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Research Report

The effect of silver-ion water on the growth of *Streptococcus mutans*

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ABSTRACT

Background: Caries is a multifactorial disease that often occurs in the oral cavity and can be caused by *Streptococcus mutans*. Various ways have been done to reduce the number of *Streptococcus mutans*, one of them is by exposing them to a material agent such as silver-ion water. Silver-ion water has been recognized as an effective material for inhibiting several pathogenic bacteria, such as *Staphylococcus aureus* and *Escherichia coli*. Thus, there is a possibility that silver-ion water can be used to reduce the number of bacteria that cause dental caries, such as *Streptococcus mutans*. **Purpose**: To determine the effect of silver ion water on the growth of *Streptococcus mutans*. **Methods:** This research is an experimental laboratory study (in vitro) with a control group post-test-only design. *Streptococcus mutans* were exposed to 0-15 ppm silver-ion water on Brain Heart Infusion Broth (BHIB) media, then Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were observed. **Results:** Silver-ion water with a concentration of 12.5 ppm was determined as MIC, and silver-ion water with a concentration of 15 ppm as MBC. **Conclusion:** Silver ion water is adequate as an antibacterial material in inhibiting (12.5 ppm) and killing (15 ppm) *Streptococcus mutans*.

Keywords: *Streptococcus mutans*; silver ionic water; MIC; MBC; Human & health

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INTRODUCTION

Caries is one of the most common diseases in the oral cavity in both children and adults, [1] where the enamel is demineralized by bacteria that produce acid and colonize to form biofilms.[2,3] The World Health Organization (WHO) reports that 60-90% of school children worldwide had caries. In addition, the results of the Basic Health Research in 2018 also mentioned that as many as 93% of early childhood in Indonesia had caries.[4] Due to the caries rate is still high, research on caries is still needed.

Streptococcus mutans is a facultative anaerobic Gram-positive recognized as the one of the main bacteria that can cause caries.[5,6] *Streptococcus mutans* is able to metabolize sucrose to produce acid and, through the enzyme glucosyltransferase (Gtf), convert sucrose into extracellular polymer glucan, which promotes biofilm formation through cellular attachment to the tooth surface and other oral microorganisms. *Streptococcus mutans* can metabolize polysaccharides into organic acids (acidogenicity) and can survive in acidic environments with low pH conditions (acidurity).[7]

Silver-ion water has been recognized as an effective material for inhibiting and killing bacteria. This nanotechnology is emerging as a rapidly growing field with many biomedical science applications and as an antimicrobial and disinfectant material that is relatively free of side effects. Silver nanoparticles have a broad spectrum of antibacterial, antifungal, and antiviral properties. In dentistry, silver nanoparticles are used to develop antibacterial materials to improve the quality of dental material for better treatment results.[8]

According to Bruta, et al. 2021, silver can reduce the number of *Staphylococcus aureus, Escherichia coli,* and this material is very effective in reducing the number of pathogenic bacteria. The potential of silver nanoparticles (AgNPs) as antibacterial materials is related to various mechanisms in inhibiting and killing various types of bacteria.[9] Thus, silver-ion water can also be used to reduce the number of bacteria that cause dental caries. There are

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some research that revealed the effect of silver ion on oral *streptococci*, however there is still limited experiment that explain the effect of silver ion water on *Streptococcus mutans* serotype C UA 159. Hence, this research aimed to reveal the effect of silver-ion water as an antibacterial material against the growth of *Streptococcus mutans*. We hope many researches reveals the effect of silver ion water may provide this potential product to reducing dental caries risk.

MATERIALS AND METHODS

This research is an experimental laboratory research (in vitro) with a control group post-test-only design with a sample size per group was 4 samples and conducted in Research Centre Faculty of Dental Medicine Universitas Airlangga. The instruments used in this study are test tubes (Pyrex, Japan), test tube racks, incubators, autoclaves, Petri dishes, oases, matches, and micropipettes. The materials used in this study are *Streptococcus mutans* serotype C strain UA 159 from the Research Center Faculty of Dental Medicine, Universitas Airlangga, silver ion water (Aquasil), Brain Heart Infusion Broth (BHIB), Mueller Hinton Agar (MHA) media, sterile distilled water.

One oase of *Streptococcus mutans* was taken, inoculated on BHIB media, and incubated for 24 hours at 37°C under anaerobic conditions.[10] The density of *Streptococcus mutans* was observed using the Mc Farland 0.5 standard (1.5 x 108 CFU/ml) through turbidity.

Streptococcus mutans were cultured in BHIB tubes containing 0-15 ppm silver-ion water and tubes containing BHIB (negative control) only, then incubated for 24 hours at 37°C anaerobically. The turbidity of the bacteria on the tube was observed visually to determine the Minimum Inhibitory Concentration (MIC). A total of 0.1 ml of solution in a clear tube was inoculated on Mueller Hinton Agar (MHA) media, leveled using a spreader, then incubated for 24 hours at 37°C anaerobically. Media with no bacteria with the smallest silver-ion water concentration is considered as the Minimum Bactericidal Concentration (MBC).

The data from this study were analyzed using tables and graphs where MIC can be determined as the smallest concentration of silver-ion water exposed to bacteria marked by clear bacterial tubes, and MBC is determined from the smallest concentration of silver-ion water exposed to bacteria marked by the absence of bacterial growth on MHA media, Moreover, MBC data also analyzed by Kruskall-Wallis and Mann Whitney test. P value <0.05 was considered statistically significant.

RESULTS

This study is a laboratory experimental study to determine the antibacterial effectiveness of silver-ion water against microbial growth of *Streptococcus mutans* with several concentrations, 0 ppm, 2.5 ppm, 5 ppm, 7.5 ppm, 10 ppm, 12.5 ppm, 15 ppm, and 1 control group (distilled water). The antibacterial effect was tested using the dilution method to determine MIC and MBC.

Table 1 showed that the silver ion-water tubes 0, 2.5, 5, 7.5, 10 ppm were turbid, while the silver ion-water tubes 12.5, 15 ppm, control (aquades only in BHIB) the tubes were clear. Based on these results, the lowest concentration seen as a clear tube (no turbidity) starts from the concentration of 12.5 ppm silver ion water.

Based on Figure 1 and 2, there were a high number of *Streptococcus mutans* colonies in the group not exposed

Table 1. Minimun inhibitory concentration test of silver-ion water on the growth of *Streptococcus mutans* in BHIB

No	Groups	Result	
		Clear	Turbid
1	Silver-ion water 0 ppm		√
2	Silver-ion water 2.5 ppm		√
3	Silver-ion water 5 ppm		√
4	Silver-ion water 7.5 ppm		√
5	Silver-ion water 10 ppm		√
6	Silver-ion water 12.5 ppm	√	
7	Silver-ion water 15 ppm	√	
8	Aquades*	√	

*Group 8 without *Streptococcus mutans*.

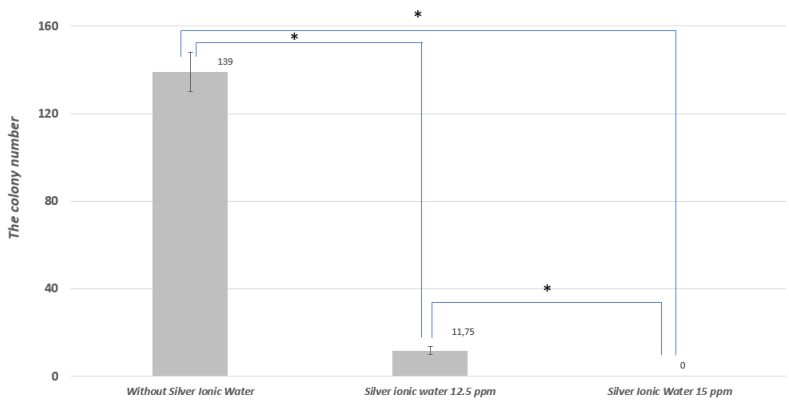

Figure 1. The effect of silver-ionic water on the colony numbers of *Streptococcus mutans* to determine Minimum Bactericidal Concentration. Asteriks indicate the significant differences (*p<0.05).

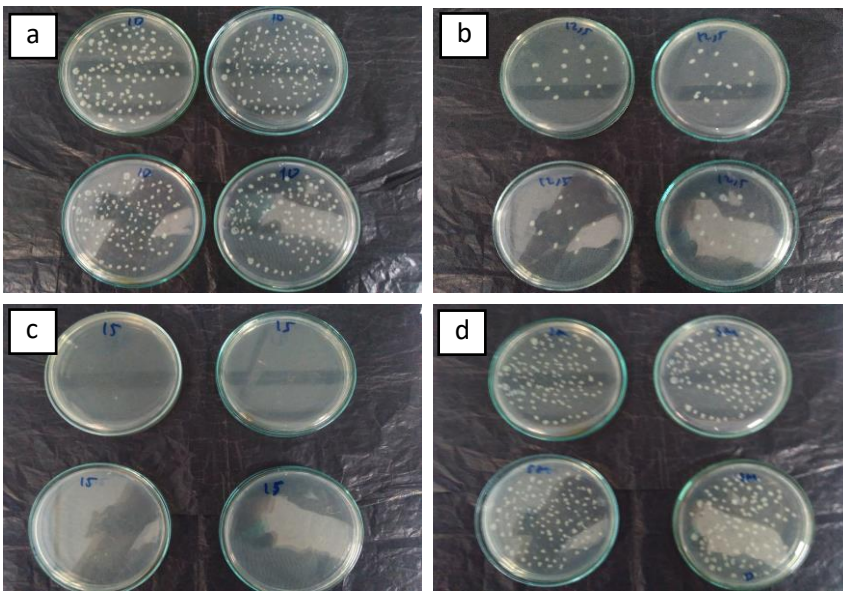

Figure 2. Bacterial colony of *Streptococcus mutans* after exposed silver ionic water 10 ppm (a), 12 ppm as Minimum Inhibitory Concentration (MIC) (b), 15 ppm as Minimum Bactericidal Concentration (MBC) (c) and without exposed silver ionic water (d).

to silver-ion water (0 ppm), as much as 139. While in the group exposed to 12.5 ppm silver-ion water, there was a low number of *Streptococcus mutans* colonies, with an average of 11.75. While in the group exposed to silver-ion water at 15 ppm, no growth of *Streptococcus mutans* colony was found. Hence, the lowest concentration of silver-ion water that causes no colony growth is 15 ppm. In addition, there are significant differences between groups not exposed to silver-ion water (0 ppm) with the groups exposed to 12.5 ppm and 15 ppm silver ion water ($p<0.05$), as well as between groups exposed to 12.5 ppm silver ion water with 15 ppm silver ion water ($p<0.05$).

DISCUSSION

Silver-ion water (Ag^+) plays an important role in inhibiting and killing the growth of pathogenic bacteria because it can easily interfere with DNA, membrane proteins, enzymes, or intracellular cofactors in bacteria to inactivate their functions.[11] Some studies mentioned that this material is effective for treating burns, urinary tract infections, central venous catheter infections, and chronic osteomyelitis.[12] In addition, silver-ion water can be applied in the form of nanoparticles and used in various biological applications such as medication, anti-bacterial, anti-fungal, and wound healing.[13]

Silver ion water is silver particles in water with a very small size (nanometers). Silver ion water can work effectively against infections because the size of silver is smaller than pathogenic bacteria and viruses, then it can penetrate the bacterial cell wall, change the structure of the cell membrane, and even cause cell death.[8] The nanoparticle size of silver-ion water is between 5-50 nm. Due to the small size of the nanoparticles, the total surface area in direct contact with silver is maximized, then the results will be effective. Some literature mentioned that the size and shape of silver nanoparticles have particular interactions with bacteria and viruses.[14]

Our results showed that silver-ion water with a concentration of 12.5 ppm can inhibit the growth of *Streptococcus mutans* characterized by clear tube, and silver-ion water with a concentration of 15 ppm can kill *Streptococcus mutans* characterized by no bacterial colonies on growth media. This result is in line with Khaydarov *et al*, explained that silver ion has been shown to have antibacterial activity against gram-positive such as *Staphylococcus aureus* and *Bacillus subtilis*.[15] Other researchers reported that silver ions have been found to be effective against and kill various types of viruses, bacteria, germs, fungi, and parasites, then this material agent is potential use in various application such as periodontal disease, gingivitis, wound healing, root canal disinfection.[16–18]

The mechanism of silver-ion water in inhibiting bacterial growth is by entering through the gap in the bacterial cell wall, because silver ion water has a very small size, then attacks DNA by binding to thiol (SH).[8,19] Silver-ion water that binds to the thiol (SH) group causes inactivation of bacteria. The Thiol group is fundamental and responsible for enzymatic activity. Some literature mentions that silver ion water enters through the cell membrane gap and then attacks DNA, disrupting the synthesis process and causing replication disorders during DNA synthesis, thus damaging DNA and inhibiting bacterial growth.[19]

In this study, we observe the growth of *Streptococcus mutans*, then it is recommended to do the experiment with the other oral pathogenic bacteria and clarify more about the

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actual mechanism, how the silver ion water can inhibit the growth of pathogenic bacteria by further experiment such as metabolomic approach, and also molecular approach such as DNA, cell membrane, enzymatic activity.

CONCLUSION

Based on the results of this research, it can be concluded that silver ion water is effective as an antibacterial material in inhibiting and killing *Streptococcus mutans*. Silver-ion water with a concentration of 12.5 ppm is the MIC, and silver-ion water with a concentration of 15 ppm is MBC.

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