Incidence of Methicillin resistance in *Staphylococcus* species and their antibiotic susceptibility pattern, in ocular samples at a tertiary care eye hospital, Hyderabad

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**Abstract:** The aim of this study is to find the cause of increase in number of cases with methicillin resistance in *Staphylococcus* isolates. A retrospective analysis was done on all *Staphylococcus* isolates which were reported at Sarojini Devi Eye Hospital, Hyderabad from January 2016 to June 2016. Total of 468 samples were tested out of which *Staphylococcus* isolates were 218. Among them, methicillin resistance was seen in 47 (21.5%) of samples. 35 (74.4%) samples were collected on outpatient basis and rest 12 (25.6%) were from inpatient. 36 patients were males and 11 patients were females. 95.74% sensitivity was seen for Moxifloxacin. Methicillin resistance in *Staphylococcus* is becoming increasingly prevalent, and ophthalmologists will see more ophthalmic MRSA infections. Microbiologist must identify Methicillin resistance in *Staphylococcus* isolates, and Ophthalmologists must adjust empirical treatment regimens where Methicillin resistance is endemic, and take steps to control emergence of resistant organisms in both inpatient and outpatient practices.

**Keywords:** MRSE, ocular, antibiotic susceptibility, MRSA, Cefoxitin.

**INTRODUCTION:**

Historically, the genus *Staphylococcus* was included with the genus *Micrococcus* in the family Micrococccaceae. There are approximately 39 species and 21 subspecies within the genus *Staphylococcus*. *Staphylococcus* species are gram positive cocci, which are catalase positive, nonmotile and non-sporo forming. Several of the coagulase-negative *Staphylococci* (CoNS) species listed may be encountered in clinical specimens[1].

The most common organism in normal and infective eye is *Staphylococcus*[2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1960, the same year that methicillin was developed, and it is still a bacterium that is frequently detected in hospitals worldwide. In the field of ophthalmology, ocular infections such as dacryocystitis, conjunctivitis and keratitis are often reported. Infectious keratitis and endophthalmitis caused by MRSA are increasing problems throughout the world. In recent years, the number of multidrug-resistant MRSA strains showing resistance to other antibiotics such as aminoglycosides, minocycline and fluoroquinolones has been on the rise[3]. In addition to being a nosocomial pathogen MRSA has become a community pathogen. Ocular Methicillin resistance *Staphylococcus* infections can be aggressive and cause severe ophthalmic disease including blindness[4]. *Staphylococcus epidermidis* has developed the same bacterial resistance as *S. aureus* and has now been termed as methicillin-resistant *S epidermidis* (MRSE). Moreover, reports have shown that MRSE can cause ophthalmic infections and blindness[3]. The aim of this study is to see the incidence of Methicillin resistance in *Staphylococcus* isolates in a tertiary care eye hospital and its antibiotic susceptibility from ocular samples.

**MATERIAL AND METHODS:**

From January 2016 to June 2016, various ocular samples were collected under aseptic conditions. The different samples were conjunctival swab, corneal scraping, AC tap, vitreous tap, etc. The samples were inoculated on Blood agar, MacConky agar, and Brain agar.
Heart infusion agar. *Staphylococcus* isolates were identified by cultural characteristics, gram stain, catalase, and tube and slide coagulase. Routine antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method for the following antibiotics disc: Chloromphenicol (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Gatifloxacin (5µg), Gentamycin (10µg), Moxifloxacin (5µg), and Tobramycin (10µg). A 0.5 Mc Farland standard suspension of isolate was made and lawn culture done on Muller Hinton Agar. Plates were incubated at 37°C for 18hrs and zone diameters were measured. All isolates were tested for methicillin resistance by cefoxitin (Cx-30µg) disc.

**RESULTS:**

Total of 468 samples were collected during six months period. Out of which *Staphylococcus* isolates were 218. Among them, methicillin resistance was seen in 47 (21.5%) of samples (table1).

Table 1: Incidence of methicillin resistance

<table>
<thead>
<tr>
<th>Methicillin resistance</th>
<th>S.aureus</th>
<th>CONS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (1.3%)</td>
<td>44 (20.19%)</td>
<td>47 (21.5%)</td>
<td></td>
</tr>
</tbody>
</table>

35 (74.4%) samples were collected on outpatient basis and rest 12(25.6%) were from inpatient. 36(76.5%) patients were males and 11(23.5%) patients were females (%).

**DISCUSSION:**

Approximately 20% of the population carries *S.aureus* persistently, 60% are intermittent carriers, and 20% are non-carriers who rarely harbor *S.aureus*. Historically, MRSA pathogens were Antibiotic susceptibility pattern of Cefoxitin resistant *Staphylococcus*

**Table 2: Antibiotic susceptibility of methicillin resistant *Staphylococcus***

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistance</th>
</tr>
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<tbody>
<tr>
<td>Chloromphenicol</td>
<td>91.48%</td>
<td>8.52%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>19.14%</td>
<td>80.86%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>46.8%</td>
<td>53.2%</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>29.78%</td>
<td>70.22%</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>31.91%</td>
<td>68.09%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>82.9%</td>
<td>17.1%</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>95.74%</td>
<td>4.26%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>80.85%</td>
<td>19.15%</td>
</tr>
</tbody>
</table>

Almost exclusively isolated from hospitals or hospital-associated facilities. However, an increasing number of MRSA cases have been reported in individuals with no known risk factors for MRSA colonization, such as admission to a hospital, surgery, and contact with a MRSA-colonized patient, intravenous drug use, or previous antibiotic exposure. These isolates, termed community acquired MRSA.

*Staphylococcus aureus* is reported to be the second most common pathogen causing bacterial
keratitis across the world. Of special concern are increasing reports of postoperative MRSA infection. MRSA keratitis, a serious and increasing complication following ophthalmic surgery, has been found after laser in situ keratomileusis and photorefractive keratotomy (PRK)[6, 7].

Coagulase-negative *Staphylococci* are the indigenous flora of our skin and mucous membrane. Coagulase-negative *Staphylococci* are isolated in large numbers from diverse ophthalmic conditions like chronic blepharitis, purulent conjunctivitis and suppurative keratitis. As they form the part of the normal flora, effective management may not be aimed at curtailing coagulase-negative *Staphylococci* (CoNS)[10].

*Staphylococcus epidermidis* has been reported with increasing frequency as a cause of bacterial endophthalmitis[11-15]. In the present study, the incidence of ocular Methicillin resistant *Staphylococcus* was 21.5%. Conjunctivitis is the most commonly reported manifestation and has been associated with long-term care units, especially in patients with neurologic impairment, nurseries and neonatal ICUs; and healthcare workers[16-18].

In South Florida by Cavuoto et al showed increased prevalence of MRSA and MRSE in conjunctivitis from 4.4% (1994-5) to 42.9% (2002-3) [19, 20]. Ching Hsi Hsiao et al.; reported the average rate of MRSA in S. aureus infections was 52.8% with a stable trend, whereas the annual ratio of CA-MRSA in ocular MRSA infections averaged 66.1% and tended to increase over the 10 year interval[21]. Aravind eye hospital reported 26% of Methicillin resistant *Staphylococcus* during the year 2012(January to December)[22]. Penny et al reported increase of S. aureus infections culture positive for methicillin resistantS aureus (MRSA) from 29.5% in 2000 to 41.6% in 2005[23]. Malathi et al.; reported 4.5% Methicillin resistant *Staphylococcus* from post-operative endophthalmitis[24].

Sotozono et al.; detected Methicillin-resistant S. aureus or MRSE was from 30 cases with ocular diseases over 5 year period from January 1996 to December 2000[25]. The S. epidermidis strains from conjunctivitis, CU, and endophthalmitis exhibited 46, 33.9, and 34.1% of oxacillin resistance respectively in a study conducted by Juárez et al. ;[26].

Antibiotic susceptibility patter of these isolates showed resistance to Ceftazidime 80.86%, Ofloxacin 70.22%, Gatifloxacin 68.09%, Ciprofloxacin 53.2%, Gentamycin 17.1%, and Chloromphenicol 8.52%. Manikandan et al.; reported resistance of Gentamycin 74.2%, Chloromphenicol 54.2%, Ciprofloxacin 40.0% and Ofloxacin 31.4%[10].

Bharathi et al.; reported the resistance in (CoNS) as Gatifloxacin 95%, Chloromphenicol 70%, Ofloxacin 43%, Gentamycin 41%, Ciprofloxacin 41%, and Ceftazidime 22%[27]. Vancomycin is known to be effective for treating MRSA/MRSE infections. MRSA isolated from ocular infections is often susceptible to chloramphenicol, fourth-generation fluoroquinolones and other antibiotics. Thus, it is preferable to use Vancomycin Ophthalmic Ointment 1% only for a short period of time and only for patients who specifically require this new drug[28].

Blanco et al.; studied Susceptibility of methicillin-resistant Staphylococci clinical isolates to Netilmicyn and other antibiotics commonly used in ophthalmic therapy and reported 27.9% MRSE and in MRSE group, 25% of the strains tested were resistant to C and MXF while 33%, 42% and 58% of the strains were resistant to LEV, AZM and TOB, respectively[29].

**CONCLUSION:**

MRSE infection accounts for 20.19% of *Staphylococcus* isolates in ocular samples in this study. They showed resistance to other drugs-Ceftazidime, Ofloxacin, Gatifloxacin and sensitive to Moxifloxacin, Chloromphenicol and Tobramycin. The incidence of Methicillin resistance in ocular samples is increasing. Antibiotic resistance among ocular pathogens is a challenge to the ophthalmologist. Vancomycin, a glycopeptide antibiotic, is known to be effective for treating methicillin resistance *Staphylococcus* infections.

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