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The effectivity of hand antiseptic against *Staphylococcus aureus*

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Abstract

This study was aimed to determine the hand antiseptic products effectivity against the growth of *Staphylococcus aureus* bacteria. The sample used were hand antiseptic products purchased from several minimarkets, markets, and obtained from several institutions in West Sulawesi by using purposive sampling technique. Samples were evaluated using the well diffusion method with three replications. Since the study data did not meet the ANOVA test requirements, the Kruskal-Wallis analysis test was used to analyze the data, then continued with the Mann Whitney test. The results showed a significant difference between hand antiseptics in preventing the growth of S. aureus bacteria, with a sig. value of 0.00<0.05. Only two of ten hand antiseptic products with the codes N8 and N10 efficiently inhibited the growth of *Staphylococcus aureus* bacteria, which is indicated by the formation of a clear zone around the paper disc saturated with hand antiseptics on the agar surface.

INTRODUCTION

COVID-19 is a global outbreak of the corona virus that is growing rapidly and spreading throughout the world. According to WHO data, this disease is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). One of the common suggestions to stop the spread of this infection is handwashing at regular intervals of time (Isbaniah et al., 2020). Hands are the body part most often associated with anything. Besides being used to touch food, hands are frequently used to grasp filthy objects that are contaminated and carry germs that can spread disease-causing (Agustiningrum 2018). Therefore, it is necessary to maintain hand hygiene as it the main transmission route and disease outbreak (Hayat and Munnawar, 2016). *Staphylococcus aureus* is one of the most prevalent harmful microorganisms discovered on hands (Jawetz *et al*. 2007). These bacteria can cause food poisoning and kill leukocytes because of the leukocidin enzyme content (Pelczar and Chan, 2014). Therefore, it is necessary to find a way to reduce the prevalence of these pathogens, which can be done by hand washing using water or hand sanitizer or hand antiseptic (Suryani *et al*. 2019).

Hand antiseptic is a hand-sanitizer that can inhibit the growth of Gram-positive and Gram-negative bacteria because it contains active substances such as alcohol and other antimicrobial ingredients. The practical way of using it without requiring water and killing germs quickly is the main advantage of hand antiseptics that attract consumers to buy these products (Rini and Nugraheni, 2018). During the pandemic, there is a surge of various hand antiseptics which offers diverse active ingredients.

Large number of antiseptic variants either contain synthetic or natural active ingredients inhibiting the growth of pathogenic bacteria circulating in the market. Thus, this study aims to

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evaluate hand antiseptic bioactivity products in the market against *S. aureus*.

MATERIALS AND METHODS

Instrument Selection Stage

The study instrument used in this study was a Vernier calipers and the inhibitory response in Table 1. The process of collecting data were obtained through several stages i.e tools and Materials Preparation, Nutrient Agar Media Preparation, Bacterial Suspension Preparation. All tools and materials were sterilized using an autoclave at a pressure of 2 atm with a temperature of 121 °C for 15 minutes. The alternative sterilizing apparatus is with oven at temperature 160–180 °C for 2 hours.

Table 1. Inhibitory Response

Average Inhibition Zone (mm)	Inhibitory Response
≤ 5	Weak
6-10	Moderate
11-20	Strong
≥ 21	Very Strong

The nutrient agar (Merck, Darmstadt, Germany) was prepared by dissolving 20 g of nutrient agar powder in a litre of distilled water. Solutions were brought to boil whilst being stirred with a magnetic stirrer to ensure homogeneity. Agar solution was sterilized in the autoclave at 121°C for 15 minutes. The sterile agar solution was then aseptically poured into disposable Petri dishes and left to solidify.

Preparation of test bacteria suspension began with taking one colony of pure *S. aureus* bacteria using an osseous needle. Followed by an inoculation on NA medium, then incubated at 37°C for 24 hours. The incubated bacterial cultures on NA slant media were suspended with 10 ml of distilled water, then homogenized using a vortex.

Hand Antiseptic Test

Hand antiseptic testing was carried out using the well diffusion method. The testing began with attaching the cylinder assay to the solidified media in a Petri dish aseptically. Then, warm media was poured into the Petri dish, and stood to solidify. Next, a sterile cotton swab was inserted into the inoculum tube containing the bacterial suspension and smeared it on the solidified NA media surface evenly. A 0.2 ml of hand antiseptic product was put into each well. Likewise, amoxicillin antibiotics (positive control) and sterile distilled water (negative control) were added into each well. The well was then incubated at 37°C for 24-48 hours while being observed. After incubation, it was observed whether an inhibition zone was formed or not. The inhibition zone was indicated by the presence of a clear zone in the area around the well. Each hand antiseptic product was tested in triplicate. The inhibition zone formed was then measured horizontally and vertically with units of millimeters (mm) using a caliper (Umaya, 2017). The diameter of the inhibition zone was measured by the following formula (Warbung, 2013):

$$\frac{(D1 - Ds) + (D2 - Ds)}{2}$$

Note:
D1: Vertical diameter (mm)
D2: Horizontal diameter (mm)
Ds: Diameter of well (mm)

Table 2. The Concentration of Active Ingredients in Hand Antiseptic Samples

Hand Antiseptic Code	Hand Antiseptic Active Ingredients		Source
	Alcohol (%)	*Chlorohexidine digluconate* (%)	
N1	-	-	Market
N2	70	-	Market
N3	70	-	Market
N4	70	-	Mini Market
N5	75	-	Mini Market
N6	-	-	Institution
N7	70	-	Institution
N8	70	-	Institution
N9	74.4	-	Institution
N10	70	0.5	Mini Market

Statistical analysis

The obtained data were analyzed using the Kruskal-Wallis and the Mann Whitney test since the data is normally distributed but not homogeneous. The conclusions were made to determine the effectivity of hand antiseptic products against the growth of *S. aureus* bacteria.

RESULTS AND DISCUSSION

In this study, 10 brands of hand antiseptics that had been tested against *S. aureus* bacteria were used. These brands included those purchased from the market (hand antiseptics encoded with N1, N2, and N3), minimarkets (hand antiseptics encoded with N4, N5, and N10), and several institutions (hand

antiseptics encoded with N6, N7, N8, and N9). Each hand antiseptic used contains different active substances which can be seen in Table 2. These differences also produce different antimicrobial effects against S. aureus bacteria.

The results of antimicrobial test using the well diffusion method showed that not all hand antiseptics exhibited an antimicrobial effect against the growth of *S. aureus* bacteria. Hand antiseptics encoded with N8 and N10 have been demonstrated to have an inhibitory effect on *S. aureus* (Figure 1). The findings indicated that the hand antiseptics N8 and N10 were both bacteriostatic, which could prevent the growth of *S. aureus* bacteria, after being incubated for 24 hours. This is demonstrated by the clean zone that has formed around the well. This is proven by the presence of a clear zone formed around the well. However, after being incubated for 48 hours, the clear zone formed on the two hand antiseptics decreased by approximately 1 mm, both for U1 (1st replication), U2 (2nd replication), and U3 (3rd replication). The results of these tests can be seen in Table 3.

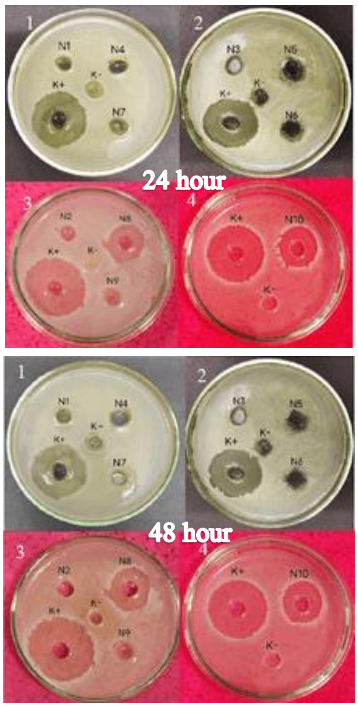

Figure 1. Inhibition test of hand antiseptic N1-N10 against *S. aureus*.

Table 3. Hand Antiseptic N1-N10 Inhibitory Zone Diameter against the Growth of *S. aureus* Bacteria with Incubation Periods of 24 Hours and 48 Hours.

Incubation Time	Hand Antiseptic Code	Inhibition Zone Diameter (mm)			
		U I	U II	U III	Average ± SD
24 Hours	N1	0	0	0	0
	N2	0	0	0	0
	N3	0	0	0	0
	N4	0	0	0	0
	N5	0	0	0	0
	N6	0	0	0	0
	N7	0	0	0	0
	N8	15.4	10	10	11.8 ± 3.11
	N9	0	0	0	0
	N10	12.6	12.2	12	12.27 ± 0.093
	K+	22.8	22.6	22.1	22.12 ± 0.97
48 Hours	N1	0	0	0	0
	N2	0	0	0	0
	N3	0	0	0	0
	N4	0	0	0	0
	N5	0	0	0	0
	N6	0	0	0	0
	N7	0	0	0	0
	N8	14	9	9	10.67 ± 2.88
	N9	0	0	0	0
	N10	12.2	12	11.8	12 ± 0.2
	K+	22	21	19.8	20.93 ± 1.10
	K-	0	0	0	0

The measurement of the hand antiseptic inhibition zone aims to determine the ability of the antimicrobial activity contained in hand antiseptics

	Chi-Square	Df	Sig
Inhibition Zone	34.727	11	.000

to inhibit the growth of *S. aureus* bacteria. Table 3 shows that the N8 hand antiseptic is bacteriostatic with the inhibitory zone of 11.8 mm at 24 hours incubation and 10.6 mm at 48 hours incubation. Meanwhile, N10 hand antiseptic has inhibition zone of 12.27 mm at 24 hours incubation and 12 mm at 48 hours incubation time.

Table 3 demonstrates that N8 and N10 hand antiseptics could inhibit the growth of *S. aureus* bacteria. They both form an inhibitory zone with a potent inhibitory response. As for the positive control, it has a very strong inhibitory response. Consequently, the hand antiseptic affected the growth of *S. aureus* bacteria. Table 4 illustrates the variation in the average diameter test of the hand antiseptic inhibitory zone against the growth of *S. aureus* bacteria.

Table 4. Inhibitory Response of Hand Antiseptic to the Growth of *S. aureus* Bacteria.

Hand Antiseptic	Average Inhibition Zone (mm) ± SD	Inhibitory Response
N1	0	No Inhibition
N2	0	No Inhibition
N3	0	No Inhibition
N4	0	No Inhibition
N5	0	No Inhibition
N6	0	No Inhibition
N7	0	No Inhibition
N8	11.8	Strong Inhibition
N9	0	No Inhibition
N10	12.27	Strong Inhibition
K+	22.13	Very strong Inhibition
K-	0	No Inhibition

The normality test showed a sig. value of 7.97 > 0.05, proving that the data was normally distributed. Meanwhile, the homogeneity test results have a significance value of 0.00 < 0.05, which indicates that they are not homogeneous. The preliminary ANOVA test reveals that although the data are not homogeneous, they are normally distributed. This suggests that the acquired data do not satisfy the criteria for the one way ANOVA test. Instead, the non-parametric Kruskal-Wallis test was used to determine the differences in the antimicrobial activity of hand antiseptics against the growth of *S. aureus* bacteria.

Table 5. Results of the Kruskal-Wallis Hand Antiseptic Test N1-N10

Table 4 shows the Kruskal-Wallis test findings, with the sig. value of 0.00 < 0.05. This proves that each hand antiseptic product from N1-N10, K+ (positive control) and K- (negative control) that have been tested has different abilities in inhibiting the growth of *S. aureus* bacteria. Due to these differences, Mann-Whitney test was used to compare all pairs of treatment averages after the analysis of variance was carried out. This was done to determine the hand antiseptic type that had the most effect on the formation of the inhibition zone against *S. aureus* bacteria. According to the Mann-Whitney test results, the hand antiseptic sig. value obtained from N1, N2, N3, N4, N5, N6, N7, N9, and negative control, was 1.000 > 0.05, indicating that there is no significant difference between their inhibitory zones for inhibiting the growth of *S. aureus* bacteria. However, the formation of inhibition zones between the hand antiseptics was significantly different from N8, N10, and positive control. The Mann-Whitney test results showed that N8, N10, and positive control all had sig. values of <0.05 specifically 0.034 and 0.037 for N8 and N10, respectively and positive control, indicating that there are differences between N8, N10, and positive control's inhibition zones for suppressing the growth of *S. aureus* bacteria.

Differences in the ability of hand antiseptics to inhibit the growth of *S. aureus* bacteria are influenced by differences in the active ingredients content. Most hand antiseptics contain antimicrobial substances in the form of alcohol which are believed to inhibit the growth of *S. aureus* bacteria (Kakroo *et al*. 2020). The study results indicate that N8 and N10 hand antiseptics are the most effective at preventing the growth of *S. aureus* bacteria. This is due to the fact that N10 contains two active antimicrobial agents, namely alcohol and chlorohexidine digluconate.

N10 consist of 70% alcohol and 0.5% chlorohexidine digluconate. According to (Asngad and Nopitasari, 2018) hand antiseptic with ± 60% to 80% alcohol content can be used to kill bacteria by coagulating and denaturing bacterial cell proteins as well as destroying cell membranes. When used as a hand antiseptic, chlorohexidine digluconate (N10) damages the cell wall and outer cell membrane, causing intracellular leakage and finally cytosolic

coagulation (Kusuma *et al*. 2019). Chlorohexidine digluconate is a derivative of chlorohexidine that crystallizes in methanol and possesses antibacterial characteristics that effectively against both Gram positive and Gram negative microorganisms. Chlorohexidine digluconate is an antimicrobial substance that reacts on the inner cell membrane after binding to the cell wall. Due to the combination of these two antimicrobial agents, the resulting inhibition zone is even greater. Kusuma *et al*. (2019) also reported that alcohol and chlorohexidine digluconate combination produced strong inhibitory response. The previous study reported that the combination of 70% alcohol and 0.5% chlorohexidine digluconate exhibited a strong inhibition zone and demonstrated the efficacy of N10 hand antiseptic in preventing the growth of *S. aureus*.

In contrast to N10, hand antiseptic N8 which also produced an inhibition zone, only consists of one antimicrobial agent, namely alcohol with a concentration of 70%. Based on the composition of the active ingredients contained, the concentration of alcohol content has met the requirements as an antimicrobial agent in hand antiseptics that can inhibit the growth of *S. aureus* bacteria. This is in line with previous study which stated that alcohol with a concentration of 70% produced weak to strong inhibitory responses (Rini and Nugraheni, 2018). These results are also in accordance with Purbosari (2021) showed that hand antiseptics which only contained an antimicrobial agent of 70% alcohol could sufficiently inhibit the growth of *S. aureus* bacteria.

Meanwhile, hand antiseptics N1, N2, N3, N4, N5, N6, N7, and N9 did not form an inhibitory zone. Each hand antiseptic has alcohol as its active ingredient in different concentrations ranging from 70%, 74.4%, 75%, and there is also a hand antiseptic do not provide the information of active ingredients used. In accordance with Kusuma *et al*. (2019), hand antiseptic containing only 70% alcohol as an active ingredient was unable to inhibit the growth of *S. aureus* due to the evaporation of alcohol during the storage, manufacture, and testing process. Evaporated alcohol will decrease its concentration, followed by the decreased inhibitory ability against bacteria and will no longer act as a bactericidal (Hamrun and Anam 2018). Ineffective hand antiseptics activity may also be the result of improper composition, and the interaction between alcohol and foreign proteins contained in the hand antiseptic mixture (Pelczar and Chan, 2014). In addition, hand antiseptics that are made not in accordance with BPOM or WHO standards can also affect the effectivity of the hand antiseptic. The absence of inhibition zones formed indicated that hand antiseptics coded N1, N2, N3, N4, N5, N6, N7, and N9 were not effective in inhibiting the growth of *S. aureus* bacteria.

CONCLUSION

Hand antiseptics that were able to inhibit the growth of *Staphylococcus aureus* bacteria were N8 and N10. The inhibition zones formed by the N8 and N10 hand antiseptics were 11.8 and 12.27 mm, respectively. Both hand antiseptics are bacteriostatic and have strong inhibitory ability. N1, N2, N3, N4, N5, N6, N7, and N9 hand antiseptics, however, were unable to inhibit the growth of *Staphylococcus aureus*.

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