



Efektivitas Daya Bunuh Antiseptik Benzalkonium Klorida dibandingkan Dengan Alkohol 70%

Septine Eka Putri^{1*}, Budi Laksono², Suhartono³

¹Program Studi Magister Epidemiologi, Fakultas Kesehatan Masyarakat, Universitas Diponegoro Semarang 50275

^{2,3}Departemen Magister Epidemiologi, Sekolah Pascasarjana, Universitas Diponegoro 50275

^{4,5}Departemen Kesehatan Lingkungan, Fakultas Kesehatan Masyarakat, Universitas Diponegoro, Semarang 50275

Info Artikel: Diterima 13 September 2023 ; Direvisi 4 September 2024 ; Disetujui 29 Mei 2025; Publikasi 29 Mei 2025



ABSTRACT

Background: One of the prevention of disease transmission can be done by maintaining hand hygiene, in addition to using soap, the use of hand sanitizer is an innovation to prevent disease transmission. Benzalkonium chloride is a chemical compound that can be used as a substitute for alcohol as an antiseptic.

Methods: This study is a true experiment with samples taken from inanimate objects in the surrounding environment using the swab method, sample identification using bacterial gram staining and effectiveness testing using the liquid dilution method to determine the MIC measured by a spectrometer and see the density of bacteria based on turbidity and solid dilution to determine MBC by looking at regrowth after 24 hours of exposure.

Result: The results of the study obtained MIC and MBC values in gram-positive and gram-negative bacterial samples occurred in 0.12% benzalkonium antiseptic which was marked by no bacterial growth and a decrease in turbidity after 24 hours of exposure. This is supported by statistical results stating that benzalkonium chloride has a different effect on inhibiting bacterial growth compared to 70% alcohol and from 130 bacterial test samples exposed to 70% alcohol, 14 samples (8 gram-negative bacteria and 6 gram-positive bacteria) experienced regrowth after 24 hours of exposure.

Conclusion : This shows that 0.12% benzalkonium is more effective in inhibiting and killing gram-positive and gram-negative bacteria in the environment.

Keywords: antiseptic; benzalkonium chloride; alcohol; bacteria; MIC; MBC

Copyright © 2025 by Jurnal Epidemiologi Kesehatan Komunitas. This is an open-access article under the CC BY-SA License (<https://creativecommons.org/licenses/by-sa/4.0>)

DOI : <https://doi.org/10.14710/jekk.v10i2.20251>

*Corresponding author, septineekap5@gmail.com

Introduction

Infectious disease is a disease caused by the multiplication or entry of microorganisms, namely bacteria, viruses, fungi, prions and protozoa into the body, causing organ damage. Disease transmission is the movement of pathogens that cause infectious diseases from exposed individuals or groups of hosts to other individuals/groups. Hand hygiene is an important aspect related to the transmission of microorganisms which have a role as objects that can bridge the interaction between skin microbiota and biometric parameters.^(1,2)

Washing hands using water or soap for 20 to 30 seconds or using alcohol-based hand sanitizers (ABHS) or non-alcohol based hand sanitizer (NABHS) as an alternative option in disease prevention, but fast and efficient use is the reason for the widespread use of hand sanitizers today, but the effectiveness of using ABHS and NABHS can be affected by the length of time you rub your hands when using a hand sanitizer and the width of the user's hands.⁽³⁻⁵⁾ Hand sanitizer products are available in various forms such as liquid or bar soap, synthetic detergents, non-alcohol based hand sanitizer (NABHS) and alcohol-based hand sanitizer (ABHS). Alcohol-based hand sanitizer and antiseptic hand soap for hand washing are 2 components of the current hand hygiene guidelines for healthcare workers recommended by the CDC.⁽⁶⁾

Recent research states that hand sanitizers containing 0,12% benzalkonium chloride (BKC) as the active ingredient were studied for long-lasting antibacterial activity against *Staphylococcus aureus* on human skin compared to 63% ethanol-based hand sanitizers. The study demonstrated significant killing of *S. aureus* on the skin up to 4 hours after application of benzalkonium chloride cleansers, compared with no persistent antibacterial activity of ethanol cleansers.⁽⁷⁾

Quaternary ammonium compounds, such as benzalkonium chloride, benzethonium chloride, and cetyl pyridinium chloride are cationic surfactants that exhibit antimicrobial activity through their adsorption onto the microbial cytoplasmic membrane, thereby damaging the structural integrity of the cell membrane.^(8,9) Therefore, they can inactivate gram-positive

bacteria and lipophilic viruses. Among the quaternary ammonium compounds, benzalkonium chloride is the active ingredient non-alcohol based hand sanitizer (NABHS) is the most commonly used.⁽¹⁰⁾

Benzalkonium chloride has several advantages over alcohol for hand disinfection: it is non-toxic, less irritating to the skin, and less flammable.⁽¹¹⁾ In fact, switching from alcohol to benzalkonium chloride hand sanitizer may lead to better hand hygiene compliance of healthcare workers⁽¹¹⁾, based on the CDC's presentation of benzalkonium chloride products as alcohol-based hand rubs that are more widely used by health workers, a study stated that surgical intensive care unit health workers found that using wet wipes containing benzalkonium chloride was as effective as using soap and water when washing hands.⁽¹²⁾

Benzalkonium has a low viscosity, high spreading power and a pH of 6.3 which tends not to cause skin irritation and has a relatively cheap price compared to other active compounds.^(13,14) In the CDC research results which showed benzalkonium had an effectiveness equivalent to washing hands with soap and in Brandon's study in 2021 stated that the Benzalkonium Chloride formulation showed -4.0 log₁₀ reduction of the SARS-CoV-2 virus in 30 seconds, and these results met the EN14476 performance standard for activity viruses against SARS-CoV-2.⁽¹⁵⁾

This study aims to find out the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) present in bacteria and are obtained directly in the environment.

Methods

The type of research in the test used in the laboratory experiment stage is a true experiment with a randomized pre-test post-test with control group design and using solid dilution and liquid dilution tests. This study was conducted to determine the level of effectiveness of benzalkonium compared to alcohol in preventing the transmission of diseases caused by bacteria on inanimate objects by looking at the minimum inhibitory level and minimum bactericidal level on bacteria, this design

compares the effect of alcohol on bacteria and benzalkonium on bacteria. Sampling was done by sweeping inanimate objects in public places (door handles, elevator buttons, public fences, and public chairs) using sterile gauze that had been moistened with buffered peptone water, the samples to be tested in this study were samples that had passed the isolation period for 18-24 hours in an incubator on agar media and there were bacteria in the sample then successfully purified based on grams of bacteria then tested with 0.2% benzalkonium chloride, 0.12% benzalkonium chloride, and 70% alcohol with 5 repetitions of the antibacterial effectiveness test using the dilution method.

a. Tools and Materials

Equipment in this study, namely test tubes, petri dishes, sterile cotton swabs, ose, measuring cups, micro pipettes, spectrometers, light microscopes, incubators, Erlenmeyer flasks, glass objects, colony counters and auto claves. The materials used in this study, namely bacterial samples, to be precise benzalkonium chloride 0,2%, benzalkonium chloride 0,12%, alcohol 70%, nutrient agar, nutrient broth, buffered peptone water, crystal violet, alcohol 96%, safranin, oil immersion, iodine solution and anhydrous alcohol.

b. Sampling

Bacterial samples were collected using the swab technique, which was carried out in public places, namely elevator buttons, door handles, stair railings and chairs using a sterile cotton swab which was inserted into buffered peptone water. Sample isolation using spread plate technique on nutrient agar media.

c. Bacterial Identification

Bacterial samples were identified based on the type of gram, then gram staining of bacteria was carried out for classification of gram bacteria. After the bacteria have been isolated, the samples are sterilized for grouping based on shape and then tested using gram staining

d. Effectiveness Test

The effectiveness test uses a dilution test to determine MIC (Minimum Inhibitory Concentration) using liquid dilution by looking at the level of turbidity measured using a spectrometer and solid dilution to determine MBC (Minimum Bacteriicidal Concentration) by looking at bacterial growth after carrying out an anti-bacterial test which is benzalkonium chloride 0,2 %, 0,12% benzalkonium chloride, and 70% alcohol.

e. Data Analysis

The effectiveness test uses a dilution test to determine MIC (Minimum Inhibitory Concentration) using liquid dilution by looking at the level of turbidity measured using a spectrometer and solid dilution to determine MBC (Minimum Bacteriicidal Concentration) by looking at bacterial growth after carrying out an anti-bacterial test which is benzalkonium chloride 0,2 %, 0,12% benzalkonium chloride, and 70% alcohol.

Result

The results of purification of bacterial samples and gram staining can be seen in table 5.2 below:

Table 1 Bacterial Purification Type

Sampling	gram positive		gram negative	
	n	%	n	%
stair railings	3	25%	3	21%
door handle	3	25%	4	29%
elevator button	2	17%	4	29%
public seat	4	33%	3	21%
total	12	100%	14	100%

Source: primary data, 2023

Based on table 1, it is known that the proportion of gram-negative bacteria (14 samples) is greater than the gram-positive bacteria, namely 12 samples, and the total colonies found in 16 public places, namely 26 bacterial colonies that have been purified.

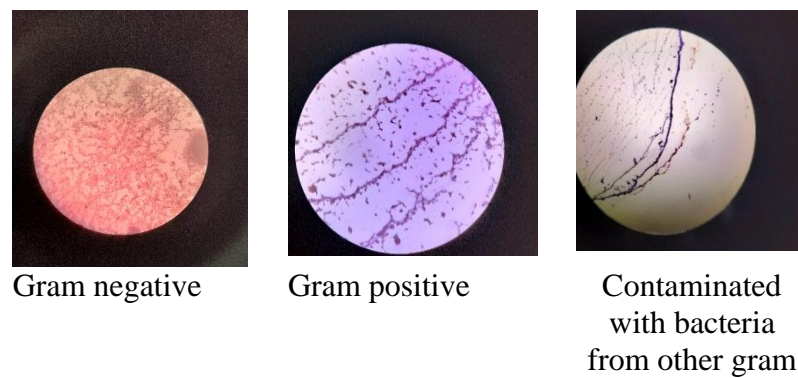


Table 2 Antiseptic Bkc 0,2%, Bkc 0,12% And Alcohol 70% Against Bacterial Inhibition Based On Turbidity

Exposure	Mean	Maximum	Minimum	Std. Deviation
70% alcohol	10.31%	79%	-62%	28,876
benzalkonium 0,12%	61.88%	92%	26%	19,413
benzalkonium 0,2%	96.13%	98%	91%	1,502
control	- 179.13%	-546%	-19%	179,992

Based on the results of liquid dilution to see the inhibitory power of the antiseptic against bacterial growth, the decrease in turbidity which showed the highest bacterial killing rate occurred in 0,2% benzalkonium chloride which was 96.13%, with the control treatment the average growth of bacteria based on turbidity was 179.13% for 24 hours with the highest maximum growth of 546% .

The maximum decrease in bacteria exposed to antiseptics occurred in 0,2% benzalkonium and the lowest was in alcohol, namely 79%, exposure to 70% alcohol still showed bacteria after exposure.

Table 3 differences in bacterial growth according to the type of antiseptic

Antiseptic Type	N	Bacterial Growth	
		+	-
Benzalkonium chloride 0,12%	130	0 (0%)	130 (100%)

Continued Table 3 differences in bacterial growth by antiseptic type

Antiseptic Type	N	Bacterial Growth	
		+	-
Benzalkonium chloride 0,2%	130	0 (0%)	130 (100%)
70% alcohol	130	14 (10,7%)	118 (89,3%)
Control	130	130 (100%)	0 (0%)

Based on the results of the solid dilution test used on 26 bacterial samples consisting of 12 gram positive bacteria and 14 gram negative bacteria, each sample was tested on 4 treatments namely the control group, 70% alcohol, 0,12% benzalkonium and 0,2 benzalkonium % repeated in 5 repetitions obtained from 130 test samples 10.7% bacteria exposed to 70% alcohol regrowth after 24 hours of exposure, based on

the experimental results of all samples exposed to benzalkonium at concentrations of 0,12% or 0. 2% no regrowth occurs. However, based on the minimum killing rate (MBC), benzalkonium 0,12 is the MBC in the results of this study with the lowest concentration of killing.

Discussion

Based on the results of an experimental study conducted on 26 samples with details of 14 gram-negative strain samples and 12 gram-positive samples, it can be seen the difference in bacterial yields after incubation by the three exposures in the sample absorption liquid dilution test with exposure to the antiseptic Benzalkonium Chloride with a concentration of 0,2% and 0,12% has a higher level of inhibition when compared to samples exposed to 70% alcohol antiseptic.

Determination of MIC by turbidimetric test is carried out by observing the turbidity of the solution in the tube, not paying attention to the color density of the solution in the tube. Bearing in mind, the higher the concentration of the color of the solution, it means that the greater the level of inhibitory activity on the growth and development of bacteria that appears in the clearer the solution. Analysis using UV/VIS spectrophotometry is a conventional method that can be used in the analysis of bacteria, enzymes, proteins, nucleotides, plasmids in addition to the purity of raw materials such as ethanol and sucrose, with a UV/VIS spectrophotometer wavelength commonly used 200-800 nm which can produce values turbidity absorption.⁽¹⁶⁾

In the solid dilution test on colony changes between before and after incubation with a maximum time of 24 hours, samples exposed to antiseptic Benzalkonium Chloride with concentrations of 0,2% and 0,12% had a decrease in growth to 0 which can be interpreted if the antiseptic has a killing rate. when compared with exposure to an antiseptic alcohol content of 70% which has a less significant change because the bacteria tested from 130 samples experienced regrowth of 14 samples. Whereas for the Man Whitney test where the test was carried out to find out the differences in the samples at each exposure, it produced data showing that in samples with 0,2% and 0,2%

Benzalkonium Chloride exposure, and based on the study of al hassan, et al stated that hand sanitizers made from benzalkonium chloride showed antibacterial activity against nine out of eleven gram-positive and gram-negative bacterial strains, while the acceptance study of the ten participants showed no signs of skin irritation or redness on its application. Therefore, this non-alcoholic based hand sanitizer was suggested as a potential alternative to alcohol based hand gels⁽¹⁴⁾.

Benzalkonium chloride is a cationic surfactant preservative of the quaternary ammonium group which has antibacterial properties, both for gram-positive and gram-negative bacteria. However, skew is more effective on gram positive bacteria. The antibacterial activity of quaternary ammonium derivatives depends on the charge density of the asymmetric N atom and the size and length of the nonpolar chain attached to the N atom. The longer the nonpolar chain, the activity increases. The mode of action of this class of preservatives is adsorption and penetration of the agent into the cell wall, then reacting with the cytoplasmic membrane (proteins and lipids) followed by membrane derangement. Cells that are in 2 membranes experience leakage and degradation of proteins and nucleic acids. The wall undergoes lysis caused by autolytic enzymes. Thus the loss of structure and integrity of the cytoplasm in bacteria which causes damage to other bacterial parts. While the mechanism of action of Benzalkonium chloride itself is to work actively on the surface of the cell by breaking down the fat on the cell membrane, the structure of the membrane becomes damaged and results in the death of germs.

The antimicrobial ability of this alcohol is to denature microbial proteins and this antimicrobial activity is effective when diluted with water around 70-80%. Higher concentrations of alcohol can reduce its ability to denature bacterial proteins because the denaturation process requires water, alcohol has an antimicrobial effect but only works on short acting, not long acting, so it is not persistent. However, the results of several studies revealed that the use of a combination of alcohol and triclosan or other antimicrobials causes persistent antimicrobial effects.

Conclusion

Results from observations of MIC (minimum inhibitory concentration)/minimum inhibitory level and MBC (minimum bactericidal concentration)/minimum killing rate on gram-positive bacteria and gram-negative bacteria, namely 0,12% benzalkonium, showed 0,12% benzalkonium is more effective as an inhibitor and kills gram positive and negative bacteria in the environment than 70% alcohol.

It is hoped that the results of this study can add information regarding chemical compounds that can be an alternative choice for hand sanitizers other than alcohol which are effective in preventing the transmission of germ-related diseases, which are less irritating and more economical than alcohol.

Based on the results of this study, further studies should be carried out regarding variations in the concentration of benzalkonium chloride against bacteria, and irritation tests should be carried out on users so that they can be used safely.

Acknowledgement

Thanks to Dr. dr. Budi Laksono, MHSc and Prof. Dr. dr. Suhartono, M.Kes who has guided this research to completion and Our thanks to JEKK for allowing us to modify templates they had developed.

References

1. Joegijantoro R. Penyakit Infeksi. 1st ed. Malang: Intimedia; 2019. xviii + 218 halaman.
2. Ameri S, Nasrollahi SA, Samadi A, Amiri F, Ahmadvand S, Yadangi S, et al. Assessment of skin microbiota and biometric parameters: a comprehensive comparison of four types of hand cleansers. *Iran J Dermatology*. 2021;24(4):306–14.
3. Price L, Gozdzielewska L, Alejandre JC, Jorgenson A, Stewart E, Pittet D, et al. Systematic review on factors influencing the effectiveness of alcohol-based hand rubbing in healthcare. *Antimicrob Resist Infect Control* [Internet]. 2022;11(1):1–22. Available from: <https://doi.org/10.1186/s13756-021-01049-9>
4. Bawankar V, Sawarkar G. Overview of sanitizer usability in COVID-19 pandemic scenario. *Indian J Forensic Med Toxicol*. 2020;14(4):6636–40.
5. CDC. Hand hygiene recommendations: guidance for healthcare providers about hand hygiene and COVID-19. 2021; Available from: <https://www.cdc.gov/handhygiene/providers/guideline.html>
6. CDC. Healthcare providers. Clean hands count for healthcare providers. Available from: <https://www.cdc.gov/handhygiene/provider/s/index.html>. Accessed May 27, 2019.
7. Bondurant SW, Duley CM, Harbell JW. American Journal of Infection Control Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application. *AJIC Am J Infect Control* [Internet]. 2021;47(8):928–32. Available from: <https://doi.org/10.1016/j.ajic.2019.01.004>
8. CDC. Hand Hygiene Recommendations Guidance for Healthcare Providers about Hand Hygiene and COVID-19. 2020;
9. Jing, J.L.J., Pei Yi, T., Bose, R.J.C., McCarthy, J.R., Tharmalingam, N., Madheswaran T. Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations. *Int. J. Environ. Res. Public Health* 17, 3326. <https://doi.org/10.3390/ijerph17093326>. 2020;
10. Golin, A.P., Choi, D., Ghahary A. Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. *Am. J. Infect. Control* 48, 1062–1067. <https://doi.org/10.1016/j.ajic.2020.06.182>. 2020;
11. Bondurant S, McKinney T, Bondurant L, Fitzpatrick L. American Journal of Infection Control Evaluation of a benzalkonium

- chloride hand sanitizer in reducing transient *Staphylococcus aureus* bacterial skin contamination in health care workers. *AJIC Am J Infect Control* [Internet]. 2020;48(5):522–6. Available from: <https://doi.org/10.1016/j.ajic.2019.08.030>
12. CDC. Hand Hygiene in Healthcare Settings. Healthcare Providers. 2021; Available from: <https://www.cdc.gov/handhygiene/%0Aproviders/index.htm>
 13. Shadman SA, Sadab IH, Noor MS, Khan MS. Development of a benzalkonium chloride based antibacterial paper for health and food applications. *ChemEngineering*. 2021;5(1):1–10.
 14. Aodah AH, Bakr AA, Booq RY, Rahman MJ, Alzahrani DA, Alsulami KA, et al. Preparation and evaluation of benzalkonium chloride hand sanitizer as a potential alternative for alcohol-based hand gels. *Saudi Pharm J* [Internet]. 2021;29(8):807–14. Available from: <https://doi.org/10.1016/j.jsps.2021.06.002>
 15. Herdt BL, Black EP, Zhou SS, Wilde CJ. Inactivation of SARS-CoV-2 by 2 commercially available Benzalkonium chloride-based hand sanitizers in comparison with an 80% ethanol-based hand sanitizer. *Infect Prev Pract* [Internet]. 2021;3(4):100191. Available from: <https://doi.org/10.1016/j.infpip.2021.100191>
 16. Ravindran A, Nirmal D, Jebalin I V BK, Pinkymol KP, Prajoon P, Ajayan J. InGaAs based gratings for UV-VIS spectrometer in prospective mRNA vaccine research. *Opt Quantum Electron*. 2022;54(9):555. doi: 10.1007/s11082-022-04002-1. Epub 2022 Jul 26. PMID: 35912403; PMCID: PMC9321284. 2022;
 17. Sun Y, Lau SY, Lim ZW, Chang SC, Ghadessy F, Partridge A, Miserez A. Phase-separating peptides for direct cytosolic delivery and redox-activated release of macromolecular therapeutics. *Nat. Chem*. 2022;14(3):274–283.