Bacterial Contamination of In-Use Ocular Medications

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Abstract: Forty in-use ocular medications from 34 patients were collected from out-patients at Dr. Agarwal’s Eye Hospital, Salem. The present study was carried out by culturing the bottle caps, the cover, nosil and a drop produced by simple inversion by standard methods. The bacterial isolates which were considered significant were identified by standard biochemical tests. Commonly Gram positive organism were significantly (71.95%) to be isolated from all medication sites than Gram negative organism (28.04%). Gram positive organism include Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, non haemolytic Streptococci, Corynebacterium and Bacillus sps. Gram negative organisms include Aeromonas spp, Serratia liquefaciens and Salmonella spp. The overall contamination rate of in-use medications / nosil contains 48 (30%) isolates, cover contains 53 (33%) isolates and cap contains 60 (37%) isolates. The results of this study indicated that ocular medication cap showed highest rate of contamination when compared to the other two. The cycle of contamination of in-use ocular medication may represent an important risk factor for bacterial keratitis in patients with ocular surface diseases.

Keywords: Ocular medications, Eye drop bottles, Ophthalmic solutions, Bacterial contamination, Duration of use.

I. Introduction

The ocular drops are the main stay of treatment for some common conditions such as allergy, infections, dry eye, glaucoma etc. These topical medications are used by pre-operative, post-operative patients respectively. In certain ocular pathologies, ophthalmic formulations need to be chronically administered in order to guarantee their efficacy (Vanrell, 2007). Most ocular medications are either in the form of an eye drops or an ointment. Eye drops are more commonly and largely used, but ointments may last longer, provide more lubrication and easier to administer. Eye drops are instilled by eye drop instillations as multiple dose bottles or in unit dose containers containing preservative system to prevent microbial growth (Pflugfelder, 2008). Ophthalmic drops are sterile preparations which are usually packed in multi-dose containers. In their uses, microbial contamination may lead to product degradation or result in ocular infection (Flores, Molillo and Crespo, 1997; Schein et al., 1992; Schein et al., 1988; Ford, Brown and Hunt, 1985). Protection of these multiple dose products against microbial contamination is usually achieved by addition of a suitable preservative system (Fazeli, Samadi and Fattahi, 2004; Furrer, Mayer and Guny, 2001; Wallhauser, 1979). Preservatives such as benzalkonium chloride and thimerosal are commonly used in ophthalmic preparations to keep the medications sterile from microorganisms and increase the usage time of eye drops (Rajpal and Glaser, 1997). The eye drops get cross-infected by contaminated instruments, hands, common towels and dropper, cap in contact with eyelids, lashes, eyebrows and facial skin. The eye drops used by in-patient and out-patient department may also get contaminated. The use of contaminated eye drops and their containers may give rise to serious ocular infections. Endophthalmitis due to eye droppers contaminated with Pseudomonas pyocyanaea (cCulloch, 1943). Serratia marcescens, Pseudomonas aeruginosa contaminate eye drop caps and droppers causing secondary keratitis have been reported (Lass et al., 1981). The eye droppers contaminated with bacteria that are used for glaucoma treatment have also been implicated in ocular infections (Templeton et al., 1982). Ophthalmic solution commonly contaminated by Acanthamoeba spp cause Acanthamoeba keratitis (Steh-Green et al., 1987; Kingston and Warhurst, 1969). The prevention of infectious diseases is certainly preferable to their treatment. Since the consequence of the contamination of these ocular medications is clinically very important, the present study was planned in a tertiary eye care hospital in Salem, with the following objectives. The aim of the present study was planned to evaluate the spontaneous contamination of in-use ocular medications, and to estimate the frequency of medication contamination.
II. Materials And Methods

Patients with ocular surface disease using ocular medication were enrolled at Dr. Agarwal’s eye hospital, Salem. Patients were contacted before an appointment and were asked to bring all in-use ocular medications. Various inclusion and exclusion criteria were used for enrolling the patients for this study. The inclusion criteria includes i) The time period of usage of these medications were not exceeding 10-14 days after consulting the ophthalmologist, ii) Re-surgery, FFA, corneal edema, dry eye and lid diseases cases and iii) Patients of the age group 20 to 80 were enrolled for this study. Patients were excluded if they were currently under treatment for a bacterial or fungal ocular infection or if an infection was present at the time of enrollment. They were also excluded if they failed to bring all their medications. The patients provided all their medications for culture. Replacements were provided free of charge. All ocular drops collected from the enrolled patients were cultured.

Medication Bacteriologic study

Sample Collection

A total of 40 in-use eye drops were collected from outpatient department of Dr. Agarwal’s eye hospital, Salem, after usage. All the collected samples were recorded for their active ingredients as well as their duration of uses. The eye drops and ointments were collected from the patients and packed in zip lock cover and sealed with micro pore plaster, marked with patient name and number with glass marking pen and immediately transferred to the microbiology laboratory. The residual contents, cover, caps and droppers of the eye samples were examined for possible microbial contamination under aseptic conditions.

Sample Processing

All ocular eye drops were cultured as follows. If cap, cover was present, a sterile swab was rotated around the inside tip and inside edge of the cap and outside edge of the cover finishing with a complete rotation around the inside rim. The swab was then inoculated on to a Sheep blood Agar (SBA), Brain Heart infusion Agar (BHA) and a Sabouraud Dextrose Agar (SDA). The medication was then inverted and one drop was allowed to fall on each of these three media. The drop was streaked on the three media plates. The bottle top/ nosil were cleaned with alcohol and were wiped. The entire bottle contents were withdrawn using a needle and sterile syringe; 0.5ml was used to inoculate each of the three plates (Sheep blood Agar, Brain Heart infusion Agar and a Sabouraud Dextrose Agar) and the remaining medications were inoculated on the nutrient broth. The bacterial plates and broths were incubated at 37°C for 24-48 hrs and the fungal plates and broths were incubated at 28°C to 30°C for 7 days. The bacterial colonies were identified by using standard biochemical test and the fungal colonies were identified by LPCB mount. The control plates were also kept at the same temperature and were incubated.

The significance of the difference between two proportions was calculated by chi-square (x2) test (Hill, 1977). Tables were mostly 2 x 2 tables with one degree of freedom. If P > 0.05, the differences observed were deemed not significant. If P < 0.05, the differences observed were deemed significant.

III. Results

Study patients

The patients investigated in this study are given in Table 1 provides about the details of the patients with ocular surface diseases and their purpose for the hospital visit.

Medications used by patients

The distribution of medications used by the patients is presented in Table 2. Forty medications were examined and 161 isolates were isolated from these medications. From this table it shows that eye drops used as lubricants (45.96%) had the high rate of contamination followed by anti-inflammatory (21.13%) and antibiotics drops (18.63%).

Medication contamination

The distribution of microorganisms cultured from the three different medication container sites such as cap, cover and nosil shows that most of the gram positive organisms isolated from the medications were coagulase negative Staphylococci which dominated the cap and nosil. Gram positive organisms were more likely to be isolated from the cover. Gram negative organisms were isolated moderately from the entire three medication sites.

The overall contamination rate of in-use medications / nosil contain 48 (30%) isolates, cover contains 53 (33%) isolates and cap contains 60 (37%) isolates (Table 3). The results of this study indicated that ocular medication cap showed highest contamination rate when compared to the other two.
Bacterial isolates from in-use ocular medications

The Bacteria most commonly isolated from in-use ocular medications were species of the genus *Staphylococcus*, accounting for 71 (44%) isolates; the species identified were 14 (9%) *Staphylococcus aureus*, 39 (24%) *Staphylococcus saprophyticus* and 18 (11%) *Staphylococcus epidermidis*. Species of the genus *Streptococcus* accounted for 14 (8.7%) of total 161 isolates; the species identified included 7 (4%) *Streptococcus pneumonia* and 7 (4%) Non-haemolytic Streptococci. The other bacteria identified included 14 (9%) *Aeromonas* spp., 17 (11%) *Bacillus* spp., 15 (9%) *Corynebacterium kutscheri*, 2 (1%) *Corynebacterium xerosis*, 6 (4%) *Micrococcus luteus*, 21 (13%) *Serratia liquefaciens* and 1 (1%) *Salmonella* spp., out of 161 isolates.

Table – 1 Total no of patients visited hospital for the cause of infection

<table>
<thead>
<tr>
<th>S. No</th>
<th>Purpose of hospital visit</th>
<th>No of patients (%total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dry eye</td>
<td>10 (29.41)</td>
</tr>
<tr>
<td>2</td>
<td>Corneal edema</td>
<td>8 (23.53)</td>
</tr>
<tr>
<td>3</td>
<td>Re-surgery</td>
<td>6 (17.64)</td>
</tr>
<tr>
<td>4</td>
<td>FFA</td>
<td>5 (14.71)</td>
</tr>
<tr>
<td>5</td>
<td>Lid diseases</td>
<td>5 (14.71)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>34</td>
</tr>
</tbody>
</table>

Table – 2 Details of medication used by study patients

<table>
<thead>
<tr>
<th>S. No</th>
<th>Types of medications</th>
<th>No. of Medication</th>
<th>Total number of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lubricants</td>
<td>17</td>
<td>74</td>
<td>45.96</td>
</tr>
<tr>
<td>2</td>
<td>Antibiotics</td>
<td>9</td>
<td>30</td>
<td>18.63</td>
</tr>
<tr>
<td>3</td>
<td>Anti-inflammatory</td>
<td>7</td>
<td>34</td>
<td>21.13</td>
</tr>
<tr>
<td>4</td>
<td>Anti-glaucoma</td>
<td>6</td>
<td>21</td>
<td>13.04</td>
</tr>
<tr>
<td>5</td>
<td>Antifungal</td>
<td>1</td>
<td>2</td>
<td>1.24</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
<td>161</td>
<td></td>
</tr>
</tbody>
</table>

Table- 3 Contamination rate of in-use medications

<table>
<thead>
<tr>
<th>S.No</th>
<th>Culture growth</th>
<th>Drops</th>
<th>Cap</th>
<th>Cover</th>
<th>Nosil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coagulase negative Staphylococci</td>
<td>28(17.39)</td>
<td>7(4.35)</td>
<td>22(13.66)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gram Positive</td>
<td>22(13.66)</td>
<td>39(24.22)</td>
<td>7(4.35)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Gram negative</td>
<td>10(6.21)</td>
<td>7(4.35)</td>
<td>19(11.80)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>60(37.3%)</td>
<td>53(33%)</td>
<td>48(30%)</td>
<td></td>
</tr>
</tbody>
</table>

IV. Discussion

In the present study, conjunctival smear of 34 patients and 40 in-use ocular medications of 14 days usage duration were collected from the outpatient department of Dr. Agarwal’s eye hospital, Salem were taken for microbiological investigations. The results of the study showed high rate of contamination of ocular medications with bacterial isolated than fungal isolates, coagulase negative *Staphylococci*, gram-positive organisms that are not a part of potential pathogens and gram-negative organisms namely *Serratia liquefaciens*, *Aeromonas* spp and *Salmonella* spp were isolated in this study. The present study shows high frequency rate of contamination in caps (37%) than in cover (33%) and nosil (30%); this difference was statistically significant. Fazeli, Samadi and Fattahi, (2004) reported high frequencies of microbial contamination were detected in the caps and the residual contents of the eye drops; however, the latter appeared to have higher incidence of contamination (12% vs. 34% for the first day samples). While several studies have shown conflicting views with the same findings of this investigation, these studies reported dropper tips as contaminated highly (Aslund, Oslon and Sandell, 1978; Hoviding and Sjursen, 1982; Stevens and Matheson, 1992; Tasli and Cosar 2001).

The scanty growth obtained by dripping in these studies may represent small amounts of bacteria surviving but not multiplying in the solutions, but it is probable that many of the bacteria isolated originate from contaminated dropper tips. The results obtained in the present study for caps are comparable to the earlier reports (Coad, Osato and Wilhelmus, 1984; Schein et al., 1992) in which caps of squeezed bottles have been proposed to act as potential reservoirs for microbial contamination. This contamination paves way to the contents through the droppers.

In this study no organism was detected on the plates of droppers in contrast to the caps and residual contents. These results obtained in this study are similar to those of the previous reports (Stevens and Matheson, 1992) in which contents were more contaminated than tips and thus it was concluded that germ desiccation and also aspiration of contaminated tips into the content may explain higher bio burden rate of the residual contents.
High contamination rates were observed in β-blockers, steroid drops, and ocular lubricants. The contamination rates of individual products cannot be accurately assessed because of the small numbers of samples taken in this study. These results are consistent with those of other workers (Schein et al., 1992). Of the eye drops tested, lubricants, anti-inflammatory and antibiotics showed higher contamination rates which were 45.96%, 21.13% and 18.63% respectively. The high bio burden load of the lubricants eye drop could be attributed to the poor aseptic condition in the preparation of this product. Since the number of the tested ophthalmic drops was not large enough, no conclusion could be derived on the statistical point of view. Schein et al. (1992) reported high rate of contamination of ocular medications, particularly with potentially pathogenic gram-negative organisms that are not part of usual conjunctival flora and founded a high rate of concordance of organisms in medications and conjunctiva to which that medication was applied. These indicate that the microbial ecology of medication and conjunctival contamination differs significantly for gram-positive to gram-negative organisms. In this present study, a high rate contamination of in-use ocular medication with gram-positive organisms that are part of usual conjunctival flora (35.16%) was reported. Apart from this, a high rate of concordance of organisms in medications and conjunctiva to which that medication was applied was also found in this study. These indicate that conjunctival contamination differs significantly for gram-positive to gram-negative organisms in the conjunctival flora.

In our study, the medication container was divided into three different sites namely cap, cover and nosil and examined for contamination rate. The usual conjunctival flora (35.16%) was isolated more in cap and gram-negative organisms such as Salmonella spp., Serratia liquefacians and Aeromonas spp., were also isolated. The Contamination was divided into contamination of the medication cap and contamination of contents. Schein et al., 1992 reported gram-positive organisms and fungi tended to be cultured only from the cap, while gram-negative organisms tended to be cultured from multiple medication sites. The presence of a pathogenic organism on a normal ocular surface is a necessary ingredient for the development of serious ocular infection. Schein et al., 1992 wished to alert ophthalmologists caring for patients with ocular surface diseases of the high rate of contamination of in-use ocular medications. The multi-dose drop in out-patient department, where a bottle is used for different patients, is a potent source of contamination. Handling the medication leads to contamination of cap and nosil, thus acting as the reservoirs for the contamination of eye drops. Ideally single-dose eye drops should be used but they cost more than multidose ones. However, application of single-dose drops is recommended for patients with eye infections. Alternatively, multi-dose bottles could be discarded after use by an infected patient.

V. Conclusion

In conclusion, it can be said that the observations made in the present study will possibly contribute substantially to the knowledge of the use of these ophthalmic preparation with care. The contamination of eye drops occur with increased length of use of bottles. Therefore it is recommended that the ophthalmic drops used in outpatient department should not be extended more than 15 days, yet care should be taken while handling and initializing the eye drops. The use of single-dose eye drops for the patients with ocular surface diseases is recommended as the multi-dose ones are cost effective. In case of multi-dose bottles being used should be discarded after use with an infected patient. The safe practice recommendations have to be adhered to very strictly in the avoidance of in-use contamination of ocular medications and prevention of any infection associated with their usage.

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References


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