Research Article

The Efficacy of Multipurpose Contact Lens Solutions were Sold in Indonesia against *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergilus niger*

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ABSTRACT

Contact Lens is a lens placed on the cornea of the eye to improve vision. Multipurpose contact lens solution/MPS (Multipurpose solution) is a desinfectans solution of contact lenses that causes of microbial keratitis. This study aims to evaluate the efficacy of antimicrobial MPS were sold in indonesia against Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus niger and determine the contact time in order to achieve maximum antimicrobial effect by the Stand Alone criteria method with variation of contact time 1 hour, 3 hours, 6 hours, and 24 hours, based on International Organization for Standardization (ISO) 14 729 guidelines. The results showed that this study indicated all of the MPS have activity as antimicrobial after exposure for 24 hours. MPS B which contained Polyhexamethylene biguanid showed the greatest decrease in the concentration of the challenge organisms with contact time was 6 hours.

Keywords: multipurpose solution, Antimicrobial Staphylococcus aureus, Pseudomonas aeruginosa, aspergilus niger and candida albicans

INTRODUCTION

Contact lens is visual aids other than glasses that is placed on the cornea of the eye to improve vision, primarily for cosmetic purpose (Eleonore, 2013). In worldwide including Indonesia, more than 125 million of people use contact lens as an alternative eyesight (Yvonne, 2010; Rumpakis, 2010). The incidence of contact lens associated complications is rare, but if severe, could lead to permanent vision loss. Microbial keratitis is the most serious and sight threatening condition associated with contact lens wear (Yvonne, 2010).

Microbial keratitis is potentially devastating complication of contact lens wears. 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. Breaks in the corneal epithelium were probably important predisposing factors to bacterial keratitis (Eleonore, 2013).

Investigations have documented that contact lens-related microbial keratitis is commonly caused by bacteria such as *Staphylococcus aureus and Pseudomonas aeruginosa* (Mohammadinia, 2012) and also caused by fungi such as *Candida albicans* and *Aspergillusniger (*Liesegang, 1980).

Multipurpose contact lens solutions (MPS) are used for cleaning and disinfecting contact lenses. 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. Despite antibacterial properties have been demonstrated, butduration of contact time between the contact lens and

MPS solution has not been determined, so it is necessary to determine the contact time between the MPS with contact lenses (Liesegang, 1980).

This study determined the efficacy of Multipurpose Contact Lens Solutions against *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* and determined the effective contact time of that solution (Jalbert, 2010).

MATERIALS AND METHODS

Five bottles of multipurpose contact lens solutions (MPS), commonly marketed in Indonesia and manufactured by 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 (Eleonore, 2013). The MPS tested are listed in Table 1.

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

Multipurpose Solution (MPS)	Active substances
A	Polyaminopropyl biguanid 0.0001 %
B	Polyhexamethylene biguanid
C	Polyhexanide
D	Polyhexamethylene biguanid
E	Polyaminopropyl biguanid 0.0001 %

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The Sterility of Laminar Air Flow

Petri dishes containing TSA medium is placed in LAF. Petri dishes were placed in an open state for 15-30 minutes. Then closed put in the incubator for 24 hoursat a temperature of 370 °C, colony growth was observed (Budiman, 2014).

The Microorganisms Used

Based on the results of literature research regarding contact lens-related central microbial keratitis, the two most common etiologic agents were *Pseudomonas sp.* and *Staphylococcus sp., Candida albicans* and *Aspergillus niger* were used as challenge organisms to determine the efficacy of MPS on a wide range of ocular pathogens, both bacteria and fungi (Eleonore, 2013).

Preparation of Microbial Suspension

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 NaCl 0.9 % to achieve a turbidity comparable to a 0.5 McFarland standard (Eleonore, 2013; Rosenthal, 2002).

One mL of the microbial solution was aliquot aseptically separate test tubes for each organism undergoing testing. They were then added with 1 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."

Positive controls for each challenge organism were created using microbial stock solutions with solution containing NaCl 0.9 %.

Negative controls were prepared by adding solution containing NaCl 0.9% to the five MPS to check for possible contaminations.

The Effectiveness Test of Antimicrobial

One mL sample were added to 1 mL each agar (*Triptone-Soya-broth and Sabourband Dextrose Broth)* then sub-cultured on an agar medium recovery (*Triptone-Soya-broth and Sabour band Dextrose Broth)*. Recovery Plate was incubated for 24 hours at a temperature of35-37° C for bacteria and 20-25° C for fungi. Colonies of microorganism were determined using the Total Plate Count and log reduction in bacterial count.

Statistical Analysis

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 (a) of 0.05. Tukey Honestly Significant Difference (HSD) test was used to determine the post-hoc differences among the variables presented.

RESULTS

LAF was evaluated to ensure that the LAF room which was used as a study room, did not give contamination of samples. The result showed in Table 2.

Table 2: The Result test of Contamination's LAF

Petri dishes	Colony (cfu/m^3)
I (cabin LAF)	0
II (around of LAF)	7

The Result showed that LAF that was used as study room meet the requirements as sterile class (Class I), that was <1 cfu/m^3 (Budiman, 2014).

Five bottles of multipurpose contact lens solutions (MPS), commonly marketed in Indonesia and categorized based on active substances.

All products were previously unopened and used for the experiment prior to the expiry dates indicated in their packaging[1].

Samplings of MPS were tested by non-probability sampling method.

Non-probability sampling method is a technique that does not provide equal opportunities or opportunities for each element or selected members of the population to be sampled (Eleonore, 2013).

Bacterial suspension obtained was determined 1.0×10^6 *colony-forming units* per milliliter (cfu/mL) determined using 0,5 *McFarland standard which* containing NaCl 0.9% (Robertson, 2007; Cano-Parra, 1999). The microorganisms were selected based on its similarity to that recommended by ISO for contact lens MPS stand alone criteria (ISO/CD 14 729).

Pure isolate test organisms were obtained in Laboratory of Microbiology, Faculty of Pharmacy, University of Padjadjaran.

Multipurpose solution to be effective if it is able to kill bacteria by ≥3 log reduction in the number of bacterial colonies such as on the recommendation the minimum disinfection time listed on the packaging.

Time recommendations on the label and packaging MPS obtained the following data:

Table 3: Content of Active Substances and Recommended Minimum Time of Multipurpose Disinfecting Solution

Multipurpose Solution (MPS)	Active Substances	Contact Time (Hours)
A	Polyaminopropyl biguanid 0.0001%	6
B	Polyhexamethylene biguanid	6
C	Polyhexanide	6
D	Polyhexamethylene biguanid	6
E	Polyaminopropyl biguanid 0.0001%	6

Results of tests carried out by counting the colony using method Total Plate can be seen in the following figure.
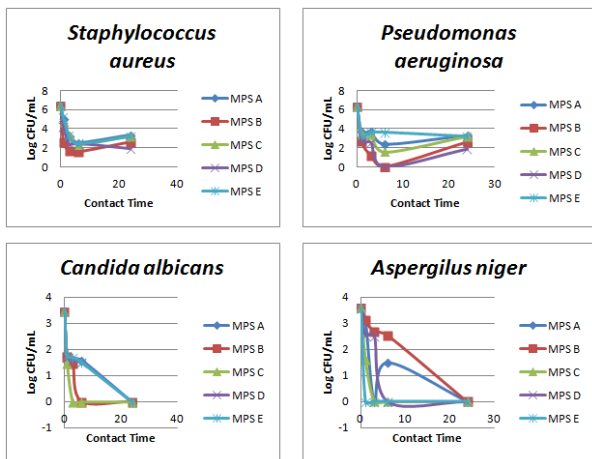

Figure 1: The contact time of MPS against microbial

The figures show that the effect of five MPS showedthat MPS have potential as antimicrobial which all MPS can kill microbial for 24 hours. MPS B has better antibacterial activity against *Staphylococcus aureus* and *Psoudomonas aeruginosa*. This was indicated by the contact time was much smaller than other MPS. Contact time of MPS B was 6 hours.

Whereas for fungi, MPS was most effective against *Candida albicans* and *Aspergillus niger* was MPS C. It can be seen from the time the contact was smaller than the other MPS. The contact time MPS C against fungi was 3 hours.

The effect of each treatment on the number of microbial can be seen in the following Figure.
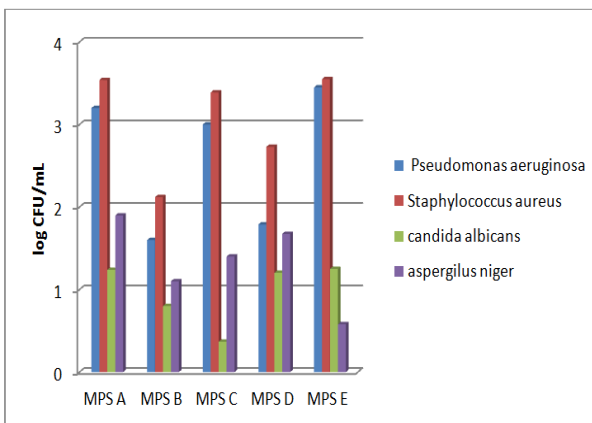

Figure 2: Average Number of Colonies of microbial

Based on the figure, MPS B was the least bacterial concetration. Whereas for fungi, the most inhibiting *Aspergillus niger* was MPS Eand for *Candida albicans* was MPS C. In Anova calculation, The Result showed that MPS B and MPS D more effective as antibacterial than other MPS. The MPS B and MPS D have the same active substances. Because of $F = 6.25 > F_\alpha = 3.259$, then Ho was rejected, that means there were differences among five MPS in term their effect on the concentration of

Pseudomonas aeruginosa. For *Staphylococcus aureus*, the result showed that MPS B and MPS D more effective as antibacterial than other MPS. The MPS B and MPS D have the same active substances. $F_{calc} = 20.39 > F_\alpha = 3.259$, H_0 was rejected, that means there were differences among five MPS in term their effect on the concentration of *Staphylococcus aureus*. It was occurred in fungi.

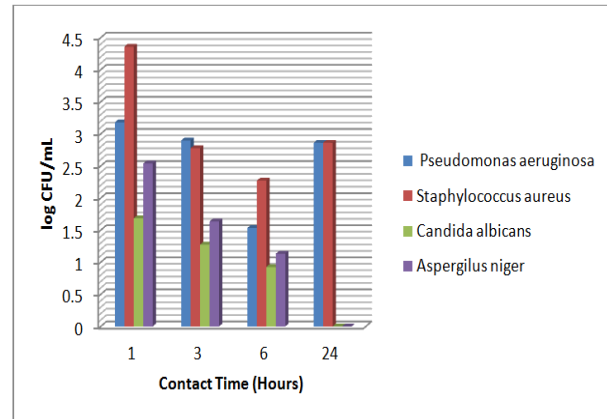The effective contact time for each MPS can be seen the folowing figure.

Figure 3: Average number of bacteria colonies of microbial

The figure showed that average of contact time for all MPS was 6 hours. The effective contact time for each MPS were 6 hours which contain in the label of MPS Solution. $F_{Calc} = 6.67 > F_\alpha = 3.49$, $H_0$was rejected, that means varying duration of exposure showed significant effects on the concentration of P. aeroginosa. For *Staphylococcus aureus*, the result showed that the effective contact time for each MPS were 6 hours which contain in the label of MPS Solution. $F_{hitung} = 7.85 > F_\alpha = 3.49$, $H_0$was rejected, that means varying duration of exposure showed significant effects on the concentration of *Staphylococcus aureus*. It was occurred in fungi.

DISCUSSION

Contact lens use has been identified in numerous studies to be a risk for ocular infection. It is known that pathogenic microorganisms can attack the eye from the contact lens to the eye, especially when contact lens unclean. Therefore, efficient disinfection of the lens is essential (Eleonore, 2013).

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 (*Pseudomonas aeruginosa*, and *Staphylococcus aureus*) by 3 log and of fungi (*Aspergillus niger* and *Candida albicans*) by 1 log at the minimum disinfection time recommended by the manufacturers (Eleonore, 2013).

The result showed that the effect of five MPS showed a significant effect all microbial. The Active Substances of MPS were Polyhexamide, polyaminopropyl biguanide and polyhexamethylene biguanide. Polyhexanide contain highly active side-charge who has the ability to damage cell membranes microbial with electrostatic interactions that are effective against a wide-range of bacteria (Eleonore, 2013). Polyaminopropyl biguanide works by interfering membranes and reducing permeability, which has a lethal effect to bacteria and then bind to bacterial DNA, alter transcription, and cause lethal DNA damage. Polyhexamethylene biguanide initially active agent interacts with the bacterial surface and then transferred to the cytoplasm and the cytoplasmic membrane.

Effects on bacteria mainly Gram-negative bacterium where the membrane acid induced that makes the increase in the fluidity and permeability then causes the release of lipopolysaccharide (Yasuda, 2003).

Tukey HSD test between the five different MPS showed that there were significant differences among the test solutions in terms of their antimicrobial effects. MPS B and D showed the greatest decrease in the concentration of the challenge organisms, followed by C and A. MPS E showed the least antimicrobial effects.

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. Results showed that the challenge organisms should be exposed to the MPS for at least 6 hours to achieve the maximal antimicrobial effect.

CONCLUSION

The results of this study indicate that all of the MPS have activity as antimicrobial after exposure for 24 hours.

MPS B which contained Polyhexamethylene biguanid showed the greatest decrease in the concentration of the challenge organisms with contact time was 6 hours.

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