ORIGINAL RESEARCH PAPER

Microbiology

INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCAL* ISOLATES FROM OCULAR SAMPLES

KEY WORDS: macrolide-lincosamide-streptogramin B resistance, cMLSB, iMLSB, ocular, clindamycin, D-test

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ABSTRACT

Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics. This study was undertaken to detect the presence of inducible clindamycin resistance among clinical isolates of *Staphylococci* from ocular samples in a tertiary care eye hospital. The detection of inducible clindamycin resistance was performed by D-test using erythromycin and clindamycin discs as per CDC guidelines. Among 217 ocular samples 100 isolates of *Staphylococci* were studied, 8 (8%) showed inducible clindamycin resistance and belonged to the iMLSB phenotype. Among the iMLSB phenotypes, 1 isolates was methicillin-resistant Staphylococcus aureus, 1 was methicillin-sensitive *Staphylococcus aureus* and 6 were coagulase negative *Staphylococci*. The test for inducible resistance to clindamycin should be included in the routine antibiotic susceptibility testing of *Staphylococci* for ocular samples, as it will help in guiding the therapy.

INTRODUCTION

Genus Staphylococcus belongs to family Micrococcaceae. There are approximately 39 species and 21 subspecies within the genus Staphylococcus. Staphylococcus species are gram positive cocci, which are catalase positive, non-motile and non-spore forming. Several of the coagulase-negative Staphylococci (CoNS) species listed may be encountered in clinical specimens[1]. Staphylococcus is the most common pathogenic organism[2].

The importance of Staphylococcus aureus (S. aureus) as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections in hospital as well as in community settings, is its extraordinary potential to develop antimicrobial resistance[3]. Many Methicillin Resistance Staphylococcus aureus (MRSA) isolates are sensitive to in vitro Clindamycin[4]. Subconjunctival injection of Clindamycin phosphate, semi synthetic antibiotic that is effective in the treatment of infections caused by Gram-positive bacteria, was found to be highly concentrated in the choroid, iris, and retina of the eye.

The Macrolide Lincosamide Streptogramin B (MLSB) is a group of antibiotics commonly used in the treatment of Staphylococcal infections, but unrestricted consumption has increased the rate of resistance to these drugs. In this field, two main mechanisms are involved:

1) Active efflux mechanism encoded, affecting macrolides and type B streptogramins, which results in MS phenotype (resistance to macrolides and group B streptogramins and susceptibility to lincosamides); and

2) Target site modification via 23S rRNA methylation which confers constitutive or inducible resistance to MLSB agents Strains with constitutive MLSB resistance (cMLSB) phenotype show resistance to all MLSB drugs without any need to an inducer. In contrast, in inducible MLSB resistance (iMLSB), exposure to a strong methylation inducer (e.g., erythromycin) results in the expression of resistance to lincosamides and streptogramins B [6]. However, it has been demonstrated that spontaneous mutations can transform iMLSB phenotype to cMLSB, without the presence of an inducer[7].

To detect iMLSB isolates, there is a particular method. A Clindamycin disc 2µg with an approximate distance of 15-20 mm is placed beside Erythromycin 15 µg (D-test) (see fig-1). By this means constitutive macrolide resistance can be distinguished from inducible resistance. Constitutively resistant strains grow up to both discs. With inducible resistance, there is a zone around the clindamycin disc which is flattened on the side next to erythromycin disc, which is the inducer[8].

There are no studies exclusively on ocular samples, this study was conducted at a tertiary care eye hospital; Hyderabad to detect inducible clindamycin resistance in ocular isolates.

MATERIAL AND METHODS:

Various ocular samples were collected under aseptic conditions. The different samples were conjunctival swab, corneal scraping, anterior chamber tap, vitreous tap, etc. The samples were inoculated on Blood agar, MacConkey agar and Brain Heart infusion broth. Plates were incubated at 37^{0}C for 18-24 hrs. Staphylococcus isolates were identified by cultural characteristics, gram stain, catalase, tube and slide coagulase.

A 0.5 Mc Farland standard suspension of isolate was made and lawn culture done on to Muller Hinton Agar for Antibiotic susceptibility testing. Cefoxitin disc (30µg) was used to test for methicillin resistance. Plates were incubated at 37^{0}C for 18-24 hrs and zone diameters were measured.

A Clindamycin (CD) disc (2µg) with an approximate distance of 15 mm is placed beside Erythromycin (Er) disc (15µg). After incubation plates were examined for flattened zone (D zone). (See fig-1)

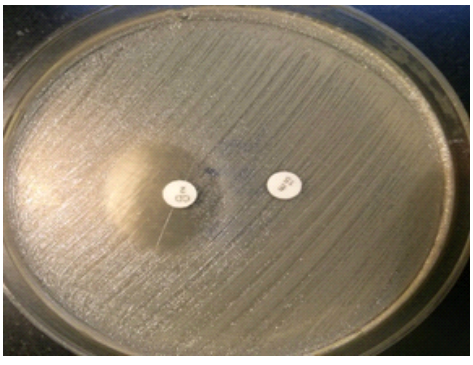

Fig 1: D test showing D zone

RESULTS

A total of 217 ocular samples were cultured. Out of 217 cultures, 109 showed growth of a pathogenic organism. Among those 109 isolates, 100 were Staphylococcal isolates.

Methicillin resistance was seen in 21 isolates of the 100 Staphylococcal isolates (21%).

Erythromycin and clindamycin was sensitive in 74 (74%) isolates.

Erythromycin resistance and clindamycin sensitive was seen in 4 (4%) isolates. Both Erythromycin and clindamycin resistances was seen in 14 (14%) isolates. D-zone was seen in 8 (8%) isolates. (See table 1)

TABLE – 1

	Er-S, CD-S	Er-R, CD-S	Er-R, CD-R (cMLSB)	D-zone (iMLSB)
MSSE	58	1	9	5
MRSE	10	3	5	1
MSSA	5	0	0	1
MRSA	1	0	0	1
Total	74	4	14	8

MSSE- Methicillin-sensitive Staphylococcus epidermidis, MRSE-Methicillin-resistant Staphylococcus epidermidis, MSSA-Methicillin-sensitive Staphylococcus aureus, S- Sensitive, R-Resistant

DISCUSSION:

Staphylococcus is the most common organism as a carrier and pathogen in any clinical sample. Similarly in ocular samples, Staphylococcus is most commonly isolated and the common species is S.epidermidis. Antibiotics used for the treatment of ocular infections include fluoroquinolones, macrolides, aminoglycosides, glycopeptides, tetracyclines, chloramphenicol, Neosporin. These are usually available as topical eye drops. Others such as amikacin, clindamycin, vancomycin, can be administered as intraocular injections, depending on the clinical condition. Other routes of administration such as subconjunctival injections are rarely used. The aminoglycosides have been the mainstay in the treatment of ocular infections. However, increasing resistance has limited their use in recent years in the treatment of Staphylococcal infections[9].

Staphylococcus was found resistant to the antimicrobial action of various antibiotics such as penicillin, methicillin, vancomycin, clindamycin and linezolid. Inducible Clindamycin resistance can be detected by D-test, automated methods like VITEK2 and molecular methods (erm gene).

Till date, scientific workers have performed studies on inducible clindamycin resistance in Staphylococci isolates from various clinical samples, however very few of them included eye samples. The present study was carried out exclusively with eye samples.

Uzunović et al[3] collected samples from both patients and carriers. They reported 6 isolates of iMLSB out of 142 isolates in both the groups. Among the 6 isolates, 5 were MSSA and 1 was MRSA. In the present study, 8 (8%) isolates out of 100 showed iMLSB and among those 5 were MSSE and rest were MRSE, MRSA, MSSA. This dominance of MSSE is because in ocular samples the most common isolate is Staphylococcus epidermidis.

Various workers have reported iMLSB from 2 - 45%. Mohammad Aghazadeh et al[6] reported 8.9% iMLSB in MRSA from pediatric samples. Gurdal Yilmaz[10]et al reported 21.9% iMLSB from various samples.

Mohammad Motamedifar[11] reported 13.4% iMLSB from all clinical samples. Kumurya[12] et al reported 2.4% iMLSB and antibiotic susceptibility of all the isolates. Aleksandra[13] et al reported 39% iMLSB, the predominant genes associated with macrolide resistance among S. aureus were the erm (C) in 55% isolates and the erm (A) in 40% isolates. Delialioglu[14] et al reported 45% iMLSB.

CONCLUSIONS

Resistance to most groups of antibiotics is increasing. Antibiotic resistance among ocular pathogens is a challenge to ophthalmologists. Macrolide containing eye drops are the most commonly used to treat ocular infections. D-test is a simple and easy way to detect inducible Clindamycin resistance. The incidence of inducible resistance varies in different places, samples, age groups, patients and carriers. So it is necessary to test for inducible resistance and note its incidence

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