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Lactobacillus sp. Identification In Caries-Affected Students In Smp Negeri 1 Sungai Pinang Kabupaten Banjar

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Abstract

Background – *Caries is an infectious disease affecting a tooth's hard tissues, which is consisted of email, dentin, and cementum. It is a multifactorial disease, initiated by the interaction of host, microorganisms/agents, substrates, and time. One of the most pathogenic agents is Lactobacillus sp. This agent is mostly found in active lesions of caries, can produce lactate acid and is an acidic-environment tolerant organism. Lead exposure is also a factor that can worsen caries. Lead is a calcium-antagonist and can hamper the metabolism of calcium in remineralization of tooth. Lead can precipitate into drinking water after being crystallized in the air, assisted by rain. This exposure is especially worrying to the community which still depends on unfiltered water from the river to fulfill their daily needs.*

Purpose – *This study was proposed to identify Lactobacillus sp. colony in caries-affected children and assess the difference between river water consuming and well-water consuming children.*

Design/methodology/approach – *This was an observational analytics with control group design study using cross sectional approach, performed in SMP Negeri 1 Sungai Pinang Kabupaten Banjar, November-December 2017. The population was 60 students, categorized into two groups (30 students each): river water consuming and well-water consuming groups. The samples' DMF-T indexes and teeth swab samples were recorded for analysis. The swab samples were then delivered to laboratories to be treated by staining and using Huccer methods to assess the Lactobacillus sp. colony.*

Findings – *The results showed that there was a significant difference between colony count of Lactobacillus sp. and DMT-index in both river water consuming and well-water consuming children.*

Research limitations – *identify Lactobacillus sp. colony in caries-affected children and assess the difference between river water consuming and well-water consuming children.*

Originality/value – *There are no specific findings regarding the types of bacteria that cause oral health problems, especially caries in South Kalimantan*

Lactobacillus sp. IDENTIFICATION IN CARIES-AFFECTED STUDENTS IN SMP NEGERI 1 SUNGAI PINANG KABUPATEN BANJAR

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ABSTRACT

Background: Caries is an infectious disease affecting a tooth's hard tissues, which is consisted of email, dentin, and cementum. It is a multifactorial disease, initiated by the interaction of host, microorganisms/agents, substrates, and time. One of the most pathogenic agents is Lactobacillus sp. This agent is mostly found in active lesions of caries, can produce lactate acid and is an acidic-environment tolerant organism. Lead exposure is also a factor that can worsen caries. Lead is a calcium-antagonist and can hamper the metabolism of calcium in remineralization of tooth. Lead can precipitate into drinking water after being crystallized in the air, assisted by rain. This exposure is especially worrying to the community which still depends on unfiltered water from the river to fulfill their daily needs. *Purpose:* This study was proposed to identify Lactobacillus sp. colony in caries-affected children and assess the difference between river water consuming and well-water consuming children. *Methods:* This was an observational analytics with control group design study using cross sectional approach, performed in SMP Negeri 1 Sungai Pinang Kabupaten Banjar, November-December 2017. The population was 60 students, categorized into two groups (30 students each): river water consuming and well-water consuming groups. The samples' DMF-T indexes and teeth swab samples were recorded for analysis. The swab samples were then delivered to laboratories to be treated by staining and using Huccer methods to assess the Lactobacillus sp. colony. *Results:* The results showed that there was a significant difference between colony count of Lactobacillus sp. and DMT-index in both river water consuming and well-water consuming children.

Keywords: caries, DMF-T index, river water consumption, well-water consumption, lead

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INTRODUCTION

Oral health is one of the main concerns in Indonesia's health development. This focus is built on the fact that a vast number of productive Indonesians have been affected by oral diseases, and this poses as an obstacle in improving many aspects of a quality life. Suffering from oral diseases also limits one's choices in participating as an active member of a lot of professional work fields in Indonesia. Not to mention that an active disease can impact one's health as a whole and hamper productivity. World Health Organization (WHO) reported as much as 60-90% children of elementary school age in the world are affected by caries, with the highest percentages found in Asia and South America. Indonesia's National Basic Health Research carried out in 2007 showed that South Kalimantan had 29,2% citizens affected by oral health problems with Decayed Missing Filled Teeth index (DMF-T index) of 6,83, and active caries number as high as 83,4%. The most recent report of 2013 delivered worsening

numbers, percentages of citizens affected by oral health problems reached 36,1%, with DMF-T index of 7,2 and D-T index as high as 2,2.

Caries is an infectious disease affecting tooth's hard tissues: enamel, dentine, and cementum. Caries is proposed as a multifactorial disease because it is caused by a number of factors: host, agent, substrate, and time. Caries will only happen if those four factors interact with each other. The initial sign of caries is demineralization of tooth's hard tissue, followed by the destruction of its organic materials. This causes bacterial invasion and further destruction of pulpal tissues, and infection spread to periapical tissues. The most pathogenic bacteria from 200 bacteria found in a plaque is *Lactobacillus sp*. (Harshanur, 1995; Kidd & Joyson, 1992; Situmorang, 2005; Nurmala, 2006).

Lactobacillus sp. is an acid-resistant microorganism because it can survive in a highly-acidic environments. This bacteria adheres to tooth's surface, mostly found in active caries, and metabolizes carbs, especially those containing glucoses and sucroses; it also produces organic acid (lactic acid) that causes drastic pH drop which will push the onset of enamel demineralization (Marsh & Martin, 2000; Cura *et al.,* 2012).

Behavioral and environmental factors also play important roles in caries pathogenesis, one of them is South Kalimantan citizen's habit of consumming river water for daily needs. This habit is not recommended because river water doesn't contain adequate minerals to protect oral health, especially accompanied by the fact that active coal mines are present near the community. The danger of excess metal exposure in drinking water is a real risk for those who aren't educated to correctly process the river water or are unreachable by PDAM (Regional Drinking Water Company).

A number of regions in South Kalimantan that are known to have coal mines is as followed: Kabupaten Banjar, Tanah Laut, Kotabaru, Tanah Bumbu, Tapin, Balangan, and Hulu Sungai Selatan. Majority of coal mines in Kalimantan use surface method that can produce coal dust. Coal dust contains elements such as lead (Pb), aluminum (Al), iron (Fe), and sulphur (S). Pb has a characteristic as calcium-antagonist and can inhibit calcium metabolism. If there's a high Pb exposure, the remineralization of tooth cannot occur optimally, and demineralization will further take place and increase the risk of caries (Moelyaningrum, 2007). Pb can enter the water bodies through Pb crystalization in rain water, and mineral stones corofication can also expose Pb to the water (Palar, 1994).

Previous studies showed that caries is not only caused by tooth's surface, cariogenic bacteria, fermented carbs, and time, but also polluters can also affect caries onset, especially in school-aged children. This study was carried out to identify, and characterize *Lactobacillus sp.* in school-aged children affected by caries, to analyze caries index, and to analyze the difference between caries index and *Lactobacillus sp.* colony counts of SMP Negeri 1 students consuming river water and PDAM. The result of this study was expected to be used as a fundamental reference for preventive education and curative choices of pathogenic bacterial infections, especially in school aged children.

METHODS

This study passed the ethical conduct of The Ethical Committee of Medical Research Ethics Dentistry Faculty University of Lambung Mangkurat Banjarmasin, Indonesia (No.064/KEPKG-FKGULM/EC/IX/2017).

Study Design

This study used observational analytics with control group design with cross sectional approach. The population in this study was SMP Negeri 1 Sungai Pinang Kabupaten Banjar students affected by caries. Samples were chosen through simple random sampling using

Slovin formula. The inclusion criterias were : females and males, 12-14 years old, affected by caries, 30 students consuming river water for daily needs, and 30 students consuming PDAM water, and consenting. The exclusion criterias were : students with systemic diseases, genetic diseases, no caries lesion, and uncooperative students. Total samples were 60 students, categorized into two groups of river water consuming-group, and PDAM water consuming-group. Plaque swabs were taken from samples' all teeth's surfaces using tooth brush. This procedure was carried out in SMP Negeri 1 Sungai Pinang Kabupaten Banjar and the specimens identification was done in Microbiology Laboratory of Faculty of Medicine University of Lambung Mangkurat and *Eijkman Institute for Molecular Biology* Jakarta from November to December 2017.

Tools and Materials

Tools used in this study were toothbrushes, diagnostic sets, neer bekken, sterile centrifuge tubes, sterile petri dishes, beaker glasses, measuring glasses, Erlenmeyer, stirring sticks, sterile ose, spiritus lamps, object glasses, sterile pipettes, sterile measuring pipettes, incubator, autoclave, staining racks, and ice thermos.

Materials used were subjects identification sheets, DMF-T index sheets, questionnaire sheets, stationary, sterile Brain Heath Infusion Broth media, sterile Nutrient Agar media, Huccer method gram-staining (1% violet crystal solution, Lugol solution, acetone alcohol, and carbol fuchsin solution), 10% H_2O_2 solution, 70% alcohol, 96% alcohol, aquadest, cottons, handscoons, masks, laboratory coats, tissues, pH indicators (litmus sheets), and ice packs.

Procedures

Study was carried out first by educating the students about proper tooth brushing technique, and followed by filling WHO-based questionnaire sheets about oral health. After that, objective examinations of intra oral conditions were done using DMF-T index sheets to fine caries-affected students. Swab samples were taken using students' toothbrushes, and then swabs were put in sterile BHIB tubes, coded, and kept in ice box before they were sent to microbiology laboratory in 24 hours.

Day I : Samples in BHIB media were transferred to NA media using 10 μl micropipettes. Colonies were counted using colony counter. Samples were spread on NA media using sterile ose and then incubated in 35-37°C anaerobic environment for 48 hours.

Day III : NA medias were examined macroscopically and possible Lactobacillus sp. colonies were counted. Possible Lactobacillus sp. colonies were catalyzed and transferred to NA medias. Medias were then incubated in 35-37°C anaerobic environment for another 48 hours.

Day V : Colonies on NA medias were examined macroscopically, possible Lactobacillus sp. colonies were catalyzed for the second time. Colonies were taken from NA medias for gram-staining using Huccer methods.

Huccer Methods

First, object glasses were cleaned using filter paper and passed over a fire to remove dirt and fats. Then, a 2-3cm circle was made under object glass using glass pencil and labeled. Preparation was made by taking one ose of 0,9% NaCl solution and one ose of bacterial colonies from NA medias, then spread and dried, and fixed by passing the preparation over a fire twice or thrice. Preparation was then inundated in 2% crystal violet for 1 minute and then washed under running water, and then inundated in lugol solution for 1 minute and washed under running water. After that, acetone alcohol was ran over preparation until the first stain

was drained, then the preparation was inundated again in 0,25% safranin solution for 30 seconds, and then washed under running water. Preparation was dried in room temperature by placing the preparation on tissues. The final results were examined under microscopes using 10x100 magnifications in emersion oil to assess bacterial shapes, structures, and characteristics. Results showed Lactobacillus sp. if they were bacilli shaped, chain-like, and gram positive (purple-stained).

Data Analysis

Data compiled from examination were checked, written down, coded and then tabulated in tables and graphs, which consisted of positive samples of Lactobacillus sp. from SMP Negeri 1 Sungai Pinang Kabupaten Banjar students affected by caries. Data were processed using SPSS: test of normality was carried out for colonies count and DMF-T index, if data were not distributed normally ($p \leq 0.05$), Wilcoxon test would be chosen to find out the difference between colonies of samples. Statistical tests would also be carried out based on categorized groups of river and PDAM water consuming students.

RESULTS

Lactobacillus sp. colonies count and DMF-T index means in river and PDAM water consuming groups were as followed:

Table 1. Lactobacillus sp. colonies count and DMF-T index means in SMP Negeri 1 Sungai Pinang Kabupaten Banjar students

	Mean	Standard Error	Standard Deviation
DMF-T	3,116	0.004	0.038
Lactobacillus sp. colonies count	209.03	30.838	238.870

According to table 1, DMF-index means of students was 3,116 and Lactobacillus sp colonies count means was 209.03 CFU/ml.

Table 2. Test of normality of Lactobacillus sp. colonies count and DMF-T index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar students

	Kolmogorov-Smirnov[a]	
	Df	Sig.
Lactobacillus sp. colonies count	60	0.000
DMF-T	60	0.002

According to table 2, data were not normally distributed ($p \leq 0.05$), and Wilcoxon test would be chosen for further analysis.

Table 3. Significance test of Lactobacillus sp. colonies count and DMF-T index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar students

	Lactobacillus sp. colonies count and DMF-T
Sig 2-tailed	0.000

Table 3 showed that there was a significant difference (p ≤ 0.05) between Lactobacillus sp colonies count and DMF-T index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar students.

Table 4. Lactobacillus sp. colonies count and DMF-T index means in SMP Negeri 1 Sungai Pinang Kabupaten Banjar river water-consuming students

River Water Consuming Group				
Lactobacillus sp colonies count		DMF-T index		Wilcoxon test
- Mean ± Standard Deviation - Standard Error	Test of Normality - df - Sig.	- Mean ± Standard Deviation - Standard Error	Test of Normality - df - Sig.	
187.43 ± 231.988 42.538	30 0.000	3,5 ± 0.038 0.007	30 0.075	0.025

According to table 4, Lactobacillus sp colonies count means in river water consuming group was 187.43 CFU/ml, test of normality showed that data were not normally distributed (p ≤ 0.05). DMF-T index means was as high as 3,5 and test of normality showed that data were normally distributed (p ≥ 0.05). Significance test chosen was Wilcoxon test, and there was a significant difference between Lactobacillus sp colonies count and DMF-index in river water consuming group.

Table 5. Lactobacillus sp. colonies count and DMF-T index means in SMP Negeri 1 Sungai Pinang Kabupaten Banjar PDAM water-consuming students

PDAM Water Consuming Group				
Lactobacillus sp colonies count		DMF-T index		Wilcoxon test
- Mean ± Standard Deviation - Standard Error	Test of Normality - df - Sig.	- Mean ± Standard Deviation - Standard Error	Test of Normality - df - Sig.	
230.63 ± 246.650 45.032	30 0.000	2,73 ± 0.037 0.006	30 0.003	0.008

According to table 5, Lactobacillus sp colonies count means in river water consuming group was 230.63 CFU/ml, test of normality showed that data were not normally distributed (p ≤ 0.05). DMF-T index means was as high as 2,73 and test of normality showed that data were not normally distributed (p ≤ 0.05). Further test chosen was Wilcoxon test, and there was a significant difference between Lactobacillus sp colonies count and DMF-index in PDAM water consuming group.

Table 6. Significance test of Lactobacillus sp. colonies count in SMP Negeri 1 Sungai Pinang Kabupaten Banjar river water consuming and PDAM water consuming students

Lactobacillus sp. colonies count		
	Test of normality - df - Sig.	**Sig.test**
River water consuming group	30 0.000	**0.403**
PDAM water consuming group	30 0.000	

Table 6 showed that both data were not normally distributed (p ≤ 0.05). Further test chosen was Mann-Whitney test, and the result showed that there was no significant difference of Lactobacillus sp colonies count between river water consuming and PDAM water consuming groups (p ≥ 0.05).

Table 7. Significance test of DMF-T index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar river water consuming and PDAM water consuming students

DMT-Index		
	Test of normality - df - Sig.	**Sig.test**
River water consuming group	30 0.003	**0.145**
PDAM water consuming group	30 0.075	

Table 7 showed that DMF-index of river water consuming group were not normally distributed (p ≤ 0.05), meanwhile data of PDAM water consuming group were normally distributed (p ≥ 0.05). Further test chosen was Mann-Whitney test and the result showed that there was no significant difference of DMF-T index between river water consuming and PDAM water consuming groups (p ≥ 0.05).

Table 8. Significance test of Lactobacillus sp. colonies count and Decayed index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar river water consuming students

River Water Consuming Group		
	Test of Normality - df - Sig.	**Sig.test**
Lactobacillus sp. **colonies count**	30 0.000	**0.008**
Decayed-Teeth **index**	30 0.003	

The table showed that both data were not normally distributed (p ≤ 0.05), so Wilcoxon test was chosen and there was a significant difference between *Lactobacillus sp.* colonies count and D-T index in river water consuming group (p ≤ 0.05).

Table 9. Significance test of Lactobacillus sp. colonies count and Decayed index in SMP Negeri 1 Sungai Pinang Kabupaten Banjar PDAM water consuming students

PDAM Water Consuming Group		
	Test of Normality - df - Sig.	**Sig.test**
Lactobacillus sp. **colonies count**	30 0.000	**0.025**
Decayed-Teeth **index**	30 0.018	

The table showed that both data were not normally distributed (p ≤ 0.05), so Wilcoxon test was chosen and there was a significant difference between *Lactobacillus sp.* colonies count and D-T index in PDAM water consuming group (p ≤ 0.05).

DISCUSSION

Results showed that DMF-T index in PDAM water consuming group was 2,73 and the Lactobacillus sp colonies count was 187.43 CFU/ml and in river water consuming group were 3,5 and 230.63CFU/ml respectively. General analysis result showed that there was a significant difference between Lactobacillus sp colonies count and DMF-T index in all samples. But Mann-Whitney test showed that there was no significant difference of DMF-T index between river water consuming and PDAM water consuming groups. Similar result was also shown from analysis of Lactobacillus sp, whereas there was no significant difference between river water consuming and PDAM water consuming groups (p ≥ 0.05).

This result might be correlated to a number of uncontrolled variables such as OH habits, diets, age of first permanent teeth. Especially because caries is a multifactorial disease, caused by host, agent, substrates, and time, and samples chosen were mainly coming from similar

socioeconomic environments. In the next proposed study, this consideration should be taken to determine more detailed inclusion and exclusion criterias as to obtain more accurate results.

The conclusion of this study was that there was no significant difference of DMF-T index between river water consuming and PDAM water consuming groups. The results of this study are expected to be useful as the foundation of strategic issues formation in health department, to improve one's life's quality and community health as a whole, especially in South Kalimantan.

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