iMLS B Staphylococci leads to the production of constitutive resistance, which in turn leads to therapeutic failure[8]. Inducible clinamycin resistance test can be detected by simple test known as Disk approximation test or D-test[9]. The optimal inter disk distance between the antibiotics is not yet clear, and the Clinical and Laboratory Standards Institute (CLSI) suggests a range of 15 to 20 mm separation of the disc[10].

Aims and Objective: The aim of the present study is to know the inducible clindamycin resistance among the MRSA and MSSA isolates from Tertiary Care Hospital.

Material and Method

Study Site and Population: This study was conducted in Department of Microbiology at Shalinitai Meghe Hospital and Research Centre, Wanadongri, Nagpur in collaboration with Jawaharlal Nehru Medical College (Datta Meghe Institute of Medical Sciences), Wardha, from November 2019 to April 2020.

This study was prospective study which includes all the sample obtained in the Department of Microbiology laboratory from the patients attending OUT patient Department and IN patient Department of Shalinitai Meghe Hospital and Research Centre with bacterial infection and confirmed by positive culture.

Isolation, Identification and AST of Staphylococcus aureus: The samples received in the microbiology lab were inoculated on Blood agar and Mac Conkey's agar, incubated for 24 hours at 37^0C for staphylococcal isolation.

Patients with clinical infection but organisms not grown in the culture or organism expect Staphylococcus auresus, who has taken antibiotic before sample collection, were excluded from the study. Total of 124 Staphylococcal species were isolated and confirmed by gram stain and various biochemical tests like catalase test, oxidase test, coagulase test, mannitol salt agar, etc. Antibiotic susceptibility test (AST) was examined on Mueller-Hinton agar (MHA) plateby using modified Kirby-Bauer disc diffusion method following CLSI guidelines[11] with commercial antibiotic discs (Hi Media).

Detection of MRSA and MSSA: Screening of Methicillin Resistant was performed by Cefoxitin (30µg) disc diffusion method and interpreted according to CLSI guideline[11]. The isolates which gave zone of inhibition(ZOI) $\leq$ 21mm were Methicillin Resistant (MRSA) and isolates with ZOI $\geq$ 21 mm were Methicillin Susceptible (MSSA).

Detection of inducible Clindamycin (D-Test): For detection of inducible clindamycin, Disc approximation method (D – test) was performed as ruled by CLSI 2019. Clindamycin disc was placed at a measured distance (between 15-26 mm) from erythromycin disc and incubated.Appearance of flattening of the clindamycin zone adjacent to the erythromycin disk ['D' shape] was classified as D-test positive.[9]

Quality Control: Quality of each test was performed by using standard protocol Staph *aureus* ATCC 25923 an *Escherichia coli* ATCC 25922 were respectively used as positive and negative control

Result

Prevalence of Inducible Clindamycin-resistance: The prevalence of Inducible Clindamycin Resistance

was found to be 13.7% among the *Staphylococcus aureus* isolated.

Frequency of Clindamycin Resistant isolates: There was no any observation of constitutive clindamycin resistance among the *Staphylococcusaureus* isolates. However, erythromycin-induced clindamycin-resistance (ICR) was observed among 17 isolates (13.7%): six (35.29%) of those ICR positive or D-effect positive (D+) isolates were susceptible to cefoxitin (MSSA) and eleven (64.71%) were resistant to cefoxitin (MRSA). However, distribution of inducible clindamycin resistance among MSSA and MRSA was statistically insignificant (p value > 0.05).

Table No. 1: Distribution of Inducible clindamycin resistance among MSSA and MRS

Methicillin-resistance	ICR screening		Total	P value
Screening	D+	D-		
MSSA	6	62	68	0.018
MRSA	11	45	56	
Total	**17**	**107**	**124**	

Discussion

Staphylococcus aureus is one of the common human pathogens capable of causing a large variety of infection.The incidence of invasive infections has been raising with emergence of community acquired (CA) and hospital-acquired (HA) methicillin-resistant Staphylococcus aureus (MRSA)[12]. In particular in penicillin-allergic patients, clindamycin is an alternative option for mild to moderate MRSA infections. Sub inhibitory concentration of erythromycin,however, is a common resistance inducer to inducible clindamycin resistance (ICR)[13,14].

The recent studies reveal that clindamycin has become an excellent drug of choice for the staphylococcal infections specially skin and soft tissue infections. It has been used as alternative drug for the penicillin allergic patients[6].

However, the differentiation of inducible-clindamycin resistance (iMLSb phenotypes) from other type of resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant *Staphylococcus aureus* isolate. Clindamycin resistance may be either of *Erm*-mediated or M*srA*-mediated. *MsrA-mediated* resistance is due to efflux-pump mechanism. *ErmA-mediated* resistance of clindamycin may be either constitutive or inducible[6].

But when induction test was performed, 17 (13.7 %) of the isolates showed D-effect, i.e. they were inducible clindamycin resistant strains (iMLSB phenotype). Sixof those strains were MSSA and eleven were MRSA. Absence of constitutive resistance but still presence of 13.7 % of inducible resistance to clindamycin provokes the importance of D test, if clinical failure is not anticipated[15]. The observation of D-effect to be more among MRSA in comparison to MSSA indicate that clindamycin may not be suitable to be used as empirical therapy against.

In the study of Michael Z. David et al[16] showed prevalence of MRSA among adult 32.81% and among children 17.49% which is greater than this study as shows prevalence of MRSA was 13.7%. Iraj Sedighi et al[17] studied reported Inducible clindamycin resistance of 37.5% of CA-MRSA isolates at the time of admission and 22.2% of HAMRSA isolates at discharge whereas in this study showed 13.71% Inducible clindamycin resistance of MRSA AND MSSA.

Another study of Pal N et al[18] showed Inducible clindamycin resistance of 43.56% in MRSA (Methicillin resiatant staphylococcus aureus), 6.93% MSSA (Methicillin sensitive staphylococcus aureus), 43.56% MRCNS (Methicillin resiatant coagulase negative staphylococcus aureus) and 6.0% MSCNS (Methicillin sensitive coagulase negative staphylococcus aureus) whereas this study showed 35.29% MSSA and 64.71% MRSA out of total Inducible clindamycin resistance. According to the study of Naima Fasih et al[19] showed Inducible clindamycin resistance of 62% among MRSA staphylococcal isolates which was greater than this study.

A studied done by Mukesh Patel et al[20] showed the presence of MLSBi in 212 (52%) of the isolates overall, with 139 (50%) MRSA and 73 (60%) MSSA isolates exhibiting MLSBi which was greater than this study as this study showed showed 35.29% MSSA and 64.71% MRSA out of total Inducible clindamycin resistance[21].

Conclusion

The incidence of inducible clindamycin resistance among the Staphylococcus aureus was high. This high rate screen among the MRSA and MSSA provokes the

necessity of performing the D- test before starting the antibiotic therapy. The observation of D-effect to be more among MRSA in comparison to MSSA indicate that clindamycin may not be suitable to be used as empirical therapy against.

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