Preoperative Conjunctival Swab Analyses for Chloramphenicol and Moxifloxacin in Normal Ocular Commensals

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Purpose: Normal microfloras of conjunctival sac can flourish and be the potential cause for postoperative intraocular infections. Precautions and measures have to be taken to prevent acute postoperative endophthalmitis, which is a challenge to treat, and has poor visual outcome. Hence this study was done to assess the sensitivity of commonly used topical antibiotics like chloramphenicol and moxifloxacin.

Material & Methods: During the period of 10 months, 727 patients were included in the study that were either planned for intravitreal injection of anti-VEGF or were undergoing cataract surgery in a tertiary eye hospital. Conjunctival swabs were obtained, subjected to standard aerobic culture, along with identification techniques and antibiotic sensitivity evaluation was done. All collected data was entered in Microsoft Excel and statistical analysis was performed.

Results: Out of 727 conjunctival swabs, 13 (1.7%) conjunctival swabs showed microbial growth. The most common organism was found to be staphylococcus epidermidis. Microbial culture and antibiotic sensitivity revealed that only 5 conjunctival swabs (38%) showed sensitivity to moxifloxacin, whereas chloramphenicol showed sensitivity in 11 conjunctival swabs (85%). The antibiotic sensitivity reports of inhibition zone for moxifloxacin and chloramphenicol were compared using paired t test and showed that bacteria were more sensitive to chloramphenicol than moxifloxacin with p value of 0.00.

Conclusion: The results of this study suggest that topical chloramphenicol was found to be more sensitive as compared to topical moxifloxacin. Hence, it is wise to choose the cheaper and more effective topical chloramphenicol over moxifloxacin.

Introduction

Most parts of the body have normal commensals, which can become pathogenic under certain conditions, and the conjunctival sac is not an exception, thanks to the immunoglobulins and complement pathway components, which includes lactoferrin, lysozymes and B-lysin in tears that help to kill microorganisms because of their antibacterial action and decrease their adherence to the ocular surface and tear film turnover, which has flushing action. However, some normal microflora of conjunctival sac can still flourish & be a potential cause for postoperative intraocular infections. Staphylococcus epidermidis is the most common organism of conjunctival microflora followed by Haemophilus species and diphtheroids occupy the third position. Staphylococcus citreus and E.coli are also found but fungi, viruses and parasites are seldom seen.

Postoperative endophthalmitis is an inflammatory condition of the eye, due to an infectious process from bacteria, fungi or on rare occasions, parasites that enter the eye during the perioperative period. As per the European Society of Cataract and Refractive Surgeons (ESCRS) guidelines for prevention and treatment of endophthalmitis following cataract surgery, the most common organisms causing postoperative endophthalmitis include gram-positive microbes such as coagulase negative Staphylococci (33-77%), Staphylococcus aureus (10-21%), and gram-negative microbes such as Pseudomonas aeruginosa. Coagulase-negative Staphylococci are the most common causes of post-cataract endophthalmitis, and Streptococci viridians is the most common cause of post-intravitreal anti-VEGF injection endophthalmitis. Recently, increasing number of cases are occurring after intravitreal injections of anti-vascular endothelial growth factor (VEGF) medication due to a dramatic increase in the number of injections annually.

Indian studies have found that the major pathogens causing acute postoperative endophthalmitis include coagulase-negative staphylococci (70%), Staphylococcus aureus (10%), and Streptococci (9%), which matches with the western data as well. Precautions and measures have to be taken to prevent acute postoperative endophthalmitis, which is a challenge to treat, and has a poor visual outcome. Pre-operative precaution for eye surgeries includes 24 hours preoperative instillation of topical antibiotics, for which we should be aware which antibiotic is sensitive for the most common organisms found in the microflora of the conjunctival sac. Previous studies revealed maximum sensitivity was found with vancomycin, gentamycin, chloramphenicol, and ciprofloxacin and maximum resistance with fusidic acid, penicillin, and oxacillin. However, the most commonly used topical ocular antibiotics are fluoroquinolones like moxifloxacin, ciprofloxacin and ofloxacin. Hence this study was planned to assess the sensitivity of commonly used topical antibiotics, chloramphenicol and moxifloxacin, on normal ocular flora.

Materials and Methods

The patients who were included in the study were either planned for intravitreal injection of anti-VEGF or were...
undergoing cataract surgery in a tertiary eye hospital. During the period of 10 months, 727 patients were included in the study after institutional ethical and scientific committee clearance. The patients planned for intravitreal injections of anti-VEGF were subjected to microbial culture and sensitivity tests, in view of chances of post-injection endophthalmitis. Patients with clinically detectable infections of the lids or conjunctiva were excluded from the study. Similarly, patients who were treated with topical or systemic antibiotics over the past 48 hours to one week were excluded from the study. Once an informed consent was obtained, all conjunctival swabs were taken by anaesthetizing the conjunctival sac with sterile 0.5% proparacaine ophthalmic solution. Samples were obtained by gently rubbing the lower fornix with a sterile cotton swab and keeping the eye lids wide apart to avoid contamination from lid margins. The collected samples were inoculated into sterile Brain Heart infusion broth and subjected to standard aerobic culture for 24 hours. Turbidity in the broth was suggestive of microbial growth as shown in Figure 1. It was then sub cultured in Chocolate agar followed by identification techniques like catalase, coagulate tests and novobiocin antibiotic tests to identify the organism. Antibiotic sensitivity of each organism for moxifloxacin and chloramphenicol were analyzed on Mueller Hinton agar as shown in Figure 2. The minimum zone size for each drug was noted in millimeter (mm). The activity of individual drug against the isolated organism was categorized by one of the three terms “Sensitive”, “Intermediate,” and “Resistant.” The difference between the minimum zone size and the zone of inhibition was calculated. If the difference was positive i.e. if zone size obtained was more than the minimum zone size of each drug, then the drug was considered sensitive. If the difference was negative, then the drug was considered resistant. If the difference was zero, it was termed intermediate. All collected data was entered in Microsoft Excel and statistical analysis was performed. A p-value less than 0.05 was considered to be statistically significant.

Results
A total of 727 conjunctival swabs were analyzed microbiologically for culture and antibiotic sensitivity. Of these, 13 conjunctival swabs (1.7%) showed microbial growth. The most common organism found was staphylococcus epidermidis in 10 conjunctival swabs (76.92%), followed by Staphylococcus aureus in 2 subjects (15.38%) and Staphylococcus saprophyticus in one subject (7.69%), as represented in Figure 3. Culture and antibiotic sensitivity report of moxifloxacin revealed that only 5 conjunctival swabs (38%) showed sensitivity to moxifloxacin. The minimum zone of inhibition size required for moxifloxacin was 24 mm. The zone of inhibition obtained for each patient with moxifloxacin and results of sensitivity are shown in Table 1. The sensitivity reports of chloramphenicol showed that, it was sensitive in 11 conjunctival swabs (85%). The minimum

![Figure 1: Showing turbidity suggestive of growth (b) showing clear Sterile Brain Heart infusion broth suggestive of no growth.](image1)

![Figure 2: Showing culture plate for antibiotic sensitivity testing with zone of inhibition](image2)

| Table 1: Sensitivity report of moxifloxacin |
|------------------------------------------|
| Minimum zone size in mm | Zone size seen in mm | Difference | Result |
|-------------------------|----------------------|------------|--------|
| 24                      | 19                   | -5         | Resistant |
| 24                      | 19                   | -5         | Resistant |
| 24                      | 28                   | 4          | Sensitive |
| 24                      | 19                   | -5         | Resistant |
| 24                      | 28                   | 2          | Sensitive |
| 24                      | 19                   | -5         | Resistant |
| 24                      | 16                   | -8         | Resistant |
| 24                      | 24                   | 0          | Intermediate |
| 24                      | 26                   | 2          | Sensitive |
| 24                      | 26                   | 2          | Sensitive |
| 24                      | 28                   | 4          | Sensitive |
| 24                      | 23                   | -1         | Resistant |
| 24                      | 20                   | -4         | Resistant |
| 24                      | 24                   | 0          | Sensitive |

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The zone of inhibition size required for chloramphenicol was 18 mm. The zone of inhibition obtained for each patient with chloramphenicol and results of sensitivity are shown in Table 2. The most commonly used topical fluoroquinolone eye drops, moxifloxacin showed sensitivity of 38%, whereas sensitivity towards chloramphenicol was found to be 85%. The mean difference in zone size seen in MH agar sensitivity report was found to be $+7.23 \pm 7.6$ mm and $-1.61 \pm 3.95$ mm for Chloramphenicol and Moxifloxacin respectively. The antibiotic sensitivity reports for moxifloxacin and chloramphenicol were compared using paired t test and showed that bacteria were more sensitive to chloramphenicol than moxifloxacin, with a p value of 0.00. The difference in zone size comparison of the two antibiotics is shown in Figure 4.

**Discussion**

 Conjunctival flora has been blamed to be the primary and most frequent source of bacteria. Therefore, evaluation of the conjunctival bacterial flora and their sensitivity pattern is of utmost importance to prevent postoperative endophthalmitis, the most dreaded complication after any intraocular surgery.

Topical chloramphenicol has been widely used in the treatment and prevention of superficial eye infections due to its broad spectrum of activity and low cost. The use of chloramphenicol in conjunctivitis cases has been recommended in earlier studies. In-vitro antibiotic sensitivity test results showed that chloramphenicol was effective against the majority of gram positive isolates and some of the gram negative bacteria, the exception being P. aeruginosa.

Chloramphenicol is used to treat conjunctivitis in general practice and ophthalmology clinics, to treat blepharitis and for prophylaxis against infection during an ocular surgery. Part of its value, is due to its low ocular surface toxicity combined with a broad spectrum of activity, low rates for development of resistance and low cost. Chloramphenicol was discovered as a natural product secreted by the bacterium Streptomyces venezuelae found in soil and compost. Chloramphenicol (D(-)-three-2-dichloracetamido-1-p-nitro-phenyl-1,3-propanediol) is bacteriostatic which acts by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. A theoretical, but not yet conclusively proved risk of chloramphenicol induced idiosyncratic aplastic anemia exists with topical ophthalmic therapy, but many studies proved that topical chloramphenicol was not a risk factor for inducing dose-related bone marrow toxicity. Peak serum concentrations > 25 mg/L was associated with bone marrow toxicity.

Moxifloxacin is a fourth-generation fluoroquinolone which works by inhibiting both DNA gyrase and topoisomerase IV. Moxifloxacin is used as 0.5 per cent eye drops in ophthalmic practice. In a study, done to assess the antibiotic sensitivity of fluoroquinolone (FQ) and non-fluoroquinolones in rabbit eye models, it was found that the FQs, moxifloxacin, and ofloxacin, were more effective than topical non-fluoroquinolones for preventing bacterial endophthalmitis whereas our work revealed that non-fluoroquinolones like chloramphenicol are more sensitive than FQ’s.

In another study staphylococcus aureus isolates were resistant to ciprofloxacin and ofloxacin but were found to be susceptible to moxifloxacin (p=0.001). The fluoroquinolones like moxifloxacin are among the newer drugs being considered as anti-tuberculosis agents as well, as they show good activity against subpopulations

| Minimum zone size in mm | Zone size seen in mm | Difference | Result |
|-------------------------|----------------------|------------|--------|
| 18                      | 6                    | -12        | Resistant |
| 18                      | 13                   | -5         | Resistant |
| 18                      | 26                   | 8          | Sensitive |
| 18                      | 26                   | 8          | Sensitive |
| 18                      | 28                   | 10         | Sensitive |
| 18                      | 26                   | 8          | Sensitive |
| 18                      | 28                   | 10         | Sensitive |
| 18                      | 25                   | 7          | Sensitive |
| 18                      | 30                   | 12         | Sensitive |
| 18                      | 35                   | 17         | Sensitive |
| 18                      | 30                   | 12         | Sensitive |
| 18                      | 29                   | 11         | Sensitive |

**Table 2: Sensitivity reports of chloramphenicol**

Figure 3: Distribution of microbial flora in conjunctival sac

Figure 4: Difference in zone size comparison of two antibiotics

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of resistant mutants. But for the ocular normal flora, our study reveals that chloramphenicol is a better choice than moxifloxacin.

In our study, the most common organism found was staphylococcus epidermidis. Microbial culture and sensitivity proved that chloramphenicol was more sensitive compared to moxifloxacin, which is also easily available and cost effective among all the antibiotics.

Conclusion
The results of this study suggest that topical chloramphenicol is a more sensitive agent compared to topical moxifloxacin for the most common organisms found in the normal microflora of the conjunctival sac. This study may act as a guide in choosing antibiotics for preoperative prophylaxis or postoperative prophylaxis and treatment.

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