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Comparison of Total Bacterial Count in Contact Lenses with Different Treatments of Contact Lens Solutions

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Abstract
The use of contact lenses continues to increase, raising attention to aspects of eye health because of their hygienic factors. The hygiene of contact lenses comes not only from the way they are treated but also from the soaking fluid. This study aimed to determine the total bacterial count on contact lenses with and without the use of immersion solutions. The research method used is a comparative descriptive research method that compares the two pairs of contact lenses with two different contact lens solutions. The total bacterial count was calculated using the total plate count (TPC) method. The number of bacterial colonies for contact lenses before being immersed in liquid A was found in dilution 10-2 with an amount of 4.4×103 CFU/mL, and after soaking, it was in dilution 10-1 with an amount of 8.5×102 CFU/mL. The number of bacterial colonies on contact lenses before being immersed in B liquid was found in 10-1 dilution with an amount of 1.3×103 CFU/mL, and after being soaked, the average colony was 3.9×104 CFU/mL. From these results, it can be seen that in contact lenses and liquid A, there is a decrease in the number of bacterial colonies, while on the other hand, in liquid B, there is an increase in the number of bacterial colonies. The causal factor is thought to originate from the composition of the solutions. However, do not rule out contamination from bottles and contact lens care. Therefore, this research can be used as a basis for hygiene education in contact lenses.

Keywords: Bacteria, Contact Lens, Solutions, Total Plate Count.

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1. INTRODUCTION

Contact lenses are visual aids that are used with or instead of glasses. Contact lenses are visual aids that are placed on the surface of the cornea so they are efficient and comfortable to use in daily activities (Ibrahim et al., 2021). Aside from being a vision aid for people with refractive errors, contact lenses also have cosmetic and therapeutic functions (Centers for Disease Control and Prevention, 2022). These functions have made contact lenses continue to gain popularity.

Worldwide, there are approximately 125 million users of contact lenses which are used to correct common visual impairments such as myopia, hypermetropia, presbyopia, and astigmatism (ResearchAndMarkets.com, 2023; Waghmare & Jeria, 2022). Not only worldwide, the popularity of contact lenses in Indonesia continues to increase. This can be seen from the increasing demand for contact lenses, especially daily disposable lenses and cosmetic lenses (Statista Market Insight, 2022). Based on research conducted by Statista (Statista Market Insight, 2022), it was found that contact lenses are preferred by young people because of their ease of use. In addition, the weather conditions in Indonesia which tend to be humid make it comfortable to use contact lenses.

Some of the factors that are the focus of attention on eye health in the use of contact lenses are continuous use of contact lenses, unhygienic contact lenses, overnight use, and complications caused by contact lens fluids (Waghmare & Jeria, 2022). The use of contact lenses that are not good, and hygiene can cause bacterial infections in the eye. Bacterial colonies on contact lenses can cause inflammation and other complaints such as red, sore, itchy, and watery eyes (Szczotka-Flynn et al., 2010).

The gap in this study arises from the lack of comprehensive comparative data on the effectiveness of different contact lens solutions in inhibiting bacterial growth. Based on research conducted by Iguban and Nanagas (Iguban & Nanagas, 2016) found contamination of contact lens solutions by pathogenic bacteria. 9% of samples taken from contact lenses, 34% of samples from contact lens containers, and 11% of samples from contact lens solutions, were contaminated with pathogenic microorganisms such as Serratia sp, Staphylococcus aureus and coagulase-negative Staphylococci. In addition, Indrayati and Amelia (Indrayati & Amelia, 2019) found that contact lens solutions with disinfectant concentrations of 0%, 0.0001%, 0%, and 20% had no inhibitory power in inhibiting the growth of Staphylococcus aureus bacteria.

Referring to these problems, it can be seen that improper contact lens hygiene can lead to bacterial contamination and eye infections. It also revealed that not all contact lens solutions are equally effective in preventing this contamination. However, there is limited research comparing the total bacterial count across various contact lens solutions, which is crucial for educating the public on proper lens care and choosing the most effective solutions. This study aimed to determine the total bacterial count on contact lenses with and without the use of immersion solutions.

2. RESEARCH METHOD

The research method that will be used in this study is a comparative research method that aims to explain the relationship between different variables through comparison and hypothesis testing (Rizki et al., 2022). This study aims to see the comparison of contact lens swabs between two soaking solutions. The research samples taken were two pairs of used contact lenses. The contact lens solutions were chosen based on the popularity of the contact lens solution chosen by the contact lens users. From the previous observation and interview with the optician, there are two contact lens solutions that are popular among the users. Therefore, those types of contact lens solutions were used in this research. The independent variables in this study are contact lenses and solutions, while the dependent variable is the number of bacterial colonies.

The instruments used in this study were: test tubes, sterile petri dishes, watch glass, spatula, Erlenmeyer, stirring rod, cotton, gauze, paying paper, mattress thread, autoclave, dry

sterilizer, laminar air flow, test tube rack, technical balance, 1000 micron micropipette, blue tip, and spirit lamp. The media and reagents used were physiological NaCl (0.85%), distilled water, and plate count agar (PCA) media.

Calculation of the total value of bacteria using the Total Plate Count (TPC) method. The TPC test was carried out aseptically to prevent unwanted contamination and was done in duplo to increase the accuracy of the results obtained (Tapotubun et al., 2024). The total plate count was calculated in 1 ml of a sample by multiplying the average number of colonies in the dish with the dilution factor used (Jamilatun & Lukito, 2024). The number of bacteria is expressed as Colony Farming Units (CFU) (Rizki et al., 2022) with the equation:

$$\text{TPC} = \text{Number of coloni} \times \frac{1}{\text{dilution factor}} \quad (1)$$

Where the number of colonies counted is the number of colonies observed on the agar plate; the Dilution factor is the factor by which the sample was diluted before plating (e.g., for a 1:100 dilution, the dilution factor is 100). Due to multiple dilutions and plating for each sample, the average CFU was calculated to minimize variation. To evaluate the results, the CFU/mL between different treatments or groups was compared to analyze trends in microbial growth.

3. RESULTS AND DISCUSSION

The samples in this experiment included both used and new contact lenses. The contact lenses were swabbed with a sterile cotton swab, and the process was repeated three times to ensure adequate sample collection. Each swab was then introduced into a series of test tubes labeled 1, 2, and 3, corresponding to serial dilutions ranging from 10^{-1}, 10^{-2}, to 10^{-6}.

For each test tube, 9 ml of sterile physiological NaCl was prepared, and 1 ml of sample from the transport media tube was added to tube number 1 to create a 10^{-1} dilution. Subsequent dilutions were made by transferring 1 ml from one tube to the next, with a code of 10^{-2} dilution. The test tube was shaken so that the solution was homogeneous and a 10^{-2} dilution was obtained. From a test tube with a dilution of 10^{-2}, it was taken with a 1 ml pipette and put into a tube that was given the code of dilution 10^{-3}. Shake the test tube again so that the solution is homogeneous. The same thing was done up to the 10^{-6} dilution. This procedure allowed for a progressive dilution of the sample, reducing the bacterial concentration for easier enumeration

Following the dilution process, 1 ml of solution from each dilution level was transferred using a sterile pipette into pre-labeled sterile petri dishes. The petri dishes were then incubated at 37°C for 24-48 hours to allow bacterial colonies to develop. This method facilitated a comparative analysis of germ counts from swabs taken from contact lenses with and without immersion liquid, providing insight into potential differences in contamination levels. The germ counts were carefully recorded in a table, offering a clear view of bacterial growth across different dilutions. Through this process, the study aimed to evaluate the efficacy of contact lens immersion liquids in reducing microbial contamination on contact lenses. Calculation of the number of germs from contact lens swabs with and without immersion liquid can be seen in the table 1 below.

Table 1. Average TVC Number of Contact Lenses with and Without Solutions

Sample	Type	Dilutions	Colony number	Average TPC (CFU/mL)
1	CL without soaked solutions	10^{-2}	44	$4.4x10^{3}$
	CL with soaked A solutions	10^{-1}	85	$8.5x10^{2}$

Sample	Type	Dilutions	Colony number	Average TPC (CFU/mL)
2	CL without soaked solutions	10^{-1}	130	$1.3x10^3$
	CL with soaked A solutions	10^{-2} 10^{-3}	170 60	$3.9x10^4$

Dilution affects the number of bacterial colonies. Based on Table 1 above, it is known that most contact lens bacterial colonies are found in just one dilution. However, in contact lenses with B solutions, it was found that two groups of colonies were found at different dilutions even though the number was getting smaller. Dilution is done to reduce the density of bacteria with the consideration that to grow bacterial colonies on limited media it is not possible to count tens of thousands of bacteria (Laili et al., 2022).

Based on the table above, it can be seen that the highest number of bacterial colonies before immersion in contact lens A was found in the 10^{-2} dilution with an amount of 4.4×10^3 CFU/mL and after being soaked it was in the 10^{-1} dilution with the amount 8.5×10^2 CFU/mL. The highest number of bacterial colonies on contact lenses before soaking in B solutions was found in 10^{-1} dilution with an amount of 1.3×10^3 CFU/mL and after being soaked the average colony was 3.9×10^4 CFU/mL. From these results, it can be seen that in contact lenses and immersion liquid A, there is a decrease in the number of bacterial colonies, while in contrast, in immersion liquid B, there is an increase in the number of bacterial colonies.

Based on the interview results, contact lens users stated that the two liquids were obtained in new condition and had a long expiration date. It is possible that the presence of bacteria is due to the ineffectiveness of the solutions. The composition of the solutions will affect its effectiveness in inhibiting bacteria. Liquid with brand A has a buffered, isotonic, and sterile liquid composition with Sodium Hyaluronate, Poloxamer, EDTA, and Polyhexanide 0.0001%. Brand A liquid specifically states that their liquid can clean, moisturize, kill germs, clean protein impurities, and maintain the quality of contact lenses during storage. Whereas solutions with brand B have a composition of Sodium Chloride, Potassium Chloride, Disodium Edetate, Polyhexanide, Poloxamer, Hypromellose, and Sodium Phosphate Buffer. Liquid B claims to clean and moisturize.

The active ingredients for removing bacteria are usually contact lens solutions with compositions such as Polyhexamethylene biguanides (PHMB), quaternary ammonium compounds, hydrogen peroxide, alcohol, sorbic acid, and thimerosal (Eryılmaz, et al., 2018). From the composition of the two solutions, it can be seen that only brand A has a disinfectant/bacterial removal composition, while brand B does not. This explains why brand A has a decrease in the number of bacterial colonies, while brand B actually has an increase in the number of bacterial colonies.

In eyes, without contact lenses, the ocular surface would be considered sterile or have a normal microorganism biota (Willcox et al., 2002). The presence of microorganisms in the eye is important because they will produce antimicrobials that play a role in the defense of the ocular surface from infection. Contamination can come from existing bacteria from the contact lens itself and also transfer from the ocular surface of the eye (Szczotka-Flynn et al., 2010). The surface of the eye is very susceptible to bacterial contamination. Bacterial contamination in the eye will be cleaned by the eye itself as a form of eye defense mechanism. Although in very small amounts, namely under 5 to 10 CFU/lens (Szczotka-Flynn et al., 2010).

The presence of contact lenses on the ocular surface can increase bacterial contamination. This is due to protein deposits produced from contact lenses (Barr et al., 1988) whose amount depends on the contact lens material itself (Szczotka-Flynn et al., 2010). In addition, the oxygen level in contact lenses will also affect the number of bacteria. Increased oxygen levels in contact lenses can reduce bacterial bonding with the ocular surface (Waghmare & Jeria, 2022).

Based on the results of the interviews, it is known that contact lens care will have a significant effect on contact lens hygiene. The number of bacterial colony numbers found to be different in each contact lens can be caused by several factors. Holding contact lenses is the main factor causing bacterial contamination (Szczotka-Flynn et al., 2010). Other factors can also play a role, such as the lens case and immersion solutions.

Contamination can come from existing bacteria from the contact lens itself and also transfer from the ocular surface of the eye (Szczotka-Flynn et al., 2010). The surface of the eye is very susceptible to bacterial contamination. Bacterial contamination in the lens case was detectable even in the presence of liquid immersion, which consisted of a mixture of bacterial, fungal, and protozoan contaminants (Clark et al., 1994). The main cause of contamination even though there is immersion fluid is biofilm (Szczotka-Flynn et al., 2010). Bacterial contamination usually begins with the initial attachment of some bacteria to a surface followed by implantation, but later, develops to form a biofilm on the contact lens surface. This biofilm shows resistance to antibiotics in washing solutions and the immune system (Indrayati & Amelia, 2019).

Soaking solutions are used to clean, lubricate, and disinfect contact lenses (Waghmare & Jeria, 2022). Therefore soaking solutions usually consist of buffers, surfactants, preservatives, lubricants, and antimicrobial agents. Contact lens solutions use polymers as disinfectants, but the function of other contact lens cleaning solutions that is preferred is as a sterile storage medium as long as contact lenses are not used (Indrayati & Amelia, 2019).

The minimum amount of bacteria in contact lens fluid has been established by ISO standards. Contact lens fluid manufacturing processes are defined in ISO 14729 and ISO 18259 (McAnally et al., 2021). Based on ISO 14759, the minimum number of inoculum bacteria present is $1x10^5 – 1x10^6$. However, soaking fluids have varying effectiveness in dealing with the transfer of bacterial contamination. Research conducted by Indrayati (Indrayati & Amelia, 2019) found that contact lens cleaning fluids with disinfectant concentrations of 0%, 0.0001%, 0%, and 20% had no inhibitory power in inhibiting the growth of Staphylococcus aureus bacteria.

Soaking solutions packaged in bottles can easily become contaminated, and become a source of microbes that contaminate the lens case, stick to contact lenses, and cause inflammatory reactions and corneal infections (Szczotka-Flynn et al., 2010). All types of contact lens solutions, including those with hydrogen peroxide composition, are contaminated even if the bottle has not been opened (Szczotka-Flynn et al., 2010). Durban et al., (1996) found that Acanthamoeba organisms were almost always found in lens cases or lens solutions. Achantamoeba is a cause of keratitis (Waghmare & Jeria, 2022). Several studies have also found that contamination from solutions can occur due to the behavior of transferring lens fluid to the box through the bottle cap (Szczotka-Flynn et al., 2010). The length of time since the bottle was opened also affects bacterial contamination. Donzis (Donzis et al., 1987) found that bacteria can be acquired immediately after 5 days from opening the bottle cap, and the risk of contamination continues to increase the longer the bottle is opened.

Bacterial contamination found on contact lenses can come from the contact lenses themselves or from external factors such as contact lens cases and solutions. Contact lens wearers should pay great consideration to cleaning and disinfection practices to decrease bacterial growth, reduce chances of biofilm formation (Hadeel T AL-Hadithi & Zahira M AL-Khani, 2023). Most of the bacterial isolates obtained from contact lens wearers had the potential to produce biofilms (Raksha et al., 2020). This bacterial contamination will be a concern because it can cause infections such as keratitis (Szczotka-Flynn et al., 2010). One solution that can be done from contact lens manufacturing is contact lens formulation and contact lens cases with anti-microbial coatings such as silver polyquats, polymeric pyridium compounds, free-radical producing agents, quorum-sensing blockers, and anti-infective agents (Weisbarth et al., 2007). Preventive measures can be taken by increasing awareness about the importance of

contact lens hygiene so that contact lens users can adopt healthy habits such as cleaning contact lenses using a special liquid (not with water); periodically dispose of the liquid in the contact lens case; cleaning and changing contact lens cases every three months (Waghmare & Jeria, 2022). These actions can reduce the risk of bacterial infections in contact lenses such as keratitis.

4. CONCLUSION

From the results of the research on examining the number of bacterial colonies using the TPC method, it can be concluded that there was a decrease in the number of bacteria in the results of examining contact lenses with immersion liquid A, amounting to 4.4 x 103 CFU/mL sample to 8.5 x 102 CFU/mL sample. Meanwhile, when examining contact lenses and immersion liquid B, the number of germs increased, namely from 1.3 x 103 CFU/mL sample to 3.9 x 104 CFU/mL sample. Future research could focus on evaluating the effectiveness of various disinfectant concentrations in contact lens solutions to determine optimal bacterial inhibition, while also investigating the mechanisms of biofilm formation and resistance on lenses and in storage cases. Long-term contamination studies could explore how bacterial levels change over time after solution bottles are opened. Additionally, research into the impact of user behavior, such as cleaning and handling practices, could provide insights into reducing contamination. Further studies might examine the use of antimicrobial coatings on lenses and cases, as well as the development of new antimicrobial agents to enhance solution efficacy.

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