

EVALUATION OF POSSIBLE MICROBIAL CONTAMINATION IN EYE DROP BOTTLES USED BY PATIENTS IN DAY TO DAY PRACTICE

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ABSTRACT

The present study was planned to evaluate the spontaneous contamination of in-use ocular medications, and to estimate the frequency of medication contamination with Conjunctiva. Forty in-use medications from 34 patients with normal ocular surface diseases were studied by culturing the bottle caps, cover and nozzle sterile. Sixty eight conjunctival smears from 34 patients were also examined for organisms. The comparison of the in-use medication with the conjunctiva of the patients using the medication showed the spectrum of microorganisms isolated from conjunctiva and the in-use ocular medications were similar indicating the same organisms is carried to the eye by the use of contaminated medication and gets lodged in the eye as the microbial flora replacing the normal flora of the eye. The most commonly isolated organism from both in-use medication and conjunctiva was *Staphylococcus saprophyticus*. We conclude that the ophthalmic drops used in outpatient department should not be extended more than 15 days. The use of single-dose eye drops for the patients with ocular surface diseases is recommended. It is important finding, thus replacement of normal flora is a significant ocular risk factor for several complications like Microbial Keratitis and Endophthalmitis.

KEYWORDS: Bacteria, Isolation, Bacterial contamination, Ocular medications, conjunctival smear.

INTRODUCTION

Ocular medications are used to treat and prevent eye diseases. The contamination of ocular medication is one of the greatest problems that ophthalmologist face today. The contamination of these ocular medications leads to deadly ocular complications such as microbial keratitis, endophthalmitis in patients with ocular surface disorder (Fazeli, Samadi and Fattahi, 2004). These contaminations may arise out due to improper handling, enabling the entry of the microorganisms via the cap, nozzle, resulting in the contamination of the interior contents. The inclusion in eye drops a suitable preservative would therefore appear necessary (Samadi *et al.*, 2009). The safe ocular practise recommends labelling on ophthalmic products suggesting that medications should not be shared among patients and use of these ophthalmic with utmost care and proper hygiene. The prevention of infectious diseases is absolutely preferable to their treatment.

Since the consequence of the contamination of these ocular medications is clinically very important, the present study was planned in a tertiary eye care hospital in Salem, with the following objectives. The aim of the present study was planned to evaluate the spontaneous

contamination of in-use ocular medications, and to estimate the frequency of medication contamination. Specifically we compared the bacteriology of contaminated medicines with that of conjunctival flora of patients. Finally we suggested the safe practises that should to be followed in the dispensing of these ocular medications which might reduce the risk of serious ocular infection.

MATERIALS AND METHODS

Patients with ocular surface disease using ocular medication were enrolled at Dr. Agarwal's eye hospital, Salem over a period of three months from August to October 2010. Patients were contacted before an appointment and were asked to bring all in-use ocular medications. Various inclusion and exclusion criteria were used for enrolling the patients for this study. The inclusion criteria includes i) The time period of usage of these medications were not exceeding 10-14 days after consulting the ophthalmologist, ii) Re-surgery, FFA, corneal edema, dry eye and lid diseases cases and iii) Patients of the age group 20 to 80 were enrolled for this study. Patients were excluded if they were currently under treatment for a bacterial or fungal ocular infection or if an infection was present at the time of enrollment.

They were also excluded if they failed to bring all their medications. The patients provided all their medications for culture. Replacements were provided free of charge. All ocular drops collected from the enrolled patients were cultured.

Medication Bacteriologic study

Sample Collection

A total of 40 in-use eye drops were collected during August to October 2010 from outpatient department of Agarwal eye hospital, Salem, after days of use (9 samples each). All the collected samples were recorded for their active ingredients as well as their duration of uses. The eye drops and ointments were collected from the patients and packed in zip lock cover and sealed with micro pore plaster, marked with patient name and number with glass marking pen and immediately transferred to the microbiology laboratory. The residual contents, caps and droppers of the eye samples were examined for possible microbial contamination under aseptic conditions.

All ocular eye drops were cultured as follows. If cap was present, a sterile swab was rotated around the inside tip and inside edge of the cap, finishing with a complete rotation around the inside rim. The swab was then inoculated on to a Sheep blood Agar (SBA), Brain Heart infusion Agar (BHIA) and Sabouraud Dextrose Agar (SDA). The medication was then inverted and one drop was allowed to fall on each of these three media. The drop was streaked on the three media plates. The bottle top/ nozzle were cleaned with alcohol and were wiped. The entire bottle contents were withdrawn using a needle and sterile syringe; 0.5ml was used to inoculate each of the three plates (Sheep blood Agar, Brain Heart infusion Agar and Sabouraud Dextrose Agar) and the remaining medications were inoculated on the nutrient broth. The bacterial plates and broths were incubated at 37°C for 24-48 hrs and the fungal plates and broths were incubated at 28°C to 30°C for 7 days. The bacterial colonies were identified by using standard biochemical test and the fungal colonies were identified by LPCB mount. The control plates were also kept at the same temperature and were incubated.

Conjunctival Bacteriology

A total of 34 patients were included in this study. A sterile cotton-tip swab moistened with sterile saline was rotated along the inferior tarsal conjunctiva while the lower lid was retracted. For each eye three swabs were taken. Two swabs were used to inoculate three culture media by C – streaking namely Sheep blood Agar (SBA), Brain Heart infusion Agar (BHIA) and Sabouraud Dextrose Agar (SDA). One swab was smeared on to a clean, dust free glass slide. The swab was then broken off and was placed in the nutrient broth. The bacterial plates and broths were incubated at 37°C for 24-48 hrs and the fungal plates and broths were incubated at 28° C to 30° C for 5 days. The bacterial colonies were identified by using standard biochemical

test and the fungal colonies were identified by LPCB mount.

The significance of the difference between two proportions was calculated by chi-square (X^2) test (Hill, 1977). Tables were mostly 2 x 2 tables with one degree of freedom. If $P > 0.05$, the differences observed were deemed not significant. If $P < 0.05$, the differences observed were deemed significant.

RESULTS

Study patients

The patients investigated in this study are given in Table 1. It provides the details of the number of patients in different categories of age in relation to the culture. A correlation was attempted between patient's age and culture results. Table 2 provides about the details of the patients with ocular surface diseases and their purpose for the hospital visit.

Conjunctival Microbiology

Table 3 gives the potential pathogens which accounted for 83 (64.84%) isolates were more likely to be cultured from the conjunctiva of patient's who use ocular medications. The rest of the bacterial isolates reported accounting for 45 (35.16%) isolates were the usual conjunctival flora. These includes coagulase negative *Staphylococci*. This difference was not statistically significant (X^2 [d.f. =2] =3.841; $P > 0.05$).

Medications used by patients

The distribution of medications used by the patients is presented in Table 4. Forty medications were examined and 161 isolates were isolated from these medications. From this table it shows that eye drops used as lubricants (45.96%) had the high rate of contamination followed by anti-inflammatory (21.13%) and antibiotics drops (18.63%).

Medication contamination

The distribution of microorganisms cultured from the three different medication container sites such as cap, cover and nozzle shows that most of the gram positive organisms isolated from the medications were coagulase negative *Staphylococci* which dominated the cap and nosil. Gram positive organisms were more likely to be isolated from the cover. Gram negative organisms were isolated moderately from the entire three medication sites; this difference was not statistically significant (X^2 [d.f. =2] =9.49; $P > 0.05$). The overall contamination rate of in-use medications / nosil contain 48 (30%) isolates, cover contains 53 (33%) isolates and cap contains 60 (37%) isolates Figure 1; this difference was statistically significant (X^2 [d.f. =2] =5.9; $P < 0.05$). The results of this study indicated that ocular medication cap showed highest contamination rate when compared to the other two.

Concordant cultures

Each patient had at least one medication contaminated at one or more medication containers sites (cap, cover and nosil). 80% of these 34 patients had the same organisms cultured from the conjunctiva to which that contaminated medication was applied. The comparison of bacterial isolates between the conjunctival smear and in-use ocular medications. 39(24.22%) *Staphylococcus saprophyticus*, 21(13.04%) *Serratia liquefacians*, 18(11.2%) *Staphylococcus epidermidis*, 17(10.56%) *Bacillus spp.*, 15(9.32%) *Corynebacterium kutscheri*, 14(8.69%) *Aeromonas spp.*, 7(4.35%) *Streptococcus pneumonia* and 6 (3.73%) *Micrococcus luteus* were isolated more in number from in-use medications than in conjunctival smear. 16 (9.38%) *Staphylococcus aureus* and 18 (14.06%) non-haemolytic *Streptococci* were isolated more in number from conjunctival smear when compared to in-use medications. 5 (3.9%) *Lactobacillus spp.*, and 6(4.69%) *Streptococcus pyogenes* were isolated from conjunctival smear. 1 (1%) *Salmonella spp.*, was isolated from in-use ocular medications (Fig 2); the differences were not statistically significant (X^2 [d.f. =2] =23.6; $P > 0.05$).

As it was not possible to determine whether the medication or the conjunctiva was the first site of contamination, we also investigated the relationship between the conjunctival cultures and contaminated drops or contents (Figure 2). 80% of these 34 patients

had the same organisms cultured from the conjunctiva to which that contaminated medication was applied. Cultures from these 34 patients yielded 83(64.84%) potentially pathogenic organisms and 45(34.62%) of coagulase negative *Staphylococci*. Gram-positive organisms 68(42.24%), 36(22.36%) of gram-negative organisms and 57(35.4%) of coagulase negative *Staphylococci* were isolated from medication containers. This differences were not statistically significant (X^2 [d.f. =2] =23.6; $P > 0.05$).

Safe Practice Recommendations

Eye drops in multiple-dose bottles and eye ointments for use in the community should be discarded four weeks after opening (unless otherwise stated). Eye drops for use in hospital wards should be discarded one week after first opening. A separate bottle can be used if required for each eye only unless there are concerns about cross-contamination. Preservative free eye drops should be discarded within seven days after opening. In eye surgery single-dose packs should be used if possible; if a multiple-dose pack is used, it should be discarded after single use. Care should therefore be taken to avoid eyedropper contact with eyelids, lashes, eyebrows and facial skin. Expiry dates must be checked, as out-of-date drops can be a source of infection. Personal hygiene like washing of hands with soap solution before and after application of eye drops should be followed very strictly.

Table: 1 Age distribution of patient's conjunctival smear in relation to culture.

S. No	Sex	Total no of Isolates						Total
		20-30	31-40	41-50	51-60	61-70	71-80	
1	Male	12	11	17	17	8	7	72
2	Female	13	2	17	9	5	10	56
Total		25	13	34	26	13	17	128

Table: 2 Total no of patients visited hospital for the cause of infection

S. No	Purpose of hospital visit	No of patients (%total)
1	Dry eye	10(29.41)
2	Corneal edema	8(23.53)
3	Re-surgery	6(17.64)
4	FFA	5(14.71)
5	Lid diseases	5(14.71)
Total		34

Table 3: Distribution of microorganism from patient's using ocular medications.

S.No	Conjunctival cultures	Total number of isolates	Percentage
1.	Usual conjunctival flora*	45	35.16
2.	Potential pathogens +	83	64.84
Total		128	

Table 4: Details of medication used by study patients.

S. No	Types of medications	No. of Medication	Total number of isolates	Percentage
1.	Lubricants	17	74	45.96
2.	Antibiotics	9	30	18.63
3.	Anti-inflammatory	7	34	21.13
4.	Anti-glaucoma	6	21	13.04
5.	Antifungal	1	2	1.24
Total		40	161	

Fig. 4.1



Figure 1: Contamination rate of in-use medications

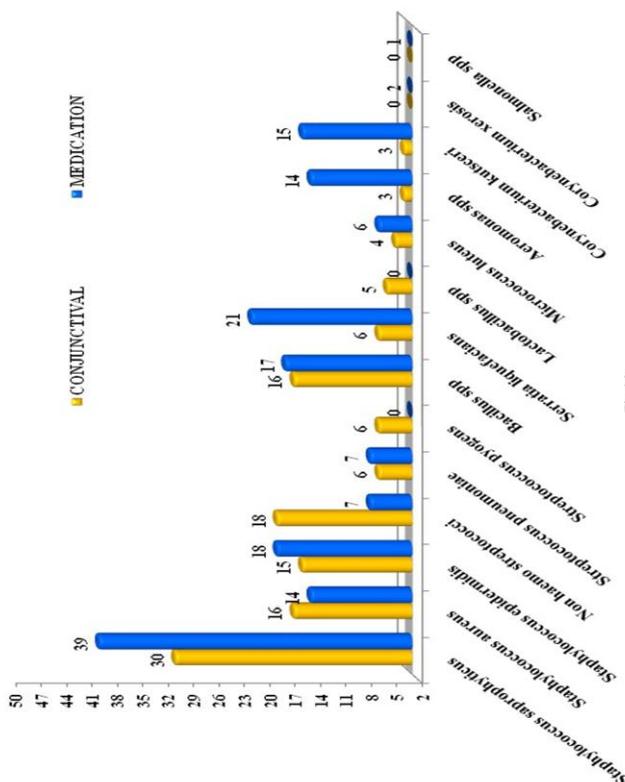


Fig 4.2

Figure 2 Comparison of contaminated medications with conjunctival flora

DISCUSSION

The results of the study showed high rate of contamination of ocular medications with bacterial isolates than fungal isolates. coagulase negative *Staphylococci*, gram-positive organisms that are not a part of potential pathogens and gram-negative organisms namely *Serratia liquefacians*, *Aeromonas spp* and *Salmonella spp* were isolated in this study.

Earlier reports have stated that the eye drops used for 1, 2, 4 or 7 days in outpatient departments microbiologically examined showed high rate of contamination with the normal skin flora, mostly coagulase negative *Staphylococci*, *Micrococci* or air-borne gram-positive spore bearing bacilli and the

pathogenic organisms such as *Staphylococcus aureus* and group A *Streptococci* (Fazeli, Samadi and Fattahi, 2004). In this study, the rate of isolated contaminants also showed an increasing trend when the duration of the usage was extended.

The present study shows high frequency rate of contamination in caps (37%) than in cover (33%) and nosil (30%); this difference was statistically significant. Handlings the eye drops such as nosil and cap in contact with eyelid, eyelashes and cheek have been proposed to act as potential reservoirs for microbial contamination. These observations are consistent with those of other workers who have made similar observations in their study. Fazeli, Samadi and Fattahi, (2004) reported high

frequencies of microbial contamination were detected in the caps and the residual contents of the eye drops; however, the latter appeared to have higher incidence of contamination (12% vs. 34% for the first day samples). While several studies have shown conflicting views with the same findings of this investigation, these studies reported dropper tips as contaminated highly (Aslund, Oslon and Sandell, 1978; Hoviding and Sjursen, 1982; Stevens and Matheson, 1992; Tasli and Cosar 2001).

The scanty growth obtained by dripping in these studies may represent small amounts of bacteria surviving but not multiplying in the solutions, but it is probable that many of the bacteria isolated originate from contaminated dropper tips. The results obtained in the present study for caps are comparable to the earlier reports (Coad, Osato and Wilhelmus, 1984; Schein *et al.*, 1992) in which caps of squeezed bottles have been proposed to act as potential reservoirs for microbial contamination. This contamination paves way to the contents through the droppers.

In this study no organism was detected on the plates of droppers in contrast to the caps and residual contents. These results obtained in this study are similar to those of the previous reports (Stevens and Matheson, 1992) in which contents were more contaminated than tips and thus it was concluded that germ desiccation and also aspiration of contaminated tips into the content may explain higher bio burden rate of the residual contents. This study reported the cap was the most frequently colonized medication site; it is possible that the cap is the initial reservoir of contamination and may lead to subsequent colonization of the contents by contamination of the dropper or ointment tip. The contamination of a medication drop or content is likely of greater importance clinically, since it guarantees delivery of the organism to the ocular surface. The moist nozzle might also serve as a reservoir for microbial contamination (Geyer *et al.*, 1995; Hoviding and Sjursen, 1982; Coad, Osato and Wilhelmus, 1984; Schein *et al.*, 1992).

High contamination rates were observed in β -blockers, steroid drops, and ocular lubricants. The Contamination rates of individual products cannot be accurately assessed because of the small numbers of samples taken in this study. These results are consistent with those of other workers (Schein *et al.*, 1992).

Of the eye drops tested, lubricants, anti-inflammatory and antibiotics showed higher contamination rates which were 45.96%, 21.13% and 18.63% respectively. The high bio burden load of the lubricants eye drop could be attributed to the poor aseptic condition in the preparation of this product. Since the number of the tested ophthalmic drops was not large enough, no conclusion could be derived on the statistical point of view. The microbial contamination arising from the four sample groups indicated similar pattern of microorganisms. The major isolated microorganisms were either those

associated with the normal skin micro flora, most notably coagulase negative *Staphylococci* and *Micrococci*, or air-borne Gram-positive spore-bearing bacilli and fungal spores. The only pathogenic organisms which were found were *Staphylococcus aureus* and group A *Streptococci*, which are also considered as normal flora. Surprisingly, no gram-negative bacteria were found in the samples. The kind of microorganisms which is found in the eye drops implies that the medications have come into contact with the eyelid of patients or hands of nursing staffs during administration; furthermore, they may have been left without closure. The identity of contaminating organisms found is consistent with those of other studies (Geyer *et al.*, 1995; Akiba *et al.*, 1996), in which it has been demonstrated that gram-positive cocci as the dominant contaminant of these eye drops.

In another study it has been shown that most of the contaminating organisms were of skin flora. The contamination rate obtained for 1 day's use drops was high (34%). Although most of the detected contaminants were gram-positive organisms, in particular *Staphylococci* and *Streptococci* that are indigenous to conjunctiva and skin should be taken into account as these organisms could be harmful to patients who have disrupted epithelial barriers or those who have immunocompromised conditions (Livingstone, Halon and Dyke, 1998)

The microbial contamination rate identified in this study was 8.69 %. While several studies shows conflicting views, Livingstone, Halon and Dyke, 1998 reported the incidence of microbial contamination for the 7 and 14 day use eye drop residues were 6.1% and 9.1% respectively when compared with the 7 days inpatient use the contamination rate were 8% and 11.7% (Douch and Davison, 1992 and Guest *et al.*, 1990). These values are very much less than the 37% contamination rate reported by Harte *et al.*, 1978.

In a study carried out on eye drops presented in glass bottles with separate droppers. Examination of the types of microbial contamination arising from the two sample groups indicates a broadly similar pattern of microorganisms. The predominant bacteria isolated were those associated with the normal skin micro flora, most notably coagulase negative *Staphylococci* and *Micrococci*. It should be noted, however, that the rate of occurrence of these bacteria was much lower than that which has been reported in the normal, healthy human conjunctiva (37%–94%). The remainder were microorganisms frequently found as contaminants in the environment (primarily bacterial and fungal spores). Gram negative bacteria were found in small numbers in samples from the 14 day patient group but were considered to be of doubtful clinical significance (Rosebury, 1994). In this present study, the eye drops in plastic bottles used after 14 days use was examined. The most common gram-positive micro organisms were *Staphylococci saprophyticus*, *S. epidermidis* and Non

haemolytic *Streptococci* and gram-negative organisms identified were *Salmonella spp.*, *Aeromonas spp.* The organisms isolated from these medications were not statistically significant.

Schein *et al.* (1992) reported high rate of contamination of ocular medications, particularly with potentially pathogenic gram-negative organisms that are not part of usual conjunctival flora and founded a high rate of concordance of organisms in medications and conjunctiva to which that medication was applied. These indicate that the microbial ecology of medication and conjunctival contamination differs significantly for gram-positive to gram negative organisms. In this present study, a high rate contamination of in-use ocular medication with gram-positive organisms that are part of usual conjunctival flora (35.16%) was reported. Apart from this, a high rate of concordance of organisms in medications and conjunctiva to which that medication was applied was also found in this study. These indicate that conjunctival contamination differs significantly for gram-positive to gram-negative organisms in the conjunctival flora.

In our study, the medication container was divided into three different sites namely cap, cover and nosil and examined for contamination rate. The usual conjunctival flora (35.16%) was isolated more in cap and gram-negative organisms such as *Salmonella spp.*, *Serratia liquefacians* and *Aeromonas spp.*, were also isolated. The Contamination was divided into contamination of the medication cap and contamination of contents. Schein *et al.*, 1992 reported gram-positive organisms and fungi tended to be cultured only from the cap, while gram-negative organisms tended to be cultured from multiple medication sites.

The presence of a pathogenic organism on a normal ocular surface is a necessary ingredient for the development of serious ocular infection. Schein *et al.*, 1992 wished to alert ophthalmologists caring for patients with ocular surface diseases of the high rate of contamination of in-use ocular medications. The present study reports that the cycle of contamination between in-use medication and conjunctiva may present important risk factors for several ocular surface diseases and the ophthalmic drops used in outpatient departments cannot be extended more than 15 days. The multi-dose drop in out-patient department, where a bottle is used for different patients, is a potent source of contamination. Handling the medication leads to the contamination of cap and nosil, thus acting as the reservoirs for the contamination of eye drops. Ideally single-dose eye drops should be used but they cost more than multidose ones. However, application of single-dose drops is recommended for patients with eye infections. Alternatively, multi-dose bottles could be discarded after use by an infected patient.

CONCLUSION

In conclusion, it can be said that the observations made in the present study will possibly contribute substantially to the knowledge of the use of these ophthalmic preparation with care. The contamination of eye drops occur with increased length of use of bottles. Therefore it is recommended that the ophthalmic drops used in outpatient department should not be extended more than 15 days, yet care should be taken while handling and initializing the eye drops. The use of single-dose eye drops for the patients with ocular surface diseases is recommended as the multi-dose ones are cost effective. In case of multi-dose bottles being used should be discarded after use with an infected patient. The safe practice recommendations have to be adhered to very strictly in the avoidance of in-use contamination of ocular medications and prevention of any infection associated with their usage.

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