

<b>Research Report</b>
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## The number of *Lactobacillus acidophilus* after using *Chlorhexidine* 2%, laser diode (405 nm), and combination of *Chlorhexidine* 2% with laser diode (405 nm)

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### ABSTRACT

**Background:** *Lactobacillus acidophilus* is gram-positive bacteria that produces acids from carbohydrates and causing dental caries. Caries treatment is done by the cavitation of teeth which is preceded by cavity disinfection. The purpose of cavity disinfection is to kill microorganisms and reduce the risk of new carious lesions. Bacterial elimination can be done using chlorhexidine and laser. Chlorhexidine is widely used for cleaning cavities but cannot remove biofilms, tissue debris and has limited elimination of bacteria in the dentinal tubules. Another way to eliminate bacteria is using Photodynamic Therapy (PDT) which consists of photosensitizer and laser. Until now there has not been a single ingredient that is considered to cleanse the cavity thoroughly. There has been no research yet that examine the number of *Lactobacillus acidophilus* after using chlorhexidine 2%, laser diode (405 nm), and combination of 2% chlorhexidine with laser diode (405 nm). **Purpose:** To compare the decreasing number of living *Lactobacillus acidophilus* after using chlorhexidine 2%, laser diode (405 nm), and combination of chlorhexidine 2% with laser diode (405 nm). **Methods:** A total of 24 samples of *Lactobacillus acidophilus* were divided into 4 groups: (I) chlorhexidine 2%, (II) chlorophyll photosensitizer and 75 seconds irradiation, (III) combination of chlorhexidine 2%, chlorophyll photosensitizer, and 75 seconds irradiation. After treatment, the sample was incubated 48 hours and the colony count was calculated for each group. Results of the analysis were carried out by ANOVA and Tukey HSD tests with  $p < 0.05$ . **Results:** The average number of group colonies (I) was 35.33 CFU/ml, (II) 16.83 CFU/ml, (III) 9.5 CFU/ml, (IV) 123.33 CFU/ml. **Conclusion:** The combination of 2% chlorhexidine with diode laser (405 nm) gives the least amount of living *Lactobacillus acidophilus* bacteria compared with the administration of 2% chlorhexidine and laser diode (405 nm).

**Keywords:** *Lactobacillus acidophilus*; chlorhexidine 2%; laser diode (405nm)

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### INTRODUCTION

Dental and oral diseases such as caries is one of the diseases that develops throughout the world, including Indonesia.<sup>1</sup> Based on Riskesdas data in 2018, the prevalence of caries reached 88.8%.<sup>2</sup> *Lactobacillus acidophilus* is gram-positive bacteria that produces acids from carbohydrates and causes a drastic decrease in pH. These bacteria are among the bacteria most pathogenic to dental caries. If caries is not treated, then the cavity formed will be deeper into the dentin or pulp and cause an inflammatory response and even cause pulp necrosis.<sup>3</sup>

Dental caries treatment is carried out by placing a cavity in the tooth cavity using a restorative material after the cavity preparation and disinfection. Cavity disinfection is done by cleaning the cavity of the accumulation of carious bacteria and caries-affected tissue to ensure that as many

microorganisms are killed so that it will reduce the risk of new caries lesions<sup>4</sup>. The elimination of these bacteria can be done using chlorhexidine, and laser. Until now there has been no material that is able to cleanse the cavity thoroughly so that it cannot use only one disinfection material<sup>5</sup>.

*Chlorhexidine* has been widely used in dentistry, especially for cleaning cavities with antibacterial properties with the ability of chlorhexidine to reduce cariogenic microorganisms. However, chlorhexidine cannot remove biofilms, tissue debris and has limited penetration into the dentine tubules to eliminate bacteria. Another therapy is Photodynamic therapy (PDT) which consists of photosensitizer and laser which are considered to have an antibacterial effect on microorganisms. In a study conducted by Ruslan (2018)<sup>6</sup>, there was an 85% reduction in Extracellular Polymeric Substance (EPS) biofilm of *Lactobacillus acidophilus* with a 405 nm diode laser and

chlorophyll photosensitizer. In a study conducted Araujo et al., (2012) <sup>7</sup> found a decrease of 37.6% *Lactobacillus acidophilus* bacteria obtained by using curcumin as a photosensitizer and a 450 nm LED laser.

Some studies show the antimicrobial effect of using only the laser is less effective than using irrigant ingredients. However, a greater bactericidal effect is obtained when using a combination of irrigant ingredients with diode lasers. Kaiwar et al. (2013) <sup>8</sup> obtained similar results when using a combination of diode lasers with irrigant ingredients with the greatest levels of disinfection compared to using only lasers or irrigant ingredients. Until now there has been no research that examined the number of *Lactobacillus acidophilus* bacteria after administration of chlorhexidine 2%, laser diode (405 nm), and combination of chlorhexidine 2% with laser diode (405 nm). It is hoped that this research can be a reference in the use of diode lasers (405 nm) combined with chlorhexidine 2% as an alternative to cavity disinfection before it is deposited with restoration materials. The purpose of this study was to determine the decrease in the number of living *Lactobacillus acidophilus* bacteria after administration of chlorhexidine 2%, laser diode (405 nm), and combination of chlorhexidine 2% with laser diode (405 nm).

**MATERIALS AND METHODS**

Ethical Clearance Certificate: No. 450/HRECC.FODM/VII/2018 from ethical committee of Faculty of Dental Medicine, Universitas Airlangga. *Lactobacillus acidophilus* bacterial preparations were obtained from the stock of *Lactobacillus acidophilus* bacteria at the Research Center of the Faculty of Dentistry, Airlangga University, Surabaya. The research design used was the Post Test Only Control Group Design. The tools used in this study are test tubes (Pyrex), micropipettes (Sokorex), Bunsen spiritus, laser diode (CNC), Petri dish (Pyrex), spreaders, osse wires, incubators (Espec), anaerobic jar (Oxoid), brander, and 96-well plate (Pyrex) microplate. The ingredients used are the bacterium *Lactobacillus acidophilus*, Brain Heart Infusion Broth (BHIB), nutrient agar, chlorophyll photosensitizer (K-Link), and chlorhexidine 2% (OneMed).

Bacterial preparations were taken using osse, put into a test tube containing BHIB and incubated at 37°C in an

incubator for 48 hours in an anaerobic atmosphere. The dosage is taken as much as 0.5 ml with a micropipette and put into a test tube containing a new BHIB to be equalized on the McFarland scale 0.5 (1.5 x 10<sup>8</sup> CFU / ml), then inserted into a microplate of 24 wells from 96-well plates, each containing 110µl containing 90µl BHIB and 20µl bacterial culture<sup>9</sup>. A total of 24 wells from 96-wellplate containing BHIB and *Lactobacillus acidophilus* bacteria were grouped into 4 groups containing 6 sample wells in each group. Group I was given chlorhexidine 2% and no irradiation. Group II was given chlorophyll photosensitizer 0.8 mg / ml and irradiated for 75 seconds. Group III was given chlorhexidine 2% and chlorophyll photosensitizer 0.8 mg / ml and irradiated for 75 seconds. Group IV is a control group containing *Lactobacillus acidophilus* bacteria without chlorhexidine 2% treatment, chlorophyll photosensitizer, and laser diode irradiation. Group I was given 90µl of chlorhexidine 2% for 75 seconds. Chlorophyll photosensitizer with a concentration of 0.8 mg / ml was taken as much as 90µl using micropipettes to groups II and III and allowed to stand for 1 minute in order to react before irradiation. Irradiation is carried out using a CNC diode laser that was previously set with 405nm wavelength and 20 mm from the surface of the BHIB media containing *Lactobacillus acidophilus*. Irradiation starts by pressing the Start button and it will last for 75 seconds. Group IV is a control group that contains *Lactobacillus acidophilus* bacteria without administration of 2% chlorhexidine, chlorophyll photosensitizer, and laser diode irradiation. Each sample was taken as much as 50µl using a micropipette to be planted on petri dish nutrient agar and leveled with a spreader. Each sample carried out incubation for 48 hours at 37°C in anaerobic atmosphere. The number of *Lactobacillus acidophilus* bacteria colonies were counted manually, and the results of each group were averaged.

Data analysis was performed using SPSS Statistics Base. The normality test was carried out with the Kolmogorov-Smirnov Test and homogeneity test using the Levene Test. If the test results show a normal distribution, the significance of the entire treatment group is done by One-Way Anova and the difference in the average of the treatment group is done by the Tukey HSD analysis test with a p of 0.05. If the results show an abnormal distribution, the significance of the entire treatment group is carried out by Kruskal-Wallis.

**Table 1.** Mean and standard deviations in the number of *Lactobacillus acidophilus* colonies

Group	N	$\bar{x}$ (CFU/ml)	SD
Chlorhexidine 2%	6	35.33	3.32666
Photosensitizer chlorophyll + laser	6	16.83	1.60208
Combination of Chlorhexidine 2% and photosensitizer chlorophyll + laser	6	9.5	1.04881
Control	6	123.33	2.80476

**Table 2.** Tukey HSD Test results

Group	1	2	3	4
1		.000*	.000*	.000*
2			.000*	.000*
3				.000*
4				

\*) Significant differences

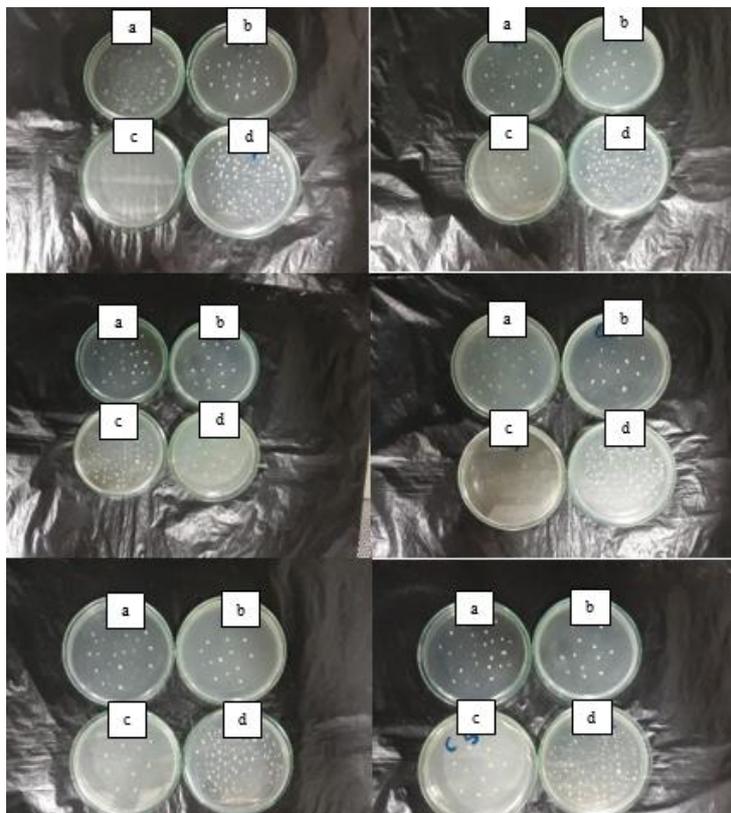
**RESULTS**

The average results and standard deviation of the total colonies of the *Lactobacillus acidophilus* bacteria can be seen in Table 1, Figure 1, and Figure 2. The results of data analysis, the Kolmogorov-Smirnov test showed  $p > 0.05$  which means the results of the study data were normally distributed. The Levene Test shows  $p > 0.05$ , which means that the data variant is homogeneous. In the One Way ANOVA test results obtained  $p$  value  $< 0.05$  which indicates that there are significant or significant differences in the number of *Lactobacillus acidophilus* colonies per group. Comparison of each group was done with the Tukey HSD Test follow-up (Table 2).

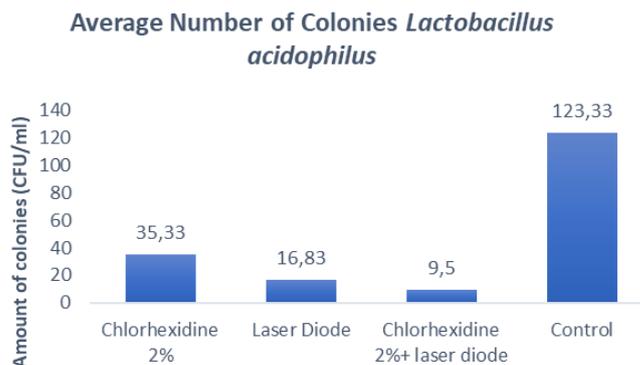
**DISCUSSION**

The aim of this study was to compare the number of *Lactobacillus acidophilus* bacteria after using chlorhexidine 2%, laser diode (405 nm), and combination of chlorhexidine 2% with laser diode (405 nm). Based on the analysis of research data, significant differences were found in all groups. This is consistent with the research of Iselinni et al. (2017)<sup>10</sup> and Shahar-Helft et al. (2011)<sup>11</sup> which states that the use of lasers combined with irrigant materials such as chlorhexidine will increase the bactericidal effect.

Chlorhexidine is part of the bis-biguanide group which has positively charged molecules. The positive charge of chlorhexidine interacts with the bacterial cell wall phosphate



**Figure 1** Results after 48 hours of incubation with group (a) chlorhexidine 2%, (b) laser diode (405 nm), (c) combination of 2% chlorhexidine and laser diode (405 nm), and (d) control.



**Figure 2.** Mean in the number of *Lactobacillus acidophilus* colonies.

group which is negatively charged resulting in a change in the osmotic balance of the bacterial cell which causes an increase in the permeability of the bacterial cell. This causes the chlorhexidine molecule to enter the bacterial cell resulting in the deposition of the cytoplasm and result in bacterial cell death<sup>12,13</sup>. Chlorhexidine is widely used for cleaning cavities but cannot remove biofilms, tissue debris and has limited bacterial elimination in dentinal tubules<sup>10,14,15</sup>.

Another therapy is Photodynamic Therapy (PDT) which consists of photosensitizer and laser. The laser used in this study is a diode laser with a wavelength of 405 nm which is used to kill bacteria. Photosensitizer used in this research is chlorophyll which has a spectrum that matches the laser diode with energy absorption at wavelengths between 400 to 700 nm<sup>16,17</sup>. Before irradiation, the chlorophyll is allowed to stand for 60 seconds so that the interaction between the chlorophyll photosensitizer and the external surface or wall of the bacterial cell is negatively charged<sup>18,19</sup>.

Chlorophyll photosensitizer with a positive charge (cation) will interact with the external surface or cell wall of the bacterium to have a negative charge (anion) and cause an electrostatic interaction. Electrostatic interactions cause the removal of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions from bacterial cell walls, resulting in disorganization of the outer membrane and an increase in the permeability of bacterial cells. Photosensitizer diffuses into the plasma membrane and binds to the bacterial plasma membrane<sup>20,21</sup>. When the photosensitizer is illuminated with a diode laser, the photosensitizer absorbs the light and causes a low-energy ground state phase transition to an excited singlet state and can experience a transition to a high-energy triplet state.

Photosensitizer in the triplet state phase reacts with biomolecules through 2 different types of reactions, namely type I and type II<sup>22</sup>. Type I reactions involve electron transfer reactions between photosensitizers and substrate molecules and produce highly reactive Oxygen Species such as superoxide anions ( $\text{O}_2^-$ ), hydroxyl radicals ( $\bullet\text{OH}$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Type II reactions involve electron transfer reactions between photosensitizers and oxygen ( $\text{O}_2$ ) and produce highly reactive oxygen singlets ( $^1\text{O}_2$ )<sup>23</sup>. Both reactions occur simultaneously. Reactive Oxygen Species and singlet oxygen will cause damage to cell membranes, disruption of cell function, disruption of cell metabolism and damage to bacterial cell DNA which results in bacterial cell death<sup>23,24</sup>.

Research shows that the greater antimicrobial effect is obtained by using a combination of irrigant ingredients with diode lasers compared to using only lasers only. In this study the lowest number of living *Lactobacillus acidophilus* bacteria in the combination group of chlorhexidine 2% and laser diode (405 nm) was 9.5 CFU / ml (7.7%). Kaiwar et al. (2013)<sup>8</sup> obtained results similar to the greatest level of disinfection when using a combination of diode lasers and irrigant materials compared to using only lasers or irrigant ingredients only. In a study conducted by Shahar-Helft et al. (2011)<sup>11</sup>, found a synergistic effect between the use of chlorhexidine with laser on bacterial growth to increase the antibacterial effect.

In addition to the reaction between photosensitizer and laser diode, the combination of chlorhexidine and laser diode will also occur in the cavitation process of chlorhexidine bubble cavitation. This cavitation induction process occurs due to diode laser exposure on a water-based medium, characterized by the formation and breakdown of water vapor. Cavitation is a state of change in the liquid phase that is being flowed from the liquid phase into the vapor phase, causing bubbles to occur because of changes in pressure. Bubble pressure from cavitation can increase the breakdown of irrigation solution molecules thereby increasing the ability of chlorhexidine disinfection to interact with phosphate groups on bacterial cell walls and cause changes in the osmotic balance of bacterial cells. Changes in osmotic balance in bacterial cells increase the permeability of bacterial cells resulting in the deposition of cytoplasm and result in bacterial cell death. This bubble pressure can also damage the biofilm of microorganisms, break down cell membranes, and clean the smear layer and debris<sup>10</sup>. With the synergistic effect between chlorhexidine and laser diodes, bacterial growth will be disrupted and antibacterial effects increased<sup>11</sup>.

In this study, it can be said that the combination of chlorhexidine 2% and diode laser (405 nm) had the greatest effect on reducing the number of *Lactobacillus acidophilus* bacteria compared to using chlorhexidine alone or laser diode alone. The combination of chlorhexidine 2% and laser diode (405 nm) has a synergistic effect resulting in an increase in antibacterial effect. Although there is a synergistic effect between chlorhexidine and diode lasers, it is necessary to do a further review of the antibacterial mechanism and interaction between chlorhexidine and diode laser photosensitizers in more detail.

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