SCIENTIFIC REPORT

Microbial contamination of preservative free eye drops in multiple application containers

M Q Rahman, D Tejwani, J A Wilson, I Butcher, K Ramaesh

Br J Ophthalmol 2006;**90**:139–141. doi: 10.1136/bjo.2005.078386

Background/aims: The majority of eye drops used in the United Kingdom contain preservatives and are bottled in plastic containers. Preservative free drops are used to avoid ocular irritation and allergies in certain individuals. The aim of this study was to investigate the incidence of microbial contamination of preservative free drops dispensed from multiusage containers.

Methods: Eye drop bottles were collected from patients attending the Tennent Institute of Ophthalmology outpatient and inpatient departments. The bottles were collected on day 3 (for inpatients) and day 7 (for outpatients) of use. The drops were inoculated onto different culture plates (chocolate agar, blood agar, fungal culture media, and enriched media) and the resulting microbial growth was identified using standard microbial identification techniques.

Results: 95 eye drop bottles were collected, containing a variety of 10 different eye drops. Significant bacterial growth was found in eight bottles. In total, seven different types of organism were identified from the eye drops. The organisms identified were *Staphylococcus aureus,* coagulase negative staphylococcus, *Bacillus spp, Serattia spp, Klebsiella oxytoca, Enterobacter cloacae,* and alpha streptococcus. *Staph aureus* was the commonest microbial organism.

Conclusion: Preservative free eye drops in multiple application containers are at risk of contamination by potentially pathogenic micro-organisms.

Most eye drops in the United Kingdom contain preservatives bottled in plastic containers.[1] Preservatives are added to avoid microbial contamination of the drops.[2–4] The addition of preservatives may cause ocular irritation, allergies, and disruption of the ocular surface.[5] Preservative free drops are prescribed for patients who are susceptible to reactions from preservatives, and to those who need frequent applications. However, microbial contamination of eye drops can lead to serious consequences with corneal infections and perforations having been previously reported.

Preservative free eye drops, that are not available commercially are supplied by Moorfields Eye Hospital Pharmacy, and stored locally in traditional glass containers designed for multidose use (fig 1). These preparations are given arbitrary time limits of use; between 1 and 7 days, based upon practical considerations of maintaining supply.

At present, preservative free eye drops in multiple application containers may be used for 3 days (inpatients) or 7 days (outpatients). This time period is arbitrary—not evidence based. A Department of Health directive recommended that "…urgent investigation…" is necessary to determine the safety period of use of preservative free eye drops dispensed in traditional glass bottle containers.[6] The aim of this study was therefore to evaluate and compare the microbial contamination arising after 3 days' and 7 days' use of preservative free eye drops in multiple application glass containers.

MATERIALS AND METHODS

Preservative free eye drops were obtained from Moorfields Eye Hospital Pharmacy, and prepared and stored into multiple application bottles by the Gartnavel General Hospital Pharmacy according to good medical practice guidelines. Eye drops were administered by nursing staff for inpatients, and by patients themselves in an outpatient setting. Used eye drop bottles were collected on either day 3 (inpatients) or day 7 (outpatients) of use. All patients gave informed consent for their used eye drop bottles to be analysed. Altogether, 10 different types of preservative free eye drop preparations were used (table 1).

The eye drop preparations were analysed both qualitatively and quantitatively.

One ml of eye preparation was added to each of two blood culture bottles (aerobic and anaerobic). Bottles were then incubated for a maximum of 14 days. If the cultures showed growth, an aliquot of broth was removed and examined as per standard laboratory procedures.

The quantitative analysis is detailed as follows: 0.1 ml aliquots of the eye preparations were dropped onto the surface of two blood agar plates, one chocolate agar plate, and two Sabouraud-dextrose agar plates. The 0.1 ml aliquots

Figure 1 Glass container designed for multidose use.

were spread over the surface of the agar plates. One blood agar plate was incubated aerobically and the other anaerobically, both at 37℃ for 48 hours, and then at 30℃ for a further 5 days. The chocolate plates were incubated at 37℃ in CO_2 for 7 days. The sabouraud-dextrose plates were incubated for 14 days at two different temperatures: one plate at 37℃, and another at 30℃. Colonies on the plates were counted at the end of the incubation period and values were given as colony forming units/litre. Any isolates were identified and then stored.

Statistical method

The 95% and 99% confidence intervals of the incidence of contamination were found using the binomial distribution. The statistical significance of the incidence in contamination between antibiotic preparations and non-antibiotic preparations, and between hospital inpatients and outpatients were compared using Fisher̀s exact test.

RESULTS

Altogether, 95 eye drop bottles were evaluated; 75 bottles were from inpatients, and 20 were from outpatients. The range of different eye drops analysed, and the number of contaminated bottles is presented in table 1.

Eight out of the 95 eye drop bottles were found to be contaminated by bacteria. This represents an overall incidence of 8.4%, with a 95% confidence interval of 3.71% to 15.9%, and a 99% confidence interval of 2.77% to 18.5%. None of the 53 antibiotic eye drop bottles showed signs of contamination, but the overall incidence of contamination in the 42 non-antibiotic bottles was 19% and the difference between these two groups was statistically significant ($p<0.01$). Hypromellose appeared to be the most likely eye drop bottle to be contaminated (60%), followed by prednisolone (21.4%) and then acetylcysteine (11.1%).

Of the 75 bottles collected from inpatients, three were contaminated (4%), whereas five out of 20 outpatient bottles were contaminated (25%). The difference between these groups was found to be statistically significant ($p<0.01$). Out of the 75 bottles examined from inpatients, 42 were non-antibiotic bottles and the contamination rate in these bottles was 12%. The contamination rate for non-antibiotic bottles collected from outpatients was 38%. The difference between these groups was found to be statistically significant ($p<0.01$). The identity of contaminating micro-organisms is presented in table 2.

It should be noted that several of the contaminated bottles grew more than one type of contaminant. The most common contaminant was *Staph aureus*, with a percentage occurrence of 4.5%.

DISCUSSION

We noticed a high incidence (8.4%) of microbial contamination of preservative free ophthalmic medications in multiple application containers during the usage period. The rate of contamination was high in non-antibiotic medications (19%). Although several studies have documented contamination of preserved eye drops, to the best of our knowledge, none has examined the contamination of preservative free drops dispensed from multiple application containers in a clinical setting. The contamination rate of preserved eye drops varies between 2.2% and 34.8%.[4 7]

Altogether, seven different organisms were detected, and only one of these (coagulase negative staphylococcus) was a normal commensal of the conjunctival flora. Contamination of eye drops can lead to serious ocular infections especially when the ocular surface defences are compromised with topical steroids. Application of contaminated eye drops may lead to potentially devastating consequences in patients with ocular surface diseases and after intraocular surgery where there are wound leaks. Templeton *et al* reported three cases post keratoplasty, in which *Serratia marcescens* keratitis developed as a result of the contamination of eye drops with this organism.[8]

Contamination was common in non-antibiotic bottles. Negative culture results from antibiotics such as gentamicin and cefuroxime may be the result of the high concentration of antibiotics, or the techniques used may not have been sensitive enough to isolate any contaminants. Previous studies on preserved eye drops have found high contamination rates in β blockers, steroid drops, and ocular lubricants.[9] One study, in which a range of preservative free eye drops were intentionally inoculated with four different micro-organisms in order to test their inherent antimicrobial efficacy, also concluded that acetylcysteine, hypromellose, and prednisolone drops are prone to contamination even in the presence of preservatives.[10] Our study has shown that contamination occurs in both inpatient and outpatient settings and that eye drops used by outpatients are more likely to be contaminated than those used by inpatients. Moreover, the highest rates of contamination were seen in non-antibiotic bottles used in an outpatient setting (38%).

Contamination of eye drops may be related to the design of multiple application containers. During administration, the pipette attached to the cap of the bottle, comes completely out of the container, and this exposes the open contents of the bottle directly. Previous studies on preserved eye drops have concluded that pathogenic Gram negative bacteria are more likely to grow in the bottle reservoir than Gram positive organisms, which are mainly commensal in the environment.[9 11] Spillage of the contents can also increase the chance of contamination. Poor technique in administering the drops is a further risk factor for contamination, especially if

Table 1 The range of eye drop bottles analysed

	Number of containers analysed	Number of contaminated bottles	% contamination
Gentamicin	20	0	0
Cefuroxime	18	0	0
Acetylcysteine	18	2	11.1
Prednisolone	14	3	21.4
Dexamethasone	7	0	0
Vancomycin	6	0	0
Hypromellose	5	3	60
Amphotericin	3	0	0
Cyclosporin	2	0	0
Fluconazole	2	0	0

Table 2 Identity of contaminating micro-organisms

Contaminating micro-oganism isolated	Number of containers contaminated	Occurrence (% of total)
Staph aureus	4	4.5
Coagulase negative staphylococcus	1	1.1
Bacillus spp	1	1.1
Serattia spp	1	1.1
Klebsiella oxytoca	1	1.1
Enterobacter cloacae	1	1.1
Alpha streptococcus	1	1.1

patients are self administering in an outpatient setting. Elderly patients, with poor vision and coordination, may inadvertently touch their eyes or skin with the pipette dropper, and on insertion of the dropper back into the container after use, may again contaminate the container. Patients who have to use these drops very frequently greatly increase the risk that any or all of the above situations may occur.

Unfortunately, there are no commercially available multiple application containers that have been proved to prevent contamination rates for preservative free medications, although several alternatives are available in other countries. In France, multiple application plastic squeeze bottles are available that contain an expurgating filter to remove preservatives before they reach the ocular surface.[12] Another design in the United States involves preserving medications in a boric acid-perborate mixture, which results in a hydrogen peroxide preservation system that decomposes on the ocular surface to water and oxygen.[12] In Germany, a specially designed container is used to deliver preservative free medications.[12 13] However, no clinical trial of these designs has been carried out to determine their effectiveness at reducing contamination, and all these designs consist of plastic, squeeze containers with screw-on caps—a design that can still result in microbial keratitis.[8 9]

Preservative free eye drops are commercially available in unit dose vials (UDVs), but their use in a domicillary setting is prohibitively expensive. Single unit preservative free drops are 1169% more expensive than the equivalent preserved eye drops.[10] It may also be inconvenient and cumbersome to carry boxes of these vials, compared with a single, multiple application container.

Preservative free eye drops in multiple application containers are at risk of contamination with potentially pathogenic micro-organisms. This may place some patients at increased risk of developing serious ocular infections. The prescription of these drops to patients with compromised ocular surface defences needs to be considered with caution.

ACKNOWLEDGEMENTS

The author would like to thank Dr G B Drummond for his help with the statistical analysis. This study was funded by a Research Endowment Fellowship Award, North Glasgow University Hospitals NHS Trust, 2004 (to KR, IB).

Authors' affiliations

M Q Rahman, D Tejwani, K Ramaesh, Tennent Institute of Ophthalmology, Gartnavel General Hospital, 1053, Great Western Road, Glasgow G12 0YN, UK
J A Wilson, I Butcher, Department of Clinical Microbiology, Western Infirmary, Glasgow G11 6NT, UK

Correspondence to: M Q Rahman, Tennent Institute of Ophthalmology, Gartnavel General Hospital, 1053, Great Western Road, Glasgow G12 0YN, UK; mmnrahman@hotmail.com

Accepted for publication 27 September 2005

REFERENCES

1 **Livingstone DJ**, Hanlon GW, Dyke S. Evaluation of an extended period of use for preserved eye drops in hospital practice. *Br J Ophthalmol* 1998;**82**:473–5.
2 **Furrer P**, Mayer JM, Gurny R. Ocular tolerance of preservatives and alternatives. *Eur J Pharm Biopharm* 2002;**53**:263–80.
3 **Barkman R**, Germanis M, Karpe G, *et al*. Preservatives in eye drops. *Acta Ophthalmol (Copenh)* 1969;**47**:461–75.
4 **Tasli H**, Cosar G. Microbial contamination of eye drops. *Cent Eur J Public Health* 2001;**9**:162–4.
5 **Pisella PJ**, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and preservative free glaucoma medication. *Br J Ophthalmol* 2002;**86**:418–23.
6 **Department of Health**. Guidance for use of opthalmic preparations in hospital and care homes. *Pharm J* 2001;**267**:307.
7 **Aslund B**, Olson OT, Sandell E. Studies on in-use microbial contamination of eye drops. *Acta Pharm Suec* 1978;**15**:389–94.
8 **Templeton WC 3rd**, Eiferman RA, Snyder JW, *et al*. erratia keratitis transmitted by contaminated eyedroppers. *Am J Ophthalmol* 1982;**93**:723–6.
9 **Schein OD**, Hibberd PL, Starck T, *et al*. Microbial contamination of in-use ocular medications. *Arch Ophthalmol* 1992;**110**:82–5.
10 **Oldham GB**, Andrews V. Control of microbial contamination in unpreserved eyedrops. *Br J Ophthalmol* 1996;**80**:588–91.
11 **Geyer O**, Bottone EJ, Podos SM, *et al*. Microbial contamination of medications used to treat glaucoma. *Br J Ophthalmol* 1995;**79**:376–9.
12 **Wilson LA**. To preserve or not to preserve, is that the question? *Br J Ophthalmol* 1996;**80**:583–4.
13 **Teping C**, Wiedemann B. [The COMOD system. A preservative-free multidose container for eyedrops]. *Klin Monatsbl Augenheilkd* 1994;**205**:210–17.