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**TOTAL COLONY CHARACTERIZATION OF AEROBIC BACTERIA OF THE
 ORAL CAVITY ON PEATLANDS WATER AND PDAM WATER.**

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ABSTRACT

Background: South Kalimantan peatland water is approximately 8000 km². Barito Kuala has 40,858 hectares or 90% of the area classified as peatland water. Society uses peatlands water with acidic pH(3.5-4.5) for everyday purposes including rinsing and brushing teeth. The acidic nature of peatland water can affect the growth of bacterial colonies in the oral cavity so that the acidic conditions of the oral cavity increases and the effect on the occurrence of dental caries. In addition PDAM water is water from river water that is processed through several stages to become clean water after filtration and disinfection stages to eliminate microorganisms. **Objective:** this study aims to determine the characterization of the number of colonies of aerobic bacteria of the oral cavity on peatland water and PDAM water. **Methods:** This study used quasi experimental method with post test only control group design. The study sample consisted of 30 respondents. Research materials were 4ml of a result of the salivary gargle on peatland water and PDAM water then the number of aerobic bacteria colonies were calculated by the method of TPC (Total Plate Count) and characterization of aerobic bacteria was done by using catalase test, test MSA and coagulase test. **Results:** This study showed that there were 1653 CFU/ml of aerobic aerobic colony, whereas in water of PDAM there were 1264 CFU/ml of aerobic colonies. In the characterization tests of aerobic bacteria colonies showed that there were *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria on gargle water of peatland or PDAM water. Independent t-test results showed significant differences in aerobic bacterial colonies in peatland water and PDAM water ($p = 0.001$)($p = 0.002$)($p < 0.05$). **Conclusion:** Based on this study it can be concluded that the number of *Staphylococcus aureus* and *Staphylococcus epidermidis* found in the oral cavity of children saliva who gargled with peatland water are higher than the number of *Staphylococcus aureus* and *Staphylococcus epidermidis* found in the saliva of children who gargle with PDAM water.

Keywords: Peatland water, PDAM water, aerobic bacteria, the number of bacterial colonies

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INTRODUCTION

Caries is an infectious disease of the teeth caused by demineralization on email interaction by lactic acid bacteria on the teeth with the aid of substrate. Dental caries usually occurs in children because children mostly consume a lot of sweet food or drinks, moreover the factor dental caries in children is also influenced by the degree of acidity of saliva, oral hygiene, the amount of food that cause caries, regularity and tooth brushing techniques.^{1,2} The main cause of dental caries is bacteria, bacteria located in the plaque will produce acid. Beside, these acids not only can lead to the destruction of enamel crystals but also as a source of energy for bacteria to grow and thrive. The acid

that attached on the tooth surface can decrease the pH into 5,5-5,2 (critical pH) within 5-10 minutes, so that in the long term it will lead to cavity formation on the tooth.³ According to RISKESDAS (2013) 36,1% of South Kalimantan society has a problem with oral and dental health in 28.9% of children's dental caries. RISKEDAS (2007) states that the Barito Kuala area is the population with the highest dental and oral health problems in South Kalimantan.^{4,5}

Indonesia has the largest peatland water among other tropical countries, which is about 21 million ha. South Kalimantan, especially 90% in the Barito Kuala region's peat swamp has a pH (3,5-4,5).^{6,7} Acidity of peatland water can leads the growth of acidogenic and acidouric bacteria that

can decrease the pH in oral cavity. Acidogenic bacteria can form acids at pH 6-6.5. Bacteria that have the highest activity is *Streptococcus* bacteria one of them is *Streptococcus mutans* bacteria because this bacteria can still produce acid at pH 4.5.³ *Streptococcus mutans* itself belongs to a group of facultative anaerobic bacteria, but these bacteria prefer to live in aerobic air.⁸ Purwandari (2015) studies suggest that there are aerobic bacterial colonies in the peatland gargle water as much as 656 bacterial colonies.⁹

Bandarmasih PDAM water is water from river water that conducted through several stages of water treatment such as sedimentation, filtration, and coagulation-flocculation to be clean water. PDAM also give disinfection to eliminate pathogen bacteria, but because there is reaction of disinfection material on dirty pipe can cause bacteria of pathogen in PDAM water not completely gone.¹⁰ Based on the results of Arditya (2015) research that in PDAM water there are *Pseudomonas*, *Enterobacter*, *E.coli*, and *Klebsiella* bacteria.¹¹

MATERIALS AND METHODS

This study used a quasi-experimental study with post test only control group design. The treatment consisted of 2 treatments which were gargling with peatland water and PDAM water, 20ml each and gargled for 30 seconds. The population in this research was students of SDN Puntik Luar 1 of Barito Kuala and SDN Melayu 5 Banjarmasin. Sampling was conducted using simple random sampling technique with the criteria for inclusion in this study were willing to participate in the study (informed consent), aged 7-10 years, free of caries, and the sex of male and female. The number of samples in this study were 30 students which divided into 15 people in each group respectively.

The samples were identified according to the predefined inclusion criteria. All samples were divided into 2 groups. First group consisted of 15 people who gargled with 20ml peatland water for 30 seconds while the second group consisted of 15 people who gargled with 20ml of PDAM water for 30 seconds. After the gargle water was released and 4ml of saliva taken to a laboratory to make the culture and identification of aerobic bacteria colonies of the oral cavity. The data obtained were analyzed using the Shapiro-Wilk normality test and parametric tests by using independent t-test.

STUDY RESULTS

The result of the culture and the calculation of aerobic bacteria colonies number in the oral cavity with the peatland water and PDAM water has shown on the figure.

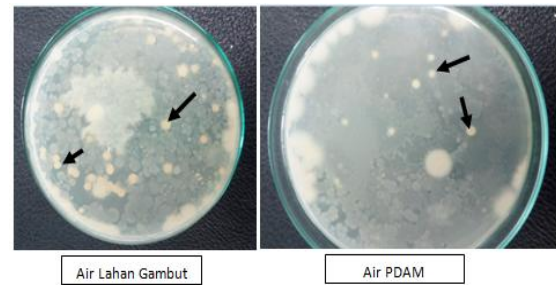


Figure 1. The culture of bacteria colonies on peatland water and PDAM water

Based on the figure above, the culture of aerobic bacteria colonies on the saliva of a children's gargle with peatland water and PDAM water shows colonies with round shape, soft bacterial surface, yellow gold and white with 4 mm diameter suspected as *Staphylococcus sp* bacteria.

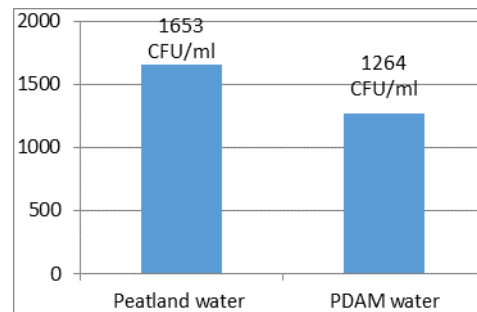


Figure 2. The average number of aerobic bacterial colonies in peatland water and PDAM water

Figure 2 shows that the calculation of the number of aerobic bacterial colonies of the oral cavity in gargle saliva rinsing with peatland water is 1653 CFU/ml and PDAM water is 1264 CFU/ml.

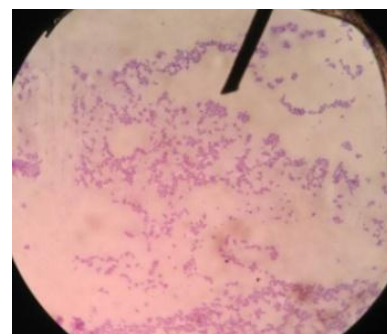


Figure 3. *Staphylococcus sp-shaped coccus bacteria.*

Figure 3 is an illustration of colonies of aerobic bacteria of the oral cavity observed by using a microscope with 100x magnification. It was found the presence of coccus-shaped, clustered and grapes-formed bacteria.

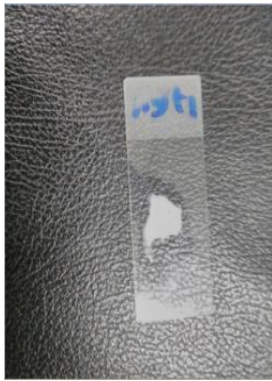


Figure 4. Catalase test results

Figure 4 shows the presence of bubbles on the catalase test in the object glass taken from a blood agar plate media when given liquid H_2O_2 . It can be concluded that the colonies of bacteria which were found is *Staphylococcus sp.*



Figure 5. The culture of *Staphylococcus sp* bacteria from MSA media.

Figure 5 shows the culture of *Staphylococcus sp* in MSA and the change in color in the media where it becomes yellow and pink. It can be concluded that bacteria found in yellow MSA media is *Staphylococcus aureus* or other *Staphylococcus* species bacteria while in pink MSA media is *Staphylococcus epidermidis* bacteria.

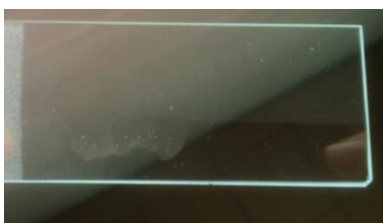


Figure 6. Coagulation Test

Figure 6 shows the presence of agglutination on the coagulation test in the object glass taken from MSA when the plasma fluid citrate was given. From the test which had been performed, it can be concluded that the bacterial colony is *Staphylococcus Aureus*.

DISCUSSION

Acidity in peatland water is caused by high cation exchange capacity resulting in very low saturation so that the deeper the peatland the more

acidic the pH become. The formation of phenolic and carboxylic acid during the decomposition of organic matter will cause high acidity of peatland.^{6,16} The oral cavity that exposed by peatland water will affect the degree of acidity of the oral cavity reaches up to critical pH of 5.5. H^+ ions contained in the peatland water binds to PO_4^{3-} ions from saliva thus forming HPO_4^{3-} . In this form HPO_4^{3-} can not balance the condition of enamel and saliva.⁶

The high acidity of the peatland water will cause damage to the teeth due to the acidity which is one of the factors that influence the growth of bacterial colonies number in the oral cavity of both aerob and anaerob. One of the bacteria that has the highest activity is the *Streptococcus mutans* bacterium, because these bacteria can still produce acid to pH 4.5. The acidity produced by peatland water will support the growth of acidogenical *Streptococcus* and *Staphylococcus* bacteria which are able to metabolize acid. The results of bacterial metabolism such as acetic acid, formic acid, lactic acid, propionate acid and pyruvic acid are used by *Streptococcus* or *Staphylococcus* bacteria as energy to perform metabolism, thus accelerating the formation of biofilm colonies.^{3,6} It is also supported by Muhtar (2017) who said that there are various colonies of biofilm bacteria on the tooth surface and two of them are *Streptococcus sp* and *Staphylococcus sp*.¹⁷

The acidic content of peatland water is not only support the tooth damage such as tooth caries but also can cause other damage such as erosion of the teeth. Tooth erosion is an erosion or the dissolution of hydroxyapatite crystals on the surface of the teeth caused by acidic substances without chemical process involving bacteria. Previous research has found that there is a minimal erosion in dental immersion with 4,2 pH water and it will increase at the pH below 4,0.¹⁹ It is also supported by Tarigan (2013) which states that the erosion can be caused by acidic drinks that have acidic properties or low pH.⁸ In this study, researchers used a peatland water that has a pH of 4,0.

In the catalase test, O_2 gas bubbles were obtained when H_2O_2 fluid was given and it was concluded that the bacteria were *Staphylococcus sp* bacteria because they could convert H_2O_2 into water (H_2O) and bubble gas (O_2). This catalase test also serves as a distinction between *Staphylococcus sp* bacteria and *Streptococcus sp* bacteria. In the coagulase test, positive agglutination or clotting on the object glass was obtained when the plasma citrate was administered which proved that the bacterias were *Staphylococcus aureus* bacteria because other *Staphylococcus* bacteria will show negative agglutination results or has no bubbles on the object glass when plasma citrate was obtained.^{14,18}

In this study, we found that *Staphylococcus aureus* bacteria in the culture of peatland water is a normal flora bacteria in the human body that contribute to the pathogenesis of dental caries. *Staphylococcus aureus* bacteria are able to produce catalase and coagulase enzymes.^{14,12} These bacteria can also ferment carbohydrates mannitol to produce acid that can lower the pH.¹²

This study also found *Staphylococcus* bacteria such as *Staphylococcus epidermidis* on peatland water culture. *Staphylococcus epidermidis* bacterium is an aerobic membrane that affects tooth damage. It has been proven by Kusumaningari (2011) who mentions that the formation of biofilms contains *Staphylococcus epidermidis* bacteria.¹⁵ This bacteria can not ferment mannitol, so that when it breed on MSA (mannitol salt agar) it will appear as pink or negative results that are different from *Staphylococcus aureus* which can ferment mannitol. At the time of the planting in MSA, those bacteria can change the media into golden yellow.^{13,18}

Based on health ministry regulation No.492/Menkes/PER/IV/2010 regarding to drinking water quality requirements which include odorless, tasteless, non carcinogenic and colorless, chlorine compounds are used in PDAM water treatment as an agent to reduce pathogenic bacteria. The water chlorination process leaves residual free chlorine. The concentration of free chlorine in water should be more than 0.2 mg/l and should not be more than 0.5 mg/l, because if there is a decrease in free chlorine concentration or <0.2 mg/l it will increase the concentration of bacterial colonies in water, whereas if the free chlorine concentration is more 0.5 mg/l it will cause toxicity to the body.¹⁰

This study found the colonies of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria in PDAM water culture, but the number of bacterial colonies from PDAM water culture is lower than the number of bacterial colonies on peatland water which was 1653 CFU/ml on peatland water and 1264 CFU/ml on PDAM water. The presence of bacteria in PDAM water due to a decrease in the concentration of free chlorine that is affected by the pipe wall reaction. The pipe wall reaction is the decrease of the concentration of free chlorine caused by the reaction with pipes supplied from the PDAM. The decrease in the concentration of free chlorine will lead to the increase of bacterial pathogens.¹⁰

Based on the research, it can be concluded that the number of colonies of *Staphylococcus Aureus* bacteria and *staphylococcus epidermidis* of oral cavity in the children's saliva gargled with peatland water is higher than the number of colonies of *staphylococcus aureus*

bacteria and *staphylococcus epidermidis* of the oral cavity in the saliva gargled with PDAM water.

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