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Bacterial contamination of eye drops in multiple application bottles at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria

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Abstract: Contamination is the presence of any substance or organism that makes a preparation impure. In Ophthalmology, different types of eye drops in multiple application bottles are in common use for both diagnostic and therapeutic purposes and their contamination, reported by several authors, may be associated with ocular irritation, disruption of ocular surface and ocular infection. We therefore decided to determine the incidence of bacterial contamination of eye drops in multiple application bottles. Eye drop in multiple application bottles dispensed to patients at the Ophthalmology clinic of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku, Awka Anambra state of Nigeria on outpatient basis were retrieved from them after 7-28 days and the left-overs in these bottles were analyzed. A total of 30 eye drop bottles were collected within the period of two months. One bottle was discarded because it contained no specimen. The incidence of bacterial contamination of all the eye drops over the 7-

28day period was 0.31 (31%). The incidence of bacterial contamination of antibiotics eye drop was 0.18(18%) compared to 0.38 (38%) for non-antibiotics eye drops. The binomial probability of contamination was 0.0186. There was no statistically significant relationship between the duration of usage of the eye drops and the percentage bacterial contamination using student t-test (P value = 0.3214).The incidence of bacterial contamination was higher in non-antibiotic-containing eye drops compared to antibiotic-containing eye drops. There was no correlation between the duration of usage and bacterial contamination of eye drops.

Key Words: Bacterial contamination, eye drops, multiple application, duration of usage.

INTRODUCTION

Contamination is the presence of any substance or organism that makes a preparation impure, or the soiling or pollution by inferior materials[1]. In Ophthalmology, different types of eye drops in multiple application bottles are in common use for both diagnostic and therapeutic purposes and their contaminations have been reported by several authors[2-8]. Some of these ophthalmic eye drops have some preservatives of various types added to them to avoid microbial contamination of the eye drops[9-11]. However, addition of preservatives may be associated with ocular irritation, allergies and disruption of ocular surface[12]. These patients who are susceptible to reactions and need frequent application may benefit from preservative free drops[13].

Contaminated eye drops in multiple application bottles poses serious risk factor for ocular infections.[4] Plastic bottles have been reported to be more commonly contaminated near the bottle cap and this has been attributed to lack of preservatives at this area.[2] The percentage of bacterial contamination for such eye drops have been reported to range from 2.2% to 34.8% [4, 8,11,14-16].

The use of contaminated eye drops can be associated with keratitis, corneal ulcers and endophthalmitis by bacteria such as *Serrate marcescens* and *Pseudomonas pyocyanea*[4, 16, 17]. Such bacterial infections are more likely to occur if the ocular epithelial barriers are compromised[18]. In addition to increasing the morbidity, contaminated eye drops can also prolong treatment and hospital stay thereby increasing the total cost of treating a particular eye disorder in a patient. It may be that the higher the magnitude of contamination (bacterial load) of the eye drop, the higher the chances of contacting infection by using the eye drop. It is also possible that the bacterial load will depend on the duration of usage of the eye drop by the patient after prescription. In all, minimizing the contamination of multiple-dose eye drops and subsequent reduction in rate of transmission of infection is an important issue in clinical ophthalmology[18].

Objectives of the study: The general objective of this study was to determine the occurrence of bacterial contamination of eye drops in multiple application bottles. The specific objectives included the following:

a) To determine the incidence of bacterial contamination of eye drops in multiple application bottles.
b) To determine the difference in incidence of contamination between the antibiotic and non-antibiotic eye drop.

c) To determine the correlation between the duration of usage and bacterial contamination of eye drops in multiple application bottles.
d) To determine the probability of bacterial contamination of eye drops in multiple application bottles.

MATERIALS AND METHODS

Eye drops (antibiotic and non-antibiotic) in multiple application bottles earlier dispensed to patients at the Ophthalmology clinic of Chukwuemeka Odumegwu Ojuwu University Teaching Hospital Amaku, Awka Anambra State of Nigeria were retrieved from them after 7-28 days of use over the two month period from 31-10-2015 to 30-11-2015. Thirty of such bottles were collected and the left-over contents analyzed for bacterial contamination. Each bottle was analyzed as soon as it was collected from the patient to minimize possible further contamination. Informed consent and ethical approval were obtained from the patients and the Ethical Committee of Chukwuemeka Odumegwu Ojukwu Univesty Teaching Hospital (COOUTH) respectively. The durations of usage (in days) of the eye drops by the patients were indicated on the bottles after retrieval as follows:

a) Eye drops that were used for 1 week before retrieval.
b) Eye drops that were used for 2 weeks before retrieval.
c) Eye drops that were used for 3 weeks before retrieval.
d) Eye drops that were used for 4 weeks before retrieval.

There was qualitative analysis of eye drops for bacterial contamination.1.0 ml of each eye drop was added to each of two blood culture bottles (aerobic and anaerobic). This was repeated for the 30 samples. Thereafter, the blood culture bottles were incubated for 14 days. Thereafter, the blood culture bottles were inspected for evidence of growth of micro-organism. For the bottles that showed evidence of growth, an aliquot of the broth was stained and examined to identify the bacteria.

Statistical analysis: The incidence of contamination of the eye drops was calculated. The difference in incidence of contamination between the antibiotics and non-antibiotics preparations was also calculated. Also the binomial probability of bacterial contamination of the eye drops was calculated using Stat Trek Online Binomial Calculator. The statistical relationship between duration of usage and incidence of bacterial contamination determined using students't-test. The statistical analysis was done using Graph Pad Prism 7.0.

RESULTS

A total of 30 eye drop bottles were collected within the period of two months. One bottle was discarded because it contained no specimen. Therefore, 29 bottles were evaluated giving coverage of 96.7%. Eleven bottles, (37.9%) contained antibiotic while 18 bottles (62.1%) contained non-antibiotic eye drops.The incidence of bacterial contamination of the eye drops over the 7-28 day period was 0.31 (31%). Also, the incidence of bacterial contamination of antibiotic eye drops was 0.18 (18%) compared to 0.38 (38%) for non-antibiotic eye drops. Therefore, the difference in incidence of contamination between the antibiotic and non-antibiotic eye drops is 10%.

Table 1 and Figure 1 show the distribution of bacterial contamination among the antibiotic and non-antibiotic eye drops. **Table 2 and Figure 2** show the relationship between the duration of usage and the presence or absence of bacterial contamination among the eye drops. There was no statistically significant relationship between the duration of usage of the eye drops and the percentage bacterial contamination using student t-test (P value = 0.3214) as shown in Table 2 and Figure 2.The binomial probability of contamination was 0.0186 as shown in Figure 3. This represents the probability of the multi-dose eye drops being contaminated by bacteria when used for a period of 7-28 days.

Table 1: Different eye drops used and the number of contaminated bottles

Name of eye drop	Interpretation	Duration of use	Organisms isolated
Beoptic N	No growth	2 wks	Nil
Chloramphenicol	No growth	3 wks	Nil
Chloramphenicol	No growth	2 wks	Nil
Flurbiprofen	No growth	4 wks	Nil
Chloramphenicol	No growth	2 wks	Nil
Atropine	Growth	2 wks	*Staph aureus*
Eyesaxoline	No growth	2 wks	Nil
Efemoline	Growth	1 wks	*Staph aureus*
Tropicamide	Growth	2 wks	*Staph aureus*
Atropine	Growth	3 wks	Mixed growth of *E.coli* and *Staph. aureus*
Chloramphenicol	Growth	2 wks	*Staph. aureus*
Atropine	Growth	2 wks	*Staph. aureus*
Dexamethasone	No growth	2 wks	Nil
Eyesaxoline	No growth	2 wks	Nil
Diclogenta	No growth	3 wks	Nil
Chloramphenicol	No growth	3 wks	Nil
Beoptic. N.	No growth	3 wks	Nil
Aristocron	No growth	2 wks	Nil
Chloramphenicol	Growth	2 wks	*Staph. aureus*
Chloramphenicol	No growth	1 wk	Nil
Flurbiprofen	No growth	1 wk	Nil
Antallarge	No growth	1 wk	Nil
Sodium chromoglycate	Growth	4 wks	Heavy growth of *E.coli*
Diclogenta	No growth	1 wk	Nil
Flurbiproten	No growth	1 wk	Nil
Visine	No growth	1 wk	Nil
Atropine	No growth	1 wk	Nil
Atropine	Growth	2 wks	Scanty growth of *Staph. aureus*
Chloramphenicol	No growth	2 wks	Nil.

Table 2: The different eye drops used, the duration, and the bacteria isolated.

Contaminated	No of weeks used	Micro organisms isolated.
Atropine	2 weeks	*Staph aureus*
Efemoline	1 weeks	*Staph aureus*
Tropicamide	2 weeks	*Staph aureus*
Atropine	3 weeks	Mixed *E. coli and Staph. aureus*
Chloramphenicol	2 weeks	*Staph aureus*
Atropine	2 weeks	*Staph aureus*
Chloramphenicol	2 weeks	*Staph aureus*
Sodium Chromoglycate	4 weeks	Heavy growth of *Staph aureus*
Atropine	2 weeks	Scanty growth of *Staph aureus*

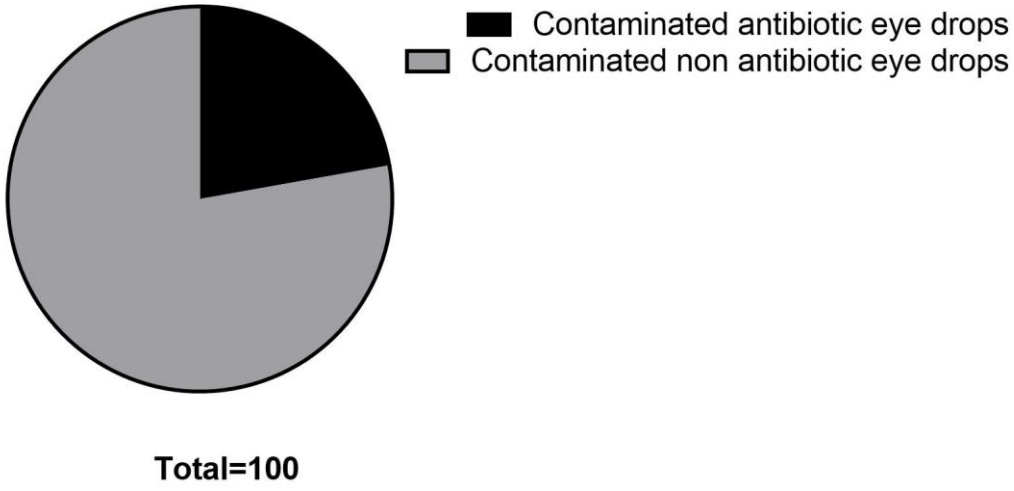

Figure 1: Pie chart showing percentage composition of the contaminated antibiotic and non-antibiotic eye drops.

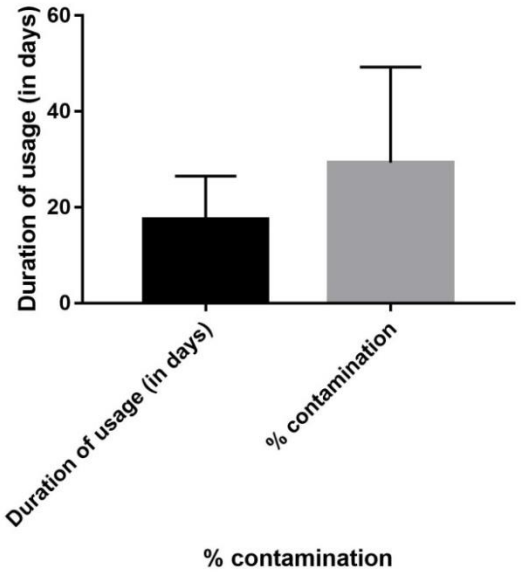

Table Analyzed	Data 1
Column B	% contamination
vs.	vs.
Column A	Duration of usage (in days)
Unpaired t test	
P value	0.3214
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.08 df=6
How big is the difference?	
Mean ± SEM of column A	17.5 ± 4.518, n=4
Mean ± SEM of column B	29.33 ± 9.968, n=4
Difference between means	11.83 ± 10.94
95% confidence interval	-14.95 to 38.6
R squared (eta squared)	0.1629
F test to compare variances	
F, DFn, Dfd	4.866, 3, 3
P value	0.2263
P value summary	ns
Significantly different (P < 0.05)?	No

Figure 2: Relationship between duration of usage of eye drops in multiple application bottles and percentage contamination of the eye drops using student t-test.

Probability of success on a single trial	0.5
Number of trials	29
Number of successes (x)	9
Binomial Probability: P(X = 9)	0.0186544004
Cumulative Probability: P(X < 9)	0.0120597723
Cumulative Probability: P(X ≤ 9)	0.0307141728
Cumulative Probability: P(X > 9)	0.9692858271
Cumulative Probability: P(X ≥ 9)	0.9879402276

Figure 3: Calculation of binomial probability of contamination using Stat Trek Binomial calculator

DISCUSSION

We noticed 0.31 (31%) incidence of microbial contamination of the multiple application ophthalmic eye drop bottles in this study during the usage period. Previous studies have recorded contamination rates of 2.2% to 34.8%.[4, 8,11-19]. The incidence of 0.31 (31%) found in the present study falls within the contamination range of these previous studies.

The rates of bacterial contamination in the present studies (31%) is higher than that of some previous studies[14-20]. The difference could be due to the fact that in those studies, the eye drops were for inpatients use while in the present study, the eye drops were used on outpatient basis which may have contributed to poor handling of the eye drops. Expectations were that the duration of use of the eye drops (7-28days) in this study could have also accounted for the high incidence of contamination. However, there was no statistically significant relationship between the duration of usage of the eye drops and the percentage bacterial contamination in the present study in contrast to a previous study where it was reported that the percentage of bacterial contamination showed an increasing trend when the duration of the usage was extended beyond seven days.[21]

The incidence of bacterial contamination of antibiotic eye drops in the present study is 0.18 (18%) compared to 0.38 (38%) for non-antibiotics eye drops. The differences in incidence could be due to difference in chemical combination of the two groups of drugs. Some authors in their study did not report microbial contamination of antibiotic eye drugs battles[13,21]. The observed disparity between this and the present study could be due to the fact that the former were on inpatient while the latter were on outpatients[13,21]. Also, the eye drops in the previous studies were used for a shorter duration of 3-14

days[13,21]. Some other studies have reported low occurrences of microbial contamination following short application periods. [14, 15].

Of the nine contaminated eye drop bottles, *Staphyticoccus aureus* was the sole contaminant in eight bottles while one bottle had a mixed growth of *E. Coli* and *Staphylococcus aureus*. The major bacterial contaminants in this study is part of the normal commensal flora of the conjunctiva or the skiin[21,22,23] .This further supports the finding that the rate of bacterial contamination of the eye drops increases with the length of usage of the eye drops since this increases the chances of contact of the application bottle tips with the normal bacterial flora of the eye. The finding is in accordance with other studies[4,18,24]. However it differs from the study by Rahman et al who found only a small proportion of the micro-organism identified to be part of the normal commensal flora when studying the contamination of unpreserved eye drops[13].

CONCLUSION

The incidence of bacterial contamination was higher in non-antibiotic-containing eye drops compared to antibiotic-containing eye drops. Poor handling of the eye drops and long usage period could be risk factors for bacterial contamination. Patients who are inexperienced or challenged physically or mentally that they cannot handle the eye drop in an aseptic manner should be assisted by competent hands. Packaging of eye drops in small volumes to last for shorter durations is advised. Also, patients should be taught the rules of handling and applying eye drops.

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