Microbiological Safety of Using Eye Drops After One Month: Contamination is A Rule or Mere Regulation?

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Abstract

Objective: The study was aimed to assess microbial contamination of the eye drop solution after using for one month.

Design: A prospective study of the sterility of sixty multi-drop residual eye drops was done. Eye drops were collected from the rural based patients, after at least one month of use. All the eye drop bottles were de-labeled and coded and sent for microbiological evaluation. Only eye drop solution was examined for contamination.

Results: None of the eye drop had any qualitative or quantitative evidence of growth after 14 days of incubation on various culture media.

Conclusion: The eye drops can be used without fear of microbiological contamination for at least six weeks after opening the vial irrespective of its contents and presence of preservatives.

Keywords: eye drops, microbial, contamination, extended use

Introduction

Extended use of the multi-drop ophthalmic eye drops is a debatable issue. For chronic ocular diseases like glaucoma and dry eye, patients have to use the eye drops lifelong. Depending upon the amount of the drug it contains, the eye drop bottles last for a variable time. It would be desirable by the patient, on account of factors like cost and frequent visits to repurchase the eye drops, that the eye drops could be used till the last drop was left. But stability, efficacy, and efficient use are among some of the limitations. Contamination of the solution is the most important concern in extended use of the eye drops. Different studies have reported incidence of contamination between zero and 37%.¹⁷ In two reports, keratitis from the contaminated bottle tip has been reported.⁵,⁶ But interestingly in most other studies contamination has not been identified to result in clinically relevant infections.⁸,⁹ Preservatives are added to eye drops to prevent microbial growth in solution, but preservatives too have a shelf life. Many drugs have become available in preservative free form and many more in between will and added to this in future. Preservative free solutions have been found to be more likely contaminated than the preservative containing eye drops.³ Instructions on eye drop containers usually advise to discard the multi-use eye drops after 4 weeks of opening. At least one study reported no statistically significant difference in rate of contamination between less than 4 weeks and less than eight weeks of use.¹⁰ Recent years has seen availability of the improved filling, packaging and safety of the eye drops. Most, except few studies about contamination and safety of the eye drops have been from the developed world countries but in developing countries usage conditions and levels of hygiene are different.⁸ In addition, cost of the unused drug adds burden to the user. The study was done to know the contamination in the commercially available eye drops after using for a month in the Indian rural set up.

Material & Methods

The study methodology is summarized in (Figure 1).

![Flowchart](http://dx.doi.org/10.7869/djo.164)
**Collection of eye-drops**

Sixty multi-drop bottles from the patients attending the eye out patient department were collected. Eye drop bottles sold only from the hospital pharmacy counter were collected for the study. During the collection phase of the eye drops, the date of sale was marked on the label of the bottle. A record of the patient’s registration number and batch number of the eye drop was maintained. A drop box was made available in outpatient area of the hospital to collect used eye drop bottles. On weekly basis collected eye drops were taken out of the box. Bottles opened at least 35 days prior and containing sufficient residual amount of medication were sorted out. Amongst these eye drop bottles, either FFS (form, fill and seal) or three piece polypropylene bottles containing antibiotic, lubricant, anti-glaucoma or anti-allergic contents were selected for inclusion in study (Table 1). External visual appearance of the bottle was observed as “clean” and “unclean”. A bottle was considered as unclean if any part of the bottle-tip, neck or body had dirty surface. After collection, all eye drops were de-labeled, coded, and kept at room temperature in a clean plastic container. These were then sent for assessment of microbial contamination. After culture results decoding was done for comparison to analyze relationship of the bottle and type of polypropylene bottle.

### Table 1: Frequency distribution of eye drops along parameters used to study relationship with microbial contamination of solution

| Parameter               | Number (Percent) |
|-------------------------|------------------|
| **Contents of eye Drops** |                  |
| Moxifloxacin 05%        | 16 (27)          |
| Carboxy-methylcellulose 0.5% | 23 (38)       |
| Timolol 0.5%            | 11 (18)          |
| Olopatadine 0.3%        | 10 (17)          |
| **Preservative**        |                  |
| Preservative free       | 16 (27)          |
| Preservative containing | 44 (73)          |
| **External Appearance** |                  |
| Unclean                 | 24 (40)          |
| Clean                   | 36 (60)          |
| **Bottle Type**         |                  |
| FFS                     | 32 (53)          |
| Three-Piece             | 28 (47)          |

**Culture**

After receiving in the laboratory, the code on the eye drop bottles were noted. The eye drop bottles were either processed immediately or refrigerated at 4 degree centigrade for a maximum of 17 hrs, before microbial evaluation. Whole process was carried out under aseptic conditions. The eye drop bottle was inverted and two drops each were directly inoculated on to the surface of two blood agar plates, one chocolate agar plate and two Sabouraud-dextrose agar plates, without touching the bottle tip. The eye drop residue was spread over the surface of the agar plates. One blood agar plate was incubated aerobically and the other anaerobically, both at 37 degree centigrade for 48 hrs, and then at 30 degree centigrade for another five days. The chocolate agar plates were incubated at 37 degree centigrade in CO2 for seven days. The Sabouraud-dextrose plates were incubated for 14 days at two different temperatures, one plate at 37 degree centigrade and other at 30 degree centigrade. After incubation the plates were examined for any growth.

For qualitative analysis, 0.5 ml of eye drop residue was added to 10 ml of thioglycollate broth and incubated at 37 degree centigrade for a maximum of 7 days and examined for the growth.

**Statistical Analysis**

Data was entered in Microsoft excel sheet 2010. Unpaired “t-test” was applied to study the relationship between microbial contamination and the type of content, presence of preservative, external appearance of the bottle and type of polypropylene bottle. The level of significance (p value) was set at <0.05.

**Results**

Collection of the eye drops, meeting the study criteria, took sixty eight days. The period since opening of the bottle and collection varied between 37 days to 54 days. Twenty four (40%) bottles had unclean, dirty neck or body. The average delay between collection and culture was 1.7 days (Range 1-5 days).

On qualitative analysis, no visible turbidity or growth was seen after seven days of incubation. None of the eye drop solution had any growth on any culture media after a period of 14 days. Absence of microbial growth was not related to the type of content (p >0.9), presence of preservative (p >0.9) or external appearance of the bottle (p >0.9).

**Discussion**

Our results on used multi-dose eye drops suggest that the microbial contamination of the solution is not the rule, even after one month. In rural Indian setup storage of eye drops may be improper and unhygienic. In our study, we noted dirty external appearance in twenty six percent of eligible bottles. Previous studies have shown wide variation in frequency of microbial contamination of eye drop after their use, and incidence of contamination varies from 0% to 37%.

The growth has even been detected in unopened bottles. A number of factors seem to be determinant of contamination of solution. Culture results may be affected by the source of sample, presence of the preservative, duration since opening, type of the drug and the usage pattern or conditions. In studies where the sample were taken from the solution only, as also the case in our study, either the contamination was not detected or had low incidence. Nentwich et al did not find any significance difference in contamination between
dropper tips and residual solution. The culture positivity rate increased by culturing the bottle tip as this seems to be the most frequently colonized site. Presence and absence of the preservative, type of the preservative and concentration of the preservative has been shown to affect the incidence of contamination. Studies have reported conflicting results and in some of published reports no such association with regard to use of preservatives or type of preservative has been identified. In our study there was no growth irrespective of the presence or absence of the preservative. Similarly, we did not find any association with regard to the type of the content, unlike many studies where increased risk of the contamination has been reported in non-antibiotic solutions. But a few have reported no contamination of non-antibiotic drops also. Danny H et al found incidence of contamination in seventeen percent of the dirty looking bottles, higher than the clean appearing bottles. In our study twenty six percent eye drops had dirty external appearance, but were found to have non-contaminated solution. Studies carried out involving glass bottles with separate dropper had reported higher incidence of contamination. In our study we included only polypropylene eye drop bottles, and there was no difference between FFS pack or three piece bottles.

The period of use and incidence of contamination has been variably reported. Livingstone et al has reported increased but statistically insignificant rate of contamination with increased duration of use. Similarly, Geyer et al have found relation between length of use and rate of contamination, but there was no clinically and statistically significant difference in usage between 4 weeks and up to 8 weeks. Many studies have noted that touching the dropper tip as important source of contamination which may become non-sterile if it is inadvertently touched with hand or comes in contact with the eye or any other surface. Instructing the users about proper and safe handling of the eye drop during use, decreases the risk of contamination of the eye drop. The duration an eye drop would last, depends upon the usage frequency, wastage and amount of the drug supplied per bottle. Technology of filling and packaging of eye drops has improved in last decade or so. Putting instruction on eye drop container “not to touch tip”; may add to patient’s education about safe usage of the drop. Wilson has correctly commented in his study that early discontinuation of the eye drop is “...based on fear not science”. Under rule 126A, schedule FF of the drugs and cosmetic act, 1940 and the drug and cosmetic law, 1945 it is mandatory to mention the statement “Use the solution within one month after opening the container” on the label of the container. The regulations were last amended nearly half a century ago. In last fifty years revolutionary changes have taken place in packaging of eye drop bottles, patient’s awareness about hygiene and awareness about health. But in light of these changes, guidelines about usage of eye-drops have not been reviewed.

Our study results suggest that multi-drop eye drops can be used for an extended period. Since, we have not identified the “maximum safe period” for extended use, but given the number of pre-culture period of exposure (up to 54 days); we believe the continued use after four weeks in chronic, non-infective conditions like ocular allergies, glaucoma and dry eye etc may be safe. However, more studies need to be taken with different groups of eye drops with different durations to determine maximum duration of sterility of eye drop solutions. In conditions with more risk of infection transmission and in post operative period the recommended regulation of using solution within one month of opening should be followed unless larger studies are taken up to prove safer use after one month.

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