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MULTIPURPOSE CONTACT LENS SOLUTIONS: FIGHTING PSEUDOMONAS AERUGINOSA INFECTIONS

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Abstract
Contact lens wear is a popular method to improve vision, with millions of users worldwide. While contact lenses are generally safe, complications can occur, and one of the most serious is microbial keratitis. This condition can lead to permanent vision loss if not properly managed. In this study, we aim to investigate the factors contributing to microbial keratitis in contact lens users, with a focus on understanding its incidence and risk factors. By gaining a better understanding of this sight-threatening condition, we can work towards improving the safety of contact lens use and preventing complications. Our findings will be valuable for both contact lens wearers and eye care professionals, enhancing awareness and guiding preventive measures.

Keywords: contact lens, microbial keratitis, vision loss, complications, risk factors

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 devasta 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-

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related microbial keratitis is commonly caused by bacteria such as clinical isolates Staphylococcus aure us and Pseudomonas aeruginosa (Mohammadinia, 2012).
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 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 clinical isolates Pseudomonas aeruginosa and Staphylococcus aureus, 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

Kode	MPS	Perusahaan	Zat aktif
A	X2	Stericon Pharma	Polyhexanide
B	O2	Omega	Polyhexamethylene biguanid
C	Re-nu	Bausch & Lomb	Polyaminopropil biguanid
D	OPTI-FREE	Alcon	Polyquaternium-1 dan Myristamidopropyl dimethylamine
E	Revitalens	Ocutea	alexidine dihydrochloride dan polyquaternium-1

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 hours at a temperature of 37 °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 clinical isolates of Pseudomonas sp. and Staphylococcus sp., (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.

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The resulting MPS + microorganism solution was termed "bio-test solution 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) then sub-cultured on an agar medium recovery (Triptone-Soya-broth). Recovery Plate was incubated for 24 hours at a temperature of 35-37o C. Colonies of microorganism was 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/m3)
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/m3 (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 nonprobability 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 x 10.8 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, and 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/MPS X2	Polyhexanide	6
B/O2	Polyhexamethylene biguanid 0, 0001%	6
C/Re-nu	Polyaminopropil biguanid	6
D/Optifree	Polyquaternium 1 dan Myristamidopropyl Dimethylamin	6
E/Revit alens	Alexidine dihydrochlorie dan polyquaternium-I	6

The Table show that the effect of five MPS showed that MPS have potential as antimicrobial which all MPS can kill microbial for 6 hours. Based on the ISO, the solution is said to be an effective multi-purpose solution when it is able to kill bacteria with ≥3 log reduction in the number of bacterial colonies.

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This means that 3 is the result log from 1000, so it is said to be effective when MPS solution capable of killing bacteria more than 1000. Because the log of the number of bacteria clinical isolates of Pseudomonas aeruginosa and Staphylococcus aureu of 7.5 log CFU / mL and 8.2 log CFU / mL, eat MPS solution Bilam said effective value of log CFU / mL (the amount of bacterial growth) of less than 4.5 log CFU / mL for the bacteria Pseudomonas aeruginosa clinical isolates and 5.2 log CFU / mL for clinical isolates of Staphylococcus aureus. From the results of calculations using the method of total plate count, it is known that all MPS solution can kill ≥3 log on contact time 3 and 6 hours. Shown in Table 4.1 and 4.2.

Table 4.1 Results Log Number of Colonies of Bacteria Log Results of Clinical Isolates of Pseudom onas aerug inosa

	0	1	3	6	24
Multipurpose Solution	Log Number of Colonies of BacteriaClinical Isolates Pseudomonas aeruginosai to Contact Ttime (Log CFU/mL)				
MPS A	7.5	5.9	4.2	4.5	4.8
MPS B	7.5	4.5	3.5	3.4	3.7
MPS C	7.5	5.8	4.4	4.3	4.7
MPS D	7.5	4.7	3.2	2.7	3.2
MPS E	7.5	4.2	3.2	2.6	3.2

Table 4.2 Results Log Number of Colonies of bacteria Staphylococcus aureus

Clinical Isolates against Time Contacts					
Log Number of Colonies of Bacteria Clinical Isolates					
MPS (Multipurpose Solution)	Staphylococcus aureus to Contact Time (Log CFU/mL)				
	0	1	3	6	24
MPS A	8.2	5.8	3.3	3.4	5.2
MPS B	8.2	4.7	2.4	2.3	2.5
MPS C	8.2	5.9	3.4	3.3	4.8
MPS D	8.2	4.8	2.4	2.1	2.1
MPS E	8.2	4.3	0	0	0

Statistical Analysis Results

The observation that consists of 5 treatments (MPS A, MPS B, MPS C, MPS D, and MPS E) and also against four groups of contact time (1, 3, 6, and 24 hours) obtained the effect of each treatment and time to the number of clinical isolates of Pseudomonas aeruginosa bacteria, the effect of each treatment and time to the number of clinical isolates of Staphylococcus aureus bacteria, analysis of variance, and Tukey test

Statistical Analysis Results MPS Treatment Effect on Bacteria Clinical Isolates of Pseudomonas aerug inosa

The effect of each treatment of the number of bacterial colonies clinical isolates of Pseudomonas aeruginosa can be seen in Figure 1:

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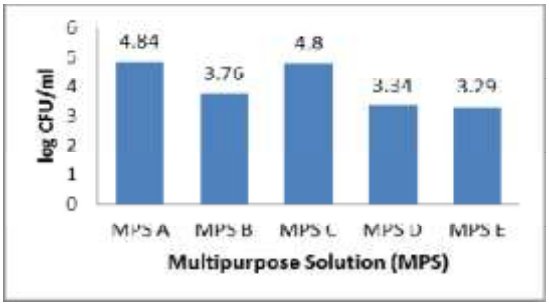

Figure 1: Average number of colonies against clinical isolates of Pseudom onas aerug inosa Treatment Solution MPS

Based on Figure 1. of the five MPS solution is used, it is known that the MPS solution which has an average value of log cfu / mL was highest in the colonies of bacteria Pseudomonas aeruginosa clinical isolates is MPS brand A solution of 4.84 log cfu / mL, while the most low is the solution MPS brand E of 3.29 log cfu / mL. This means that contact lens multipurpose solution that is most effective in killing the bacteria Pseudomonas aeruginosa clinical isolates are MPS solution E. This is because MPS solution E contains two active substances, namely alexidine dihydrochloride and polyquaternium-1

To see whether or not there is a different between the effects of each treatment, then performed a statistical test Analysis of Variance (ANOVA). Based on the results of statistical calculations ANOVA test using SPSS 20 software to determine the effect of solution MPS different samples to the concentration of Pseudomonas aeruginosa. So it can be concluded that with a 95% confidence level ($\alpha = 0.05$) obtained value of $F = 5.374$, because of $F = 5.374 > F\alpha = 3.06$ then H0 is rejected, it means there difference of 5 treatments of antimicrobial activity of contact lens multipurpose solution (Multipurpose Solution) is available in a shopping center in Bandung to the concentration of the bacteria Pseudomonas aeruginosa clinical isolates.

To know the difference between 5 MPS solution is then conducted further tests using Tukey test, so it can be concluded that with a 95% confidence level ($\alpha = 0.05$) there are significant differences between the MPS solution A and D MPS, MPS A and MPS E, C and MPS D MPS, MPS and the MPS C and E.

Statistical Analysis Results Effect (time) against Clinical Isolates of Pseudomonas aeruginosa bacteria

The average number of bacterial colonies clinical isolates of Pseudomonas aeruginosa in each treatment can be seen in Figure 2.

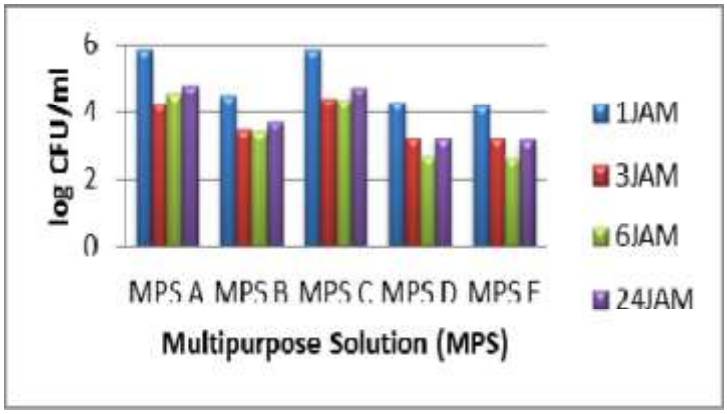

Figure. 2 Effect of sample solution MPS different to the number of bacterial colonies with the increase in contact time on clinical isolates of bacteria Pseudomonas aureginosa

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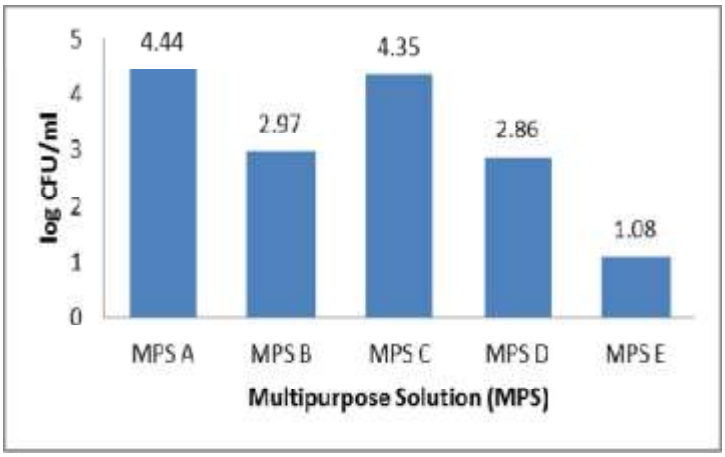
Based on Figure. 2 shows that the five MPS solution with a wide range of contact time showed a significant effect on the bacteria Pseudomonas aeruginosa clinical isolates.
Where the value of log cfu / mL each MPS lowest tend to look at the contact time of 6 hours except on MPS A, so that it can be concluded that the most effective MPS solution in the 6th hour. Based on the chart above the contact time of 24 hours the number of bacterial colonies increased clinical isolates of Pseudomonas aeruginosa ,.because if MPS has interacted with the outside air, the ability of active substances to disinfect contact lenses are limited by time resulting in decreased effectiveness or suffer recontamination.
Statistical Analysis Results Effect (time) against Clinical Isolates of
Staphylococcus aureus bacteria the effect of each treatment on the number of Staphylococcus aureus clinical isolates can be seen in the following Figure. The average number of bacterial colonies clinical isolates of Staphylococcus aureus each treatment can be seen in Figure 3.



Based on Figure 3 of the five MPS solution is used, it is known that the MPS solution which has an average value of log cfu / mL was highest in the colonies of bacteria Staphylococcus aureus clinical isolates are MPS A solution that is equal to 4.44 and the average of the lowest namely MPS E is 1.08. This means that contact lens multipurpose solution that is most effective in killing the bacteria Staphylococcus aureus is the solution MPS E compared to other MPS solution.
To see whether or not there is a difference between the effects of each treatment, then performed a statistical test Analysis of Variance (ANOVA).
Based on the results of statistical calculations ANOVA test can be concluded that with a 95% confidence level (α = 0.05) obtained value of F = 3.481, because of F = 3.481 <Fα = 3.06 then H0 is rejected, it means there perberdaan of 5 treatments antimicrobial activity of the solution multipurpose contact lens (multipurpose solution) is available in a shopping center in Bandung to the concentration of
Staphylococcus aureus.
To find out where the difference lies between 5 MPS is then conducted further tests using Tukey test. Based on the results of statistical calculations Tukey test can be concluded that with a 95% confidence level (with α = 5%), there is a difference between MPS A and MPS E as well as MPS C and MPSE.

Statistical Analysis Results Effect (time) against Clinical Isolates of
Staphylococcus aureus bacteria

The average number of bacterial colonies clinical isolates of Staphylococcus aureus in each treatment can be seen in Figure 4:

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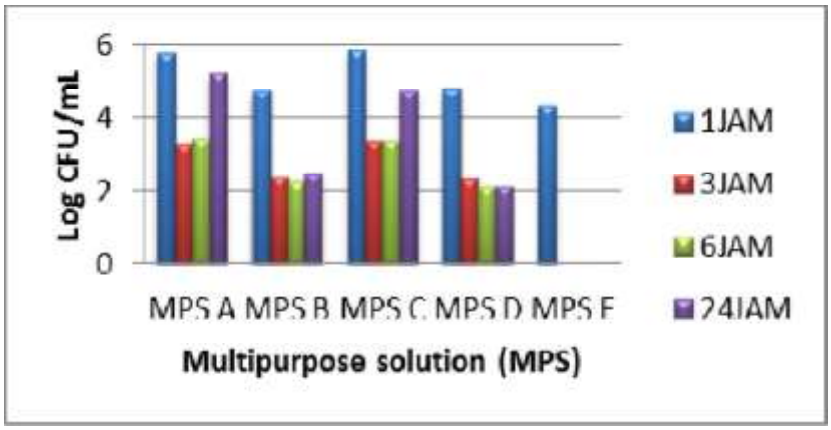

Figure 4: Effect of sample solution MPS different to the number of bacterial colonies with the increase in contact time on clinical isolates of Staphylococcus aureus From Figure 4: shows that the effect of the five MPS from various contact time showed a significant effect on Staphylococcus aureus. Therefore we can conclude that the most effective antimicrobial solution in the 6th hour.

Based on the chart above the contact time of 24 hours increased the number of colonies of Staphylococcus aureus, this is because when the MPS has interacted with the outside air, the ability of active substances to disinfect contact lenses are limited by time resulting in decreased effectiveness or suffer recontamination

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 affectivity of locally available contact lens disinfecting solutions against common contact-lensrelated ocular pathogens. According to this standard for standalone primary acceptance criteria, disinfecting solution must be able to reduce the starting concentration of bacteria (Pseudomonas aeruginosa, and Staphylococcus aureus) by 3 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 sidecharge 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 lip polysaccharide (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.

Turkey 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.

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Conclusion and Suggestion:

Conclusion:

Based on these results it can be concluded that all of the multipurpose solution (Multipurpose Solution / MPS) on the market are effective against the bacteria of clinical isolates of Pseudomonas aeruginosa and Staphylococcus aureus. The contact time is most effective for 6 hours in accordance with a recommendation of the International Organization for Standardization (ISO)

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

Suggestion:

From the results of this study advised to test the antimicrobial effectiveness of the multipurpose solution (Multipurpose Solution) contact lenses by variations in temperature or humidity against clinical isolates Pseudomonas aeruginosa and Staphylococcus aureus or pathogenic microorganisms cause keratitis

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