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The *In Vitro* Anti-Microbial Activity of Multipurpose Contact Lens Solutions against Standard Strains of Common Ocular Pathogens: The Effect of Duration from First Use

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Abstract

Objective: This study aims to determine the *in vitro* anti-microbial efficacy of opened multi-purpose contact lens solutions on common contact lens-related ocular pathogens. Specifically, this study intend to compare the log reduction in microbial concentration when exposed to newly opened, 5 months- opened, and 10 months–opened multi-purpose contact lens solutions.

Methods: This is a single-blind controlled experiment that evaluated five locally available multi-purpose contact lens solutions (MPS) in terms of their antimicrobial efficacy towards common contact lens-related ocular pathogens using the stand alone criteria. Newly opened, 5-month old, and 10 –month old multipurpose contact lens solutions were compared based on their effect in reducing microbial concentration at 6 hours of exposure.

Results: Multi-purpose contact lens solutions (MPS) containing polyquaternium-1 and myristamidopropyl dimethylamine (MAPD) as well as polyhexamide reduced the bacterial concentrations by 3 log and fungal concentrations by 1 log, enabling them to fulfill the stand alone criteria for disinfecting solutions. This antimicrobial efficacy was most evident with newly-opened contact lens solutions, followed by those opened for five months. Those which were opened for 10 months showed limited anti-microbial activity for both bacteria and fungi.

Conclusion: Multipurpose contact lens solutions demonstrated variability in their antimicrobial activity, which are significantly affected by the kind of MPS used and the duration from the date of first use. Multi-purpose contact lens solutions (MPS) containing polyquaternum and MAPD are preferred due to its broad spectrum efficacy and effectivity. They must be utilized before their expiration date since results have shown a decrease in anti-microbial activity with an increase in duration from first use. This is to prevent contact-lens related ocular infections brought about by exposure to humid climate.

Keywords: Contact lens; Multipurpose contact lens solutions; Microbial keratitis; Silicon hydrogels

Introduction

Contact lens wear continues to be a popular method of vision correction, with an estimated 125 million contact lens wearers [1]. New lens polymers that promote ocular health, longer-lasting comfort, and flexible wearing modalities attract new wearers and contribute to the success of this form of vision correction [2]. However, complications due to contact lens wear affect roughly 5% of contact lens wearers each year [3]. They range from self-limiting to sight threatening, which require rapid diagnosis and treatment to prevent vision loss.

Recent studies have shown that the widespread use of contact lenses has resulted in an increased incidence of microbial keratitis worldwide [3]. The most predominant microbial pathogens are *Pseudomonas aeruginosa*, gram-positive organisms, [4] and *Acanthamoeba* [5]. Various factors have been reported as being responsible for contact lens-related ocular infections. The use of contact lenses past the replacement date causes the highest risk of developing microbial keratitis. Overnight wear of lenses and poor lens hygiene significantly increases the risk of microbial keratitis, respectively [6]. Patient compliance is also a major issue surrounding the use of contact lenses because patient noncompliance often leads to contamination of the lens, storage case, or both.

Contaminated contact lens solutions are primary causes of contact lens-related microbial keratitis. Contact lens solutions may harbor organisms due to unsanitary usage by the contact lens wearer and prolonged exposure to humid climates. In a study by Stapleton in 2007, they concluded that climatic conditions, particularly those in the tropics, play a role in disease severity in contact lens-related microbial keratitis [7]. Severe contact lens-related microbial keratitis was more likely to occur in warmer, humid regions [7]. In the Philippines, where humidity is relatively increased at 75-88%, the recommended storage duration of an opened contact lens solution has not yet been established [8].

Multipurpose contact lens solutions are dual-purpose liquids that both clean and disinfect contact lenses. Multipurpose solutions were previously marketed as "no-rub" solutions, because they are designed

to adequately clean and disinfect lenses with a simple rinse-and-store method, eliminating the need to mechanically rub the lenses to remove lens deposits [9]. In recent years there has been a global recall of at least one brand of a multipurpose contact lens solution because of an outbreak of eye infections associated with the product [10].

The International Organization for Standardization (ISO) has established microbiological requirements and test methods for products and regimens for hygienic management of contact lenses with methodology and acceptance criteria for stand-alone disinfecting solutions (ISO/CD 14729). According to this standard for stand-alone primary acceptance criteria, a disinfecting solution must be able to reduce the starting concentration of bacteria (*Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) by 3 log and of fungi (*Fusarium solani* and *Candida albicans*) by 1 log at the minimum disinfection time recommended by the manufacturers [11]. However, in most countries and even in the Philippines, *Escherichia coli*, an entero-bacteria which is of the same family as that of *Serratia marcescens*, is a more commonly isolated ocular pathogen as compared to the latter [12].

At present, with the advent of silicon hydrogels, there is an increasing demand for contact lenses in the Philippine market, both for refractive and cosmetic purposes. There is also a concomitant rise in the number of contact lens multipurpose solutions available in the local market for routine cleaning and disinfecting of contact lenses. Due to financial constraints, most contact lens wearers, particularly those in the marginalized sector, would devise ways to decrease their daily expenses. They would often utilize contact lens solutions even after their expiry date, which are usually three months from the date of opening. To date, there has been no local clinical data available with regard to the anti-microbial efficacy of expired multi-purpose contact lens solutions in a humid environment such as in the Philippines.

This study aims to determine the *in vitro* anti-microbial efficacy of opened multi-purpose contact lens solutions on common contact lens-related ocular pathogens. Specifically, this study intend to compare the log reduction in microbial concentration of *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeroginosa* (ATCC 27853), *Escehricha coli* (ATCC 25922), *Candida albicans* (ATCC 14053), and *Fusarium solani* (ATCC 36031) when exposed to newly opened, 5 months-opened, and 10 months-opened multi-purpose contact lens solutions.

Methodology

This research is a single-blind controlled experiment that shall evaluate locally available expired multi-purpose solutions in terms of their anti-microbial efficacy towards common contact lens-related ocular pathogens. This study was conducted at the Microbiology Laboratory of the Institute of Ophthalmology of the University of the Philippines-Manila.

The test solutions

Five bottles of multipurpose contact lens solutions (MPS) commonly marketed in the Philippines and manufactured by five different companies were evaluated. The MPS used were All Comfort Plus (Opto-Pharma), All Clean Soft (Avizor), Optifree Express (Alcon), Solocare Aqua (Ciba Vision), and Septocare (Ashford). The active ingredients in these MPS are itemized in Table 1. These test solutions were then categorized based on their identified disinfecting ingredient. The investigator performing the microbiological procedures was blind with regard to the brand of multi-purpose solution during the duration of the research.

Code	Contact Lens Solution	Manufacturer	Active Disinfectant
A	ALL Comfort Plus	Opto-Pharma	Polyaminopropyl biguanide 0.00015%
B	All Clean Soft	Avizor	Polyhexanide 0.0002%
C	Optifree Express	Alcon	Polyquaternum-1 0.001%, Myristamidopropyl Dimethylamine 0.0005%
D	Solocare Aqua	Ciba Vision	Polyhexanide 0.0001%
E	Septocare	Ashford	Thimerosal 0.001%

Table 1: Characteristics of the Multi-purpose Contact Lens Solutions (MPS) Used.

Multipurpose contact lens solutions that were used as controls were previously unopened and unused. The control contact lens solutions were also used prior to the expiry dates indicated in their packaging. Bottles were first examined to check if they were indeed untampered and sterile. They were then assigned code letters that will be used for the duration of the study. After coding, the solutions were transferred into sterile test tubes using aseptic techniques and were then properly labeled. The opened multi-purpose contact lens solutions were then kept closed and stored at room temperature. They were eventually re-opened for testing at five months and ten months. These five month old and ten month old MPS were compared with the newly-opened control contact lens solutions in terms of in vitro anti-microbial efficacy.

The challenge organisms

Based on the result of literature search regarding contact lens-related central microbial keratitis, the two most common etiologic agents are *Pseudomonas sp.* and *Staphylococcus sp.* [13,14]. These, along with *Eschericha coli*, *Fusarium solani* (filamentous fungi) and *Candida albicans* (yeast) were used as challenge organisms to determine the anti-microbial activity of multipurpose contact lens solutions on a wide range of ocular pathogens, both bacteria and fungi. The challenge organisms used and their American Type Culture Collection (ATCC) codes are seen in Table 2. These organisms were also chosen based on its similarity to the recommended challenge organisms by the International Organization for Standardization (ISO) for stand-alone disinfecting solutions (ISO/CD 14729). Standard isolates of these organisms were obtained in collaboration with the Microbiology section of the Institute of Ophthalmology.

Challenge Organism	Code
Staphylococcus aureus (gram positive cocci)	ATCC 25923
Pseudomonas aeroginosa (gram negative bacilli)	ATCC 27853
Escharicha coli (gram negative bacilli/ Enterobacteria)	ATCC 25922
Candida albicans (yeast)	ATCC 14053

Fusarium solani (filamentous fungi)	ATCC 36031

Table 2: The Challenge Organisms and their ATCC Codes.

The stand-alone criteria

The efficacy of the multipurpose contact lens solutions were then tested against the five microbiological isolates using the stand alone criteria for determination of contact lens disinfection efficacy. The stand-alone criteria measure the innate antimicrobial activity of the disinfecting solution alone. For the MPS that were employed in this study, they were marketed as a "no rub, no rinse" contact lens solution, which therefore indicates that they are formulated to pass the stand alone criteria. For a disinfectant to qualify for the stand alone criteria, it must have >3 log reduction of bacterial count and >1 log reduction of fungal concentration at regimen soaking time [15].

Inoculation and Microbial Culture

Stock solutions

Using microbial standard isolates obtained from the Institute of Ophthalmology, microbial suspensions using Mueller-Hinton broth was adjusted to contain 1.0×10^8 colony-forming units per milliliter (cfu/mL) bacteria and fungi, as determined by using the McFarland standards. These microbial suspensions were then termed as the "stock solutions". McFarland standards are used as reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range.

Turbidity standard for inoculum preparation

To standardize the inoculum density for this susceptibility test, a barium sulfate standard equivalent to a 0.5 McFarland standard was used. It was prepared by adding 0.5 ml aliquot of 0.0048 mol/L $BaCl_2$ to a 99.5 ml of 0.18 mol/L H_2SO_4 with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified using a VITEK colorimeter. The absorbance at 625 nm should be 0.8 to 0.10 for the 0.5 McFarland standard.

The barium sulfate suspension was transferred in 6 ml aliquots into screw cap tubes which were tightly sealed and stored in the dark at room temperature. The suspension was vigorously agitated on a vortex mixer before each use and inspected for a uniformly turbid appearance. A positive control (crystal violet) and negative control (saline) were used to compare turbidity.

Preparation of stock solutions (Direct colony suspension method)

A microbial colony of the same morphological type was selected from an agar plate culture of the microbial standard strains. The top of each colony was touched with a wire loop and the growth transferred aseptically to a tube containing 15 ml of Mueller-Hinton Broth to achieve a turbidity comparable to a 0.5 McFarland standard. This resulted in a suspension containing approximately 1×10^8 cfu/ml for each test organism. Comparison of the broth culture with the McFarland standard was done using a colorimeter and under adequate lighting using a card with a white background and contrasting black lines.

Bio-test MPS solutions

Three (3) mL of the prepared suspension of organism (stock solution) was aliquoted aseptically in five separate test tubes for each organism undergoing testing. They were then added with 3 ml of each test multi-purpose contact lens solution to achieve a 1:1 concentration. The stock solutions were exposed to the MPS solutions for 6 hours, which was the recommended duration of exposure to achieve maximal antimicrobial efficacy. The resulting MPS+microorganism solution was termed "bio-test solutions". The samples were then vortexed to ensure adequate dispersion.

Moreover, negative controls for each challenge organism were created using microbial stock solutions with only saline solution as an additional ingredient. This is to determine if indeed the stock solutions prepared were able to give 10^8 microbial colonies when plated in recovery media. Positive controls on the other hand, were prepared by adding saline with the five MPS contact lens solution to check for possible contaminations.

Susceptibility testing and neutralization

Three (3) mL aliquots of bio-test solutions were taken for viability counts at 6 hours of exposure. Samples were then neutralized with three (3) ml of Dey-Engley neutralizing broth and vortexed vigorously. They were then incubated at 35°C for 3 hours then plated onto recovery agar plates (Dey-Engley agar) in triplicate.

Recovery plates were incubated for 24 hrs at 35°C for bacteria and at room temperature for fungi. Colonies were then counted using the approximate plate counting method and the log viability reductions were calculated. All of the experiments were carried out in triplicate.

To determine if an opened multipurpose solution was still as effective disinfectant as opposed to a newly-opened one, their anti-microbial efficacy were analyzed at five months and ten months after initial use. Newly-opened bottles of the five multi-purpose solutions served as the control treatment. Similar inoculation and culture methods stated above were utilized for analysis of anti-microbial effect. All experiments were done in triplicate.

Statistical analysis

A statistical software from the Research Development Core Team (2011) R 2.14.0 was used to compute the significant effects and differences among variables [16].

The Two-way Analysis of Variance (ANOVA) was used to determine the factors affecting the concentration challenge organisms (log cfu/ml) with a level of significance (α) of 0.05. Tukey Honestly Significant Difference (HSD) test, meanwhile, is a single-step multiple comparison procedure generally used in conjunction with an ANOVA to find which means are significantly different from one another. It was used to determine the post-hoc differences among variables presented.

Results

The initial microbial concentration was 10^8 cfu/ml determined using the 0.5 McFarland standard. The multipurpose solution was considered to be an effective bactericidal when it has >3 log reduction of bacterial count and >1 log reduction of fungal concentration at regimen soaking time, thus qualifying for the stand alone criteria as set by the ISO for disinfecting solutions.

All negative controls (microbial stock solutions+saline solution) had 108 microbial colonies when plated in recovery media. Positive controls (saline+MPS) on the other hand, had no growth on culture which verifies the sterility of the MPS contact lens solutions tested.

Two-way ANOVA showed that the concentration of all the challenge organisms were significantly affected by the duration since first use of the multi-purpose contact lens solution ($p<0.001$). Meanwhile, the kind of multipurpose contact lens solution was found to have significant effects ($p<0.001$) on the concentration of Staphylococcus aureus, Pseudomonas aeroginosa, and *Escherichia coli*, but not *Candida albicans* and *Fusarium solani*.

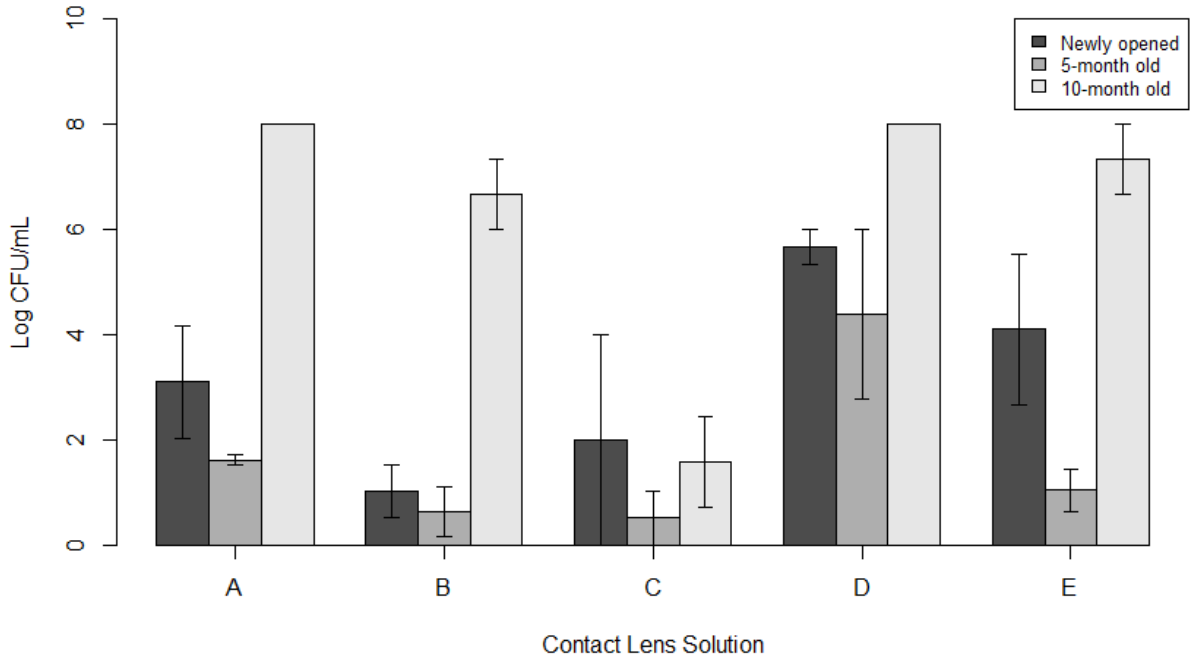

Figure 1: Effect of Duration of Opening and kind of MPS on the concentration of *Staphylococcus aureus.*

The kind of MPS used and the duration of opening were shown to have significant effects on the concentration of *Staphyloccus aureus* ($p<0.001$) as seen in Figure 1. Based on the results of the Turkey HSD test, MPS C and B were shown to have the greatest inhibitory effect, particularly when newly opened and up to 5 months from the date of opening. The anti-microbial effect of the test solutions were found to be reduced at 10 months from opening date.

Meanwhile, as seen in Figure 2, the kind of MPS used and the duration of opening were also shown to have significant effects on the concentration of *Pseudomonas aeroginosa* ($p<0.001$). Tukey HSD test revealed that MPS A and E had poor inhibitory effect on the challenge organism. The anti-microbial effect of the test solutions were found to be reduced at 5 and 10 months from opening date when compared to newly opened ones.

The kind of MPS used ($p<0.01$) and the duration of opening ($p<0.001$) were also shown to have significant effects on the concentration of Escherichia coli as shown in Figure 3. Tukey HSD test showed that MPS C and D greatly decreased the microbial concentration, especially when newly-opened test solutions were utilized. The inhibitory effect on Escherichia coli decreased when using 5-month and 10-month old solutions.

The microbial concentration of *Candida albicans* in Figure 4 was observed to be significantly affected by the duration of opening ($p<0.001$) of the MPS solution, but not by the kind of MPS solution used. However, microbial load was found to be more inhibited by MPS B, C and D, especially when using newly opened and 5 month old MPS solution.

On the other hand, the microbial concentration of *Fusarium solani* was observed to be significantly affected by the duration of opening ($p<0.001$) of the MPS solution, but not by the kind of MPS solution used as seen in Figure 5. Four of the five month old test MPS solutions showed a decreased microbial load compared to newly-opened ones. Moreover, microbial load was found to be more inhibited by MPS B and D.

Discussion

Contact lens use has been identified in numerous studies to be a risk for ocular infection. 3 Pathogenic microorganisms may be transferred quite easily from the contact lens to the eye, especially when improperly cleaned and used. Ocular infection in contact lens wearers is associated with microbial contamination of their care products, such as contact lens solutions and inadequate cleaning of their lenses [17].

In this study, we used the stand alone criteria from the International Organization for Standardization (ISO/CD 14729) to determine the affectivity of locally available contact lens disinfecting solutions against common contact-lens-related ocular pathogens. According to this standard for stand-alone primary acceptance criteria, a disinfecting solution must be able to reduce the starting concentration of bacteria (*Serratia marcescens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) by 3 log and of fungi (*Fusarium solani* and *Candida albicans*) by 1 log at the minimum disinfection time recommended by the manufacturers [11]. Although not required by ISO guidelines, we utilized *Escherichia coli*, an entero-bacteria, in lieu of *Serratia Marcescens*, since the former is more commonly isolated in our setting and was found to contaminate contact lens accessories stored in bathrooms [12].

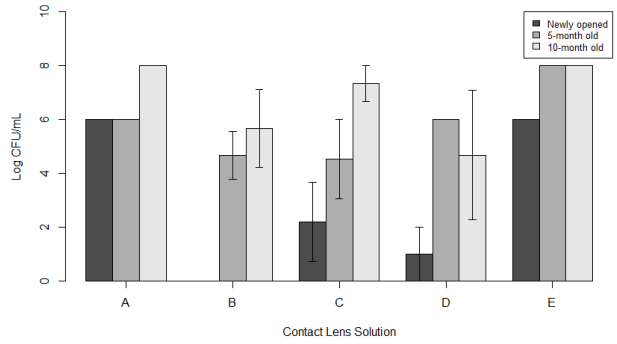

Figure 2: Effect of Duration of Opening and kind of MPS on the concentration of *Pseudomonas aeroginosa*.

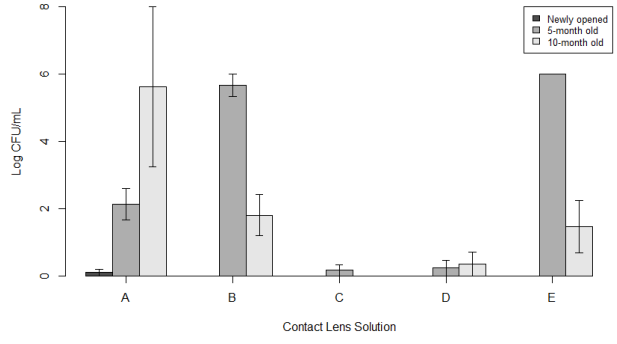

Figure 3: Effect of Duration of Opening and kind of MPS on the concentration of *Escherichia coli*.

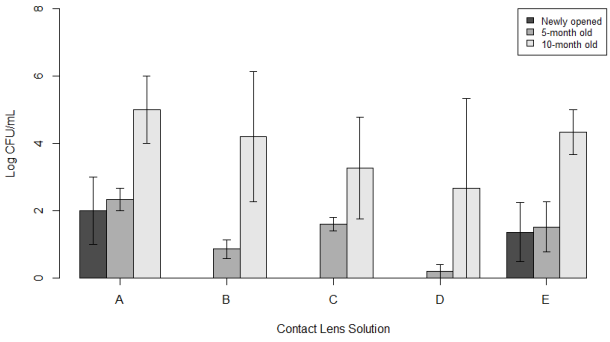

Figure 4: Effect of Duration of Opening and kind of MPS on the concentration of *Candida albicans*.

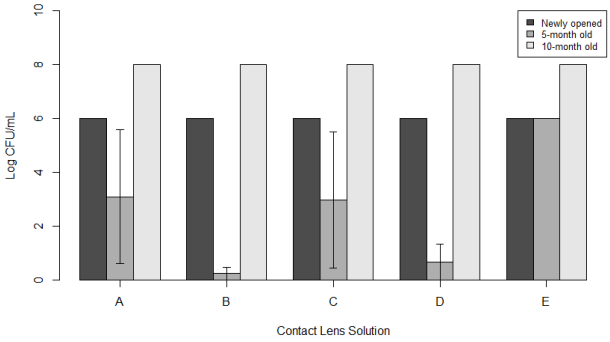

Figure 5: Effect of Duration of Opening and kind of MPS on the concentration of *Fusarium solani*.

This study, in particular, tested five bottles of multi- purpose contact lens solutions (MPS) commonly marketed in the Philippines and manufactured by five different companies (Table 1). The five MPS contained different kinds of active disinfectants, namely: one with polyhexa-methylenebiguanide (MPS A), two with polyhexanide (MPS B and D), one with thimerosol (MPS E), and one containing polyquaternium-1 (MPS C).

Our results showed that the MPS with polyquaternium-1 and MAPD (MPS C) showed the greatest decrease in the concentration in most of the challenge organisms, followed by those containing polyhexanide (MPS B and D). For *Fusarium*, however, those containing polyhexanide as a main ingredient showed greater anti-microbial effect than MPS C. MPS containing polyaminopropyl-biguanide (MPS A) and thimerosol (MPS E), on the other hand, were less effective in diminishing microbial concentration.

In this study, MPS C showed the highest antimicrobial activity against most of the bacteria and fungi tested, which was consistent with findings from other studies [12,14]. This might be attributed to the synergistic action of the two antimicrobial agents, polyquad and Myristamidopropyl Dimethylamine (MAPD) contained in the said

solution. Polyquad is a quaternary ammonium-based antimicrobial agent providing predominantly antibacterial properties, whereas MAPD has a wider spectrum of antimicrobial activity, particularly for fungi [12].

Those with polyhexamide (MPS B and D) were also comparable to MPS C in terms of significantly lowering the microbial concentration of the challenge organisms. They were even observed to have a greater inhibitory effect on *Fusarium solani*, a virulent and pathogenic filamentous fungi. Polyhexanide, a member of the biguanide disinfectant family, contains highly-charged active sites that have the ability to disrupt microbial cellular membranes by electrostatic interaction which were most effective against a wide-range of microorgansims.

Meanwhile, those containing polyaminopropylbiguanide (MPS A) and thimerosol (MPS E) were found to have the least anti-microbial effect on the bacteria and fungi used in this study.

In terms of the duration of opening of multipurpose contact lens solutions and its effect on anti-microbial efficacy, we observed that greatest microbial inhibitory effect for most of the challenge organisms was for the newly-opened MPS, followed by the 5 month old contact lens solution. Specifically, anti-microbial activity of 5-month old MPS were comparable to newly opened ones when dealing with *Staphylococcus aureus*, *Candida albicans*, and *Fusarium solani*. MPS that were already opened for 10 months showed limited anti-microbial activity for all challenge organisms and may suggest a higher risk for microbial keratitis when used for contact lens care.

These results further strengthen the findings that exposure of MPS to humid climates past its recommended duration of usage of three months may cause a decrease in its antimicrobial efficacy, thus maybe considered as a predisposing factor to ocular infections.

Limitations of this research include the following: (1) the utilization of a single representative bottle of each multipurpose contact lens solution; (2) the sampling of the contact lens solution at two points in time (at 5 months and 10 months) after opening; and (3) the *in-vitro* design of this study.

Conclusion

Multipurpose contact lens solutions demonstrated variability in their antimicrobial activity, which are significantly affected by the kind of MPS used and the duration from the date of opening. Moreover, multi-purpose contact lens solutions with broad spectrum efficacy and effectivity, such as those containing polyquaternum and MAPD, are preferred to prevent contact lens related ocular infections.

In this study, anti-microbial action was noted to diminish when using five month-old and ten month-old multi-purpose contact lens solutions. Thus, it is recommended that multi-purpose contact lens solutions must be discarded after its expiration date since anti-microbial activity decreases with increase duration from opening. Moreover, since this study has an in-vitro design, further studies should also be made to evaluate the antimicrobial effectivity of these contact lens solution in an actual patient population.

Disclosure

The authors of this study have no financial interest nor received any financial support from the companies that manufactured.

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