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Evaluation of fungal and bacterial contaminations of patient-used ocular drops

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The aim of the present study was to investigate the incidence of fungal and bacterial contaminations of in-use eye drop products in the teaching department of ophthalmology, Imam Khomeini Hospital, Ahvaz. Two hundred and eighty seven eye drop bottles were randomly collected at the end of day 1, day 2, day 3, day 4 and day 7 of use. The eye drop residues, swabs from internal caps and droppers were inoculated onto MacConkey agar, blood agar and Sabouraud’s dextrose agar. The identification of the recovered organisms was accomplished using standard microbial identification techniques. The incidence of microbial contamination of in-use eye drop products was 17.8%, with the highest rate (24.6%) and the lowest rate (9.0%) noted with day 1 and day 3 samples, respectively. The most contaminated part of the eye drop products was the caps (45.9%) followed by droppers (41.0%) and residual contents (13.1%). Considering mendicants contents, those with pilocarpine (41.7%) had the highest rate of contamination followed by atropine (31.8%), tropicamide (28.6%) and betamethasone (23.3%). Our study revealed the potential risk of contamination of in-use eye drop products in hospitals, but we did not find a direct relationship between usage duration and contamination rate.

Keywords eye drop product, microbial contamination, saprophyte, bacteria, fungi

Introduction

Microbial infections are gaining in importance in recent years. The majority of eye infections are exogenous in nature and have been related to indoor air microbial pollution [1]. These infections are attributed to contaminants, which are introduced or even produced in the environment [2]. Previous studies have shown that there is a correlation between seasonality and microbial contamination rates [3]. The pollution of indoor environments, especially hospitals, has achieved great importance. Several authors have reported airborne infectious diseases due to indoor air quality [1,4].

Microbes may infect the cornea, orbit and other ocular structures. Microbial keratitis and endophthalmitis are two well-known eye diseases. This may be caused by the implanting of microorganisms into cornea or other parts of the eye from the environment or by the user. Known causative agents include; Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Proteus species, Klebsiella species, Candida albicans, Nocardia species, Fusarium species, Aspergillus species, and dematiaceous fungi [5–7]. An association between keratitis and contact lens (CL) wearers has been documented [5]. Contaminated CL solutions have been reported as important risk factors for eye disease.

It has been demonstrated that the incidence and degree of microbial contamination of eye drop
products were associated with the duration of use [8,9]. The rate of bacterial contamination was reported to be 34.8% in opened bottles [10]. Others noted high frequencies of fungal contamination in the caps of the eye drop products [9]. The majority of these used in Iran contain preservatives and are bottled in plastic containers. Preservatives are used to avoid microbial contamination but some microbes can grow in such products.

To contribute to the knowledge of the effect of contaminated indoor air on eye drop products, this study was carried out. We investigated the incidence of fungal and bacterial contaminations of in-use eye drop products in the teaching department of ophthalmology, Imam Khomeini Hospital, Ahvaz. The investigation was also aimed to compare the microbial contamination of the residual content, droppers and caps of eye drop products.

Materials and methods

Sample collection

In the present study 287 in-use eye drop bottles were collected from the Department of Ophthalmology, Imam Khomeini Hospital, Ahvaz between 5 March and 5 June 2006. All eye drop products are available commercially in Iran. The bottles were collected randomly on day 1, day 2, day 3, day 4, and day 7 of use. All bottles were opened several times during usage ranging from 2–6 times per day. Eye drop products contained; tetracaine, sulfacetamide, tropicamide, vancomycin, ceftazidine, cephalosporine, ciprofloxacin, gentamicin, betamethasone, naprin, pilocarpine, timolol, amikacin, naphazoline, chloramphenicol, phenylephrine, homatropine and atropine. All bottles were immediately transferred after the indicated day of use to the medical microbiology laboratory and processed in a few hours to minimize contamination during testing process. Thirty-four unopened eye drop products (two of each type of medication) were also used for comparative controls.

Methods

The aliquots of one drop of the residual liquid from each bottle of eye drops were aseptically cultured on MacConkey agar (Himedia, India) and blood agar (Merck, Germany) for bacteria and Sabouraud's dextrose agar (SDA; Merck, Germany) for fungi. Sterile cotton swabs moistened with sterile water were used for sampling the inside of caps and droppers. These samples were also cultured on the MacConkey agar, blood agar and SDA medium.

MacConkey and blood agar cultures were incubated at 37°C for 24 h, while the SDA was aerobically incubated at 25–27°C for one week. After incubation, loops containing growth from the developing colonies were microscopically examined and bacterial and fungal colonies were subcultured to the same media. Fungal isolates were identified based on colony morphology on SDA and CHROMagar Candida (CHROM-Magar Candida Company, Paris, France), microscopic morphology on slide cultures, and differential tests [11,12]. Bacterial isolates were also identified using standard microbial identification techniques [13].

Results

In our study the patients’ profiles using the eye drop products were as follows; tetracaine and timolol were employed for routine eye examination, reduction of ocular pressure, removal of corneal and conjunctival foreign bodies. The antibiotics, i.e., sulfacetamide, vancomycin, ceftazidine, chloramphenicol, amikacin, naphazoline and betamethasone were used as prophylaxis for post-operative infections and inflammations. Ciprofloxacin, gentamicin and cephasoline were usually employed in the treatment of the corneal ulcer. Tropicamide, atropine, phenylephrine and homatropine were also used as pupillary dilator in fundoscopy and pilocarpine for miosis. All products were in polyethylene bottles containing ammonium preservative benzalkonium chloride (BZK) at concentrations varying from 0.004% to 0.02% w/v. The microbial contamination of 287 samples was studied at the end of day 1, 2, 3, 4 or 7 days of use. The incidence of fungal and bacterial contamination of eye drops is presented in Table 1. The contamination rates of caps, droppers and residual contents are shown in Table 2. The highest and lowest contamination rates were 24.6% and 9.0% on days 1 and 3, respectively. The most contaminated part of the eye drop bottles was the caps (45.9%) followed by dropper (41.0%) and residual contents (13.1%) (Table 1).

Table 3 shows the contamination rates of different eye drop products. Pilocarpine (41.7%), atropine (31.8%), tropicamide (28.6%), betametasone (23.3%) and homatropine (21.1%) had the highest overall rates of contamination. The identities the wide range of fungi and bacteria detected in the contaminated eye drop products are presented in Table 4. All fungal isolates were soil saprophytes with exception of C. albicans and Rhodotorula rubra, which are part of the normal human flora. In some cases, fungi were recovered from both caps and residual eye drop contents (1 case), both cap and dropper (1 case) and
dropper and residual contents (1 case). The majority of bacterial isolates were either human flora or soil saprophytes. *Bacillus cereus* (37.7%) was the most common isolated organism, followed by coagulase negative, *Staphylococcus epidermidis*. In addition, *Bacillus subtilis*, Diptheroid species and *Nocardia* species were also recovered (Table 4). Sulfacetamide was the only antibacterial agent contaminated by *Nocardia* spp. In seven bottles, *B. subtilis* was recovered from both caps and droppers and in two cases *B. subtilis* and *S. epidermidis* were also recovered from residual contents.

**Discussion**

There is always the risk that eye drop products will become contaminated with microorganisms as a result of touching the cap while opening it for usage, contact of dropper with the ocular tissues and environmental factors. Sauer et al. believe the cycle of contamination between in-use medications and conjunctivae, may represent an important risk factor for eye infection [14]. In the present study, although all drops contained BZK, 17.8% of them were contaminated with different types of microorganisms. Tasli et al. [10] report the contamination rate in eye drop products containing BZK was 34.4%. An investigation of 200 eye drop products showed a 57% contamination rate [9]. Hovding and Sjursen studied the microbial contamination of 638 in-use multidose eye drop bottles [15] and reported that 12.9% were bacterial contaminated [15]. In addition, Schein et al. [16] in Baltimore described a contamination rate of 29% and Rahman et al. [17] believed that preservative-free eye drop products are at risk of contamination by pathogenic microorganisms. BZK is a preservative and antimicrobial agent used in eye drop solutions at concentrations of 0.004% to 0.02% w/v. Previous published reports have shown that drop solutions containing this preservative do not possess adequate antimicrobial properties [5,8]. Fazeli et al. have claimed that duration of use influences the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Incidence of fungal and bacterial contamination of 287 samples of eye drop products.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of use (Day)</td>
<td>Cap</td>
</tr>
<tr>
<td>1 (n=57)</td>
<td>4 (7.0%)</td>
</tr>
<tr>
<td>2 (n=36)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>3 (n=67)</td>
<td>2 (3.0%)</td>
</tr>
<tr>
<td>4 (n=37)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>7 (n=90)</td>
<td>4 (4.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

1Total contamination incidence from all eye drop products.
2Residual liquid from each eye drop bottle.

Table 2 | Contamination rates of caps, droppers and the residual liquid from each eye drop bottle of 287 samples of eye drop products. |
<table>
<thead>
<tr>
<th>Duration of use (Day)</th>
<th>Uncontaminated</th>
<th>Contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fungi</td>
<td>Bacteria</td>
</tr>
<tr>
<td>1 (n=57)</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>2 (n=36)</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>3 (n=67)</td>
<td>61</td>
<td>3</td>
</tr>
<tr>
<td>4 (n=37)</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>7 (n=90)</td>
<td>71</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>20</td>
</tr>
</tbody>
</table>

*Significant difference from the first day (*x*² test, *p* < 0.03).

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degree of contamination [9]. However in the present study the incidence of contamination declined with use.

In this investigation we noted a high frequency of contamination associated with the caps (45.9%), followed by dropper (41.0%) and residual contents (13.1%). Fazeli et al. also detected a high frequency of cap contamination [9]. Caps are usually contaminated by airborne microorganisms and through multi-use handling, and it is possible that the caps of squeezed bottles may act as potential reservoirs of microbial contaminants. The contamination noted in the drops from the residue in the bottles may have been caused by contact with the dropper tip during collection of samples.

In the present study, fungal contamination was largely due to airborne flora with Aspergillus flavus, Penicillium spp. and Cladosporium spp. the most common recovered fungi. However, other fungi were isolated from the eye drops including Aspergillus niger, Gliocladium spp., Acremonium spp. and Alternaria spp. The incidence of airborne fungal contamination varied widely, depending on season, temperature and relative humidity. Regarding the seasonality, Khuzestan, the location of this study, is a subtropical province in Iran and the ambient temperatures reach their peak from March through September. As we collected our samples during spring, the weather could offer an ideal environment for the growth of fungi. Martins-Diniz et al. found a relatively high number of airborne fungi in areas near to a hospital in Araraquara, southeastern Brazil including C. albicans and Rhodotorula rubra, constituents of normal human flora [4]. Both of these yeastlike fungi were recovered from eye drop solutions in our investigation and since they are associated with humans, the contamination was probably due to contact with the skin or eyes during drops application. The major isolated bacteria were either those associated with the normal skin flora (coagulase negative) or airborne Gram-positive spore-bearing bacilli. These results are similar to those of the earlier reports [9,10,17]. Contact with the eyelid of patients, nursing hands during administration and airborne may be results of contamination.

In conclusion even though eye drop contamination is a concern in hospitals, there is not always a direct relation between duration of usage and contamination rate.

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References


