The Antimicrobial Efficacy of Multipurpose Contact Lens Solutions on Standard Strains of Common Ocular Pathogens

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ABSTRACT

Objective: To compare the antimicrobial effects of locally available multipurpose contact lens solutions (MPS) on the growth of standard strains of contact lens-related ocular pathogens and to establish the recommended duration of exposure to these solutions to achieve maximal antimicrobial efficacy.

Methods: This study, a single-blind controlled experiment, evaluated five locally available MPS in terms of their antimicrobial efficacy towards common contact lens-related ocular pathogens, such as *P. aeroginosa*, *S. aureus*, *E. coli*, *F. solani*, and *C. albicans*, using the stand alone criteria. Microbial viability counts were obtained at serial durations: after 1 hour, 3 hours, 6 hours, and 12 hours of exposure.

Results: MPS containing polyquaternium-1 and myristamidopropyl dimethylamine (MAPD) and 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 as mandated by ISO/CD 14729. This antimicrobial efficacy was most evident at 6 hours of exposure to the challenge organisms. MPS containing polyquaternium-1 and MAPD also have the broadest spectrum of effectivity against gram-negative and gram-positive bacteria, and *C. albicans*. All MPS tested have poor microbial activity against *F. solani*.

Conclusion: Multipurpose contact lens solutions demonstrated variability in their antimicrobial activity. MPS with broad spectrum efficacy and effectivity, such as those containing polyquaternum and MAPD, are preferred to prevent contact lens-related ocular infections.

Keywords: Contact lens, Multipurpose contact lens solutions, Microbial keratitis, Infectious keratitis, Silicon hydrogels

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Contact lens is a lens placed on the cornea of the eye to improve vision, primarily for cosmetic purposes. Complications due to contact lens wear affected roughly 5% of contact lens wearers each year. They ranged from self-limiting to sight threatening, that require rapid diagnosis and treatment to prevent vision loss. Excessive contact lens wear, particularly overnight wear, was associated with most of the safety concerns.

Microbial keratitis is a common and potentially devastating complication of contact lens wear. A large segment of current contact lens research is directed towards the treatment and prevention of conditions resulting from contact lens contamination and colonization by foreign organisms. The most predominant microbial pathogens were *Pseudomonas aeruginosa* and gram-positive organisms.[1] Breaks in the corneal epithelium were probably important predisposing factors to bacterial keratitis. More recent studies have also found an increased risk of microbial keratitis with silicon hydrogel materials.[2,3]

Various factors have been reported as being responsible for contact lens-related ocular infections. These included microbial contamination of contact lens solutions and poor contact lens hygiene by wearers. Compliance was a major issue surrounding the use of contact lenses because patient noncompliance often led to contamination of the lens, storage case, or both.

Multipurpose contact lens solutions (MPS) are dual-purpose liquids that both clean and disinfect contact lenses. They are also called "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.

Proponents of MPS said they were less expensive and easier to use than hydrogen peroxide-based solutions, and, therefore, contact lens wearers were more likely to use them properly. But 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.[4]

Despite the number of researches done with regards to the antibacterial properties of available contact lens solution, the recommended duration of contact time between used contact lens and the cleaning solution has yet to be determined. The recommended length of soaking is deemed important since the degree of antibacterial effect is related to contact time.

Moreover, 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 climate. 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.

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, 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.[5] However, in most countries and even in the Philippines, Escherichia coli, an enterobacteria which is of the same family as that of Serratia marcescens, is a more commonly isolated ocular pathogen when compared to the latter.[6]

Thus, evaluation of the antimicrobial activity of the locally available contact lens solutions is an interesting area of research. This study determined the antimicrobial effects of locally available multipurpose contact lens solutions (MPS) on contact lens-related ocular pathogens. Specifically, it compared the antimicrobial efficacy of these solutions on the growth of the ocular pathogens and established the most effective length of exposure of these solutions to the pathogens.

METHODOLOGY

This was a single-blind controlled experiment that evaluated the locally available multipurpose solutions in terms of their antimicrobial efficacy towards common contact lens-related ocular pathogens. The study was conducted at the Microbiology Laboratory of the Institute of Ophthalmology, 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. These test solutions were categorized based on their identified disinfecting ingredient. The investigator performing the microbiological procedures was blinded with regards to the brand of multipurpose solution during the duration of the study. The MPS tested are listed in Table 1.

Table 1: Characteristics of the multipurpose contact lens solutions (MPS) tested.

Code	MPS	Manufacturer	Active Disinfectant
A	**ALL Comfort Plus**	Opto-Pharma	Polyaminopropyl-biguanide 0.00015%
B	**All Clean Soft**	Avizor	Polyhexamide 0.0002%
C	**Optifree Express**	Alcon	Polyquaternium-1 0.001%, Myristamidopropyl dimethylamine 0.0005%
D	**Solocare Aqua**	Ciba Vision	Polyhexamide 0.0001%
E	**Septocare**	Ashford	Thimerosol 0.001%

All products were previously unopened and used for the experiment prior to the expiry dates indicated in their packaging. Bottles were first examined to check if they were indeed untampered and sterile. They were assigned code letters used for the duration of the study. After coding, the solutions were transferred into sterile test tubes using aseptic techniques and properly labeled.

The Challenge Organisms

Based on the results of literature search regarding contact lens-related central microbial keratitis, the two most common etiologic agents were *Pseudomonas* sp. and *Staphylococcus* sp.[1,2] These, along with *Escherichia coli*, F*usarium solani* (filamentous fungi), and *Candida albicans* (yeast), were used as challenge organisms to determine the antimicrobial activity of MPS on a wide range of ocular pathogens, both bacteria and fungi. These organisms were also chosen based on its similarity to the recommended challenge organisms by the 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.

The Stand Alone Criteria

The efficacy of the MPS were tested against the five microbiological isolates using the stand alone criteria for determination of contact lens disinfection efficacy. The stand alone criteria measured the innate antimicrobial activity of the disinfecting solution alone. The MPS that were employed in this study were marketed as a "no rub, no rinse" contact lens solutions, which indicated that they were 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.[7]

Inoculation and Microbial Culture

1. Stock Solutions

Using microbial standard isolates obtained from the Institute of Ophthalmology, microbial suspensions using Mueller-Hinton broth was adjusted to contain $1.0x10^8$ 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 were used as reference to adjust the turbidity of bacterial suspensions so that the number of bacteria would be within a given range.

A. 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 BaCL2 to a 99.5 mL of 0.18 mol/L H2SO4 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.

B. 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 $1x10^8$ 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.

2. Bio-Test Solutions

Three (3) mL of the prepared suspension of organism (stock solution) was aliquoted aseptically in four separate test tubes for each organism undergoing testing. They were then added with 3 mL of each test MPS to achieve a 1:1 concentration. The stock solutions were exposed to the MPS at serial durations of after 1 hour, 3 hours, 6 hours, and 12 hours of exposure. The resulting MPS + microorganism solution was termed "bio-test solutions." The samples were then vortexed to ensure adequate dispersion.

Positive controls for each challenge organism were created using microbial stock solutions with only saline solution as an additional ingredient. This was to determine if the stock solutions prepared were able to give 10^8 microbial colonies when plated in recovery media. Negative controls were prepared by adding saline to the five MPS to check for possible contaminations.

3. Susceptibility Testing and Neutralization

Three (3) mL aliquots of bio-test solutions were taken for viability counts at serial durations of after 1 hour, 3 hours, 6 hours, and 12 hours of exposure. Samples were neutralized with three (3) mL of Dey-Engley neutralizing broth and vortexed vigorously. They were incubated at 35°C for 3 hours and 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 counted using approximate plate counting method and log viability reductions calculated. All of the experiments were carried out 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.[8]

The two-way analysis of variance (ANOVA) was used to determine the factors affecting the concentration of challenge organisms (log cfu/mL) with a level of significance (α) of 0.05. Tukey Honestly Significant Difference (HSD) test was used to determine the post-hoc differences among the 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 positive controls (microbial stock solutions +saline solution) had 10^8 microbial colonies when plated in recovery media. Negative controls (saline +MPS) had no growth on culture, verifying the sterility of the MPS tested.

MPS B, C, and D were able to reduce the bacterial concentrations by 3 log and fungal at 1 log, thus enabling them to fulfill the stand-alone criteria. This antimicrobial effect was most evident at 6 hours of exposure of challenge organisms to the MPS.

Two-way ANOVA revealed that the concentration of challenge organisms (log cfu/mL) was significantly affected by the kind of MPS used ($p<0.001$) and length of exposure to MPS ($p<0.001$). There was a significant difference in the concentration of challenge organisms when exposed to the five different MPS at different exposure times.

Tukey HSD test between the five different MPS showed that there were significant differences among the test solutions in terms of their antimicrobial

effects (Figure 1). MPS C and B showed the greatest decrease in the concentration of the challenge organisms, followed by D and A. MPS E showed the least antimicrobial effects.

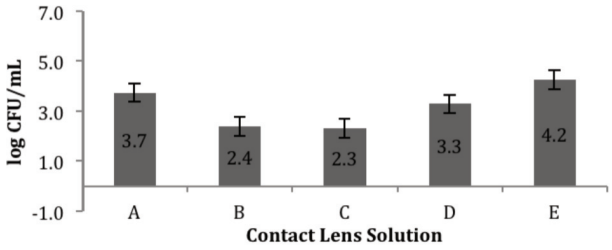

Figure 1. Effect of the kind of MPS on concentration of challenge organisms.

Tukey HSD test showed that there were significant differences when comparing the durations of exposure at 1 hour, 3 hours, 6 hours, and 12 hours to the susceptibility of the challenge organisms used (Figure 2). Results showed that the challenge organisms should be exposed to the MPS for at least 6 hours to achieve the maximal antimicrobial effect.

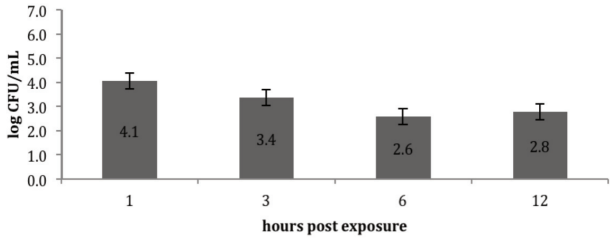

Figure 2. Effect of time exposure to MPS on the concentration of challenge organisms.

The concentration differences among the five challenge organisms when exposed to various MPS showed that *Escherichia coli* was most susceptible to the antimicrobial effects, followed by *Candida albicans*, *Pseudomonas aeroginosa* and *Staphylococcus aureus*. (Figure 3). *Fusarium solani* appeared to be the least sensitive among the five challenge organisms.

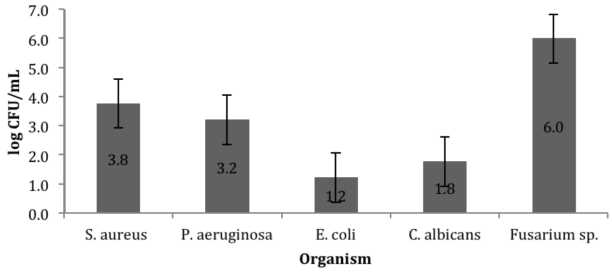

Figure 3. The differences in concentration of challenge organisms when exposed to MPS.

The effects of five MPS and varying duration of exposure showed significant effects on the concentration of S. aureus when varying the kind of MPS used ($p<0.001$) but not in the duration of exposure (Figure 4). MPS C was most effective against *S. aureus* followed by B.

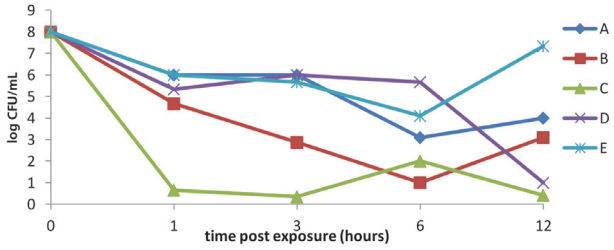

Figure 4. Effects of different MPS and length of exposure on the concentration of *Staphylococcus aureus*.

The effects of five MPS and varying duration of exposure showed significant effects on the concentration of P. aeroginosa when varying the kind of MPS used ($p<0.001$) and when differing the duration of exposure ($p<0.05$) (Figure 5). P. aeroginosa was most susceptible to MPS C, B, and D, with maximal antimicrobial effects seen after 12 hours of exposure.

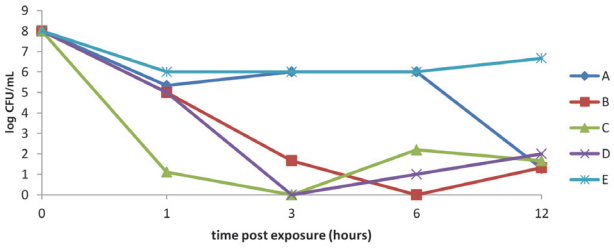

Figure 5. Effects of different MPS and length of exposure on the concentration of *Pseudomonas aeroginosa*.

The effects of five MPS and varying duration of exposure showed significant effects on the concentration of *E. coli* when varying the duration of exposure to MPS ($p<0.05$) (Figure 6). Exposing *E. coli* to MPS for 6 hours resulted in the least concentration of the microorganism. There was no significant difference among the five MPS in terms of their effects on the concentration of *E. coli*.

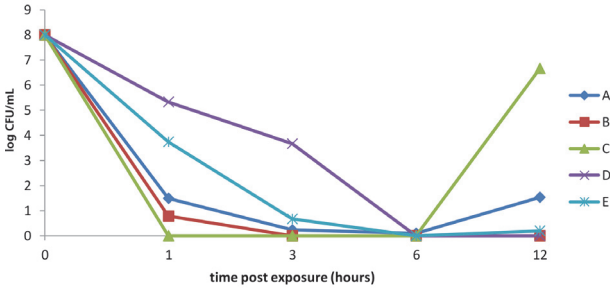

Figure 6. Effects of different MPS and length of exposure on the concentration of *Escherichia coli*.

The effects of five MPS and varying duration of exposure showed significant effects on the concentration of *C. albicans* when varying the duration of exposure to MPS ($p<0.001$) (Figure 7). Exposing *C. albicans* to MPS for at least 6 hours resulted in a significant decrease in the concentration of the microorganism. There was no significant difference among the five MPS in terms of their effects on the concentration of *C. albicans*.

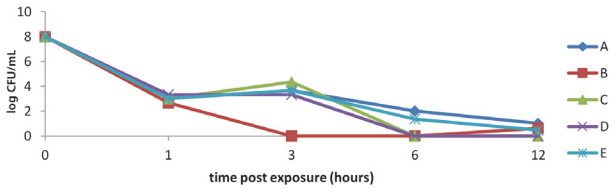

Figure 7. Effects of different MPS and length of exposure on the concentration of *Candida albicans*.

The effects of five MPS and varying duration of exposure showed no significant differences among the test solutions and the duration of exposure in terms of their effects on the concentration of *F. solani* (Figure 8). The five MPS were found to be least effective in reducing the microbial counts. However, a greater than 1 log reduction in fungal concentration for all MPS was acceptable using the stand alone criteria from ISO.

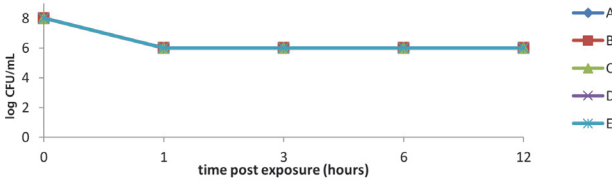

Figure 8. Effects of different MPS and length of exposure on the concentration of *Fusarium solani*.

A summary of the effectivity of the different MPS against the five challenge organisms are listed in Table 2. MPS C, containing polyquaternium-1 and myristamidopropyl dimethylamine (MAPD), was found to be most effective against bacteria and *C. albicans*. Those containing polyhexamide (MPS B and D) showed good microbial activity against *P. aeroginosa* and *C. albicans*. MPS D, however, had fair microbial activity against *E. coli* when compared to MPS B. MPS A (polyaminopropyl biguanide) and MPS E (thimerosol) had poor antimicrobial activity against several microorganisms.

Table 2. Summary of the effectivity of different MPS on the challenge organisms.

MPS	Good Microbial Activity	Fair Microbial Activity	Poor Microbial Activity
A Polyaminopropyl biguanide 0.00015%	• *Escherichia coli*	• *Candida albicans*	• *Staphylococcus aureus* • *Pseudomonas aeroginosa* • *Fusarium solani*
B Polyhexamide 0.0002%	• *Pseudomonas aeroginosa* • *Escherichia coli* • *Candida albicans*	• *Staphylococcus aureus*	• *Fusarium solani*
C Polyquaternium-1 0.001%, Myristamido-propyl dimethylamine 0.0005%	• *Staphylococcus aureus* • *Pseudomonas aeroginosa* • *Escherichia coli* • *Candida albicans*		• *Fusarium solani*
D Polyhexamide 0.0001%	• *Pseudomonas aeroginosa* • *Candida albicans*	• *Escherichia coli*	• *Staphylococcus aureus* • *Fusarium solani*
E Thimerosol 0.001%	• *Escherichia coli*	• *Candida albicans*	• Staphylococcus *aureus* • *Pseudomonas aeroginosa* • *Fusarium solani*

Good Microbial Activity: ≤2 log cfu/mL, Fair Microbial Activity: > log 2 but ≤ log 4 cfu/mL, Poor Microbial Activity: > log 4 cfu/mL.

DISCUSSION

Contact lens use has been identified in numerous studies to be a risk for ocular infection. It is known that pathogenic microorganisms may be transferred quite easily from the contact lens to the eye, especially when improperly cleaned and used. Therefore, efficient disinfection of the lens is essential.

Several studies have demonstrated that ocular infection in contact lens wearers was associated with microbial contamination of their contact lens care products and inadequate cleaning of their lenses.[9-10] Thus, it was imperative to determine and verify the antimicrobial activity of locally available soft contact lens disinfecting solutions in the Philippines, in order to prevent contamination and the transmission of infection, which may lead to blindness. The optimal contact lens care system should provide a balance

of minimal ocular toxicity and efficacy in terms of disinfection against a wide spectrum of ocular pathogens, particularly those that were commonly isolated from contact-lens-related infections.

In this study, we used the stand alone criteria from the International Organization for Standardization (ISO/CD 14729) to determine the effectivity of locally available contact lens disinfecting solutions against common contact-lens-related ocular pathogens. According to this standard for stand alone primary acceptance criteria, 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.[5] Although not required by ISO guidelines, we utilized *Escherichia coli*, an entero-bacteria, in lieu of *Serratia marcescens*, since the former was more commonly isolated in our setting and was found to contaminate contact lens accessories stored in bathrooms.[11]

This study also tested five bottles of multi-purpose contact lens solutions (MPS) commonly marketed in the Philippines and manufactured by five different companies (see Table 1). The five MPS contained different kinds of active disinfectant: one polyhexamethylenebiguanide (MPS A), two polyhexamide (MPS B and D), one thimerosol (MPS E), and one containing polyquaternium-1 (MPS C). Significant differences were observed among the five MPS in terms of their antimicrobial effects on the five challenge organisms, particularly *Staphylococcus aureus* and *Pseudomonas aeroginosa*. Our results showed that the MPS with polyquaternium-1 and MAPD (MPS C) showed the greatest decrease in the concentration of the challenge organisms, followed by those containing polyhexamide (MPS B and D). Moreover, MPS containing polyamino-propylbiguanide (MPS A) and thimerosol (MPS E) 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.[12-16] This might be attributed to the fact that it contained two antimicrobial agents, polyquad and MAPD. Polyquad is a quaternary ammonium-based antimicrobial agent providing predominantly antibacterial properties, whereas MAPD has a wider spectrum of antimicrobial activity, particularly for fungi.[10] However, *Fusarium solani*, a filamentous fungi, showed limited susceptibility when exposed to the five MPS, probably because of its high virulence and pathogenicity.

Those with biguanide-based antimicrobial agents, such as polyhexamide (MPS B and D) and polyaminopropylbiguanide (MPS A), were able to significantly lower the microbial concentration of the challenge organisms. These disinfecting agents contained 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 bacteria. Those with polyhexamide were found to be more effective than the polyaminopropylbiguanide. These differences in antimicrobial activity might be due to differences in disinfecting agent concentration and other solution qualities, such as viscosity and ionic balance of the solution, as well as other extrinsic factors that might have diminished quality control during the manufacturing process. Finally, the challenge organisms were found to be least susceptible to the MPS containing thimerosol (MPS E). Thimerosol is an organo-mercury compound with established antiseptic and antifungal properties.

When comparing the spectrum of microbial activity of the MPS, our results showed that MPS C, containing polyquaternium-1 and myristamidopropyl dimethylamine, was found to be most effective against gram-negative and -positive bacteria, as well as *C. albicans*. Those containing polyhexamide (MPS B and D) showed good microbial activity for *P. aeroginosa* and *C. albicans*. MPS D, however, had fair microbial activity against *E. coli*, probably due to its decreased concentration of polyhexamide. Most of the challenge organisms, such as *S. aureus*, *P. aeroginosa*, and *F. solani* had poor susceptibility to MPS A and E, indicating a limited antimicrobial spectrum for these solutions; thus, increasing the risk for possible ocular infections.

In terms of length of exposure of the challenge organisms to the five MPS, there was a statistically significant difference in the concentration of challenge organisms when exposed to varying durations to the MPS, particularly for *P. aeroginosa*, *E. coli*, and *C. albicans*. It was also established that the recommended minimum exposure time to achieve effective antimicrobial activity was at least 6 hours.

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