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The effect of *lactobacillus reuteri* probiotic to improve the amount of il 23 and il 22 cytokine on *mus musculus* of *postpartum* model induced by *staphylococcus aureus*

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Abstract. The childbed infection was an inflammation of all the genitalia organ on childbed period caused by aerobic and anaerobic bacteria. One of them was *Staphylococcus aureus* which could attack and survive to live in epithelial and endothelial cells, and recognized by *Antigen Presenting Cell (APC)*. It would release cytokines as a trigger for T naïve activation to produce Th1, Th2, Treg and Th 17. Interleukin-23 (IL 23) issued by APC would make T naïve differentiated into Th17 which released IL17 and IL 22 as a natural medicine being an antibiotic alternative studied. One of them was probiotic (*Lactobacillus reuteri*). This research aimed to prove the effect of *L. reuteri* probiotic to improve the amount of IL-23 and IL-22 cytokines to *Mus musculus* of childbed model induced by *S. aureus*. The research method was *True Experimental through post test Only Control group* in vivo the tenth day 40 Balb/c pregnant mice divided into 8 groups K-HI, K-HIII, K + HI, K + HIII, P1HI, P1HIII, P2HI and P2HIII (the first and third day of negative control group, the positive/given *S. aureus* on day 1 and day 3, the first and third day of *L. reuteri* group, the first and third day of *L. reuteri* + *S. aureus* group). Then, performed the surgery to obtain cardiac blood. Furthermore, it would produce blood plasma after doing centrifugation followed by measurement of IL 23 and IL 22 amount using ELISA method kit with catalog number M2300 and M2200 of R & D brand. The data were analyzed using *Independent Sample T test* through SPSS software for Windows 23. The comparison result between the negative control (K-) and the treatment group on the first day proved that the amount of IL 23 and IL 22 cytokines were increased. The comparison between the observed groups on the first day proved that the amount of IL 23 and IL 22 cytokines were increased. The result of comparative experiment between P1 and P2 on the first day the amount of IL 23 cytokine was increased, but the amount of IL 22 cytokine was not increased. It was not statistically significant. Meanwhile, the comparison results on the third day proved that the amount of IL 23 and IL 22 cytokines were increased, but in P1 group the amount of IL 22



cytokine was decreased. The giving of *L. reuteri* probiotic could increase the amount of IL 23 and IL 22 cytokines on childbed mus musculus induced by *S. aureus*. Therefore, probiotics could be useful during postpartum period to improve the body immunity so that the infection during childbed period could be avoided.

1. Introduction

The childbed infection case is 13% higher because the comparison of operative childbirth / Sectio caesarea is more occurred than vaginal childbirth. It is through medical indications or on-demand. It is an inflammation of all genitalia organ on childbed period caused by aerobic and anaerobic bacteria. One of them is *Staphylococcus aureus* (*S. aureus*) which spreads through the perineum wound and endometrial surface[1]. It has polysaccharide capsules or thin films that play a role in virulence of infection bacteria originating from external sources such as open wounds from the mucosa (vaginal mucosa etc.)[1].

The infection treatment of *S. aureus* initially uses antibiotics. However, in recent studies it could be found that *S. aureus* isolated from the hospital is generally resistant to antibiotics in circulation, more than 85% of patients are resistant to oxacillin³. Therefore, one of the natural medicinal substances as an alternative of antibiotics widely studied is a probiotic which is normal flora bacteria in the mouth, gastrointestinal tract, and urogenital tract obtained through fermented probiotic intake (eg; cheese, yogurt, olive oil)[2].

One of the various types of probiotic species is *Lactobacillus reuteri* (*L. reuteri*) which is not found in all individual. Therefore, dietary supplements are needed to keep body immunity. *L. reuteri* is useful for the women health as the treatment of vaginal and urinary infections. In other studies, *L. reuteri* can inhibit the production of IL-12, IL-6, TNF- α pro-inflammatory cytokines, and B7.2 (CD86) expression in dendritic cells which stimulates the regulator of T[3] cell differentiation. In another study, the giving of *L. reuteri* in mice of sepsis model can significantly reduce the degree of intestinal inflammation[4]. One of *L. reuteri* strains is *L. reuteri* ATCC 6475 as an antiinflammatory treatment including the reducing of TNF- α . *L. reuteri* ATCC 6475 has also been known to increase the production of proinflammatory cytokines[5], [6].

2. Methodology

In this research, the experimental animals were the tenth day of 40 pregnant female *Mus musculus* Balb/c strain obtained from Biotech Laboratory of Malang State Islamic University, divided into 8 groups. Two days adaptation was done to them. Then, did the treatment on the 12th day until the third day of childbirth. Each group includes K1, K2, K3, K4, K5, K6, K7 and K * (the first and third day control groups, the groups administered *S. aureus* in the first and third day, *L. reuteri* group of first and third days, *L. reuteri* + *S. aureus* group of first and third days).

Probiotic used in this research was *L. reuteri* type with ATCC 6475 strain obtained from American Type Culture Collection (Manassas, VA 20108 USA), then did breeding in Mrsbroth's media. *L. reuteri* was given through oral sonde at a dose of 1×10^7 CFU /mouse as much as 250 μ l/mouse every day (once daily) from the 13th days of pregnancy to the first and third days of post partum.

Staphylococcus aureus bacteria were the type of gram-positive bacteria obtained from the Microbiology Laboratory of Medical Faculty of Brawijaya University then bred using nutrient broth and rinsed using NaCl. Furthermore, they were inducted into the postpartum *Mus musculus* vaginal with intra-vaginal using 1cc syringe which replaced the needle with a cut surflo $\frac{3}{4}$ part. It was at dose of 5×10^7 as much as 200 μ l/mouse at 0 to 12 hours of post partum or immediately after childbirth.

IL 23 cytokine amount was observed from blood plasma taken from cardiac blood systematically. Then, it was measured using Elisa kit method with catalog number M2300 of R & D brand.

IL 22 cytokine was observed from blood plasma taken from cardiac blood systematically. Then, it was measured using Elisa method kit with catalog number M2200 of R & D brand.

All data of research results were analyzed using statistical analysis with significance level $p \leq 0.05$ and level of trust 95% using SPSS software vs. 23.0 data tested normality using Saphiro-Wilk test followed by parametric test of T test (independent sample t test).

3. Result and Discussion

- a. The test results of the comparison between the group of *L. reuteri* probiotic and the giving *L. reuteri* + *S. aureus* probiotic group on the 1st day

Table 1. the comparison between the group of probiotik *L. Reuteri* given and *L. reuteri* + *S. aureus* Probiotics given on the 1st day

Observation groups	IL-23 amount (pg/mL)		IL-22 amount (pg/mL)	
	mean \pm SD	p - value	mean \pm SD	p - value
P1	100.70 \pm 7.73	–	63.44 \pm 2.70	–
P2	129.33 \pm 15.1	0.015	73.64 \pm 8.13	0.055
	4			

Note:

P1= The group given the *L.reuteri* treatment

P2 = The group given *L.reuteri* + *S. aureus* treatment

It showed that there was a significant difference ($p\text{-value} = 0.015 < \alpha$), the mean of IL-23 amount in *Mus musculus* of post partum model between *L. reuteri* probiotic group (P1) was 100.70 ± 7.73 pg/mL and *L. reuteri* + *S. aureus* probiotic group (P2) was 129.33 ± 15.14 pg/mL. It meant that the giving of *L. reuteri* + *S. aureus* probiotic bacteria was more able to increase the amount of IL-23 than only giving of *L. reuteri* probiotic in *Mus musculus* of post partum model.

It showed that there was no significant difference ($p\text{-value} = 0.055 > \alpha$), the mean of IL-22 amount in *Mus musculus* of post partum model between *L. reuteri* probiotic given group (P1) was 63.44 ± 2.70 pg/mL and *L. reuteri* bacteria + *S. aureus* probiotic given group (P2) was 73.64 ± 8.13 pg/mL. It meant that the giving of *L. reuteri* + *S. aureus* probiotic bacteria was more able to increase IL-22 amount than only giving of *L. reuteri* in *Mus musculus* of post partum model although it was not statistically significant.

Interleukin 23 could play a role in the development and maintenance of autoimmune balance, IL 23 had the ability to increase T cells producing *interferon* γ and proliferation⁴. The formation of IL 23 was occurred in dendritic cells, the cascade cytokine process would activate the immune cells which were necessary to participate in the eradication of any pathogen[1], [8]

IL 22 was an important cytokine for modulating tissue response during inflammation and played an important role in inflammation. These cytokines were expressed by many types of lymphocytes including innate and adaptive immune systems. They included CD 4 T cells, especially TH 17 and Nk cells, LTI cells, and LTI - like receptor[9]

In *S.aureus* infection, there would be an increasing of proinflammatory cytokines produced by Th1 and Th17 cells. Th17 cells were cells secreted IL 22 which could increase the production of β -Defensin later played a role in bacterial cleansing. Administration of *S. aureus* in childbed *Mus musculus* model could increase the amount of IL 22. By passing cascade signals from STAT 3, these cytokines induced proliferation and anti-apoptotic pathways, and helped preventing tissue damage and assisted in repairing[10].

The induction response of immune system of probiotic was occurred after interacted with gastrointestinal immunity, it was *peyer patch*. The interaction caused the increasing of cytokines' secretion needed to make the adequate immune response. If cytokines' induction run smoothly so when there was an odd thing enter into body, it would be fight by systemic immune optimally[11]

- b. The test results of the comparison between *L. Reuteri* probiotic given group with *L. reuteri* + *S. aureus* probiotic given group on the 3rd day

Table 2. The comparison the observation groups on the 3rd day

Observation groups	IL-23 amount (pg/mL)		IL-22 amount (pg/mL)	
	mean \pm SD	<i>p</i> - value	mean \pm SD	<i>p</i> - value
K+	81.54 \pm 4.27	–	53.53 \pm 4.10	–
P1	108.31 \pm 12.06	0.006	60.83 \pm 10.76	0.252
P2	170.33 \pm 31.36	0.001	82.01 \pm 13.50	0.007

Note:

K+ = The group given *S.aureus* treatment

P1 = The group given *L.reuteri* treatment

P2 = The group given *L.reuteri* + *S. aureus* treatment

It showed that there was a significant difference (p -value = 0.006 $< \alpha$), the mean of IL-23 amount in *Mus musculus* of post partum model between the positive control group (K+) was 81.54 \pm 4.27 pg/mL and *L. reuteri probiotic* given group (P1) was 108.31 \pm 12.06 pg/mL, there was a significant difference (p -value = 0.001 $< \alpha$), the mean of IL-23 amount in *Mus musculus* of post partum model between the positive control group (K+) was 81.54 \pm 4.27 pg/mL and *L. reuteri* + *S. aureus probiotic* bacteria group (P2) was 170.33 \pm 31.36 pg/mL. It meant that the giving of *L. reuteri* + *S. aureus probiotic* bacteria was more able to increase IL-23 amount than only giving of *S. aureus* bacteria in *Mus musculus* of post partum model.

It showed that there was no significant difference (p -value = 0.252 $< \alpha$), the mean of IL-22 amount in *Mus musculus* of post partum model between the positive control group (K+) was 53.53 \pm 4.10 pg/mL and *L. reuteri probiotic* group (P1) was 60.83 \pm 10.76 pg/mL. However, there was a significant difference (p -value = 0.007 $< \alpha$), the mean of IL-22 amount in *Mus musculus* of post partum model between the positive control group (K+) was 53.53 \pm 4.10 pg/mL and *L. reuteri* + *S. aureus probiotic* group (P2) was 82.01 \pm 13.50 pg/mL. It meant that the giving of *L. reuteri* + *S. aureus probiotic* bacteria was more able to increase IL-22 amount than only giving *S. aureus* bacteria in *Mus musculus* of post partum model.

The complications of genetic infections due to *S. aureus* in the childbed mom mostly was occurred on the third day. It was the most common vaginal pathogen which often became the one of the bacteria most commonly contributed to infection. Its colonization in the vaginal mucous membrane could cause *Toxic Shock Syndrome* which affected maternal mortality[11].

Based on the statistical analysis, the mean value of P2 group had higher amount of IL 22 cytokine if it compared with the other groups (82.01 \pm 13.50), it was proven that the increasing amount of IL 22 would work on the eradication of pathogens due to *S. aureus* bacteria. It would infect on the third day.

Along with this study found that in AD patients (*Atopic Dermatitis*) *Staphylococcal enterotoxin B* and the concentration of α -toxin could induce the production of IL 22 in PBMC and the isolated CD4 + T cells. The secretion of IL 22 increased with stimulation of α -toxin in keratinocytes and T cells. IL 22 increased significantly in α -toxin stimulation when compared with the psoriasis and healthy group patients[12].

c. The test result of the comparison between the days of IL-23 amount

Table 3. The comparison between the days of IL-23 amount (pg/mL)

Obsevation group	the 1 st day	the 3 rd day	<i>p</i> - value
K-	49.45 \pm 5.07	60.56 \pm 2.87	0.009
K+	76.51 \pm 2.92	81.54 \pm 4.27	0.100
P1	100.70 \pm 7.73	108.31 \pm 12.06	0.328
P2	129.33 \pm 15.14	170.33 \pm 31.36	0.057

Note:

K- = The Negative Control Group (no treatment)

K+ = the Group given *S.aureus* treatment

P1 = The group given the *L.reuteri* treatment

P2 = the Group given *L.reuteri* + *S. aureus* treatment

It showed that there was a significant difference ($p\text{-value} = 0.009 < \alpha$), the mean of IL-23 amount between the 1st day of observation was 49.45 ± 5.07 pg/mL and the 3rd day was 60.56 ± 2.87 pg/mL the negative control group (K-). It meant that on the 3rd day of observation showed the increasing of IL-23 amount. There was no significant difference ($p\text{-value} = 0.100 > \alpha$), the mean of IL-23 amount between the 1st day of observation was 76.51 ± 2.92 pg/mL and the 3rd day was 81.54 ± 4.27 pg/mL of the positive control group (K+). It meant that the 3rd day of observation showed the increasing of IL-23 amount although it was not statistically significant. There was no significant difference ($p\text{-value} = 0.328 > \alpha$), the mean of IL-23 amount between the 1st day of observation was 100.70 ± 7.73 pg / mL and the 3rd day was 108.31 ± 12.06 pg/mL of *L reuteri* probiotic group (P1). It meant that at the 3rd day observation showed the increasing of IL-23 amount although this increasing was not statistically significant. There was no significant difference ($p\text{-value} = 0.057 > \alpha$), the mean of IL-23 amount between the 1st day of observation was 129.33 ± 15.14 pg/mL and the 3rd day was 170.33 ± 31.36 pg/mL of *L reuteri* + *S. aureus* probiotic given group (P2). It meant that at the 3rd day of observation showed the increasing of IL-23 amount although it was not statistically significant.

The amount of IL 23 cytokine on the third day was increased if compared with the first day. *Lactobacillus* probiotics were able to induce immune cells in the intestinal mucosa which later the immune cells would be homing into the genitourinary lymphoid tissue, so that reactive antigen immune cells in the area also increased and they would react to the local antigen[7].

Th17 cells were the distinct derivatives of CD4+ T cells marked by releasing of IL 17. In addition, Th17 also secreted IL 22 as a member of IL 10. The expression of IL 22 began with a signaling change in the growth of factor β in IL 6 and other proinflammatory cytokines. The production of IL 22 would depend on the production of IL 23[2].

This depended on the given dose, where doses were low ± 100 μg / ml, the pro-inflammatory immune response (Th1 and Th17) reached a maximum value at 12 hours after exposure, while at high doses ± 1000 μg / ml it would achieve maximal value at 24 hours after exposure then decrease.

d. The test result of the comparison between the day of IL-22 amount

Table 4. The comparison between the day of IL-22 amount (pg/mL)

Obeservation group	the 1 st day	the 3 rd day	<i>p</i> - value
K-	27.27 \pm 3.28	38.81 \pm 4.04	0.004
K+	49.40 \pm 2.66	53.53 \pm 4.10	0.142
P1	63.44 \pm 2.70	60.83 \pm 10.76	0.654
P2	73.64 \pm 8.13	82.01 \pm 13.50	0.329

Note :

K- = The negative control group (no treatment)

K+ = The group given *S.aureus* treatment

P1 = The group given *L.reuteri* treatment

P2 = The group *L.reuteri* + *S. aureus* treatment

It showed that there was a significant difference ($p\text{-value} = 0.004 < \alpha$), the mean of IL-22 amount between the 1st day of observation was 27.27 ± 3.28 pg/mL and the 3rd day was 38.81 ± 4.04 pg/mL of the negative control group (K-). It meant that on the 3rd day of observation showed the increasing of IL-22 amount. There was no significant difference ($p\text{-value} = 0.142 > \alpha$), the mean of IL-22 amount between the 1st day of observation was 49.40 ± 2.66 pg/mL and the 3rd day was 53.53 ± 4.10 pg/mL of the positive control group (K+). It meant that on the 3rd day of observation showed the increasing of IL-22 amount although it was not statistically significant. There was no significant difference ($p\text{-value} = 0.654 > \alpha$), the mean of IL-22 amount between c of observation was 63.44 ± 2.70 pg/mL and the 3rd day

was 60.83 ± 10.76 pg/mL of *L reuteri probiotic* given group (P1). It meant that on the 3rd day of observation showed the reducing of IL-22 amount although it was not statistically significant. There was no significant difference ($p\text{-value} = 0.329 > \alpha$), the mean of IL-22 amount between the 1st day of observation was 73.64 ± 8.13 pg/mL and the 3rd day was 82.01 ± 13.50 pg/mL of *L reuteri* + *S. aureus probiotic* group (P2). It meant that on the 3rd day of observation showed the increasing of IL-22 amount although it was not statistically significant.

In normal condition, there was a normal flora in the vagina which consisted of a large number of bacteria, especially lactobacilli (90-95%) and other bacterial species with a smaller percentage. Lactobacilli maintained the normal ecosystem of the vagina by preventing the growth, adhesion, and expansion of the pathogens entering through the vagina. Normal flora was an important factor in anti-infection defense of the vaginal ecosystem. The number of normal flora was changeable during a woman's life affected by hormones (including during pregnancy and after childbirth), sexual activity and illness[8], [13].

L. reuteri produced a broad-spectrum antibiotic through the fermentation of a glycerol organism called reuterin, it inhibited the growth of some Gram-negative and Gram-positive bacteria which was harmful to cause the desired antimicrobial effect[3], [12]

4. Conclusion

The administrated of *L. reuteri* probiotics in *Mus musculus* of childbed model induced by *S. aureus* can increase the amount of IL-23 and IL-22 cytokines.

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