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MICROBIAL EVALUATION OF NORMAL SALINE USED BY CONTACT LENS WEARERS

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Contact lens solutions should only be used for four weeks after first opening but this practice is not always followed. Therefore, the present study was conducted to determine the duration that contact lens wearers could use their normal saline without microbial contamination. Two brands of normal saline, Opticare™ and Klean & Kare™, were used by 30 contact lens wearers on alternate days. Samples were collected weekly for microbial evaluation. On an average, the duration of use without microbial contamination was four weeks. Half of the participants were able to use both bottles of their normal saline without microbial contamination for at least four weeks after first opening and this included nine participants who were able to use for at least eight weeks. The brands of normal saline, the frequency of use, the place where the normal saline was stored or used were not significantly related to the duration of contamination-free period. Of the 27 samples tested, 11 grew gram-positive bacteria and 16, gram-negative bacteria. The most common bacteria found were Staphylococcus aureus and Pseudomonas species. None of the samples had Acanthamoeba spp. and no eye infection or irritation was reported. It was concluded that on an average, a bottle of sterile normal saline can be used for at least four weeks after first opening. Some users may be able to extend this expiry date to eight weeks, depending on the way the solution was used.

Keywords: *Contact lens, Normal saline, Microbial, Expiry date*

INTRODUCTION

All ophthalmic preparations must be sterile as the eyes, especially abraded or damaged cornea, are very sensitive and prone to infections (Allwood 1994). Non-sterile ophthalmic products could cause blindness (Turco 1996). The microorganism that is of most concern is *Pseudomonas aeruginosa* (Turco 1996; Cheng *et al.* 1999).

Multiple dose ophthalmic products must always contain antimicrobial agents, which are intended to destroy or limit the growth of microorganisms inadvertently introduced into the product during usage (Swanson and Barlett 1993). However, no preservative or combination of preservatives has been proven as absolutely effective against *Pseudomonas*

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spp. (Turco 1996). Commonly used preservatives have caused allergic reaction and ophthalmologists prefer to use single dose unpreserved ophthalmic products although this may be unattractive economically.

Contact lens solutions are also considered as ophthalmic products although these are not used for therapeutic purposes. To reduce cost, many contact lens solutions are manufactured in large volume intended for multiple applications. Popular brands of contact lens solutions in the Malaysian market include Opticare™, Klean & Kare™, Bausch & Lomb™, and CIBA Vision™. Most standard references recommend that such products should only be used for two to four weeks after opening depending on their formulation (Engle 1993; Lund 1994). However, this practice is not always followed perhaps owing to economic reasons (Wakelin 1995). Isotonic sterile normal saline is the basic solution used for rinsing, thermally disinfecting and storing of soft contact lenses (Engle 1993). This solution is available either in the preserved or preservative-free form. The preservatives commonly used are thiomersal and chlorhexidine with sorbic acid-preserved products promoted for sensitive eyes. Preservative-free normal saline solutions are available in unit-of-use containers. Some people prepare their own preservative-free saline using salt tablets and USP purified water but this practice is not recommended because of the possible development of *Acanthamoeba* keratitis (Engle 1993).

A study in Singapore found that contact lens wear was one of the major risk factors for bacterial keratitis (Tan, Lee and Lim 1995). Another study in Canada also found that 12% of patients with corneal ulcers had bacterial infection associated with the use of contact lenses (Cheung and Slomovic 1995). Lack of hygiene in handling contact lenses and its products, use of tap water to clean the lenses, going swimming without removing the contact lenses, failure to dry lens-storage cases, and the use of wrong contact lens products or non-sterile saline solutions have been identified as risk factors to the development of microbial keratitis in contact lens wearers (Engle 1993; Houang *et al*. 2001; Fan *et al.* 2002).

In view of possible bacterial infections associated with the use of contact lens, the present study is conducted to examine possible microbial contamination of normal saline under normal conditions of usage by contact lens wearers. The main aim is to determine the duration that contact lens wearers could use their normal saline without microbial contamination and to identify common microorganisms that may contaminate such solutions.

METHODS

Two brands of preservative-free normal saline, Opticare™ and Klean & Kare™, commonly used for cleaning or rinsing contact lenses were evaluated in this study. A total of 30 contact lens wearers participated in this study. The participants filled a questionnaire on basic demographic data and information on the usage of the normal saline solutions for the care of their contact lenses. These included the frequency of use, place of use and storage of the normal saline solutions.

Each participant was given two bottles of normal saline (one of each brand). The participants used the two bottles of normal saline on alternate days as specified on the labels of the bottles. Participants were briefed on how to take the weekly samples from each bottle including the method of hand washing and swabbing the nozzle of the bottles with alcohol before taking the samples. The first sample from each of the 500 ml normal saline was collected in the laboratory and if the result was positive, it was replaced by a new bottle and the same procedure was repeated. Participants were provided with two sterile tubes weekly to collect 10 ml of the remaining normal saline from each of the two normal saline bottles, for microbial testing.

Samples were collected and tested every week for at least eight weeks or until there was microbial growth, whichever occurred earlier. Samples were tested for the presence of bacteria and *Acanthamoeba* spp. At the end of the eight-week study period or when samples submitted by the participant showed microbial growth, the remaining normal saline was returned. A sample from the returned bottle of normal saline was then cultured in a tube of sterile nutrient broth to confirm that the microbial contaminant obtained in the weekly samples was from the solution in the bottle and not introduced by the participant when collecting the weekly samples.

Two bottles of each brand of normal saline evaluated were kept in the laboratory as controls. These bottles were opened once a day and a small quantity of the solution was discarded each time as though being used to cleanse contact lenses. Weekly samples were also collected and tested for microbial growth.

Microbial contamination of the bottle content was considered as positive only if samples obtained from two consecutive weeks were culture positive plus the sample obtained directly from the bottle was also culture positive. Samples collected were incubated in nutrient broth at 35°C for 2 to 3 days. Subcultures were made onto agar plates and colonies

grown were identified by standard bacteriological techniques (Hugo 1994).

To test for the presence of *Acanthamoeba* spp., samples were centrifuged and the specimen at the bottom of the tube was inoculated onto pre-treated non-nutrient agar containing *E. coli.* This was incubated for 2 to 3 days and then the culture where the specimen had been inoculated was scrapped and examined under a microscope (Martinez 1985).

If no sample from a particular bottle was available for testing, the last known sample tested, which did not have any microbial growth was taken as the week that the contact lens wearer could use the solution without microbial contamination. This means that the estimation of the duration that a contact lens wearer could use the normal saline without microbial contamination was more conservative than the actual duration, which could be longer if samples were still available for testing.

Possible factors associated with the contamination of normal saline were analysed using Pearson's chi-square test. Wilcoxon matched pairs signed rank sum test and sign test were used to check within subject difference.

RESULTS AND DISCUSSION

A total of 30 participants who wear contact lens were recruited into the study. All the participants were between 20 to 30 years old and all except two were female. One participant was a lecturer while the rest were university or college students.

The mean duration of use of the normal saline without microbial contamination was 4.6 ± 2.9 weeks with a median at 4.0 weeks and a mode at 8.0 weeks. From Figure 1, 35 of the 60 (58.3%) normal saline solutions (50% of Opticare™ and 66.7% of Klean & Kare™) could be used without microbial contamination for at least four weeks. These included 21 (35%) normal saline solutions (33.3% of Opticare™ and 36.7% of Klean & Kare™) that did not have any microbial contamination even after eight weeks of usage by the contact lens wearers. Whereas, the remaining 25 bottles (41.7%) were contaminated before four weeks of usage with nine bottles (15%) showing contamination after being used for only two weeks.

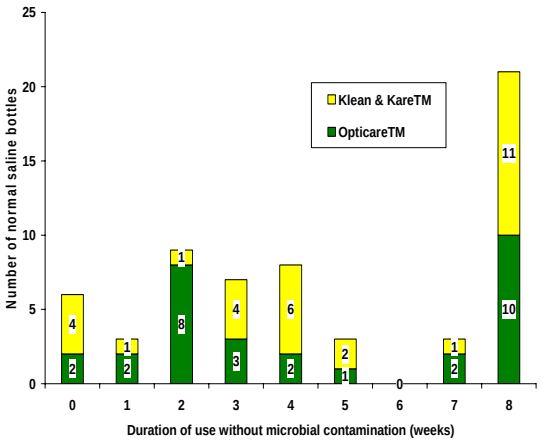

Fig. 1: Duration of use of two brands of normal saline solution by contact lens wearers, without microbial contamination.

All the four bottles that were used as controls (two bottles of Opticare™ and two bottles of Klean & Kare™) did not show any microbial growth at the end of the eight-week study period.

The results showed that half of the participants (15 out of 30) were able to use both bottles of normal saline without microbial contamination for at least four weeks after first opening. This included nine participants who were able to use for at least eight weeks. It appeared that the standard recommendation for using the normal saline for only four weeks after first opening would apply to about 50% of the users. However, three contact lens wearers contaminated their normal saline within two weeks of usage.

The duration of use of the two brands of normal saline by individual participants before microbial contamination occurred was compared using the Wilcoxon matched pairs signed rank sum test and sign test. Both tests showed no statistically significant within subject difference in the duration of use of the two brands of normal saline (p value = 0.468 and 0.481, respectively). This indicates that contamination of the normal saline is user-dependent and not brand-dependent. In other words, it could be related to the way the solutions were used by the individual contact lens wearer.

Table 1 compares the proportion of samples that could remain free from microbial contamination for up to four weeks under different conditions. The brands of normal saline, the frequency of use, the place where the normal saline was stored or used were not significantly related

to the duration of use of the normal saline without microbial contamination.

Table 1: Variables that may be associated with microbial contamination of normal saline

Independent variables	Number of participants	Samples without microbial contamination (%)		X^2 value (p value)
		< 4 weeks	> 4 weeks	
Brand of normal saline:				
Opticare™	30	50	50	1.714
Klean & Kare™	30	33.3	66.7	(0.190)
Frequency of use:				
Once a day	24	37.5	62.5	0.079
More than once a day	34	41.2	58.8	(0.778)
Place of storage:				
Bathroom	12	58.3	41.7	1.642
Bedroom/living room/ cupboard	45	37.8	62.2	(0.200)
Place of use:				
Sink in bathroom/kitchen/ dining room and bedroom	39	41.0	59.0	0.059
Bedroom table	16	37.5	62.5	(0.808)

Collins *et al.* (1994) reported that the rate of microbial contamination was related to the type of disinfection solution used but the brand of normal saline used in the present study had no effect on the contamination rate. This may be because the two brands tested in this study are of similar quality and also they both contain only 0.9% sodium chloride, without any preservative. The frequency of use and the place where the normal saline was used were also not related to the incidence of microbial contamination. However, Fan *et al.* (2002) reported that contact lens wearers with microbial keratitis were significantly more likely to clean their lenses in the toilet. These authors also believed that the practice of hygiene in the care of contact lenses is still the most important factor in the prevention of contact-lens related infection. One of the participants in the present study had microbial contamination in her first bottle of normal saline within one week of usage. This participant admitted that she dropped the cap of the normal saline bottle into the sink. She was given the second set of normal saline and was able to use it up to eight weeks without any microbial contamination. This emphasizes that care should be observed when using the normal saline to prevent contamination of the nozzle or any part that is in contact with the normal

saline, which may lead to contamination of the content in the bottle. The importance of hygienic use of the solutions is further shown by the absence of contamination in the control bottles that were sampled in the laboratory by the researchers, up to the end of the eight-week period.

Of the 60 bottles of normal saline used in this study, 21 did not show any microbial contamination even up to eight weeks of usage. Nine were empty before the eighth week and hence, no further sample was available for testing. In addition, three bottles were lost in follow-up as the participants did not submit the samples. The remaining 27 bottles that were contaminated were tested to identify the types of microorganisms present. A variety of microorganisms was identified from these 27 samples of normal saline. 11 samples grew gram-positive bacteria and 16 samples grew gram-negative bacteria. These included *Staphylococcus aureus* (7 samples), *Pseudomonas* species (5 samples with 2 containing *Pseudomonas aeruginosa*), *Enterobacter* sp. (3 samples), *Klebsiella* sp. (2 samples), *Bacillus* sp. (2 samples) and *Staphylococcus epidermidis* (2 samples). One sample each was found to be contaminated with *Stenotrophomonas maltophilia, Acinebacter* sp., *Salmonella* sp. and *Serratia marcescens*. Two of the samples were contaminated with a mixture of *Pseudomonas aeruginosa and Klebsiella* spp.

In general, the bacteria found in the contaminated samples of normal saline were those commonly present in the environment. Other studies (Houang *et al.* 2001; Ephigenia *et al.* 2003) have reported that gram-negative bacteria are the predominant microbes found in unpreserved saline while gram-positive bacteria are more frequently found in preserved saline (Sweeney *et al.* 1999). Similarly, in this study on unpreserved normal saline, there were more samples contaminated with gram-negative than gram-positive bacteria although *Staphylococcus aureus* was the most commonly detected microbe.

Both *Pseudomonas aeruginosa* and *Staphylococcus aureus* are common causes of ophthalmic problems such as microbial keratitis and conjunctivitis (Hugo 1994; Cheng *et al.* 1999; Ephigenia *et al.* 2003). However, none of the participants in our study reported any eye infections or irritation, including the 15 participants who were using normal saline that were positive for microbial growth. This could be because the participants were asked to stop using the contaminated normal saline as soon as microbial growth was observed. This was normally done within one week of contamination and also infections are more likely to occur in lens wearers with corneal damage. Other studies

have also reported the use of contaminated solutions by asymptomatic contact lens wearers (Wilson *et al.* 1990).

None of the study samples had *Acanthamoeba* spp. This is encouraging as this microorganism poses a major risk factor for eye infections (Houang *et al.* 2001). *Acanthamoeba* spp. live worldwide in soil and fresh and salt water. It was found in the domestic water environment of 8% of homes in a Hong Kong study (Houang *et al.* 2001). The absence of *Acanthamoeba* spp. in the normal saline used in this study probably implies that these solutions have been adequately sterilised and were not contaminated by tap water during use.

One of the limitations in this study is that the microbial contamination detected could be from the environment, introduced into the sample during collection or specimen handling in the laboratory. In addition, the microorganisms detected could be present only on the orifice of the dispenser tip of the normal saline bottles and may not necessarily indicate that the bottle content is contaminated (Wilson *et al.* 1990). This possibility was reduced by considering the culture result of two consecutive samples and also by culturing the sample left behind in the normal saline bottles. Another unavoidable limitation is that, if a minute number of microorganisms is present in the normal saline these may not be sampled. A more accurate method of evaluating the presence of microorganisms is to filter the whole content of normal saline and then to culture the filter. This is not possible as the aim of this study is to determine whether the recommended expiry date of normal saline can be extended up to eight weeks therefore, the filtration method would render the solution not usable after each sampling.

CONCLUSION

Common microorganisms identified from the contaminated normal saline solutions included *Staphylococcus aureus* and *Pseudomonas* spp., but none had *Acanthamoeba* spp. It may be concluded that on an average, a bottle of sterile normal saline can be used for the rinsing of contact lens without microbial contamination for at least four weeks after opening as recommended by most standard references. However, some users may be able to extend this expiry date to eight weeks with proper hygiene care. On the other hand, a small number of users would contaminate their normal saline within two weeks of use. These users should be counselled further on the hygienic use and care of the contact lens solutions.

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