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Effectiveness of Various Antiseptic Hand Soaps in Reducing Skin Bacterial Counts

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ABSTRACT

Hands are the body part most frequently exposed to environmental bacteria and play an important role in disease transmission. Handwashing with antiseptic soap is an effective method for reducing bacterial contamination on the skin. Antiseptic soaps containing chloroxylenol, povidone-iodine, and chlorhexidine gluconate are widely used due to their broad-spectrum antimicrobial activity. However, the comparative effectiveness of these three antiseptic agents remains unclear. This experimental study employed a group pre-test and post-test design involving 27 students from the Faculty of Dentistry, Trisakti University. The respondents were divided into three groups based on the type of antiseptic soap used: 0.5% chloroxylenol, 7.5% povidone-iodine, and 0.5% chlorhexidine gluconate, with nine respondents in each group. The number of bacterial colonies on the hands was assessed before and after handwashing using the swab method. Data were analyzed using the Wilcoxon signed-rank test and the Kruskal–Wallis test. All three antiseptic soaps demonstrated a significant reduction in bacterial colony counts. In the chloroxylenol group, the median colony count decreased from 100 CFU to 22 CFU ($p = 0.018$); in the povidone-iodine group, from 11 CFU to 0 CFU ($p = 0.008$); and in the chlorhexidine gluconate group, from 200 CFU to 61 CFU ($p = 0.008$). Based on the percentage reduction, povidone-iodine showed the greatest decrease (99.74%), followed by chloroxylenol (79.30%) and chlorhexidine gluconate (51.97%). However, comparisons of bacterial colony reduction among the three groups showed no statistically significant difference in effectiveness ($p = 0.481$).

Keyword: Antiseptic soap; chlorhexidine gluconate; chloroxylenol; bacterial colony; povidone iodine.

INTRODUCTION

The skin is the largest organ of the human body and serves as the primary barrier against various environmental factors. In addition to functioning as a physical protective barrier, the skin also provides a habitat for diverse microorganisms that constitute the skin microbiome. The microbiome consists of resident flora that colonize the skin surface and transient flora acquired from the surrounding environment, both of which contribute to maintaining the balance of the body's defense system (Ma et al., 2024). Hands are among the body parts most frequently exposed to environmental contaminants and therefore have a high risk of microbial contamination. Daily activities, such as touching objects, shaking hands, using public facilities, and contacting contaminated surfaces, can facilitate bacterial transfer to the hands and contribute to the chain of disease transmission. Therefore, maintaining hand hygiene is one of the most effective measures for preventing the spread of microorganisms (Fitriyani & Murlistyarini, 2022; Sari et al., 2023; Kartika et al., 2017; Meena et al., 2019; Costa, 2024).

The World Health Organization (WHO) recognizes hand hygiene as a key component of infection prevention and control. In addition to removing visible dirt, handwashing helps reduce the number of microorganisms attached to the skin, thereby lowering the risk of disease transmission. The effectiveness of handwashing is influenced by several factors, including handwashing technique, duration, type of soap used, and individual skin conditions (Ratmaja et al., 2023; Zulfa & Patricia, 2023; Kim et al., 2015).

Soap is a cleansing product containing surfactants that facilitate the emulsification of oils and removal of contaminants from the skin surface. In addition to conventional soaps, various antiseptic soaps containing

antimicrobial active ingredients have been developed to enhance hand-cleaning effectiveness. These products not only remove contaminants mechanically but also inhibit or eliminate microorganisms present on the skin surface. Common antiseptic agents used in hand soaps include chloroxylenol, povidone-iodine, and chlorhexidine gluconate (Alum, 2024; Jalaluddin et al., 2023; Abbas et al., 2016; Ayuningtyas et al., 2021; Nuswantoro et al., 2020).

Chloroxylenol is a phenolic compound with antimicrobial activity against various Gram-positive and Gram-negative bacteria. Its mechanism of action involves protein denaturation and disruption of bacterial cell membrane integrity, leading to bacterial cell death (Kristy et al., 2021). Povidone-iodine is an iodine complex widely used as an antiseptic due to its broad-spectrum antimicrobial activity. It functions by releasing free iodine, which oxidizes proteins, enzymes, and essential structural components of microbial cells. Damage to these components disrupts cellular metabolism and ultimately results in microbial death (Eggers et al., 2018; Riza et al., 2019). Chlorhexidine gluconate is an antiseptic with broad antimicrobial activity and a relatively prolonged residual effect compared with several other antiseptic agents. Its mechanism involves electrostatic interactions between positively charged chlorhexidine molecules and negatively charged bacterial cell membranes, resulting in membrane disruption and leakage of cellular contents (Eggers et al., 2018; Rundle et al., 2019; Pratiwi et al., 2022).

Previous studies have demonstrated that antiseptic soaps are effective in reducing bacterial counts on the hands. Riza et al. (2019) reported that chloroxylenol 4.8% and povidone-iodine 7.5% had comparable effectiveness in reducing bacterial counts after handwashing procedures. Meanwhile, Aftab et al. (2023) found that chlorhexidine gluconate effectively reduced microbial counts on the skin due to its strong antimicrobial activity.

The urgency of this research lies in the need for evidence-based recommendations regarding hand hygiene products, particularly amid increased attention to handwashing practices during infectious disease outbreaks. Understanding the comparative effectiveness of commonly available antiseptic soaps can assist consumers and healthcare professionals in selecting appropriate hand hygiene products. This study contributes to the existing literature by providing direct comparative evidence regarding the effectiveness of three commonly used antiseptic agents in a nonhospital setting.

The novelty of this research lies in its direct comparison of three widely used antiseptic agents within a standardized handwashing procedure using concentrations commonly found in consumer products. Furthermore, this study employed a pre-test and post-test design by measuring bacterial counts before and after handwashing to directly evaluate the reduction achieved by each antiseptic soap.

Although previous studies have demonstrated the effectiveness of individual antiseptic ingredients, research directly comparing the effectiveness of chloroxylenol, povidone-iodine, and chlorhexidine gluconate in reducing skin bacterial counts remains limited. Therefore, further comparative investigations are needed to evaluate the relative effectiveness of these antiseptic soaps. This study aimed to determine the effectiveness of various antiseptic hand soaps in reducing skin bacterial counts and to analyze differences in effectiveness among soaps containing chloroxylenol, povidone-iodine, and chlorhexidine gluconate. The findings are expected to provide scientific evidence regarding the selection of effective antiseptic soaps for maintaining hand hygiene and serve as a basis for future research on the antimicrobial activity of various antiseptic agents.

METHOD

This study employed an experimental research design using a pre-test and post-test group design. The research was conducted in May 2026 at the Microbiology Laboratory, Faculty of Medicine, Trisakti University, West Jakarta. The study involved 27 students from the Faculty of Dentistry, Trisakti University. Respondents were selected based on predetermined inclusion and exclusion criteria, including active students who were willing

to participate, had no skin diseases or hand wounds, were not sensitive to antiseptic agents, and had not previously washed their hands or used antiseptic products before sample collection.

The sampling technique used was purposive sampling, in which respondents were selected based on the established criteria. The respondents were divided into three groups according to the type of antiseptic soap used, with nine respondents in each group. Data collection involved obtaining informed consent, collecting hand swab samples before and after handwashing, and counting bacterial colonies after incubation. The collected data were analyzed using SPSS software to evaluate the effectiveness of different antiseptic hand soaps in reducing skin bacterial counts and to compare the effectiveness among the tested antiseptic agents. Statistical analysis was performed using the Wilcoxon signed-rank test and the Kruskal–Wallis test because the data did not meet the assumption of normal distribution based on the normality test.

RESULTS AND DISCUSSION

Table 1. Distribution of study subject characteristics (n=27)

Variabel	Frequency (n)	Percentage (%)
Age (years)		
18 years old	12	44,4%
19 years old	14	51,9%
20 years	1	3,7%
Gender		
Male	12	44,4%
Women	15	55,6%

Based on table 1 above, the majority of respondents were 19 years old, namely 14 respondents (51.9%) and the average respondent who participated in the study was female (55.6%).

Table 2. The effectiveness of various types of antiseptic hand soap in reducing the number of skin bacteria

Variabel	Types of antiseptic soaps		
	<i>Chloroxylonol 0,5%</i>	<i>Povidone Iodine 7,5%</i>	<i>Chlorhexidine Gluconate 0,5%</i>
Decrease in the number of bacteria			
Median Pre	100	11	200
Median Post	22	0	61
Value p	0,018	0,008	0,008

Uji Wilcoxon Signed-Rank Test

Based on the results of table 2 using the Wilcoxon Signed-Test, it was found that 0.5% *chloroxylonol* soap had a median handwashing result of 100 CFU to 22 CFU, then in *povidone iodine* soap 7.5% it was 11 CFU to 0 CFU while in *chlorhexidine gluconate* soap 0.5% of 200 CFUs became 61 CFUs. All soap groups experienced a statistically significant decrease in the number of colonies after washing hands using antiseptic soap, namely *chloroxylonol* type soap 0.5% obtained a p value of 0.018, while *povidone iodine* soap 7.5% and *chlorhexidine gluconate* 0.5% had a p value of 0.008.

Table 3. Percentage decrease in the number of bacterial colonies in different types of antiseptic hand soap

Variabel	Types of antiseptic soaps		
	<i>Chloroxylonol</i> 0,5%	<i>Povidone Iodine</i> 7,5%	<i>Chlorhexidine</i> <i>Gluconate</i> 0,5%
Total colony bacteria			
Pre	1.681	779	1.653
Post	348	2	794
Decrease (%)	79,30%	99,74%	51,97%

Based on the results of the table above, it was found that the percentage decrease in the number of different bacterial colonies between soaps with the largest percentage decrease was in *Povidone Iodine* soap 7.5%, which was 99.74%, followed by *Chloroxylonol* 0.5% of 79.30%, and *Chlorhexidine Gluconate* 0.5% of 51.97%.

Table 4. Differences in the effectiveness of different types of antiseptic hand soap on reducing the number of skin bacteria

Variabel	Types of antiseptic soaps			Value p
	<i>Chloroxylonol</i> 0,5%	<i>Povidone Iodine</i> 7,5%	<i>Chlorhexidine</i> <i>Gluconate</i> 0,5%	
Decrease in the number of bacteria				
N	9	9	9	
Δ CFU median	86	11	133	0,481

Uji Kruskal Wallis-Test

Based on the results of the *Kruskal Wallis-Test* test in table 3, it was found that the median decrease from the 0.5% *chloroxylonol* soap was 86 CFU, then for *povidone iodine* soap 7.5% was 11 CFU, while in *chlorhexidine soap* 0.5% was 133 CFU. The result of the p value obtained was 0.481 which means that no statistically significant difference in effectiveness was found between the three types of antiseptic soap in reducing the number of bacterial colonies.

This study showed that all types of antiseptic soaps, namely chloroxylonol 0.5%, povidone iodine 7.5%, and chlorhexidine gluconate 0.5%, were able to significantly reduce the number of skin bacterial colonies after hand washing. These results were proven by the Wilcoxon Signed-Rank Test which showed a $p < 0.05$ value in all treatment groups. These findings indicate that the use of antiseptic soap is effective in reducing bacterial contamination of hands and supports the importance of handwashing practices as one of the infection prevention strategies (Ladyani et al., 2024).

In the 0.5% chloroxylonol soap group, there was a decrease in the number of colonies with a median value before hand washing of 100 CFU to 22 CFU. These findings are in line with research by Riza et al. (2019) who reported that chloroxylonol 4.8% was effective in reducing the number of bacteria after hand washing. The antimicrobial activity of chloroxylonol is obtained through the mechanism of protein denaturation and damage to the bacterial cell membrane thereby inhibiting the survival of microorganisms (Kristy et al., 2021).

Then the povidone iodine soap group of 7.5% showed the number of colonies from 11 CFUs to 0 CFUs. These results are in line with the research of Eggers et al. (2018) which showed that povidone iodine 7.5% has strong bactericidal activity against various microorganisms. Povidone iodine works by releasing free iodine that

oxidizes proteins, nucleic acids, and other important components in microbial cells thus causing cell death (Lepelletier et al., 2020).

The largest decrease was found in the chlorhexidine gluconate soap group of 0.5%, from 200 CFU to 61 CFU. These results are similar to the research conducted by Aftab et al. (2023) which stated that chlorhexidine gluconate is effective in reducing the number of microorganisms on the skin. The mechanism of action of chlorhexidine gluconate involves the interaction between the positive charge of chlorhexidine molecules with the negatively charged bacterial membrane resulting in damage to the cell membrane (Thangavelu et al., 2020).

Although all soap groups showed a significant decrease in the number of bacterial colonies, the results of the *Kruskal-Wallis* test showed no statistically significant difference in effectiveness between the three types of antiseptic soap ($p=0.481$). Meanwhile, based on the percentage reduction results of each soap, *the type of povidone iodine* soap 7.5% showed the result of reducing the number of bacterial colonies with the largest number of bacterial colonies, which was 99.74%, followed by *chloroxylenol* 79.30% and *chlorhexidine gluconate* 51.97%.

The results of this study support the research of Vallejo et al. (2018) which reported that chlorhexidine 4% and chloroxylenol 3% had comparable bactericidal effectiveness as well as the study conducted by Riza et al. (2019) which showed no significant difference between chloroxylenol 4.8% and povidone iodine 7.5% in reducing the number of bacteria in the hands.

The absence of a significant difference in effectiveness can be influenced by several factors. Variations in skin microbiota between individuals are one of the factors that play a role. The amount and type of bacteria present in the hands are influenced by daily activities, environmental exposure, personal hygiene habits, age, gender, and skin conditions. In addition, the skin's natural antimicrobial activity affected by pH, skin temperature, and lactic acid levels can affect the number of bacteria that survive after handwashing (Zapka et al., 2017; Boxberger et al., 2021; Hayashi et al., 2021).

This research also has limitations, among which is the relatively small number of samples so that the statistical power of the research is limited. In addition, respondent activity prior to sampling could not be fully controlled, allowing for variations in the number of initial bacteria between individuals. And the relatively similar characteristics of respondents in terms of age also limited the results of the study to a wider population. Clinically, the results of this study show that *chloroxylenol* 0.5%, *povidone iodine* 7.5%, and *chlorhexidine gluconate* 0.5% can be used as an antiseptic soap option to maintain hand hygiene because all three have been shown to be effective in reducing the number of skin bacterial colonies.

CONCLUSION

Antiseptic soaps containing 0.5% chloroxylenol, 7.5% povidone-iodine, and 0.5% chlorhexidine gluconate were proven to be effective in reducing bacterial colony counts after handwashing. The percentage reductions in bacterial colony counts were 79.30% for chloroxylenol, 99.74% for povidone-iodine, and 51.97% for chlorhexidine gluconate, respectively. Among the three antiseptic soaps, povidone-iodine demonstrated the highest percentage reduction in bacterial colony counts (99.74%). Therefore, all three antiseptic soaps may be considered potential options for supporting hand hygiene practices.

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Effectiveness Of Various Antiseptic Hand Soaps In Reducing Skin Bacterial Counts

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ABSTRACT

The hands are the part of the body that is most often exposed to bacteria from the environment and plays an important role in the spread of disease. Washing hands using antiseptic soap is one of the effective ways to reduce bacterial contamination on the skin. Antiseptic soaps containing chloroxylenol, povidone iodine, and chlorhexidine gluconate are widely used because they have broad-spectrum antimicrobial activity. However, the difference in the effectiveness of the three antiseptic ingredients is still not known for sure. This study is an experimental research with a group pre-test and post-test design conducted on 27 students of the Faculty of Dentistry, Trisakti University. Respondents were divided into three groups based on the type of antiseptic soap used, namely chloroxylenol 0.5%, povidone iodine 7.5%, and chlorhexidine gluconate 0.5% with each group consisting of 9 respondents. The number of bacterial colonies on the hands was checked before and after hand washing using the swab method. Data analysis will be carried out using the Wilcoxon Signed-Rank Test and the Kruskal-Wallis Test. All three types of antiseptic soaps showed a significant decrease in the number of bacterial colonies. In the chloroxylenol group, the median number of colonies decreased from 100 CFU to 22 CFU ($p=0.018$), in the povidone iodine group decreased from 11 CFU to 0 CFU ($p=0.008$) and in the chlorhexidine gluconate group decreased from 200 CFU to 61 CFU ($p=0.008$). Based on the percentage decrease of each soap, the largest result was obtained in povidone iodine soap, which was 99.74%. Meanwhile, the comparison of the decrease in the number of colonies between groups showed that there was no statistically significant difference in effectiveness ($p = 0.481$). Antiseptic soaps with the active ingredients chloroxylenol, povidone iodine, and chlorhexidine gluconate have been shown to be effective in reducing the number of bacterial colonies after hand washing. The percentage decrease was 79.30% in chloroxylenol, 99.74% in povidone iodine, and 51.97% in chlorhexidine gluconate, respectively, with povidone iodine showing the highest decrease. All three types of antiseptic soap can be considered as an option for maintaining hand hygiene.

Keyword: Antiseptic soap, chlorhexidine gluconate, chloroxylenol, bacterial colony, povidone iodine

INTRODUCTION

The skin is the largest organ of the human body which serves as the main barrier against various environmental factors. In addition to acting as a physical protector, the skin is also a habitat for various microorganisms that make up the skin microbiome. The microbiome consists of resident flora that settles on the surface of the skin and transient flora obtained from the surrounding environment, both of which play a role in maintaining the balance of the body's defense system. Hands are the part of the body that is most often exposed to the environment so it has a high risk of being contaminated by various types of microorganisms. Daily activities such as touching objects, shaking hands, using public facilities, and contact with contaminated surfaces can cause the transfer of bacteria to the surface of the hands and the chain of disease

transmission has occurred. Therefore, maintaining hand hygiene is one of the most effective measures to prevent the spread of microorganisms. (1) (2) (3) (4) (5) (5)

The World Health Organization (WHO) said that hand hygiene is one of the main components in infection prevention and control. In addition to removing dirt, the handwashing process also helps reduce the number of bacteria attached to the skin so that it can reduce the risk of disease transmission. The effectiveness of hand washing is also influenced by various factors, such as hand washing techniques, the duration of hand washing, the type of soap used, and the skin condition of each individual. (6) (7) (8)

Soap is a cleaning product composed of surfactants that are able to emulsify fat and remove dirt from the surface of the skin. In addition to the usual types of soaps, there are currently many antiseptic soaps that contain antimicrobial active ingredients to increase the effectiveness of hand cleaning. The use of antiseptic soaps aims not only to mechanically clean dirt, but also to inhibit or kill microorganisms present on the surface of the skin. Some of the antiseptic ingredients commonly used in hand soap include (9) (10) (11) (12) (13) (13) chloroxylonol, povidone iodine, and chlorhexidine gluconate.

Chloroxylonol is a phenolic compound that has antimicrobial activity against various Gram-positive and Gram-negative bacteria. The mechanism of action of chloroxylonol includes protein denaturation and disruption of bacterial cell

bacterial cell death. (14) Povidone iodine is an iodine complex that is widely used as an antiseptic because it has a broad spectrum of antimicrobials. This compound works by releasing free iodine capable of oxidizing proteins, enzymes, and important structural components in the cells of microorganisms. Damage to these cell components results in the disruption of metabolic function and eventually leads to the death of microorganisms. Then (15) (16) chlorhexidine gluconate soap is an antiseptic that has extensive antimicrobial activity and a relatively longer residual effect than other antiseptics. (17) Chlorhexidine works through electrostatic interactions between positively charged molecules and negatively charged bacterial cell membranes causing membrane damage and cell content leakage. (15) (18)

Various studies have shown that antiseptic soap is effective in lowering the number of bacteria on the hands. Research by Riza et al. reported that chloroxylonol 4.8% and povidone iodine 7.5% were equally effective in lowering the number of bacteria on hands after the handwashing procedure. Meanwhile, research by Aftab (16) et al. shows that chlorhexidine gluconate is effective in reducing the number of microorganisms on the skin through its powerful antimicrobial activity. (19)

Although various studies have proven the effectiveness of each antiseptic ingredient, studies that directly compare the effectiveness of chloroxylonol, povidone iodine, and chlorhexidine gluconate against reducing the number of skin bacteria are still limited. Therefore, further research is needed comparing the effectiveness of the three types of antiseptic soaps. This study aims to determine the effectiveness of various types of antiseptic hand soap on reducing the number of skin bacteria and analyze the difference in effectiveness between soaps containing chloroxylonol, povidone iodine, and chlorhexidine gluconate. The results of the study are expected to provide scientific information on the choice of effective antiseptic soap for maintaining hand hygiene and become the basis for further research on the antimicrobial activity of various antiseptic materials.

METHOD

This study uses the type of experimental research study with the design of pre-test methods and post-test group design. This research will be carried out in May 2026 at the Microbiology Laboratory, Faculty of Medicine, Trisakti University, West Jakarta. This research was conducted in May 2026 with a total of 27 students who are students of the Faculty of Dentistry, Trisakti University, which began by determining respondents based on predetermined inclusion and exclusion criteria, including active students of the Faculty of Dentistry, Trisakti University who are willing to participate in the research, who do not have skin diseases, wounds on the hands, sensitive to antiseptic materials and who have previously washed their hands or used antiseptic materials.

The sampling technique in this study is to use purposive sampling techniques to select respondents who meet the criteria that have been made, which means that not all respondents have the same opportunity. Respondents were then divided into 3 groups with each group consisting of 9 respondents based on the type of antiseptic. Data analysis is carried out in stages, starting from the informed consent signature, then swab sampling before and after washing hands and then counting the number of colonies after the media is incubated. The results of the swab collection will be analyzed using the SPSS program to assess the effectiveness of various types of antiseptic hand soap on reducing the number of skin bacteria and the difference in the effectiveness of different types of antiseptic hand soap on reducing the number of skin bacteria. The analysis used was the Wilcoxon Signed-Rank Test and the Kruskal Wallis-Test because the results of the normality test were not normally distributed.

RESULTS AND DISCUSSION

Table 1. Distribution of study subject characteristics (n=27)

Variabel	Frequency (n)	Percentage (%)
Age (years)		
18 years old	12	44,4%
19 years old	14	51,9%
20 years	1	3,7%
Gender		
Male	12	44,4%
Women	15	55,6%

Based on table 1 above, the majority of respondents were 19 years old, namely 14 respondents (51.9%) and the average respondent who participated in the study was female (55.6%).

Table 2. The effectiveness of various types of antiseptic hand soap in reducing the number of skin bacteria

Variabel	Types of antiseptic soaps		
	<i>Chloroxylonol 0,5%</i>	<i>Povidone Iodine 7,5%</i>	<i>Chlorhexidine Gluconate 0,5%</i>
Decrease in the number of bacteria			
Median Pre	100	11	200
Median Post	22	0	61
Value p	0,018	0,008	0,008

Uji Wilcoxon Signed-Rank Test

Based on the results of table 2 using the Wilcoxon Signed-Test, it was found that 0.5% chloroxylonol soap had a median handwashing result of 100 CFU to 22 CFU, then in povidone iodine soap 7.5% it was 11 CFU to 0 CFU while in chlorhexidine gluconate soap 0.5% of 200 CFUs became 61 CFUs. All soap groups experienced a statistically significant decrease in the number of colonies after washing hands using antiseptic soap, namely chloroxylonol type soap 0.5% obtained a p value of 0.018, while povidone iodine soap 7.5% and chlorhexidine gluconate 0.5% had a p value of 0.008.

Table 6. Percentage decrease in the number of bacterial colonies in different types of antiseptic hand soap

Variabel	Types of antiseptic soaps		
	<i>Chloroxylonol 0,5%</i>	<i>Povidone Iodine 7,5%</i>	<i>Chlorhexidine Gluconate 0,5%</i>
Total colony bacteria			
Pre	1.681	779	1.653
Post	348	2	794
Decrease (%)	79,30%	99,74%	51,97%

Based on the results of the table above, it was found that the percentage decrease in the number of different bacterial colonies between soaps with the largest percentage decrease was in Povidone Iodine soap 7.5%, which was 99.74%, followed by Chloroxylonol 0.5% of 79.30%, and Chlorhexidine Gluconate 0.5% of 51.97%.

Table 3. Differences in the effectiveness of different types of antiseptic hand soap on reducing the number of skin bacteria

Variabel	Types of antiseptic soaps			Value p
	<i>Chloroxylonol 0,5%</i>	<i>Povidone Iodine 7,5%</i>	<i>Chlorhexidine Gluconate 0,5%</i>	
Decrease in the number of bacteria				
N	9	9	9	
Δ CFU median	86	11	133	0,481

Uji *Kruskal Wallis-Test*

Based on the results of the *Kruskal Wallis-Test* test in table 3, it was found that the median decrease from the 0.5% *chloroxylenol* soap was 86 CFU, then for *povidone iodine* soap 7.5% was 11 CFU, while in *chlorhexidine soap* 0.5% was 133 CFU. The result of the p value obtained was 0.481 which means that no statistically significant difference in effectiveness was found between the three types of antiseptic soap in reducing the number of bacterial colonies.

This study showed that all types of antiseptic soaps, namely *chloroxylenol* 0.5%, *povidone iodine* 7.5%, and *chlorhexidine gluconate* 0.5%, were able to significantly reduce the number of skin bacterial colonies after hand washing. These results were proven by the *Wilcoxon Signed-Rank Test* which showed a $p < 0.05$ value in all treatment groups. These findings indicate that the use of antiseptic soap is effective in reducing bacterial contamination of hands and supports the importance of handwashing practices as one of the infection prevention strategies. ⁽²⁰⁾

In the 0.5% *chloroxylenol soap group*, there was a decrease in the number of colonies with a median value before hand washing of 100 CFU to 22 CFU. These findings are in line with research by Riza *et al.* who reported that *chloroxylenol* 4.8% was effective in reducing the number of bacteria after hand washing. The antimicrobial activity of *chloroxylenol* is obtained through the mechanism of protein denaturation and damage to the bacterial cell membrane thereby inhibiting the survival of microorganisms. ⁽¹⁶⁾ ⁽¹⁴⁾

Then the *povidone iodine soap group* of 7.5% showed the number of colonies from 11 CFUs to 0 CFUs. These results are in line with the research of Eggers *et al.* which showed that *povidone iodine* 7.5% has strong bactericidal activity against various microorganisms. ⁽¹⁵⁾ *Povidone iodine* works by releasing free iodine that oxidizes proteins, nucleic acids, and other important components in microbial cells thus causing cell death. ⁽²¹⁾

The largest decrease was found in the *chlorhexidine gluconate* soap group of 0.5%, from 200 CFU to 61 CFU. These results are similar to the research conducted by Aftab *et al.* which stated that *chlorhexidine gluconate* is effective in reducing the number of microorganisms on the skin. The mechanism of action ⁽¹⁹⁾ of *chlorhexidine gluconate* involves the interaction between the positive charge of *chlorhexidine* molecules with the negatively charged bacterial membrane resulting in damage to the cell membrane. ⁽²²⁾

Although all soap groups showed a significant decrease in the number of bacterial colonies, the results of the *Kruskal-Wallis* test showed no statistically significant difference in effectiveness between the three types of antiseptic soap ($p=0.481$). Meanwhile, based on the percentage reduction results of each soap, the type of *povidone iodine* soap 7.5% showed the result of reducing the number of bacterial colonies with the largest number of bacterial colonies, which was 99.74%, followed by *chloroxylenol* 79.30% and *chlorhexidine gluconate* 51.97%.

The results of this study support the research of Vallejo *et al.* which reported that *chlorhexidine* 4% and *chloroxylenol* 3% had comparable bactericidal effectiveness as well as the study conducted by Riza *et al.* which showed no significant difference between *chloroxylenol* 4.8% and *povidone iodine* 7.5% in reducing the number of bacteria in the hands. ⁽²³⁾ ⁽¹⁶⁾

9 The absence of a significant difference in effectiveness can be influenced by several factors. Variations in skin microbiota between individuals are one of the factors that play a role. The amount and type of bacteria present in the hands are influenced by daily activities, environmental exposure, personal hygiene habits, age, gender, and skin conditions. In addition, the skin's natural antimicrobial activity affected by pH, skin temperature, and lactic acid levels can affect the number of bacteria that survive after handwashing. (24) (25) (26)

1 This research also has limitations, among which is the relatively small number of samples so that the statistical power of the research is limited. In addition, respondent activity prior to sampling could not be fully controlled, allowing for variations in the number of initial bacteria between individuals. And the relatively similar characteristics of respondents in terms of age also limited the results of the study to a wider population. Clinically, the results of this study show that *chloroxylenol* 0.5%, *povidone iodine* 7.5%, and *chlorhexidine gluconate* 0.5% can be used as an antiseptic soap option to maintain hand hygiene because all three have been shown to be effective in reducing the number of skin bacterial colonies.

CONCLUSION

2 Antiseptic soap type chloroxylenol 0.5%, povidone iodine 7.5% and chlorhexidine gluconate 0.5% have been proven to be effective in reducing the number of bacterial colonies after hand washing. The percentage decrease in the number of bacterial colonies was 79.30% in chloroxylenol, 99.74% in povidone iodine and 51.97% in chlorhexidine gluconate, respectively. Among the three types of antiseptic soaps, povidone iodine showed the highest percentage reduction in the number of bacterial colonies by 99.74%. Thus, all three types of antiseptic soaps can be considered as options for maintaining hand hygiene.

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