WORLD VIEW

Microbial contamination of multi-use ophthalmic solutions in Kenya

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Background/aims: Contaminated ophthalmic solutions represent a potential cause of avoidable ocular infection. This study aimed to determine the magnitude and pattern of microbial contamination of multi-dose ocular solutions at the Department of Ophthalmology, University of Nairobi, at the Kenyatta National Hospital, Kenya.

Methods: 101 vials were obtained for microbial examination after an average use of 2 weeks. The dropper tip and the residual eye drop were examined for contamination. The specimens were cultured, the number of colonies counted, the organisms identified and susceptibility testing to selected antimicrobial agents was done.

Results: Six (6%) of the 101 analysed vials were contaminated: 4/77 vials (5%) from a multi-user setting and 2/24 vials (8%) from a single user setting. Three contaminations (3/38, 8%) occurred in vials from the eye ward, another three (3/59, 5%) in vials from the outpatient clinic. Most bacteria identified belonged to the normal commensal flora of the eye. Isolated contaminants were micrococci (n = 2), *Staphylococcus epidermidis*, *Haemophilus* sp, *Bacillus* sp and a Gram negative rod. The dropper tip was more often contaminated (n = 6) than the residual solution (n = 1), and only one vial showed a contamination of both the drop and the tip.

Conclusion: Our data show a contamination rate of 6%, which is in the lower range of data published on the contamination of eye drops elsewhere (0.07% to 35.8%).

Contaminated eye drops and other ophthalmic solutions are a potential cause of ocular infection. They can be associated with keratitis[1] and corneal ulcers[2] and carry the risk of transmitting opportunistic micro-organisms,[3 4] as well as pathogenic organisms, such as *Pseudomonas aeruginosa* and *Serratia marcescens*.[1] The published contamination rate of in-use ophthalmic solutions varies widely in the literature from 0.07%[5] to 35.8%.[3] Apart from the risk of infection, bacterial contamination of eye drops may alter the pH of the solution and therefore reduce the efficacy of the drug.[6]

In order to prevent contamination, most preparations contain antimicrobial substances, unless the solution itself has an antimicrobial effect. These substances aim at preventing or inhibiting the growth of micro-organisms which increase the risk of infection or degradation of the drug. The self sterilising effect of eye drops caused by the presence of preservatives has been discussed controversially.[7] Preservatives must meet several requirements: (1) to be compatible with other ingredients; (2) to be efficient during the entire duration of use of the eye drops; and (3) to be non-toxic to the eye. Commonly used preservatives of ophthalmic solutions are benzalkonium chloride, which also works as a detergent and therefore increases the penetration of the active ingredient of the drug; thiomersal; chlorhexidin; parahydroxy benzoate; phenylmercuric nitrate; EDTA; chlorobutanol; benzyl alcohol; phenyl ethyl alcohol; and parabens.[8 9] As preservatives interfere with the metabolism and inhibit the growth of micro-organisms, they may have similar effects on human cells, explaining potential cytotoxic effects and inflammatory cell responses.[6] The antimicrobial activity is important for the rate of infection resulting from contamination during the process of instillation. Contact with fingers or lids, ciliaries, conjunctiva and cornea are possible causes of contamination even if instilled by healthcare professionals. Plastic bottles have been reported to be more commonly contaminated near the bottle cap. This has been attributed to a lack of preservative at this area.[4] In a clinical study 220 in-use medications of 101 patients with non-microbial ocular surface disorders were examined by cultivating the bottle caps. The authors concluded that a cycle of contamination between in-use medications and conjunctiva may present an important risk factor for microbial keratitis in patients with ocular surface disease.[10] The occurrence of bacterial ocular infection such as keratits and endophthalmitis transmitted by contaminated eye droppers has been reported.[11] In a recent study the authors noted that some cases of bacterial keratitis in Iran are thought to be due to contaminated eye drops used on multiple patients.[12] Brudieu *et al* found a big difference in contamination rates between vials used by ophthalmological patients (17.7%) and vials used by medical and gerontological patients (35.8%). A positive correlation was also found for vial contamination and the duration of use. Vials containing an antimicrobial agent were less likely to be contaminated than vials without antimicrobials. However, no clinically relevant infection through such vial contamination was identified.[3] In a study comparing the contamination rate of drops used in an eye department and a nursing home no difference was found but the authors stated that the residual eye drop is more often contaminated than the tip of the bottle. They also noticed the presence of Gram negative organisms in the nursing home.[7] Most studies, however, found the bottle tips to be more often contaminated than the solution.[2 4 13] Therefore, topical eye medications may present a potential risk of infection, especially if the ocular epithelial barrier is compromised. Minimising the contamination of eye drops and the transmission of infections is an important issue in clinical ophthalmology. Several regulations and suggestions have been made in this context. Some suggest discarding eye drops in multiple use containers in a domestic setting after 4 weeks of use and after 1 week in

Abbreviation: BHI, brain heart infusion

Table 1 Preservatives (n = 101)

Preservative	Number of medications	% of medications
Chlorbutol IP 0.5%	18	18
Benzalconiumchloride 0.01%	59	58
Phenylmercuric nitrate IP 0.001%	11	11
Chlorhexidine acetate	1	1
Thiomersal IP 0.005%	3	3
None	9	9
Total	101	

hospital wards.[14] With this in mind, we conducted the following cross sectional study and analysed 101 ophthalmic solutions examining bacterial and fungal contamination.

MATERIALS AND METHODS

In total, 101 containers were obtained for microbial examination. We distinguished between topical medications used by a single patient and those used by several patients as well as between different settings. All specimens were taken to the Department of Microbiology, University of Nairobi at the Kenyatta National Hospital, Kenya, and analysed the same day in order to limit the effects of storage time and mimic the clinical situation as closely as possible. Only eye drops with a minimum use of 7 days were included, again to get a cross sectional view of the medication used at the Department of Ophthalmology. The microbial analysis was performed on both the dropper tip and the residual eye drop for each container. The specimens were obtained and cultured according to the following protocol:

- A sterile cotton swab was moistened in sterile brain heart infusion (BHI) before wiping the nozzle tip of the eye drop containers and then used to inoculate the culture plates.
- The vials were inverted and one drop was directly inoculated on each of the media and then spread across the plates.

All media except the Sabouraud agar plates were incubated at 37℃ for 48 hours and evaluated after 24 and 48 hours. The blood-agar, chocolate-blood agar plates were incubated in a microaerophile environment.[15] The Sabouraud agar plates were incubated at 30℃ for up to 10 days and evaluated for growth on days 1, 5 and 10. The BHI broth was also incubated at 37℃ and subcultured on blood agar after 24 hours. All culture media except Sabouraud dextrose agar (BioMérieux, F) were obtained from Biotec Laboratories Ltd, UK.

A significant growth was considered a growth on the main inoculation site or on two or more streaks on the plate. The BHI was analysed for changes in colour and turbidity of the media.

The colonies on solid media were counted and all organisms identified by microscopy after Gram staining and biochemical tests. Bacteria were tested for susceptibility of antimicrobial agents by the disc diffusion method using Mueller-Hinton agar as media and antimicrobial discs (tetracycline, penicillin, erythromycin, imipenem, vancomycin, gentamicin, chloramphenicol, norfloxacin, ciprofloxacin, cefazolin, cefotaxime) for testing.

Cross tab analysis using exact Fisher's test (SPSS for Windows 14) was performed to determine statistically significant differences between the multi-user, single user eye drops, and for different settings (outpatient clinic, ward and theatre).

RESULTS

A total of 101 medications were analysed; 77 from a multi-user setting and 24 from a single user setting; 59 specimen were obtained from the outpatient clinic, 38 from the ward and four from the minor theatre. Table 1 shows the different, commonly used preservatives found in the analysed specimens. The vials were grouped in five different categories: mydriatics, tetracaine, antibiotics, steroids, others. Overall, six (6%) of the 101 analysed vials were contaminated (table 2) at the bottle tip alone or with additional contamination of the solution. A contamination of the solution only without additional contamination of the tip was not found in any specimen. Within the five categories the rate of contamination varied between 0% for antibiotics and 10% for tetracaine.

The dropper tip was more often contaminated (n = 6) than the residual solution (n = 1). One bottle showed contamination of both the dropper tip and the medical solution. The contamination rate of antibiotic medication was 0 out of 18 (0%) while six (7%) of the 83 non-antibiotic vials were contaminated.

Most of the identified organisms were part of the normal skin or conjunctival flora. Gram positive organisms were cultivated from four of the six contaminated medications (67%) and two contaminated medications grew Gram negative organisms (33%). Two out of the six contaminated medications showed heavy growth with many colonies (33%) while the other four yielded only a single colony on the main inoculum.

The contamination was 4/77 (5%) for the multi-user setting and 2/24 (8%) for the single user setting ($p>0.5$). A contamination of 3/38 (8%) was found in vials obtained from the eye ward. Three of the 59 bottles taken from the outpatient department (5%) were contaminated. No specimen obtained from the minor theatre was found to be contaminated ($p>0.5$ in all cases). No fungal contamination was found in any specimen.

No significant difference in contamination was found between locally produced and imported drugs ($p>0.5$). The

Table 2 Eye medications and contamination (n = 101)

Eye medication	Number tested	Contaminated	Tip contaminated	Tip/drop contaminated	Drop contaminated	Contaminants
Mydriatics	56	4 (7%)	3 (5%)	1 (2%)		*Haemophilus* spp, *Bacillus* spp, *Micrococcus* spp, *Staphylococcus epidermidis*
Tetracaine	10	1 (10.0%)	1 (10.0%)			*Micrococcus* sp
Antibiotics	18	0				
Steroids	14	1 (7%)	1 (7%)			Gram negative rod
Others	3	0				
Total	101	6 (6%)	5 (5%)	1 (1%)		

Table 3 Contaminated medications and preservatives

Eye medication	Bottle size/ tube size	Preservative	Contaminant
Tropicamide 0.8% with phenylephrine 5%	5 ml	Chlorbutol IP 0.5%	*Micrococcus* sp
Cyclopentolate 1%, Phenylephrine 10%	5 ml	None	*Bacillus anthracoides*
Tropicamide 1%	5 ml	Phenylmercuric nitrate IP 0.001%	*Staphylococcus epidermidis*
Tropicamide 1%	15 ml	Benzalconiumchloride 0.01%	*Haemophilus* sp.
Tetracaine 0,5%	15 ml	Benzalconiumchloride 0.01%	*Micrococcus* sp
Dexamethasone Phosphate 0.1%	5 ml	Benzalconiumchloride 0.01%	Gram negative rod

contamination for these two groups was 1/12 (8%) and 5/89 (6%) respectively.

None of the medications grew more than one type of bacterium. Three of the 101 bottles (3%) were found to be past the expiry date. None of these showed any bacterial growth. Three other bottles did not have a clean and legible label, which might suggest improper handling. However, none of these bottles was contaminated.

Specimens where only little solution was left at the time of analysis were not contaminated any more often than medications with a large amount of residual solution.

All identified bacteria were susceptible to imipenem, vancomycin, gentamicin and ciprofloxacin, which proved to be the most powerful antibiotic in this study. On the other hand some commonly used antibiotics in Nairobi showed some lack in effectiveness. Two of six bacteria were resistant to chloramphenicol, tetracycline, penicillin and cefotaxime (see table 4).

DISCUSSION

We noticed a microbial contamination of 6/101 (6%) of in-use multiple application dispensers. The mean contamination rate of preserved eye drops described in the literature varies widely from 0.07%[5] to 35.8%.[3] Four of the six contaminated eye drops only showed one single colony. These can be regarded as occasional contamination[16] and may be of limited clinical relevance.

Five different micro-organisms were detected. As the containers were analysed on the day of collection, our results are likely to represent the specific clinical situation of that day. A slow self sterilising effect does not prevent the transmission of micro-organisms from one patient to another if the medication is used frequently with different patients—a situation not uncommon for an ophthalmic outpatient department.

Four of six of the identified organisms were Gram positive and 2/6 Gram negative. Most of the organisms were part of the normal commensal flora of the conjunctiva or the skin. The resident flora of the conjunctiva and eyelid mainly comprises of Gram positive bacteria, including coagulase negative staphylococci, *Corynebacterium* spp, *Propionibacterium* spp, as well as *Staphylococcus aureus*, *Bacillus* spp, *Micrococcus* spp and *Enterobacter* spp.[17 18] This is in accordance with several other published studies.[7 13 16] However, it differs from results published by Rahman *et al*,[19] who found only a small proportion of the micro-organisms identified to be part of the normal commensal flora when studying the contamination of unpreserved eye drops.

Table 4 Antibiotic susceptibility pattern of bacterial isolates (n = 6 tested)

	Susceptible	Intermediate	Resistant
Tetracycline	4		2
Penicillin	3	1	2
Erythromycin	4	2	
Imipenem	6		
Vancomycin	6		
Gentamicin	6		
Chloramphenicol	4		2
Norfloxacin	5		1
Ciprofloxacin	6		
Cefazolin	5		1
Cefotaxime	4		2

A cycle of contamination between the lids and dropper tips was suggested by Schein *at al*.[10] The contamination of eye drops and eye drop dispenser with the same micro-organism, especially Gram negative, has been described by the same group. This represents a potentially serious risk for ocular infection, especially in cases of compromised corneal epithelium as in extensive contact lens wear, ocular trauma or the use of topical steroids.

In this study pathogenic organisms were rare and showed limited growth that probably did not represent a clinically relevant risk of infection.

The contamination rates of the setting of the eye ward (3/38, 8%) were not significantly different from the setting in the outpatient department (3/59, 5%). even though patient characteristics were different in the two settings: in the outpatient department, mostly mydriatics and local anaesthetics are used for diagnostic purposes, whereas in the eye ward many inpatients received specific topical eye treatment. Mydriatics, especially in the outpatient department, are used frequently and used quickly as part of routine eye examinations.

We did not find any significant difference in the contamination rate of eye drops/dropper tips and the residual volume which was left in the bottle. This supports the previously described self sterilising effect of many eye medications. We therefore conclude that the risk of transmitting micro-organisms may depend on the frequency of shared eye medications in a multi-user setting.

Another relevant factor might be differences in the administration of eye drops. In the setting of the Department of Ophthalmology, University of Nairobi, Kenyatta National Hospital, Nairobi, Kenya, this task is performed by ophthalmologists and postgraduate students in the outpatient department and nurses in the eye ward. This regular involvement of qualified eye care personnel might contribute to the overall low rate of contamination.

The design of the containers might also influence contamination. Only bottles with a tip attached to the bottle itself were analysed in this study. The bottle tips were more often contaminated (n = 6) than residual drops (n = 1), with contamination of both the tip and the residual solution appearing in only one specimen. These results are similar to the ones

reported in earlier studies.[11 13 16] One reason for this pattern to be considered is the antimicrobial activity of preservatives or of the solution itself in antibiotic drops. Such antimicrobial effects, however, may not act sufficiently on the tip itself as the contact time is limited. Further, the tip provides a large surface for contamination from ocular structures or hands. Even dried crusts can sometimes be found on the bottle tips. The removal of such remnants with a sterile swab might further reduce the contamination rate.[4] However, the contamination of the solution itself has to be regarded as clinically more relevant since these get in direct contact with the patient's eye.

It is not possible to establish the exact contamination rates of each individual product in this study as the total number of specimens is not sufficient and the variety of different medications too great for this purpose. However, the contamination rates of commercially available (5/89, 6%) and locally produced (1/12, 8%) medications were comparably low in our study. This is particularly relevant for the situation in developing and emerging nations where drugs are increasingly produced locally to reduce costs.

The number of expired eye drops, 3/101 (3%), in our study is relatively low in comparison to the 20% of the bottles examined by Wessels *et al*.[5] We recommend not storing any open bottles in the back of drawers or on top shelves but always keeping them handy and limited to those actually needed. Furthermore, we recommend noting the date of first opening on each container, as the duration of use might be another and possibly more relevant parameter in this context rather than the expiry date.[5]

Conclusion

The results of this study support the importance of a proper set of rules and the correct handling and application of eye medications in multi-user settings. Healthcare providers and patients should be carefully trained and informed as to how to administer eye medications. Patients who are unable to use eye drops in an aseptic way because of age or other physical (for example, poor vision) or mental limitations should be assisted by competent relatives or caretakers.

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REFERENCES

1 **Mayo MS**, Schlitzer RL, Ward MA, *et al*. Association of Pseudomonas and Serratia corneal ulcers with use of contaminated solutions. *J Clin Microbiol* 1987;**25**:1398–400.
2 **Donzis PB**. Corneal ulcer associated with contamination of aerosol saline spray tip. *Am J Ophthalmol* 1997;**124**:394–5.
3 **Brudieu E**, Duc DL, Masella JJ, *et al*. Bacterial contamination of multi-dose ocular solutions. A prospective study at the Grenoble Teaching Hospital. *Pathol Biol (Paris)* 1999;**47**:1065–70.
4 **Clark PJ**, Ong B, Stanley CB. Contamination of diagnostic ophthalmic solutions in primary eye care settings. *Mil Med* 1997;**162**:501–6.
5 **Wessels IF**, Bekendam P, Calvin WS, *et al*. Open drops in ophthalmology offices: expiration and contamination. *Ophthalmic Surg Lasers* 1999;**30**:540–6.
6 Perry HD, Donnenfeld ED. Issues in the use of preservative-free topicals. *Manag Care* 2003;**12**:39–41.
7 **Raynaud C**, Laveran H, Rigal D, *et al*. Bacterial contamination of eyedrops in clinical use. *J Fr Ophtalmol* 1997;**20**:17–24.
8 **Zanen A**. Prevention of infections in the ophthalmology office. *Bull Soc Belge Ophtalmol* 1996;**260**:9–16.
9 **Sklubalova Z**. Antimicrobial agents in eyedrops. *Ceska Slov Farm* 2004;**53**:107–16.
10 **Schein OD**, Hibberd PL, Starck T, *et al*. Microbial contamination of in-use ocular medications. *Arch Ophthalmol* 1992;**110**:82–5.
11 **Coad CT**, Osato MS, Wilhelmus KR. Bacterial contamination of eyedrop dispensers. *Am J Ophthalmol* 1984;**98**:548–51.
12 **Jalali R**, Zinolabedini F, Moradi M, *et al*. Bacterial contamination rate of eyedrops: comparison of a hospital and a private outpatient center in Kermanshah, Iran. *Insight* 2004;**29**:12–4.
13 **Geyer O**, Bottone EJ, Podos SM, *et al*. Microbial contamination of medications used to treat glaucoma. *Br J Ophthalmol* 1995;**79**:376–9.
14 *British National Formulary*. London: British Medical Association and Royal Pharmaceutical Society of Great Britain, 1997:441.
15 **Schaller UC**. *Untersuchungen zur mikrobiologischen Probengewinnung und Transport bei bakteriellen Entzündungen des äußeren Auges*. Munich: Ludwig-Maximilians-Universität, 1998.
16 **Hovding G**, Sjursen H. Bacterial contamination of drops and dropper tips of in-use multidose eye drop bottles. *Acta Ophthalmol (Copenh)* 1982;**60**:213–22.
17 **Ta CN**, Chang RT, Singh K, *et al*. Antibiotic resistance patterns of ocular bacterial flora: a prospective study of patients undergoing anterior segment surgery. *Ophthalmology* 2003;**110**:1946–51.
18 **Leong JK**, Shah R, McCluskey PJ, *et al*. Bacterial contamination of the anterior chamber during phacoemulsification cataract surgery. *J Cataract Refract Surg* 2002;**28**:826–33.
19 **Rahman MQ**, Tejwani D, Wilson JA, *et al*. Microbial contamination of preservative free eye drops in multiple application containers. *Br J Ophthalmol* 2006;**90**:139–41.

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