A Study of Susceptibility Patterns of Ocular Bacterial Flora to Topical Antibiotics in Patients Undergoing Anterior-Segment Intraocular Surgery

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ABSTRACT

Endophthalmitis is an ocular inflammation resulting from the invasion of infectious agents into the posterior segment of the eye which may cause partial or complete loss of vision. The source of infecting microorganisms is often existing flora from the conjunctival surface. This prospective study was carried out to determine and study the susceptibility patterns of ocular bacterial flora to topical antibiotics in patients undergoing anterior-segment intraocular surgery, at Pt. B.D. Sharma, PGIMS, Rohtak, India. A total of 266 bacterial strains were isolated from two hundred and fifty patients undergoing anterior-segment intraocular surgery. Antimicrobial susceptibility using the modified Stokes disk diffusion technique showed that Gram-positive organisms were the commonest isolates, followed by Gram-positive and Gram-negative bacilli. Gatifloxacin followed by amikacin provided the best broad-spectrum coverage of ocular bacterial flora. (J Infect Dis Antimicrob Agents 2008;25:73-9.)

INTRODUCTION

Endophthalmitis is an ocular inflammation resulting from the introduction of infectious agents into the posterior segment of the eye. The incidence of intraocular infection after anterior segment intraocular surgery has sharply declined over the past 40 years with the advent of newer antimicrobial agents, better and more effective sterilization practices, and improved surgical techniques.1 Such infection frequently causes irreversible damage to delicate photoreceptor cells of the retina. Despite aggressive therapeutic and surgical intervention, endophthalmitis generally results in partial or complete loss of vision, often within a few days of inoculation.2
The conjunctival sac is never free from microorganisms. Most of the organisms normally present are commensals including *Staphylococcus epidermidis*, *Propionibacterium acne*, *Moraxella catarrhalis*, and *Corynebacterium* spp.. These commensals from the surrounding skins and the conjunctivae are known to harbour organisms capable of causing endophthalmitis. Recent studies using molecular epidemiological techniques have demonstrated that these flora are the most likely source of contamination. These studies have demonstrated that an organism isolated from the vitreous was genetically indistinguishable from the isolate recovered from the patient’s eyelid, conjunctiva, or nose in most cases of endophthalmitis. Therefore, given the ability of surface flora to enter the eye during surgery, many of the prophylactic techniques to decrease the risk of endophthalmitis aim to suppress their numbers. The optimal choice of preoperative topical antibiotic is based on a variety of factors including the spectrum of bacteria recovered, the rapidity with which the antibiotic eliminates the organism from the conjunctival surface, the duration of action, the penetration and toxicity of the antimicrobial, the antibiotic susceptibility pattern, and the cost. Although narrow-spectrum antibiotics such as bacitracin, vancomycin, and cefazolin have retained Gram-positive effectiveness over the years 1993-2001, the resistance of ocular bacterial isolates to topical fluoroquinolones, the most commonly used agents for prophylaxis of endophthalmitis is increasing. Bacterial resistance can be limited by an judicious prophylactic use of antibiotics, by cycling antibiotics, by culturing patients with serious infections to obtain bacterial identification and susceptibility profiles and by adhering to recommended antimicrobial agents.

The resistance pattern of various bacteria to different antibiotics changes constantly. Since no such study on ocular isolates had been conducted in this institution, this prospective study was carried out to determine the susceptibility patterns of ocular bacterial flora to topical antibiotics in patients undergoing anterior-segment intraocular surgery.

**MATERIALS AND METHODS**

This study was carried out in Departments of Ophthalmology and Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak, India. Two hundred and fifty patients undergoing anterior-segment intraocular surgery were included in the study. Informed consent was taken from all the patients. Those patients with the following criteria were excluded from the study:
1. Patients with clinical evidence of any type of local (eye) or systemic infection.
2. History of recent use of topical or systemic antimicrobial agents in the previous 15 days.
3. History of allergy to iodine or topical antimicrobial agents.
4. History of previous intraocular surgery or penetrating injury to eye.
5. History of intake of steroids or other immunosuppressive agents.
6. Any history of malignancy.
7. Pregnant patients.

Conjunctival swabs were obtained from the eyes to be operated upon, one day prior to surgery.

**Method of sample collection**

The patient was asked to look up, and the inferior conjunctival fornix of the eye to be operated upon was swabbed with two sterile cotton tipped applicators without touching the eyelid margins or lashes. The swabs were then inoculated in a tube containing glucose broth. The sample was then transported to Department of Microbiology within two hours of collection. Patient details were maintained separately. In order to reduce
operator sampling bias, a standardized sampling
technique was used for all study patients. After the
sample collection, routine preoperative and operative
protocols were followed.

**Processing of samples**

With each conjunctival swab collected, a Gram-
stained smear was prepared and examined under the
microscope. The other conjunctival swab was used
to inoculate the culture plates of blood agar, chocolate
agar, and MacConkey’s agar. Blood agar and
chocolate agar plates were incubated in an atmosphere
of 5-10 percent CO₂. Any growth obtained was
identified on the basis of Gram-staining, cultural
characteristics and biochemical reactions, following
standard protocols.¹⁰

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing of all the
bacterial isolates was performed using the modified
Stokes disk diffusion technique with commercially
available antimicrobial disks. The bacterial isolates were
tested for all commonly used antimicrobial agents in
Department of Ophthalmology, namely, cefazolin (30
μg), ceftazidime (30 μg), gentamicin (10 μg), tobramycin
(10 μg), amikacin (30 μg), neomycin (30 μg),
ciprofloxacin (5 μg), ofloxacin (5 μg), norfloxacin (10
μg), levofloxacin (5 μg), gatifloxacin (5 μg), sparfloxacin
(5 μg), lomefloxacin (5 μg), erythromycin (5 μg),
tetracycline (10 μg), chloramphenicol (5 μg), and
vancomycin (25 μg).

**RESULTS**

The age range of patients randomly selected in
this study was 3 to 88 years. The mean age of the
patients was 60.34 ± 13.42 years. Of the 250 subjects
enrolled in the study, 129 (52%) were females and
121 (48%) were male. There were 152 (61%) patients of rural origin, and 98 (39%) patients of urban
origin. Two hundred and twenty-five patients had
swabs with positive cultures, and 25 patients had
swabs taken that failed to grow any microorganisms.
Of the 225 eyes, a total of 266 bacterial strains were
isolated resulting in a mean of 1.18 bacterial strains
per eye. Of the 266 bacterial strains, the most common
isolates (223, 84%) were Gram-positive cocci, followed
by 27 (10%) Gram-positive bacilli and 16 (6%) Gram-
negative bacilli. Coagulase-negative staphylococci
(CoNS) were the most commonly isolates, accounting
for 193 (73%) of all 266 isolates. The pattern of
distribution of micro-organisms is summarized in Table
1. Of the 16 isolates of Gram-negative bacilli,
*Pseudomonas aeruginosa* was the most common
isolates (9, 56%), followed by *Citrobacter freundii*

**Table 1. Pattern of distribution of microorganisms.**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>193</td>
<td>73</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2. Distribution of polymicrobial flora.

<table>
<thead>
<tr>
<th>Combination of organisms</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci and S. aureus</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci and Gram-negative bacilli</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci and Corynebacterium spp.</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci and viridans streptococci</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Viridans streptococci and Corynebacterium spp.</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Gram-negative bacilli and Corynebacterium spp.</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>S. aureus and Corynebacterium spp.</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Of the 225 culture-positive patients, a single isolate was recovered from 184 (82%) patients, whereas polymicrobial flora was observed in 41 (18%) of the patients. The commonest combination was that of CoNS and *Staphylococcus aureus* (Table 2).

More than 90 percent of the CoNS isolates were susceptible to gatifloxacin, sparflxacin, amikacin, gentamicin, and vancomycin (Table 3). *S. aureus* was most susceptible to gatifloxacin (98%) amongst the fluoroquinolones, and to gentamicin (97%) among the aminoglycosides. All the 16 Gram-negative bacilli recovered were susceptible to gatifloxacin, whereas only 81 percent of these isolates were susceptible to amikacin and gentamicin.

**DISCUSSION**

There has been a decreasing incidence of postoperative endophthalmitis over the past several decades that can be attributed to several factors including prophylactic use of antiseptics and antibiotics, emphasis on improved operative techniques, and awareness of factors contributing to postoperative infection. Since untreated endophthalmitis results in compromised visual function and morbidity to the eye at best, prevention of endophthalmitis is important.

Preoperatively, the most important source of potentially infectious organisms is the patient’s own natural conjunctival and skin flora. Up to, 75 percent of cultures from samples taken from normal eyes have been found to be positive for *S. epidermidis*, *S. aureus*, and various streptococci. A similar pattern has been found in eyes with endophthalmitis. Studies by various authors also confirm that periocular microbes serve as a potential causal agent for postoperative endophthalmitis. Given the ability of surface flora to enter the eye during surgery, many of the prophylactic techniques used to decrease the risk of endophthalmitis aim to suppress bacterial numbers and to limit the growth of microorganisms entering the eye.

We conducted this study to determine the susceptibility patterns of ocular bacterial flora to topical antimicrobial agents. There was no detectable growth of bacteria on culture of conjunctival swabs in 25 (10%) of the patients. The 90 percent culture positivity rate of the patients in this study is comparable to the study by Ta and colleagues who recovered bacterial growth in 82 percent of patients undergoing intraocular surgery. A total of 266 bacterial strains were cultured from 225 eyes, resulting in a mean of 1.18 bacterial strains isolated...
Table 3. Antibiotic susceptibility patterns of various ocular flora to commonly used topical antimicrobial agents.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Drug*</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Neomycin</th>
<th>Vancomycin</th>
<th>Cefazolin</th>
<th>Chloramphenicol</th>
<th>Tetacycline</th>
<th>Erythromycin</th>
<th>Ceftazidime</th>
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<td>Coagulase-negative</td>
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<tr>
<td>staphylococci</td>
<td>S</td>
<td>96</td>
<td>2</td>
<td>92</td>
<td>05</td>
<td>88</td>
<td>08</td>
<td>82</td>
<td>09</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>100</td>
<td>96</td>
<td>97</td>
<td>93</td>
<td>91</td>
<td>66</td>
<td>81</td>
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<td>67</td>
<td>67</td>
<td>64</td>
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<td>Staphylococcus aureus</td>
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<tr>
<td>Gram-negative bacilli</td>
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</table>

S: susceptible, I: intermediately susceptible, NA: not applicable

*The number denotes the percentage.
per eye. This finding is similar to the study conducted by Ta and colleagues, who isolated 143 bacterial strains from 99 eyes, thus giving a mean of 1.44 bacterial strains isolated per eye.\(^1\)

We found that the most common organisms colonizing the eye were CNS (73%). This finding is consistent with previously published studies.\(^{14-17}\) Kasper and colleagues, isolated 162 bacterial strains, of which 124 were CoNS.\(^18\) Similarly, Ta and colleagues obtained a total of 143 organisms, of which 112 (78%) were CoNS isolates.\(^5\) Corynebacterium spp. was the second most common isolate, followed by \textit{S. aureus} (8%), Gram-negative bacilli (6%), and streptococci (3%). These results are consistent with the study by Kasper and colleagues.\(^18\) In this study, there was no isolate of CoNS which was found to be resistant to vancomycin. This result is consistent with the previously published studies.\(^{1,18,20,21}\) We found that aminoglycosides including tobramycin (99%), amikacin (98%), and gentamicin (97%), remained highly effective against CoNS. Kowalski and colleagues described the susceptibility rate of CoNS isolates to amikacin and gentamicin was 95 percent and 82 percent, respectively.\(^19\) Ta and colleagues reported the susceptibility rate of CoNS to amikacin and gentamicin was 99 percent each.\(^1\)

This study suggests a disturbingly high resistance rate of CoNS to most of the fluoroquinolones tested. The resistance rate to ciprofloxacin, ofloxacin, and norfloxacin was found to be 13 percent, 16 percent, and 36 percent, respectively. These findings are consistent with a few recent studies showing an increasing Gram-positive bacterial resistance to fluoroquinolones in isolates from patients with keratitis. In the study by Ta and colleagues, the resistance rate of CoNS to ciprofloxacin and ofloxacin was found to be 15 percent and 16 percent, respectively.\(^{1,7,8}\) Other recently available fluoroquinolones including gatifloxacin, sparflloxacin, levofloxacin, and lomefloxacin were highly effective against Gram-positive microorganisms with the resistance rates being 2 percent, 3 percent, 4 percent, and 9 percent, respectively.

**CONCLUSION**

In conclusion, in this study none of the patients developed endophthalmitis postoperatively within a six-week follow-up period. Gatifloxacin was found to provide excellent broad-spectrum coverage against conjunctival bacterial flora. However, one must consider the possibility that the results of the in vitro antimicrobial susceptibility may not match the in vivo effectiveness of the antimicrobial agent tested, as for example, the high concentrations of the commonly used antibiotics with frequent topical application or by subconjunctival injection might change the relative efficacy of the various agents in vivo. Because of the widespread prevalence of antibiotic resistance, ophthalmologists should carefully select antibiotics that are most effective in minimizing ocular colonization with resistant organisms. Despite there being little scientific evidence in support of perioperative prophylactic antibiotics adjunctively with anterior-segment intraocular surgery, their use is continuing and is based on the assumption that reducing the conjunctival bacterial flora reduces the risk of endophthalmitis.

**References**

3. Ariyasu RG, Nakamura T, Trousdale MD, Smith R.


