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# Prevalence and antimicrobial susceptibility pattern of staphylococci and streptococci causing ocular infections from a tertiary eye care hospital, South India.

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## Abstract

**Gram positive cocci viz., streptococci and staphylococci are found to be more predominant among ocular infections. Treatment of bacterial eye infections may engross empirical therapy with topical ophthalmic broad spectrum antibiotic formulations which is a prevailing practice among ophthalmologists. Ocular samples were collected from patients attending a tertiary eye care hospital, Coimbatore, south India from a total of 1802 ocular samples viz., Gram positive cocci (n=329), other bacteria & actinomycetes (n=561) and fungi (n=716), were isolated. Out of 329 Gram positive cocci, a total of 200 isolates viz., *Streptococcus pneumoniae* (n=76), coagulase negative staphylococci (CoNs) (n=49), *Staphylococcus aureus* (n=40), *S. viridans* (n=33) and *S. pyogenes* (n=2). The results revealed that the patients belonging to the age group between 60-80 years were most affected and male preponderance was observed. The isolates were subjected to antibiogram analysis with 15 different antibiotics according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic viz., ampicillin, ofloxacin, gatifloxacin, levofloxacin, cefotaxime, cefazolin, piperacillin and vancomycin were found to effective against Gram positive cocci ocular infections.**

## Key words:

Eye, Infections, Staphylococcus, Streptococcus, Antibiotic susceptibility.

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## Introduction

Eye is a unique and necessary organ that is constantly exposed to the external environment. Infection and inflammation of the ocular regions may lead to blindness if prompt and appropriate therapy is not instituted [1]. Suppurative keratitis or corneal ulcer can cause corneal opacity & perforation and is the second most common cause for monoocular blindness in developing countries after cataract. Depending on the geographical location, the aetiological factor may be fungus or bacterium [2].

Corneal ulcer involves the disruption of the epithelial layer with involvement of the corneal stroma [3]. Bacterial endophthalmitis can occur following the introduction of an infectious agent into the posterior segment of the eye, causing intraocular infection and inflammation that despite appropriate therapeutic intervention frequently results in visual loss. It occurs more frequently following cataract surgery, the most commonly performed type of intraocular surgery. Intraocular lens implantation and penetrating keratoplasty (corneal transplantation) are the common ocular surgical procedures with a greater potential for intraocular bacterial contamination through wound leaks and subsequently lead to high risk of acute postoperative endophthalmitis [4].

The defence mechanisms present in the eye prevents the adverse activity of microorganisms within the ocular surface [5] and the infection due to trauma and other invasive procedures modify the normal flora leading to ocular infections [6]. Gram positive cocci are the most common opportunistic pathogens as a primary cause of bacterial eye infections. Staphylococci are normal inhabitants of skin and mucous membrane and have overcome most of the antimicrobial agents.

The genus Staphylococcus includes many pathogenic species in which *Staphylococcus aureus* and coagulase negative *S. aureus* (CoNS) are most important. Streptococci are normally found in respiratory, urinary and gastrointestinal tract, among which *S. pneumoniae* & *S. viridans* are common amongst ocular pathogens [7]. Coagulase-negative staphylococci are responsible for about 70% of post-operative endophthalmitis, followed by *S. aureus*, *S. viridans*, other Gram positive and Gram negative bacteria [4].

In most of the cases when the pathogen is not yet known, the choice of antimicrobial agents is commonly made empirically. Bharathi et al. [8] stated that an understanding of aetiological agents is vital to institute therapy and prevention of the disease entity and therefore it is necessary to carry out laboratory investigations including direct microscopy and culture. Once the organism has been identified, the effective antimicrobial agent is chosen according to the results of antibiotic susceptibility testing.

The data of *in vitro* antibiotic susceptibility testing will aid the clinicians to device an appropriate empirical treatment strategies for treating the ocular infections [9]. The purpose of this study was to analyse the prevalence of Gram positive cocci in ocular infections among the patients attending a tertiary eye care hospital, south India and to determine the *in vitro* susceptibility of these isolates to commonly prescribed antibiotics for ocular infections.

## Materials and Methods

### *Location and duration of study*

This study was carried out from September 2012 to December 2013 among the patients attending the Ocular Microbiology Department at Aravind Eye Hospital and Post Graduate Institute of Opthalmology, Coimbatore, Tamilnadu, India.

### *Collection and processing of ocular samples*

The samples were collected from cases suspected of having ocular infection after a thorough examination by an ophthalmologist. Conjunctival swabs from the preoperative screening were collected using a sterile swab under aseptic conditions. The corneal scrapings were collected from patients with clinically suspected keratitis using a sterile Kimura's spatula by an ophthalmologist.

The aqueous tap and vitreous tap specimens from the endophthalmitis cases were obtained by an ophthalmologist through aspiration with the help of sterile tuberculin syringe and 22 gauge needles. A part of the specimen was subjected to direct microscopy after Gram staining and the rest was inoculated in brain heart infusion broth (BHI) & streaked on 5% sheep blood agar and chocolate agar plates and incubated at 37°C for 24-48 h. A culture was considered positive if growth was obtained in at least two media [10,11,12].

### *Identification and characterization of Gram positive cocci*

The isolates were subjected to Gram staining for the presumptive identification of the cocci in chains and clusters. The isolates of staphylococci were analysed for coagulase production and mannitol fermentation. Streptococci were further tested for optochin sensitivity and bile solubility. Speciation of the isolates was carried out using standard biochemical test viz., indole production, methyl red, Voges-Proskauer test, citrate utilisation, oxidase test, catalase production test, glucose fermentation, nitrate reduction and esculin hydrolysis [12,13]. Representative isolates were further reconfirmed with KB004 HiStaph™ identification kit for CoNS and KB005A HiStrep™ identification kit for Streptococcus sp.

### *Antimicrobial susceptibility testing*

Antibiotic susceptibility test was performed on Muller Hinton agar in accordance with the procedure outlined by CLSI [14]. A total of fifteen antibiotics (HiMedia, Mumbai, India) belonging to eight different classes viz., Aminobenzyl penicillin (ampicillin; 2 µg), fluroquinolones (ciprofloxacin; 5 µg, ofloxacin; 5 µg, gatifloxacin; 5 µg, moxifloxacin; 5 µg & levofloxacin; 5 µg), cephalosporins (cefotaxime; 30 µg, cefuroxime; 30 µg & cefazolin; 30 µg), ureidopenicillin (piperacillin; 10 µg), glycopeptides (vancomycin; 30 µg), chloromphenical (10 µg), tetracycline (30 µg) and aminoglycoside (tobramycin; 10 µg & gentamycin; 10 µg), at varying concentrations were used for the analysis.

For inoculum preparation, few colonies of the test isolates were transferred aseptically into sterile BHI broth and incubated at 35°C for 2 to 5 h. The broth culture was adjusted to the turbidity equivalent of 0.5 McFarland standards. Sterile Muller Hinton agar plates (with 5% sheep blood for streptococci) was inoculated, the discs were placed and incubated as per the standard procedures [14]. After incubation, the plates were observed for the zone of bacterostatis. The reading was performed by measuring the diameter of the inhibiting zone around the disc, in agreement with the CLSI criteria for all antibiotics, with one of three resulting grades: resistant, intermediately sensitive, or susceptible. *S. pneumoniae* (ATCC 49619) and *S. aureus* (ATCC 29213) were used in every test for quality control. For the confirmation of methicillin resistance among *S. aureus*, disc diffusion test was performed with cefoxitime (30 µg) and oxacillin (1 µg) discs [14].

## Results

From 1802 culture positive ocular samples processed during the 16 months study period, 329, 561 and 716 accounted for Gram positive cocci, other bacteria & actinomycetes and fungi, respectively. Of 329 Gram positive cocci, 116 (35.25%), 112 (34.04%), 40 (12.15%), 36 (10.94), and 25 (7.59%) were from corneal ulcer/keratitis, preoperative screening, endophthalmitis, other infected cases (secondary infections, pus, scleral abscess, intra ocular lens culture, sutures, buccal space infection, etc.) and other cultures (mainly corneal button, eye ball and scleral rim), respectively.

From table 1, it is evident that highest number (n=60) of *S. pneumoniae* were isolated from corneal ulcer cases. Further, *S. pneumoniae* was the most frequent (n=133) Gram positive bacteria isolated during the study period. However, CoNS (n=20) was the major causative agent of endophthalmitis and also the second most frequently isolated Gram positive bacteria (n=92) next to *S. pneumoniae*. Among other infected cases, *S. aureus* was isolated in higher number (n=13) when compared to other isolates. *S. viridans* occurred more frequently in corneal ulcer (n=17), followed by preoperative screening (n=12) and endophthalmitis (n=10). Also, 2 isolates of *S. pyogenes* were obtained from corneal ulcer.

Overall, a total of 133, 92, 56, 46 and 2 isolates of *S. pneumoniae*, CoNS, *S. aureus*, *S.viridans* and *S. pyogenes*, respectively were obtained in the study. From these isolates, a total of 200 strains viz., 76 *S. pneumoniae*, 49 CoNS, 40 *S. aureus*, 33 *S.viridans* and 2 S. pyogenes isolates were subjected for further analysis. Among the selected cases with Gram positive cocci eye infections, male preponderance (n=123) was noted. The age groups of the target patients ranged between 1 month and 80 years and it was found that the individuals between 60 and 80 years was most affected with Gram positive cocci eye infections (Table 2).

***Table 1:** Types and number of isolates of Gram positive cocci from ocular infections.*

| Gram positive cocci isolates | Type of eye infection | | | | | |
|---|---|---|---|---|---|---|
| | Corneal ulcer | Preoperative screening | Endophthalmitis | other Infected cases | Other cultures | Total (%) |
| *S. pneumoniae* | 60 | 57 | 4 | 9 | 3 | 133 (40.42%) |
| CoNS | 15 | 33 | 20 | 8 | 16 | 92 (27.96%) |
| *S. aureus* | 22 | 10 | 6 | 13 | 5 | 56 (17.02%) |
| *S. viridans* | 17 | 12 | 10 | 6 | 1 | 46 (13.98%) |
| *S. pyogenes* | 2 | - | - | - | - | 2 (0.60%) |
| Total (%) | 116 (35.25%) | 112 (34.04%) | 40 (12.15%) | 36 (10.94%) | 25 (7.59%) | 329 (100%) |

***Table 2.** Age wise distribution of the target patients with Gram positive cocci eye infections.*

| Age group | Male n=123 | Female n=77 | Total number of individuals (n=200) |
|---|---|---|---|
| 0-20 | 12 | 6 | 18 (9%) |
| 20-40 | 22 | 9 | 31 (15.5%) |
| 40-60 | 25 | 27 | 52 (26%) |
| 60-80 | 64 | 35 | 99 (49.5%) |

The antibiotic susceptibility analysis (Figure 1 and Table 3) revealed that all the isolates of streptococci were susceptible to aminobenzyl penicillin, ureidopenicillin, fluroquinolones, cephalosporins & glycopeptides, except 1 & 8 isolates of *S. pneumoniae* were intermediately susceptible to cefotaxime & chloramphenical, respectively.

Exactly, 10 & 4 isolates of *S. pneumoniae*, 4 & 22 isolates of *S. viridians* and 1 isolate of *S. pyogenes* were intermediately susceptible & resistant, respectively to tetracycline. Except 6 isolates of *S. viridians*, all the isolates of streptococci were sensitive to tobramycin.

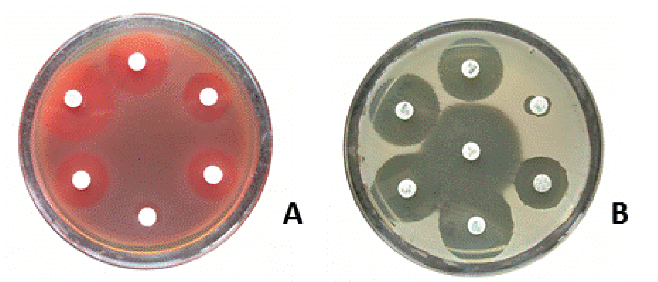


***Figure 1.** Representative antibiogram pattern of the Gram positive cocci isolates from ocular infections. a) S. pneumonia; b) S. aureus.*

All the streptococcal isolates were completely resistant to gentamycin. Among the 49 isolates of CoNS tested, 6 & 2 isolates were intermediately susceptible to ciprofloxacin & moxifloxacin, respectively 1 isolate each was resistant to tetracycline and gentamycin, respectively.

Of 40 isolates of *S. aureus* analysed, 4, 1 and 1 isolates were intermediately susceptible to ciprofloxacin, moxifloxacin and tetracycline, respectively.

Precisely, 5 and 7 isolates of *S. aureus* were resistant to tobramycin and gentamycin, respectively and 2 isolates of *S. aureus* were resistant to rest of the antibiotics. Upon further analysis of the resistant isolates of *S. aureus*, a total of 2 isolates were determined to be MRSA.

***Table 3.*** *Antibiotic sensitivity of Gram positive cocci (n=200) from ocular infections.*

| | **Streptococci (n=111)** | | | | | | | | **Staphylococci (n=89)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates** | *S. pneumoniae* **N=76 n (%)** | | | *S. viridans* **N=33 n (%)** | | | *S. pyogenes* **N=2 n (%)** | | **CoNS N=49 n (%)** | | | *S. aureus* **N=40 n (%)** | | |
| Sensitivity pattern | S | I | R | S | I | R | S | R | S | I | R | S | I | R |
| AMP[1] (2µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| CIP[2] (5 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 43 (87.75) | 6 (12.24) | - | (34) 85 | 4 (10) | 2 (5) |
| OFX[3] (5 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| GAT[4] (5 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| MXF[5] (5 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 47 (95.92) | 2 (4.08) | - | 37 (92.5) | 1 (2.5) | 2 (5) |
| LVX[6] (5 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| CTX[7] (30 µg) | 75 (98.68) | 1 (1.32) | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| CXM[8] (30 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| CFZ[9] (30 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| PIP[10] (10 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| VAN[11] (30 µg) | 76 (100) | - | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| CHL[12] (10 µg) | 68 (89.47) | 8 (10.53) | - | 33 (100) | - | - | 2 (100) | - | 49 (100) | - | - | 38 (95) | - | 2 (5) |
| TET[13] (30 µg) | 62 (81.57) | 10 (13.16) | 4 (5.26) | 7 (21.21) | 4 (12.12) | 22 (66.67) | 1 (50) | 1 (50) | 47 (95.92) | - | 2 (4.08) | 37 (92.5) | 1 (2.5) | 2 (5) |
| TOB[14] (10 µg) | 76 (100) | - | - | 27 (81.81) | - | 6 (18.18) | 2 (100) | - | 49 (100) | - | - | 35 (87.5) | - | 5 (12.5) |
| GEN[15] (10 µg) | 0 | - | 76 (100) | 0 | - | 33 (100) | 0 | 2 (100) | 47 (95.92) | - | 2 (4.08) | 33 (82.5) | - | 7 (17.5) |

CONs=Coagulase negative staphylococci; N=number of isolates tested; S=Sensitive; I=Intermediate; R=Resistant; [1]Ampicillin; [2]Ciprofloxacin; [3]Ofloxacin; [4]Gatifloxacin; [5]Moxifloxacin; [6]Levofloxacin; [7]Cefotaxime; [8]Cefuroxime; [9]Cefazolin; [10]Piperacillin; [11]Vancomycin; [12]Chloromphenical; [13]Tetracycline; [14]Tobramycin; [15]Gentamycin

## Discussion

Most of the ocular bacterial infections are due to Gram positive bacteria than Gram negative bacteria [15]. The present study primarily focused on Gram positive cocci causing eye infections. A total of 1802 samples were collected from patients attending tertiary care eye hospital between September 2012 and December 2013. The predominant isolates were *S. pneumoniae* (40.42%) followed by CoNS (27.96%). Kaliamurthy [15] reported that Staphylococcus spp. (64.5%) followed by Streptococcus spp. (12.3%) as the most common Gram positive cocci isolated from keratitis. Staphylococci being the predominant Gram positive cocci among the eye infections have been reported by many authors [5,7,16,17,18,19]. Although in the current analysis, *S. pneumoniae* was frequently isolated from corneal ulcer and preoperative screening, CoNS was the most frequently isolated bacteria from endophthalmitis cases, which is in accordance to Kaliamurthy et al. [15], Callegen et al. [20] and Durand [21]. Similar to our findings, Leck et al. [22] reported that in India streptococci accounted for 46.8% of corneal ulcer followed by staphylococci (26.8%). In the present study, of 200 patients, individuals between 60-80 years (49.5% of 200) and 40-60 years (26% of 200) were most affected with Gram positive cocci eye infections and similar results have been observed by Sreenivasan et al. [23], Muluye et al. [24] and Tesfaye et al. [25]. Male preponderance (123 of 200) of Gram positive cocci eye infection which was observed in the present study was also reported by several authors [15,24,26,27]. The development of antibiotic resistance to the commonly used topical antibiotics is an important problem worldwide. Therefore antibiotic susceptibility analysis would reveal the varying resistance pattern among the isolates and will help the clinicians to design an appropriate treatment regimen. In the present study all the streptococci were resistant to gentamycin. Among *S. pneumoniae* (n=76), 5.26% were resistant to tetracycline and 10.53% & 13.6% were intermediately susceptible to chloramphenical and tetracycline, respectively. Among *S. viridians* (n=33) 66.67% and 18.8% were resistant to tetracycline and tobramycin, respectively. Exactly, 4% of *S. viridians* were intermediately susceptible to tetracycline. Of the two isolates of *S. pyogenes* tested, one was resistant to tetracycline. The resistance to gentamycin and tobramycin in the present study correlates with the reports of Chalita [28]. In the present study, the recommended antibiotics for streptococcal ocular infections were ampicillin, the fluroquinolones tested, cefotaxime, cefazolin, piperacillin and vancomycin. The highest efficacy of fluroquinolones against Gram positive streptococci has been reported by Kaliamurthy et al. [15], Ramesh et al. [17], Reddy et al. [7] and Hofling-Lima et al. [29]. All the antibiotics analysed were effective against CoNS (n=49) isolates. Exactly, two isolates each were resistant to tetracycline and gentamycin, respectively. Also, 6 and 2 isolates of CoNS were intermediately susceptible to ciprofloxacin and moxifloxacin, respectively. Sensitivity of CoNS to most of the antibiotics has also been demonstrated by Shifraew et al. [19] and Bharati et al. [5]. But, Manikandan et al. [30] reported that 11.4% of CoNS were resistant to the entire antibiotic tested. Of 40 isolates of *S. aureus* analysed, 4, 1 and 1 isolates were intermediately susceptible to ciprofloxacin, moxifloxacin and tetracycline, respectively. Further, 5 and 7 isolates were resistant to the aminoglycosides viz., tobramycin and gentamycin, respectively and similar results were reported by Bharati et al. [5]. Of 40 isolates of *S. aureus*, exactly, 2 isolates were identified to be MRSA. Rajaduraipandi et al. [31] reported 8 MRSA from a total of 20 *S. aureus* isolated from conjunctival sample. Kang et al. [32] reported 57.6 % of MRSA from ocular infection in Taiwan. Vola et al. [33] reported 56 MRSA isolates from 566 *S. aureus* isolates from ocular infections. Nithya et al. [34] reported 33 MRSA of 127 *S. aureus* from ocular infections in south India.

Antibiotic resistance among ocular pathogens is increasing in parallel with the increase seen over the years in bacteria associated with systemic infections [35]. Antibiotic resistance in *S. aureus* can be attributed to horizontal gene transfer, primarily in hospitals as the selective pressures for resistance are maximum [36]. The emergence of antibiotic-resistant bacteria can thus be attributed to the use of antibiotics. Reduction in prescribing antibiotic is one of the effective ways to lessen the selection pressure and to prevent overuse and misuse of antibiotics, an up-to-date practical guideline, on the basis of case-by-case scientific data, for proper antibiotic prescribing should be developed [37]. Van Bambeke et al. [38] stated that antibacterial resistance in *S. pneumoniae is* increasing worldwide, affecting principally beta-lactams and macrolides followed by fluoroquinolone. The complete resistance of pneumococci, viridians streptococci and *S. pyogenes* to gentamycin was noted in the study. Salles et al. [39] reported that ribosomal mutation can result in aminoglycoside resistance *in vitro* but most resistance in the clinic is mediated by aminoglycoside modifying enzymes (AMEs).

Nuermberger and Bishai [40] reported that appropriate use of antibiotics and the introduction of the pneumococcal conjugate vaccines appear to offer the most promise for limiting the spread of drug resistance among pneumococci.

It could be concluded that, Gram positive cocci frequently causes ocular infection and the occurrence of two isolates of MRSA and emergence of intermediately susceptible and resistant strains must be given due consideration in this part of the country. In the present study, *in vitro* susceptibility analysis confirmed that ampicillin, ofloxacin, gatifloxacin, levofloxacin, cefotaxime, cefazolin, piperacillin and vancomycin demonstrated highest efficacy against Gram positive cocci isolates. These antibiotics may be considered as first line drug of choice for treating Gram positive cocci ocular infections as far as this region of study is concerned.

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