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The effectiveness test of 0.9m nacl solution and 0.2% chlorhexidine gluconate on bacterial growth in the oral cavity of students batch 2018 at medical faculty, Universitas Kristen Indonesia

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Abstract
Mouth rinse that is natural, safe, cost-effective, readily available and culturally acceptable is required as an adjunct to routine tooth brushing to combat dental diseases. Gargling with 0.9 M NaCl solution and Chlorhexidine Gluconate 0,2% can reduce the number of bacteria in the oral cavity. The purpose of this study was to determine the effectiveness of using 0,9M NaCl solution and Chlorhexidine Gluconate 0, 2% for decreasing the number of germs in the oral cavity of FK UKI Class 2018 students. This study was conducted experimentally by wiping the oral cavity of students before and after treatment. Thirty-two student samples met the inclusion criteria. The results showed quantitatively, Chlorhexidine Gluconate 0.2% could reduce the number of bacteria by 89.25%, while 0.9M NaCl solution was only able to reduce the number of bacteria by 27.05%, with the results of T dependent test p <0.005, which shows both solutions are effective in reducing the number of germs in the oral cavity. The results of the identification of this study found positive Gram round bacteria. The conclusion from this study shows that both types of mouthwash can significantly reduce the number of bacterial colonies in the mouth, and saltwater rinse can be used as an adjunct to routine mechanical plaque control for the prevention of oral diseases.

Keywords: mouthwash, chlorhexidine gluconate 0, 2%, NaCl 0,9M, bacterial, dental caries

Introduction

The oral cavity is the easiest part of the body for bacteria to colonize. Bacteria are single-celled microorganisms that can live as independent organisms (free-living) or parasites (dependent on other organisms for life). Bacteria in the oral cavity will ferment carbohydrates and then produce acids that lower the pH. A decrease in pH below 5.5 can cause bone demineralization in the tooth matrix, which causes dental caries [2; 3]. The dominant cariogenic microorganism in the oral cavity is Streptococcus mutans. In addition, there are also Lactobacillus organisms that have an impact on the formation of caries. Streptococcus plays a role in the early stages of caries by damaging the outer enamel, and then Lactobacillus will take over the role in deep caries [4].

The 2016 Global Burden of Disease Study estimated that oral disease affects at least 3.58 billion people worldwide, with dental caries being the most common of all conditions assessed. Globally, an estimated 2.4 billion people suffer from dental caries, and 486 million children suffer from caries in milk teeth. In most low- to middle-income countries and with increasing urbanization and changing living conditions, the prevalence of oral disease continues to increase mainly due to inadequate fluoride exposure and poor access to primary oral health care services. According to 2013 Riskesdas, there was an active caries prevalence increase throughout Indonesia by 9.8% in 2013 compared to 2007. Almost all provinces in Indonesia experienced an increase in active caries in 2013. The prevalence of caries in South Sulawesi increased, 29.1%, but the highest caries prevalence was in West Kalimantan, 71.7%. The province with the lowest caries prevalence was West Nusa Tenggara, 31.1% and only increased by 0.5% from 2007 [6]. Many ways can prevent cariogenic microorganisms that cause caries, one of which is using mouthwash.

Mouthwash is an antiseptic liquid preparation usually used to clean the mouth and teeth or freshen breath [1]. The efficacy of various commercially available mouthwashes has been tested and proven. The majority of over-the-counter alcohol-based mouthwashes have strong side effects. However, affordability for daily use of mouthwash, especially in remote areas, is still quite tricky. Mouthwashes that are natural, safe, cost-effective, readily available, and culturally acceptable are essential for promoting oral health in Indonesia.

Saltwater has been used by countries such as Egypt, Greece, and China to treat gum disease since 2700 BC. Using saltwater as a mouthwash can improve the pH balance in the mouth, thus making the environment more alkaline [7]. Bacteria can thrive in an acidic environment so that saltwater can prevent the growth of bacteria, especially in the mouth. Research conducted by Aravinth et al. stated that gargling with a salt solution with a minimum concentration of 0.8 M could reduce the number of bacteria in the mouth. This study used 0.8 M NaCl solution as the treatment group and 0.2% Chlorhexidine as the control group.

Salt can promote healing, reduce inflammation, and ease swelling, and it does not irritate mucous membranes, unlike over-the-counter mouthwashes. Saltwater is also isotonic, which means it contains the same salts and minerals as our bodies and in the same concentrations. Thus, this study was planned to investigate whether saltwater effectively reduces the number of bacteria present in the mouth. Chlorhexidine was the most commonly used anti-plaque and caries agent and was therefore used to control for comparison. The study aimed to assess and compare the effectiveness of a saltwater rinse with a chlorhexidine mouth rinse in reducing oral disease that causes microbial counts in the mouth.

Based on the background described above, several problems can be formulated, including the following: a) How is the effectiveness of gargling with 0.9 M NaCl solution and

0.2% Chlorhexidine Gluconate solution to decrease the number of bacteria in the oral cavity?; b) How is the difference in effectiveness between 0.9M NaCl solution and 0.2% Chlorhexidine Gluconate in reducing the number of bacteria in the oral cavity? With the aim of research to determine the effectiveness of 0.9M NaCl solution to decrease the number of bacteria in the oral cavity and compare it with mouthwash Chlorhexidine Gluconate 0.2%.

Literature Review

Plaque is a collection of bacteria that is tightly attached to the tooth surface [5]. Dental plaque is a soft layer consisting of microorganisms that adhere tightly to the tooth surface and is colourless. Plaque is the leading cause of cavities (dental caries) and inflammation of the gums (gingivitis). Based on its location on the gums, dental plaque is divided into two types, namely supragingival plaque and subgingival plaque. Supragingival plaque is located above the edge of the gum, which consists of two layers, namely the outer layer and the inner layer. The inner layer contains many Gram-negative bacteria, and the outer layer contains many Gram-positive bacteria. Subgingival plaque is located below the gum edge, between the tooth and the gum sulcus wall and the periodontal pocket [8].

Factors that influence the formation of dental plaque are the physical environment, fiction, or friction by chewed food and the influence of diet [9]. The process of formation of dental plaque begins with the formation of a pellicle on the tooth surface. The pellicle is a thin deposit of salivary glycoproteins on the tooth surface that forms a few seconds after brushing the teeth. After pellicle formation. Streptococcus mutans, Streptococcus Bovis, Streptococcus sanguis, and Streptococcus salivarius proliferate, forming a matrix consisting of extracellular polysaccharides such as levan and dextran of attachment of bacteria to the tooth surface will occur. Plaque is getting thicker because of the bacteria adhesion to the plaque's outer surface, which causes the phenomenon of attachment of bacteria other than the Streptococcus group or what is often referred to as the "succession phenomena". The older the plaque in the mouth, the environment in the plaque will become anaerobic [10].

Dental plaque can cause odontogenic infections (periodontal abscess), complicated tissue abnormalities (dental caries), and tooth-supporting tissue abnormalities (gingivitis and periodontitis). Dental caries dissolves the enamel and root surface (demineralization) by acids resulting from carbohydrate metabolism fermented in the diet by bacteria that colonize the tooth surface [11]. Dental caries is caused by teeth (host), substrate, time, and microorganisms. The dominant cariogenic microorganism in the oral cavity is Streptococcus mutans. In addition, there are also Lactobacillus organisms that have an impact on caries formation [12]. Streptococcus plays a role in the early stages of caries by damaging the outer enamel, and then Lactobacillus will take over the role in deep caries [13].

Much can be done to prevent caries. Knowing the cause is essential to understand how to prevent it. Improving dental health has become the main goal in dentistry since it is known that dental plaque is a factor that dominates the cause of tooth loss due to caries and periodontal disease. Plaque formation on the tooth surface can be limited either by preventing or regularly cleaning plaque. Plaque control can be done by mechanically cleaning plaque and chemical processes with antibacterial agents, significantly suppressing S. mutans. Brushing teeth is a mechanical method, and gargling with mouthwash is a chemical method for controlling plaque control and is the first step to control caries and periodontal disease. Currently, plaque prevention is equipped with active ingredients that contain natural or synthetic ingredients as antibacterial agents. These antibacterial ingredients are available in the form of toothpaste and mouthwash [14].

Bacteria is a prokaryotic microorganism (does not have a nuclear membrane) and reproduces by dividing. Bacteria divide in a short time. Most bacteria divide within hours or days. Under favourable conditions, it duplicates every 20 minutes. In general, bacteria are 0.5-5 m in size, but certain bacteria can be up to 700 m in diameter. Bacteria are the most abundant microbes in the oral cavity. It is estimated that there are more than 100 million bacteria per ml of saliva with more than 600 different species [15]. The mouth and throat are places where there is a large population of microorganisms. Wet and warm mucous membranes are good places for microbial growth. Bacteria found in the oral cavity are usually beneficial, including the diphtheroid, bacteroids, lactobacilli, and micrococci. Streptococcus and Staphylococcus are potential pathogens and are associated with oral disease. In children, with the development of teeth, various strains of Streptococcus appear. Some strains favour growth sites on tooth surfaces, including Streptococcus sanguis and Streptococcus mutans, while others adhere to the oral and gingival epithelial surfaces, namely Streptococcus salivarius [12].

Some bacteria in the oral cavity include a) Lactobacillus appears in the oral cavity in the first year of a child's life. Lactobacillus is a gram-positive facultative anaerobic bacterium in the form of a bacillus. These bacteria ferment carbohydrates into acid and survive in an acidic environment [16; 17]; b) Streptococcus is a heterogeneous group of bacteria. Streptococcus is a gram-positive bacterium with a spherical shape, a distinctive shape/arrangement like a chain during its growth period. This bacterium is widely distributed in nature. These bacteria produce various extracellular substances and enzymes; c) Actinomyces is gram-positive, anaerobic, acid-resistant, does not form spores, grows slowly to form long, branching filaments, and is non-motile. Most Actinomyces bacteria are normal flora, but they will become pathogenic bacteria if found in areas, not their habitat. Types Actinomyces Israeli, Actinomyces viscosus, Actinomyces naeslundii, and occasionally Actinomyces odontolyticus [16].

Factors that affect the growth of bacteria in the mouth: a) The drugs referred to here are any drugs that can affect the immune system. Oral antiseptic drugs and systemic antibiotics can reduce the number of bacteria in the oral cavity [5]; b) Age affects the complexity and distribution of normal oral flora. Generally, the complexity of the oral flora increases with age. At birth, the oral cavity is sterile, then contaminated by bacteria from the mother. In adolescence, the normal flora of the mouth reaches its peak of complexity [5]; c) Several diseases, dental and oral diseases and systemic diseases affect the secretion of saliva. Disorders of the salivary glands (such as application, hypoplasia, atrophy), diabetes mellitus, impaired kidney function, nervous system disorders such as multiple sclerosis, diarrhoea, fever and inflammation result in decreased salivary secretion. In addition to the above diseases, decreased salivary secretion can also be caused by emotional disorders, vitamin deficiency and hormonal changes.

In contrast, Parkinson's disease increases salivary secretion [5]; d) The use of dental prostheses can reduce the amount of

saliva in 10-40% of dental prosthesis wearers. In addition to experiencing a decrease in saliva, some dental prostheses users also experienced an amount increase in saliva at the beginning of use. Dental prostheses also cause the accumulation of food debris which can become food for bacteria [5]; e) Life habits of a person affect the secretion of salivary glands. Chewing gum and hard foods can cause mechanical stimulation. Smoking, consuming foods and drinks that are too acidic, too alkaline, or contain alcohol can cause chemical stimulation [6]; f) Dental and oral hygiene varies for each individual depending on the ability to maintain it, both in terms of time, frequency, and method. If oral hygiene is not maintained, the remaining food and epithelial remnants in the oral cavity will become good nutrients for bacteria [5]; and g) Foodstuffs containing much sucrose left in the mouth can be easily fermented by bacteria, thereby potentially increasing bacterial growth. Mineral deposits of food residue mixed with saliva, especially on the back teeth and crowded teeth, accumulate bacteria that later form tartar [5].

The use of mouthwash aims to inhibit the growth of bacteria because it functions as an antiseptic that has antibacterial properties. Antiseptics are compounds that can inhibit the growth or reproduction of bacteria without destroying the whole. Mouthwash, based on its benefits, is divided into two types, namely as a cosmetic that provides freshness to the mouth and breath, cleaning, removing bad breath and as a treatment for the treatment of diseases of the mucosa or gingiva, prevention of dental caries, dental and oral care and periodontal disease. Another benefit of mouthwash is that it can reach the most difficult areas to clean with a toothbrush, so using a toothbrush alone is not enough to clean the oral cavity perfectly [11].

Chlorhexidine or Chlorhexidine Gluconate (CHG) is a disinfectant and antiseptic used for skin disinfection before surgery and for sterilizing surgical instruments [12]. Chlorhexidine Gluconate is also used to clean wounds, prevent dental plaque, treat fungal infections in the mouth, and keep urinary catheters from becoming clogged [13].

Possible side effects include skin irritation, tooth discolouration, and allergic reactions [13]. It can cause eye problems in direct contact. Use in pregnancy appears to be safe. Chlorhexidine Gluconate is also effective against various microorganisms but does not inactivate spores [18]. Chlorhexidine Gluconate is active against gram-positive, and gram-negative organisms, facultative anaerobes, aerobes and yeasts and this substance is very effective against Gram-positive bacteria (in a concentration of 1 g/l). Significantly higher concentrations (10 to more than 73 g/ml) are required for Gram-negative bacteria and fungi. Chlorhexidine Gluconate (CHG) is not effective against poliovirus and adenovirus [12].

Using a CHG-based mouthwash in regular dental care can help reduce plaque build-up and improve mild gingivitis. Such mouthwashes also have several side effects, including damage to the lining of the mouth, discolouration of teeth, tartar build-up, and taste disturbances [14]. Extrinsic tooth staining occurs when a chlorhexidine rinse has been used for four weeks or more [19]. Chlorhexidine contains cations that interact with the anionic components of toothpaste, such as sodium lauryl sulfate and sodium monofluorophosphate, and form salts with low solubility and antibacterial activity.

The term salt is an ancient word that has several variations in English and related languages. The name for the crystallization of sodium chloride is Halite, taken from the Greek word hals which means salt. The name of this mineral was given by E.F Glocker in 1847 [15].

In chemical use, salt can refer to other compounds of metals and nonmetals, so words like "copper salt" or "magnesium salt" refer to chlorides, carbonates, sulfates and others, of copper or magnesium [15]. Salt for human consumption comes in different forms: coarse salt (such as sea salt), refined salt (table salt), and iodized salt. It is solid and crystalline, white, light pink, or grey produced from seawater [15]. Chloride and sodium ions are the two most abundant components in salt, needed by all living things in trace amounts. Salt is involved in the regulation of water content in the body. However, too much salt increases the risk of health problems, including high blood pressure [15].

Sodium chloride, also known as table salt, is the chemical compound with the molecular formula NaCl, representing a 1:1 sodium and chloride ions ratio. With molar masses of 22.99 and 35.45 g/mol, 100 g NaCl contains 39.34 g Na and 60.66 g Cl. These compounds are the salts that most influence the salinity of the ocean and extracellular fluids in many multicellular organisms. As the main component of table salt, sodium chloride is often used as a seasoning and food preservative.

The formation of water-insoluble glucan from sucrose is the most significant factor in accumulating streptococcus mutants on soft surfaces. The formation of water-insoluble glucan by extracellular glucosyltransferase from Streptococcus mutans 6715 was strongly stimulated by various mono- or divalent cations. The enzyme preparation contained in fractionated ethanol can catalyze the formation of water-insoluble glucan [19]. Streptococcus mutans has been considered a significant cause of dental caries in humans. The synthesis of water-insoluble glucan (WIG) from sucrose by glucosyltransferase from Streptococcus mutants has been considered essential in caries development. The extracellular activity of WIG-GTase by PS-14 strain was influenced by salt, although the role of salt in GTase activity is not well understood. The salt effect may be due to perturbation and increased cell membrane permeability, altered GTase, enzyme stabilization, and release of cell boundary GTase [17].

Research Method

This study was experimental with a pre-test and post-test-controlled group design because the authors compared the two results after the treatment was given in this study. This research was conducted at the Microbiology Laboratory of the UKI Medical Faculty. The research was carried out from July 2019 to September 2019. The target population in this study were all students of the UKI Medical Faculty. The affordable population in this study were FK UKI students Batch 2018. The determination of the number of samples in this study was calculated using the Federer formula:

$$(n-1).\ (t-1) \geq 15$$

Information
N: Sample size
T: Number of Groups
(N-1) (T-1) 15
(N-1) (2-1) 15

N 16 (the number of samples is at least 16 people in each

treatment) The tools and materials used in this research are markers, Bunsen fire, laboratory glassware, scales, incubator, microscope, sterilizer, stopwatch, mouthwash containing 0.2% Chlorhexidine Gluconate, 0.9M NaCl solution, sheep's blood, distilled water, Disinfectant, Alcohol 96%, Crystal purple carbol, Lugol, Fuksin Water, Emergence oil, Xylol. The stages in this study are as follows: a) Making a 0.9M NaCl solution following the following formula

$$M = \frac{g}{Mr} \; x \; \frac{1.000}{mL}$$

Information
M: Molarity
g: Mass (grams)
Mr: Molecular weight (NaCl = 58.44 mol)

For 1 litre of NaCl solution with a concentration of 0.9 M, the weight of the dissolved salt is 52.6 grams. How it works: 52.6 grams of coarse salt dissolved in 1000 ml of warm water.
Preparation of blood agar media The blood agar media prepared in this study were produced by Oxoid Ltd. So the method of making blood agar media refers to the standards that have been given. Method of preparation: a) Nutrient Agar as much as 8 grams is dissolved in 400 ml of distilled water; b) Then the agar material is heated in an Erlenmeyer until the medium is completely dissolved; c) The media was sterilized using an autoclave at a pressure of 1 atm and a temperature of 121˚ C for ± 15 minutes; and d) After the temperature of the media dropped until it felt lukewarm, 17 ml of sheep's blood was added to the Erlenmeyer. Then the media was poured into sterile Petri dishes and allowed to freeze. Production of MSA (Mannitol Salt Agar) media - Oxoid Ltd produced the blood agar media prepared in this study. So the method of making blood agar media refers to the standards that have been given. The materials used consisted of 10 grams of peptone, 10 grams of mannitol, 15 grams of agar, 75 grams of sodium chloride, 0.25 grams of Phenol red. Method of manufacture: a) The material is dissolved in 500 ml of distilled water, then heated until the material is completely dissolved; b) The media was sterilized using an autoclave at a pressure of 1 atm and a temperature of 121˚ C for ± 15 minutes; c) The media is cooled until it feels lukewarm, then poured into a sterile petri dish; and d) The media is allowed to freeze (to solidify).
Stages of labour; a) A total of 32 students were divided into two groups, each consisting of 16 people in group A (gargling with 0.9M NaCl solution) and treatment group B (rinsing with Clorehexidine Gluconate 0.2%); b) Groups A and B rinse their mouths with distilled water for 30 seconds; b) Then both groups rinsed their mouths with sterile mouthwash solution for 30 seconds; c) After that, do a swab around the oral mucosa with a sterile swab; d) The sterile swab was then polished into blood agar media (KA code for group A, and KB for group B); e) Labels are given according to the serial number of the sample, for example, KA1, KB1, etc.; f) So that the blood is incubated in an incubator for 24 hours, at a temperature of 37oC for the growth of germs; g) Furthermore, observing and counting the number of colonies on the blood agar plate; h) Gram stain test is performed, to determine the type of bacteria; and g) If the identification results show gram (+), continue with planting on MSA media. After the data is collected, data processing is carried out with the following steps: editing, coding, entry and cleaning.
The analysis technique of this research uses SPSS (Statistical Package for the Social Science). The first test used was the Shapiro Wilk normality test to determine whether the two data were normally distributed. If the data were not normally distributed, the Wilcoxon test was carried out, which aims to determine whether there is a decrease in the number of germs from the two treatments and test whether the hypothesis is accepted. Then a descriptive test was conducted to determine the average number of germs in the oral cavity before and after treatment, and then a paired T-test was performed to determine whether there was a significant difference between the treatment group with 0.9M NaCl and the treatment group with 0.2% Chlorhexidine Gluconate.

Result and Discussion
This research was conducted from August 2019 to September 2019 at the UKI Medical Faculty microbiology laboratory with a sample of 32 UKI Medical Faculty students in 2018.
All research samples met the inclusion criteria. Based on the research subject, data can be obtained, which is displayed in tabular form. Characteristics of respondents based on age at most 19 years old, namely 53.1%, followed by 18 years old 34.3%, and 20 years old 12.6%.
Characteristics of respondents based on gender 50% female and 50% male.

Table 1: Characteristics of respondents

Gender	n	%
Male	16	50%
Female	16	50%

In the group gargling with 0.9M NaCl solution, the average number of bacteria before treatment (pre-test) was 265.18 ± 112.45, while in the gargling group, 0.2% Chlorhexidine Gluconate solution was 162.25 ± 110.87. The test results showed a statistical difference in the number of bacteria between the two groups before treatment (p=0.014).

Table 2: The average number of bacteria before treatment (pre-test)

Treatment Group	Average number of bacteria before treatment (mean±SD)	p-value
NaCl 0.9M	265,18 ± 112,45	*p = 0,014*
Clorehexidine Gluconate 0.2%	162,25 ± 110,87	

In the group gargling with 0.9M NaCl solution, the average number of bacteria after treatment (pre-test) was 104.59 ± 118.29, while in the gargling group, 0.2% Chlorhexidine Gluconate solution was 17.44 ± 18.21. The test results showed that either NaCl 0.9 M or Chlorhexidine Gluconate 0.2% effectively reduced germs in the oral cavity.

Table 3: Average number of bacteria before and after gargling with 0.9M NaCl solution and 0.2% Chlorehexidine Gluconate

Treatment Group	Average number of bacteria (mean±SD)		P-value
	Before (mean ± SD)	After (mean ± SD)	
NaCl 0,9M	265,18 ± 112,45	104,59 ± 118,29	*p=0.001*
Clorehexidine Gluconate 0,2%	162,25 ± 110,87	17,44 ± 18,21	*p=0.000**

The results of this study indicate that gargling with Chlorhexidine Gluconate 0.2% or NaCl 0.9 M mouthwash effectively reduces the number of bacteria in the oral cavity because the number of bacteria in the oral cavity decreases significantly, which is proven by the test. Statistical t-paired test with the results of Sig.(2-tailed) p = 0.000, (p<0.05) for gargling treatment with Chlorhexidine Gluconate 0.2% and Sig.(2-tailed) p = 0.001, (p<0.05) for treatment with 0.9 M NaCl. Quantitatively, 0.9 M NaCl solution reduced the number of bacteria in the oral cavity by 27.05% and 0.2% chlorhexidine gluconate solution was able to reduce the number of bacteria in the oral cavity by 89, 25%.

From the results of the analysis of bacterial identification (Table 4), it was found that all the bacteria in the oral cavity samples were round (coccus) and purple (gram +). The results of bacterial culture in blood agar showed that all bacteria taken from oral samples did not hemolyze completely, and for the MSA test (Mannitol Salt Agar), only 25% of the total samples had bacteria that could be identified, which can be seen in samples A.5, A. 7, A.10, A.11, A.15, B.14, B.15, B.16.

Table 4: Results of identification of bacteria in the oral cavity

Identification	Results	n	%	Description
Form	Round	32	100%	
	stem	-	-	
Gram	+	32	100%	
	-	-	-	
MSA	+	7	25%	
	-	25	75%	

The results of the statistical test before treatment (pre-test) showed that there was a significant difference in the number of bacteria between the groups that would rinse their mouth with 0.9M NaCl (265.18 ± 112.45) and 0.2% Chlorhexidine Gluconate (162.25 ± 110.87) (table 3), and this is shown by the results of statistical tests in the form of mean comparison independent t-test, which shows that there is a significant difference (p=0.014).

The paired t-test showed a statistically significant decrease in the number of bacteria after gargling in the 0.9M NaCl solution group and the 0.2% Chlorhexidine Gluconate solution gargling group. Table 4 shows the number of bacteria in the group gargling with 0.9M NaCl solution before treatment (pre-test) was (265.18 ± 112.45) and after treatment (post-test) became (104.59 ± 118.29) with p = 0.001 (p<0.05) and in the gargling group the 0.2% Chlorhexidine Gluconate solution before treatment (pre-test) was (162.25 ± 110.87) and after treatment (post-test) it became (17.44 ± 18, 21) with p= 0.000 (p<0.05). Chlorhexidine Gluconate 0.2% was very effective in reducing the number of bacteria in the oral cavity, which quantitatively this decrease was 89.25%, and based on statistical tests (table 4) obtained significant results (p=0.000).

The bonding of chlorhexidine with molecules in the oral cavity, such as polysaccharides and glycoproteins found in saliva, will inhibit the growth of bacteria on the tooth surface and prevent dental plaque from forming. Chlorhexidine can denature proteins (glycoproteins in saliva) so that there is no adhesion between proteins with the oral mucosa and the surrounding area, including the tooth surface. Thus there will be no bacterial colonization that can cause plaque formation on the teeth [12]. The results of this study are in line with research conducted by Ajeng Destian Suparwi and Amit Parashar in the journal of the Department of Periodontic Institute of Dental Science India. Chlorhexidine Gluconate is an oral antiseptic that can inhibit bacterial growth, resulting in a decrease in the number of bacteria in the oral cavity and prevent dental plaque [21]. Amir Parahasar explained that Chlorhexidine Gluconate is an antimicrobial with a broad-spectrum effect and effectively reduces the number of gram-positive bacteria, gram-negative bacteria, aerobic bacteria, anaerobic bacteria, fungi, and viruses [22].

The results of this study are also in line with research conducted by Aravinth et al. in the journal Indian Society of Pedodontics and Preventive Dentistry in 2017. Research conducted by Aravinth et al. explains that NaCl solution can reduce the risk of bacterial formation in the oral cavity and dental plaque formation. if rinsed regularly every day and gargling with NaCl was as effective as Chlorhexidine Gluconate 0.2% This study revealed a statistically significant reduction in plaque scores (p = 0.00), S. mutans saliva (p = 0.00), L. acidophilus (p = 0.00), A. actinomycetemcomitans (p = 0.00), and P. gingivalis (p = 0.00) counted by saline rinse [12].

According to research conducted by Mukasa et al., salt can affect the number of bacteria in the mouth because the formation of water-insoluble glucan from sucrose has been the most significant factor in increasing the accumulation of streptococcus mutants on the tooth surface. The synthesis of water-insoluble glucan (WIG) from sucrose by glucosyltransferase from Streptococcus mutants has been considered essential in caries development. The extracellular activity of WIG-GTase by PS-14 strain was influenced by salt, although the role of salt in GTase activity is not well understood. The salt effect may be due to perturbation and increased cell membrane permeability, altered GTase, enzyme stabilization, and release of cell boundary GTase [17]. The salt solution has a different way of working with Chlorhexidine Gluconate to inhibit the growth of oral bacteria. In saline solutions of <0.6M concentration, the surrounding environment is hypotonic [12]. Oral bacteria can pump energy ions from adenosine triphosphate by respiratory enzymes found in the mesosomes. Water moves into the cells by osmosis, providing a favourable aqueous environment for the growth and reproduction of oral bacteria. At high concentrations of saline solution, the solute concentration in the surrounding solution is more significant than in the cytoplasm of oral bacteria. Water moves out of the cell by osmosis. Oral bacteria become dehydrated and eventually die within a minute [12].

This study obtained different numbers of bacteria caused by several factors, such as food and drinks consumed every day. Foods that contain lots of sucrose carbohydrates trigger

the growth of bacteria in the oral cavity. In addition to food, oral hygiene factors are also crucial in maintaining oral health, such as the habit of brushing teeth twice a day, in the morning after breakfast and at night before going to bed. Another thing that must be done is to brush teeth with four exactly five perfects, such as the right tool, the proper method, the right time, the right target, and mouthwash. These four rights and five perfects will prevent the accumulation of bacteria in the oral cavity, the formation of dental plaque, and dental disease.

Conclusion

Based on the results of research conducted at the Microbiology Laboratory of the UKI Medical Faculty, it was concluded that there was a significant difference between the number of bacteria before and after gargling with 0.9M NaCl solution (p = 0.001) and 0.2% Chlorhexidine Gluconate (p = 0.000). Quantitatively, Chlorhexidine Gluconate 0.2% can reduce the number of bacteria by 89.25% while the 0.9M NaCl solution can only reduce the number of bacteria by 27.05%. Dental and oral disease prevention can be done by brushing teeth twice a day, in the morning after breakfast and at night before going to bed, and gargling with 0.2% Chlorhexidine Gluconate mouthwash after brushing teeth, or using 0.9 M NaCl solution as an alternative. It is necessary to do further research using a bactericidal mouthwash containing iodine or fluoride and include a history of sample habits.

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