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DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS FROM A TERTIARY CARE HOSPITAL IN BIJAPUR, KARNATAKA STATE OF INDIA

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ABSTRACT

The present study aimed at in-vitro detection of macrolide resistant phenotypes of methicillin resistant *Staphylococcus aureus* and interpretation of susceptibility tests to guide therapy. The study included 100 strains of Staphylococcus aureus out of which 67 strains were methicillin sensitive *Staphyloccus aureus* (MSSA) and 33 strains were methicillin resistant *Staphyloccus aureus* (MRSA). Overall 20 (20%) strains of *Staphylococcus aureus* exhibited inducible resistance to clindmycin by double disc diffusion test . Of these 20 strains, 15(75%) were methicillin resistant, while 5 (25%) were methicillin sensitive.

Keywords: - Methicillin Resistant *Staphyloccus aureus* (MRSA), Inducible Clindamycin Resistance, Double Disc Diffusion test.

Key Messages: -

1. Detection of Methicillin Resistance in ***Staphyloccus aureus*** .
2. Detection of inducible Clindamycin resistance by double disc diffusion before starting therapy.

INTRODUCTION

Clindamycin is considered as an useful alternative drug in penicillin allergic patients and in the treatment of skin and soft tissue infections caused by *Staphylococcus aureus.* It has excellent tissue penetration (except for central nervous system), accumulates in abscesses, no dosage adjustments are required in presence of renal diseases.[1] Good oral absorption of clindamycin makes it an attractive option for use in outpatients or as follow-up treatment after intravenous therapy (de-escalation).[2]

Methicillin resistant *Staphylococcus aureus* (MRSA) are increasingly being reported as multidrug resistant with high resistance to macrolides (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin) leaving very few therapeutic options.[3] Newer antibiotics like vancomycin, linezolid, quinupristin, dalfopristin have been advocated in the management of such isolates , but the recent reports of resistance to these agents raise real concerns over how long this uniform susceptibilities will hold good.[3-5] Interestingly,all vancomycin intermediate *Staphylococcus aureus (VISA)* and vancomycin resistant *Staphylococcus aureus (VRSA)* isolates reported so far from United States have been sensitive to trimethoprim – sulfamethaxazole and tetracycline raising the probability that the older agents may have some efficiency in the treatment of MRSA infection.[3]

For years, macrolides have been used as an alternative to the use of penicillins and cephalosporins in the treatment of infections caused gram positive bacteria, but the worldwide development of macrolide resistance has now limited the use of these antibiotics.[6] Lincosamides (clindamycin) are generally not recommended in erythromycin resistant *Staphylococcus aureus* as they share a similar mode of action and have common resistance mechanisms. However, recent studies on treatment of erythromycin resistant S,aureus using clindamycin show the possible role of clindamycin in some of erythromycin resistant isolates, as there are multiplicity of resistance mechanisms and diversity of phenotypic expression.[7-10]

Staphylococcus aureus can be resistant to erythromycin through either erm gene or msrA genes. Strains with erm mediated erythromycin resistance may possess inducible clindamycin resistance but may appear susceptible to clindamycin by disc diffusion.[1] So if this inducible clindamycin resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in patients with true clindamycin sensitivity.[2] So the present study is aimed at in-vitro detection of clindamycin susceptibility in macrolide resistant phenotypes of S. aureus.

MATERIAL AND METHODS

A total of 100 *Staphylococcus aureus* isolates were studied which were collected from pus, blood, wound swabs and sputum between April 2011-June 2011. The isolates were identified as *Staphylococcus aureus* by gram stain, slide and tube coagulase test, mannitol fermentation test, phosphatase test, deoxyribonuclease test. Methicillin resistance was tested on mueller - hinton agar with 4% sodium chloride using cefoxitin disc by kirby-bauer disc diffusion method. Plates were incubated 37^0 C for 24 hrs. A zone size of 18mm or more was considered sensitive as per CSLI guidelines.[11]

Erythromycin and clindamycin double disc susceptibility testing

Inducible clindamycin resistance was detected by double disc diffusion test using 15µg erythromycin disc placed 20mm from the centre of 2µg clindamycin disc. Inhibition of circular zone around the clindamycin disc was considered positive for inducible resistance (Flattening of the zone towards erythromycin disc).[12]

RESULTS

Out of 100 strains of *Staphylococcus aureus*, 67 were methicillin sensitive *Staphylococcus aureus* (MSSA) and 33 strains were methicillin resistant *Staphylococcus aureus* (MRSA). Overall 20 strains of *Staphylococcus aureus* (20%) exhibited inducible resistance to clindamycin. Of these 20 strains 15(75%) were methicillin resistant while 5(25%) were methicillin sensitive.

DISCUSSION

Resistance to macrolide, lincosamide, streptograminB (MLS_B) antibiotics most commonly results from acquisition of erythromycin resistant methylase genes (erm genes) which encode enzymes that methylate the 23S rRNA. The overlapping binding sites of macrolide, lincosamide, streptograminB in 23S rRNA account for cross-resistance to the three classes of drugs. Expression of MLS_B resistance can be constitutive or inducible. In inducible resistance, the bacteria produce inactive mRNA that is unable to encode methylases. The mRNA becomes active only in the presence of a macrolide inducer. By contrast, in constitutive expression, active methylated mRNA is produced even in the absence of an inducer.[6] The strains harbouring an inducible erm gene are

resistant to the inducer but remain susceptible to the non-inducer macrolide and lincosamide, but the use of such non-inducer antibiotic such as clindamycin, can lead to selection of constitutive mutants at frequencies of 10^{-7}cfu.[9,1o] Besides methylation of the target site, active efflux of macrolides is encoded by plasmid borne msr (A) gene which has specificity for 14 and 15 membered macrolides (erythromycin, azithromycin, clarithromycin) and type B streptogramin. Clindamycin is neither an inducer nor a substrate for the pump, and thus the strains are fully susceptible to this antimicrobial.[6]

In our study 20% of the strains exhibited resistance to clindamycin, while it was 24% in the study done by R Goyal et al.[13] So our observation suggest that, susceptibility testing of *Staphylococcus aureus* should include the disc diffusion induction test, which will facilitate the identification of isolates exihibiting inducible or constitutive resistance to MLS_B phenotypes. Patients with infections caused by such isolates can probably be treated with clindamycin without expectation of resistance developing during therapy.

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Fig: Flattening of zone towards erythromycin disc was considered positive for inducible resistance.
Fig No 1 : Positive

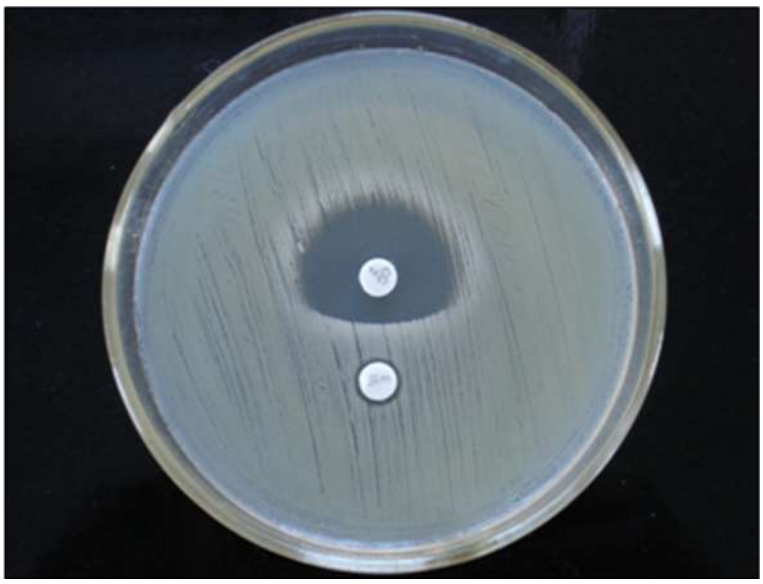

Fig No 2 Negative