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ORIGINAL RESEARCH

# Microbial Contamination of Extended Use Ophthalmic Drops in Ophthalmology Clinic

Shee Wen Chua[1,2]
Mushawiahti Mustapha[1]
Kon Ken Wong[3]
Malisa Ami[4]
Aida Zairani Mohd Zahidin[5]
Rona Asnida Nasaruddin[1]

[1]Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; [2]Ophthalmology Department, Faculty of Medicine and Health Science, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia; [3]Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; [4]Sunway Specialist Center Damansara, Petaling Jaya, Selangor, Malaysia; [5]Gleneagles Hospital, Kuala Lumpur, Kuala Lumpur, Malaysia



**Purpose:** The objectives of this study were to determine the prevalence of microbial contamination of multi-user preserved ophthalmic drops (POD) in Ophthalmology Outpatient Clinic (OOC), to compare the rate of contamination between the dropper tip and the residual contents in the bottle, and to identify the contaminating organisms.

**Methods:** This was an observational cross-sectional study using a convenience sampling method conducted in the OOC of Universiti Kebangsaan Malaysia Medical Center, Malaysia. The samples of POD bottles were divided into groups obtained after 14 days (T14) and after 30 days (T30) of use. The contamination rate at the dropper tip and in the residual contents was determined and the contaminating organisms were identified.

**Results:** A total of 140 of 149 extended-use POD bottles were included. The prevalence of contamination was 30%. There was a statistically significant difference in the rate of contamination between samples T14 and T30 (19% and 11%, respectively; $p$=0.046). Proparacaine and tropicamide showed higher contamination rates in the T14 samples ($p$=0.027 and $p$=0.497, respectively) than in the T30 samples. The site of contamination was higher at the dropper tip than in the residual contents ($p$>0.05). Coagulase-negative *Staphylococcus* species were the most frequently identified contaminants (89%).

**Conclusion:** The dropper tip was more contaminated than the residual contents, and coagulase-negative *Staphylococcus* species, which are common commensal flora of the ocular conjunctiva and skin, were the most frequently identified organisms.

**Keywords:** contamination rate, extended period, preserved ophthalmic drops, multi-user

## Introduction

In ophthalmology outpatient clinics (OOC), a single bottle of preserved ophthalmic drops (POD) is often used on multiple patients[1,2] over an extended period of time in order to decrease the cost to the hospital.[3–7] In the OOC of Universiti Kebangsaan Malaysia Medical Center (UKMMC), Malaysia, ophthalmic drops are discarded once or twice a month regardless of how much content remains. Some of the frequently used PODs are Alcaine 0.5% (proparacaine hydrochloride) (15 mL dispenser), Mydriacyl 1% (tropicamide) (15 mL dispenser), and Mydfrin 2.5% (phenylephrine hydrochloride) (8 mL dispenser). All three are manufactured by Alcon® [Novartis Corporation (Malaysia) Sdn. Bhd., Petaling Jaya, Malaysia] and have a similar preservative, benzalkonium chloride (B.A.K) (0.01%), to prevent contamination via inhibiting the growth of microorganisms by interfering with their metabolism.[8,9] However, few studies have shown that the addition of B.A.K or thimerosal to ophthalmic drops is not sufficient to ensure its sterility.[4,10] In particular, the use of these drops in multiple patients and over an extended period of

Correspondence: Mushawiahti Mustapha
Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, 56000, Malaysia
Tel +60391455981
Email drmusha@gmail.com

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time carries a potential risk of avoidable ocular infections and cross contamination.[9,11,12]

In addition, there is a risk of contamination at any point during the handling of ophthalmic drops, including during drop instillation.[4,13,14] A multi-use eyedrops policy has been established in Murray, Utah, to provide instructions for the safe handling and administration of eyedrops that could reduce the risk of contamination.[1]

The reported contamination rate in previous studies varied widely from 2.2 to 70%.[3–6,9,13] In the OOC of UKMMC, Malaysia, ophthalmic drops are discarded once or twice a month regardless of how much content remains. However, the contamination rate in the OOC of UKMMC is currently unknown. There is also no published study on the contamination rate associated with the use of POD in multiple patients in Malaysia. The literature reviews were mainly from temperate countries, but Malaysia is a tropical country.[1,3,12] As the contamination rate may vary depending on the environmental conditions, it is important to study and understand the contamination rate in tropical countries as well.

The objectives of this study were to determine the prevalence of microbial contamination of multi-user POD in OOCs in our institution, to compare the contamination rate between the dropper tip and residual contents in the bottle and to identify the contaminating organisms.

## Methods

This was an observational cross-sectional study conducted on topical ophthalmic drops used in the UKMMC OOC. The PODs tested were Alcaine 0.5% (proparacaine hydrochloride) (15 mL dispenser) and Mydriacyl 1% (tropicamide) (15 mL dispenser). They are both manufactured by Alcon® and have a similar preservative composition of 0.01% of B.A.K.

The sample size was calculated using the convenience sampling method, based on the assumption of a contamination rate of 10% as determined in a similar study by Livingstone et al United Kingdom, which found a contamination rate of 9.1% at 14 days.[5] The number of samples required to adequately compare the contamination rate of the dropper tips and the residual contents was determined using Cohen's effect size with a power of 80% at a two-tailed α value of 0.5 and a standard deviation of 50%. According to the calculation, at least 64 samples were required for each arm.

The inclusion criteria for this study were newly opened POD bottles (15 mL dispensers of Alcaine 0.5% and Mydriacyl 1%) used in OOC. Each bottle was randomly assigned a number (1, 2, 3, … to 149), and this number was used to label the bottle and its cap. The dropper tip of each newly opened bottle was swabbed with a dry swab under aseptic conditions. This sample was considered as the control sample (T0). It was placed in a nutrient broth, which was used as a transport medium, and cultured on a nutrient agar plate in the microbiology laboratory. Control samples cultured on the nutrient agar plate were monitored for up to 8 days, and bottles found to be contaminated at the beginning of the study were excluded from the study and discarded. Non-contaminated bottles continued to be used on patients for an extended period of time. Labeled PODs were collected prior to discarding from the OOC. Based on the opening and discard dates, the collected PODs were conveniently categorized into 14-day used (T14 samples) or 30-day used (T30 samples). Exclusion criteria included a broken seal on new bottles, contamination of the control sample (T0), a missing cap or tip on the day of collection, and bottles used in cases of infection. A total of 72 samples of T14 and 68 samples of T30 were collected.

The same investigator took the sample swab from the new bottle (T0) and on the days of collection (T14 or T30). The dry swab was placed in a nutrient broth used as a transport medium before being sent to the microbiology laboratory for culture at all two time points. In addition, at T14 or T30, the ophthalmic drop bottles were sent to the microbiology laboratory. A total of 140 samples of nutrient broth (Thermo Fisher Scientific, USA) containing the dry swab and the residual contents of the 140 bottles were inoculated onto blood agar, chocolate agar, MacConkey agar, and Sabouraud dextrose agar plates (Thermo Fisher Scientific, USA). All culture plates were incubated in an incubator (Memmert, Germany) with 5% $CO_2$ at 37°C. Cultures were evaluated daily for growth by the same microbiologist until day 8 inoculation. When positive growth was observed on the culture agar plates, Gram staining was performed and observed under the microscope to determine Gram-negative and Gram-positive bacteria. Gram-positive cocci bacteria were identified based on the result of catalase and coagulase reactions. Gram-negative bacteria were further tested with a series of biochemical assays using triple sugar iron, urea, citrate and motility. Analysis of the observed biochemical reactions was used to identify the causative organism.[15] All samples of nutrient broth and bottles were subsequently discarded.

IBM Statistical Package for the Social Sciences (SPSS) software version 26 was used. Descriptive statistics were used to analyze the data. Pearson's Chi-square test was used to assess differences in contamination rates at T14 and T30, and the general linear regression test was used to assess contamination risk. *P*-values of <0.05 were considered to indicate statistical significance.

## Results

Of the original 149 ophthalmic drop bottles, 140 were finally included in the analysis: Four bottles in the control group (T0) were excluded because they were contaminated, and five labeled bottles were missing on the day of collection.

As shown in Table 1, there were no statistically significant intergroup differences between the T14 and T30 samples in terms of the type of ophthalmic drops used (Alcaine vs Mydriacyl).

The overall prevalence of contamination was 30% (42/140), as shown in Table 2. Cumulatively, there was a significant difference in the rate of contamination between the T14 (19%) and T30 (11%) samples ($p$=0.046). Proparacaine was associated with a significantly higher contamination rate in the T14 samples than in the T30 samples (20% vs 7%; $p$=0.027), but the tropicamide eyedrop showed no significant difference in its contamination rate between the T14 and T30 samples ($p$=0.497).

The dropper tip was associated with a much higher contamination rate than the residual bottle contents (50% vs 33%; $p$>0.05). 17% of samples had contamination at both sites. There was no significant difference between sites of contamination in any POD.

Table 3 reveals that the contaminants identified were mainly coagulase-negative *Staphylococcus* sp. (*CONS*) (89%), followed by gram-negative rod species (7%). Other contaminants were *Micrococcus* sp. and *Acinetobacter* sp. (4%).

Table 4 shows that there was a 3% (Cox & Snell $R^2$=0.031, $p$=0.041) predictor of risk of POD contamination using a simple logistic regression test. The result showed that a longer period of POD use was a negative and marginally significant (B=−0.757, S.E.=0.381, $p$=0.047) predictor of POD contamination. However, no significance was found for the types of PODs.

**Table 1** Distribution of Multi-User PODs in 2 Extended Periods of Usage

| PODs | T14 (n,%) | T30 (n,%) | *p*-value |
|---|---|---|---|
| **Type of PODs** | | | 0.862 |
| Proparacaine | 36 (25.7) | 33 (23.6) | |
| Tropicamide | 36 (25.7) | 35 (25.0) | |
| **Total** (n=140) | 72 (51.4) | 68 (48.6) | 0.114 |

**Notes:** T14, Ophthalmic drops used for 14 days; T30, Ophthalmic drops used for 30 days.

**Table 2** PODs Contamination in 2 Extended Periods of Usage

| PODs | T14 (n,%) | T30 (n,%) | *p*-value |
|---|---|---|---|
| **Contamination** | 27 (19.3) | 15 (10.7) | 0.046* |
| **Type of PODs** | | | 0.248 |
| Proparacaine$^+$ | 14 (20.3) | 5 (7.2) | 0.027* |
| Tropicamide$^+$ | 13 (18.3) | 10 (14.1) | 0.497 |
| **Sites of contamination** | | | 0.901 |
| Dropper tip$^+$ | 13 (31.0) | 8 (19.0) | 0.747 |
| Residual contents$^+$ | 9 (21.4) | 5 (11.9) | 1.000 |
| Both sites$^+$ | 5 (11.9) | 2 (4.8) | 0.666 |

**Notes:** $^+$Contamination; *$p$-value statistically significant <0.05 using Pearson's Chi-square test.

## Discussion

The prevalence of contamination rate in the UKMMC OOC was 30%. The contamination rate reported in other studies varies considerably (2.2% to 70%).[3–6,9,13] A study in the United Kingdom with a total of 636 samples of eyedrops from bottles used in multiple patients and over a long period of time found that there was little to no association between contamination rate and the duration of bottle use.[5] They reported that the contamination rate was 6% at 7-day use and 9% at 14-day use ($p > 0.1$).[5] Based on their results, they concluded that a single bottle of eyedrops can be used for up to 14 days without posing a risk to patient health.[5] Our study found a higher contamination rate at T14. The difference between the studies could be due to other factors associated with microbial contamination such as handling technique, hygiene practices, instillation angle, and bottle geometry.[6,7,11] In

**Table 3** Identified Contaminants from the Contaminated PODs

| Identified Contaminants | T14 (n) | T30 (n) | Total, n=42 (%) |
|---|---|---|---|
| *CONS* | 25 | 12 | 37 (89%) |
| Other organisms | | | |
| *Micrococcus sp.* | 1 | 0 | 1 (2%) |
| Gram-negative rod | 1 | 2 | 3 (7%) |
| *Acinetobacter sp.* | 0 | 1 | 1 (2%) |

**Note:** $p$-value CONS with others organisms using Pearson's Chi-square test: 0.227.
**Abbreviations:** CONS, coagulase-negative *Staphylococcus* species; *sp.*, species.

**Table 4** Association Between Type of Eyedrops and Extended Period Usage of Eyedrops with Contamination Rate

| Contamination | B (S.E.) | Wald[a] (df) | OR (95% CI) | *p*-value |
|---|---|---|---|---|
| Type of PODs | 0.250 (0.376) | 0.443 (1) | 0.393 (0.615, 2.681) | 0.506 |
| Extended period of usage | −0.757 (0.381) | 3.946 (1) | 3.971 (0.222, 0.990) | 0.047* |
| Constant | | 4.193 (1) | 4.402 | Ref. |

**Notes:** *$p$-value statistically significant <0.05 using simple logistic regression test; [a]Simple logistic regression test.
**Abbreviations:** B, coefficient for the constant; S.E., standard error; df, degree of freedom for each variables; OR, odd ratio; Ref., reference.

addition, the previous study was conducted in a temperate country while our study was conducted in a tropical country.

We studied two types of eyedrops - Alcaine (proparacaine hydrochloride) and Mydriacyl (tropicamide) containing similar percentage of preservative B.A.K. and manufactured by the same company. Proparacaine is commonly used as a local anesthetic and in examinations such as measurement of intraocular pressure and fluorescein staining, whereas tropicamide is less commonly used in the clinic for pupillary dilation.[4,9,13] In addition, proparacaine is usually instilled by the examining clinician under a slit lamp, whereas tropicamide is usually instilled by a clinic assistant or nurse while the patient is sitting in the waiting area. Therefore, the contamination rate between these two eyedrops may vary depending on the frequency of use and handling technique. In our study, similar results were found as in studies from Kenya and Ethiopia,[4,9] where contamination rates were higher for bottles of local anesthetic than mydriatics, which was explained by the frequent use of anesthetic drops. Literature reviews reported that tetracaine or proparacaine (10% to 20%) had a higher contamination rate than mydriatics (5% to 7%).[9,13]

Our study found that the dropper tips had a higher contamination rate than the residual bottle contents, although the difference was not significant. This is in agreement with the findings of other published reports,[6,8,13] except for one published study which reported opposite results.[9] It is expected that the dropper tip is more contaminated as it is the most exposed part and comes in direct contact with the patient's eye. Although B.A.K. has antimicrobial activity, the contact time at the dropper tip was not sufficient for the preservative to exert its antimicrobial activity.[4,5,9] In addition, a few studies have shown that the addition of B.A.K. or thimerosal to ophthalmic drops is not sufficient to ensure their sterility.[4,10]

Poor technique in the administration of ophthalmic drops is considered a potential contamination risk, even when instilled by healthcare professionals.[4,12,16] The risk of contamination is increased by physical contact with the dropper tip while opening the bottle, contact of the tip with ocular tissue, and exposure to environmental contaminants if the bottle is left uncapped.[4] Because proparacaine is an anesthetic, it is more commonly used when examining patients (measuring intraocular pressure or performing the Seidel's test) or before a minor procedure such as removing a suture.[4,9,13] The examining clinician may instill proparacaine under a slit lamp, and there is a high likelihood that the dropper tip will come into physical contact with the patient's ocular tissue or adnexa. There is also the possibility of the dropper tip accidentally coming into contact with the slit lamp surface during drop instillation. All of this may explain the higher contamination rate associated with proparacaine.

Our study had shown that the commonly identified contaminants were resident flora found in the conjunctiva, eyelid, and skin consisting mainly of Gram-positive bacteria, including coagulase-negative *Staphylococcus* sp. and *Micrococcus* sp. and a smaller percentage of Gram-negative rod bacteria. This suggests that the contaminated ophthalmic drops may have come into contact with the ocular surface of patients or the fingers/hands of health care professionals, as reported in previous studies.[4,12,16]

In 2012, a multi-use eyedrops policy was established in Murray, Utah, which included instructions for safe handling and administration of eyedrops to reduce the risk of contamination.[1] To further reduce the rate of contamination of PODs at our center, the guidelines can be adopted as part of the hygiene protocol for instillation of ophthalmic drops: wash hands thoroughly before administering eyedrops, tilt the patient's head slightly back, pull the lower eyelid away from the eye to form a pocket, instruct the patient to look up, place the dropper directly over the eye, and avoid contact between the dropper tip and any part of the eyelid, eyelashes, or the eye itself.[1] Regular, periodic review of the drop instillation protocol may be beneficial in reducing the rate of POD contamination.

Other factors have also been shown to contribute to a lower rate of POD contamination. It has been reported that increasing the instillation angle of PODs to 90 degrees instead of 45 degrees decreases the contamination rate from 83% to 22%.[11] In addition, PODs with rounded nozzle tips have been reported to fail to prevent the flow of solution to the side of the bottle and have a higher contamination rate than smaller nozzle tips.[6] All PODs in our study had rounded nozzle tips.

One of the limitations of the present study was user bias, as the samples collected for the study were labeled from the beginning. As a result, users were likely to be more cautious in using the ophthalmic drops, which underestimated the true prevalence of microbial contamination in PODs. Another limitation was that mobilization of the labeled PODs may have occurred in the OOC, resulting in missing bottles on the day of collection. Our study showed that only 3% of the risk could be predicted by the type of PODs and longer duration of use. Therefore, we would like to suggest further studies to address the various factors that could contribute to POD contamination, such as handling technique, the angle of instillation and bottle geometry. Other factors such as the temperature and humidity at which the PODs were stored, the frequency of use of the PODs in each patient and the number of patients per POD bottle may also be investigated. The physical appearance of the PODs tips and inner sides of the caps on the day of collection can also provide additional information to determine whether or not contamination is present.

Overall, the present results are consistent with previously reported findings showing that prolonged use of PODs by multiple users alone does not affect contamination rates.[5,10,17]

## Conclusion

The dropper tip was more contaminated than the residual bottle contents, and the main contaminant was coagulase-negative *Staphylococcus* species, which are common commensal flora of the ocular conjunctiva and skin.

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## Disclosure

This manuscript has not been presented elsewhere. No competing interests exist for any authors.

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