



# In Vivo Study, Effect Combination of *Lactobacillus spp* and Doxycycline to Eliminate of *Vibrio cholerae*

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**Abstract.** Infection by *Vibrio cholerae* is a major public health problem confronting developing countries. One of possible solution for the problems could come from probiotic which have been reported to reduce diarrhea. Aim of the research to determine effect combination of *Lactobacillus spp* and Doxycycline to eliminate of *Vibrio cholerae* in vivo. This research was an experimental study. Twenty five male Swiss Webster mouse were divided into five groups: K1 (negative control), K2 (positive control), K3 (treated by Doxycycline), and K4 (treated by *Lactobacillus spp*) and K5 (treated by combination of *Lactobacillus spp* and Doxycycline). Mouses are infected by *Vibrio cholera* until symptom arise and then treated for 3 days. Interval between treated *Lactobacillus spp* and Doxycycline was an hour. Feces was cultured on TCBS (Thiosulfate-citrate-bile salts-sucrose) media and then the colonies were counted by Total Plate Count method (TPC). Test of One-Way Anova ( $\alpha < 0,05$ ) and post-hoc test ( $\alpha = 0,05$ ) was applied. LSD post hoc test showed there were significantly difference between the group K1, K2 and K4, but K3 dan K5 were not significant. Group which treated by combination of *Lactobacillus spp* and Doxycycline reduced number of *Vibrio cholerae* lowest than another groups but not significant than treated by Doxycycline only. *Lactobacillus spp*. Could eliminate *Vibrio cholerae* because it changed intraluminal acidity, produced antimicrobial substance, competition to absorb nutrition and attachment at the intestinal enterocyte receptor. Need longer interval between treated *Lactobacillus spp* and Doxycycline. One hour interval not enough for *Lactobacillus spp* to work in gastrointestinal tract.

**Keywords:** In vivo study · *Lactobacillus spp* · *Vibrio cholerae*

## 1 Introduction

Infection by *Vibrio cholerae* (*V. cholerae*) is a major public health problem confronting developing countries [1]. The disease is characterized by a devastating watery diarrhea

which leads to rapid dehydration, and death occurs in 50 to 70% of untreated patients [2]. Drug of choice which was recommended by WHO to eliminate *V. cholerae* was Doxycycline [3, 4]. There were many reported about resistention Doxycycline to *V. cholerae* [5–7]. One of possible solution for the problems could come from probiotic as supplement therapy which have been reported to reduce diarrhea [8].

## 2 Materials and Methods

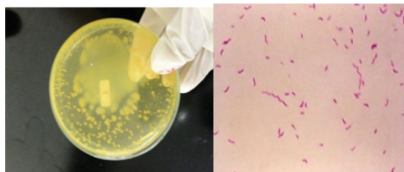
This research was an experimental study. Twenty five male Swiss Webster mouse were divided into five groups: K1 (negative control), K2 (positive control), K3 (treated by Doxycycline), and K4 (treated by *Lactobacillus spp*) and K5 (treated by combination of *Lactobacillus spp* and Doxycycline). Mouses were infected by *Vibrio cholera* until symptom arise and then treated for 3 days. Doxycycline dose for each mouse was 39 mg/kg body weight of mouse and volume of *Lactobacillus spp* was 2 ml. Interval between treated *Lactobacillus spp* and Doxycycline was an hour. This is accordance with the gastric emptying time of mouse. Feces was cultured on TCBS (Thiosulfate-citrate-bile salts-sucrose) media and then the colonies were counted by Total Plate Count (TPC) methodpoured into K2 to K5 bottles according to the specified concentration and the entire group is sterilized with autoclaves. Mixed starter cultures that are ready to be mixed into soy milk in K1 to K5 with a ratio of 1:9 or as much as 25 ml and incredied at 37 °C in a CO<sub>2</sub> incubator with CO<sub>2</sub> levels of 7.5% for 1x24 hours. Once the yoghurt is ready, each group is diluted repeatedly and calculated the number of colonies by the *Total Plate Count* method.

The number of colonies that have been obtained is analyzed using the SPSS statistics program version 25. All data is tested for normality with *Saphiro Wilk* test. Then continued with *the ANOVA* test and *the Post Hoc Man Whitney* test so that differences between groups can be assessed for their significance [9].

## 3 Results and Discussion

The aim of reidentification to determine that bacterial was *V.cholerae* exactly, base of macroscopic and microscopic examination beside standard.

Based on Fig. 1, result of macroscopic examination in TCBS agar: button shaped, yellow colonies of 1–2 mm diameter and result of microscopic examination: Gram negative, coma shape. All of the results suitable for *V. cholerae* [10, 11].



**Fig. 1.** Result of microscopic and macroscopic examination.

**Table 1.** Average of colony amount which growth in petri disk

Mouse	Treatment				
	Negative control (K1)	Positive control (K2)	Doxycycline (K3)	<i>Lactobacillus spp</i> (K4)	<i>Lactobacillus spp</i> + Doxycycline (K5)
1	0	750	189	594	143
2	0	740	172	596	148
3	0	664	164	568	124
4	0	778	185	612	152
5	0	760	178	542	171

*Lactobacillus spp* could decreased amount of *V. cholerae*. Combination *Lactobacillus spp* + Doxycycline could decreased amount of *V. cholerae* more than Doxycycline only.

**Table 2.** The result of ANOVA test

Colony amount	F	p
All of the treatment group	805.385	.000

Based on normality test in Table 1 (*Shapiro – Wilk test*), distribution of the data was normal (homogen). Then the data was tested with *One way Anova* test and post- hoc test (Tables 2 and 3).

LSD post hoc test showed that there were significantly difference between the group K1-K5. Group which treated by *Lactobacillus spp* reduced number of *V. cholerae* significantly but lower than treated by Doxycycline only. Group which treated by combination of *Lactobacillus spp* and Doxycycline reduced number of *Vibrio cholerae* lowest than another groups but not significant than treated by Doxycycline only.

The group which treated by *Lactobacillus spp*. Only could reduce amount of *V. cholerae* significant than positive control. It was caused that *Lactobacillus spp*. Could produce lactic acid and antimicrobial component (bacteriocin, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and diacetyl). Lactic acid changed acidity of intestine mucosa so that inhibit the growth of *V. cholerae*, because *V. cholerae* usually growth in alkaline environment (pH 8.5–9,5). Bacteriocin could attached on cell wall and membrane of pathogen bacteria so that cytoplasm membrane could be unstable, material from cell core released and then the cell death [12].

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which product by *Lactobacillus spp*. Caused oxidation of bacterial cell and destruct structure of protein cell. Protein synthesis disrupt so that cell death. Meanwhile, diacetyl will bind with *arginine-binding* protein, it causes disrupt of protein synthesis [13]. The group was given Doxycycline only, more significant than was given *Lactobacillus spp* only. Doxycycline was the first line drug for *V. cholerae* infection which recommended by WHO. Doxycycline was a broad spectrum antibiotic.

**Table 3.** Post-hoc test for testing between treatment group

Group	Group	Sig.
K1	K2	.000
	K3	.000
	K4	.000
	K5	.000
K2	K1	.000
	K3	.000
	K4	.000
	K5	.000
K3	K1	.000
	K2	.000
	K4	.000
	K5	.070
K4	K1	.000
	K2	.000
	K3	.000
	K5	.000
K5	K1	.000
	K2	.000
	K3	.070
	K4	.000

Doxycycline was a bacteriostatic which inhibition of protein synthesis through bounded to ribossom 30s and blocking the entry of the tRNA-amino acid complex of pathogenic bacteria, so that pathogenic bacteria cannot survive and eventually die [7].

Combination of *Lactobacillus spp.* And doxycycline gives the lowest results. This is due to the bacteriostatic properties of doxysklin and *Lactobacillus spp.* It produces antimicrobial substances that are also bacteriostatic, so both work synergistically to kill *Vibrio cholerae* [14, 15]. The provision of this combination needs to be considered the way and time of its giving. How to give it by giving *Lactobacillus spp.* First then 1 h after that only given doxycycline. The provision of this 1-h time lag is related to the time of emptying the mice stomach about 1–2 h [12]. In addition, doxycycline should not be given in conjunction with *Lactobacillus spp.* If doxycycline is given in conjunction with *Lactobacillus spp.* or first, then doxycycline will kill *Lactobacillus spp.*, so *Lactobacillus spp.* It cannot work on the intestinal mucosa. Therefore, the provision of time lag is very beneficial, because it gives *Lactobacillus spp.* a chance. to work on the intestinal mucosa. However, in this study, the possibility of a time interval of 1 h is not enough, because in the

results of the data analysis obtained the results of giving a combination of *Lactobacillus spp*. And doxycycline is not significant to the administration of doxycycline alone.

## 4 Conclusion

Although a combination of *Lactobacillus spp*. And doxycycline is not effective in inhibiting the growth of *Vibrio cholerae* compared to doxycycline alone, but clinically judging from the consistency of feces, the results are dry and hard which means very good. In addition, from TPC, the average number of colonies of *Vibrio cholerae* that grows is lowest. In this study found differences in the color of the colony of *Vibrio cholerae* that grows, so more research is needed. If you want to know the best time interval to suppress the optimal growth of *Vibrio cholerae* colony, then it takes a time interval of more than one hour. In addition, further research is also needed on the relationship of vibrio cholerae amounts with their infectious power and the benefits of *Lactobacillus spp* combination and doxysklin to reduce fluid deficiency due to dehydration.

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