

The Influence of Probiotic *Lactobacillus reuteri* on Changes in Levels of Cytokines IL 23 Puerperal First Day on MUS Musculus Exposed to Bacteria *Staphylococcus aureus*

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Abstract

Puerperal infection is inflammation of all genitalia in the puerperium caused by aerobic and anaerobic bacteria, one of which is *Staphylococcus aureus* which can attack and survive in epithelial cells and endothelial cells, then recognized by the Presenting Cell Antigen (APC). The APC will secrete cytokines as triggers for the activation of Thais, Th1, Th2, Treg and Th17. Interleukin-23 (IL 23). The purpose of this study was to prove the effect of *L. reuteri* probiotics on increasing levels of IL-23 cytokines in puerperal mice induced by *S. aureus* on the first days.

True Experimental research method post test Only Control group in vivo 40 tails Balb/c pregnant 10th day was divided into 4 groups K-, K+, P1, P2. Then performed surgery to take blood from the heart which will then produce blood plasma after centrifugation followed by measurement of IL 23 levels by the ELISA kit method with no. Catalog M2300 R & D brand

Analysis of data using the Independent Sample T Test with the help of SPSS For Windows 23 software. The results of the comparison between the negative control (K-) with the treatment group on the first day proved that an increase in IL 23 cytokine levels occurred, a comparison between groups observation on the first day proved to be an increase in IL 23 cytokine levels, the results of a comparison test between P1 and P2 on the first day there was an increase in IL 23 cytokine levels an increase but not statistically significant the administration of *L. reuteri* probiotics can increase the levels of cytokines IL 23 in puerperal mice induced by *S. aureus* therefore probiotics can be useful in the puerperium to increase body immunity to prevent infection during the puerperium

Keywords: *Lactobacillus reuteri*, *Staphylococcus aureus*, IL 23.

Introduction

The incidence of puerperal infection is 13% higher because operative delivery/cesarean cesarea is compared to vaginal delivery while operative delivery is currently occurring mostly due to medical indications and on its

own request. The puerperal infection is inflammation of all genitalia in the puerperium caused by aerobic and anaerobic bacteria, one of which is *Staphylococcus aureus* (*S. aureus*) which spreads through the perineal wound and endometrial surface.⁽¹⁾

S. aureus has polysaccharide capsules or thin membranes that play a role in bacterial virulence. Infections originating from external sources such as open sores from the mucosa (vaginal mucosa etc.).⁽²⁾

Treatment of infections due to *S. aureus* bacteria initially using antibiotics but in recent studies found that *S. aureus* isolated from hospitals are generally resistant

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to circulating antibiotics, more than 85% of patients experience resistance to oxacillin. Therefore natural remedies as an alternative antibiotic that have been studied are one of them is probiotics namely normal flora bacteria in the mouth, gastrointestinal tract and urogenital tract obtained through fermented probiotic intake (for example cheese, yogurt, olive oil). The results of a study by Evrard, that the effect of probiotics on dendritic cells at high treatment doses on *Lactobacillus rhamnosus* (*L. rhamnosus*) Lcr35 causes monocyte immune responses in humans resulting from immature dendritic cells. Induction increase in the production of Th1/Th17 proinflammatory cytokines such as TNF, IL 1 β and IL 23. The produced IL 23 will make Th0 differentiate into TH 17 which releases IL 17 and IL 22 cytokines as proinflammatory cytokines.⁽³⁾

In reproductive health services the use of *L. reuteri* probiotics as a treatment for urinary tract infections, vulvovagina candidiasis, bacterial vaginosis in humans while the target of a recent infectious disease therapy through differentiation from Th 17 cells on stimulation of IL 23 cytokines which will produce IL cytokines 22 so that the researchers wanted to know the effect of *L. reuteri* probiotics as prevention of puerperal infection as evidenced by increased levels of IL 23 and IL 22 cytokines in postpartum mice induced by *S. aureus* ⁽⁴⁾

Material and Method

- a. **Animal Try:** The experimental animals in this study were *Mus musculus* strain Balb/c pregnant female 10th day obtained from Biotech Laboratorial Laboratory of Malang State Islamic University as many as 40 animals, divided into 4 groups. The animals were adapted for 1 day. first day and control groups, first and third day *S. aureus* groups, *L. reuteri* first groups, group *L. reuteri* + *S. aureus* first day.
- b. **Probiotic *Lactobacillus reuteri*:** Probiotics used in this study were *L. reuteri* with ATCC 6475 strain obtained from the American Type Culture Collection (Manassas, VA 20108 USA), then cultured in Mrsbroth media. *L. reuteri* is given through oral sonde at a dose of 1 x 10¹⁰ CFU/mouse as much as 250 μ l/mouse every day (once a day) from gestational age 13 days to post partum on the first day.
- c. ***Staphylococcus aureus* bacteria:** It is a type of gram-positive bacteria obtained from the

Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya, then cultured with a nutrient broth and rinsed with NaCl, then induced into the vagina of the postpartum mice intravaginally with a 1cc syringe which is replaced with a lo section of surfloo. dose of 5 x 10⁷ as much as 200 μ l/mice at 0 to 12 hours post partum or immediately after giving birth

- d. **Measurement of IL cytokine levels 23:** Observed from blood plasma taken from cardiac blood which is carried out systematically then measured levels of IL 23 with the Elisa kit method with no. Catalog M2300 R & D brand
- e. **Statistical Analysis:** All research data were analyzed statistically with significance level $p \leq 0.05$ and 95% confidence level using SPSS software vs 23.0. The data were tested for normality using the Saphiro-Wilk test followed by the parametric test T test (independent sample t test)

Findings:

- a. **Comparison result of the control group with treatment (-) on the first day:** Based on the results of the free sample t test (independent sample t test) data IL-23 levels in the puerperal model mice are displayed in full in the table below

Table 1 comparison if control groups (-) with treatment on day 1

Observation Group	IL Level-23 (pg/mL)	
	Mean \pm SD	p - value
K-	49.45 \pm 5.07	-
K+	76.51 \pm 2.92	0.000
P1	100.70 \pm 7.73	0.000

Information:

- K- = Negative Control Group (no treatment given)
- K + = group given *S.aureus* treatment
- P1 = group given *L.reuteri* treatment

Showed that there was a significant difference (p -value = 0,000 <) the average IL-23 level in puerperal mice between negative control groups (K-) (49.45 \pm 5.07 pg/mL) and positive control groups (K +) (76.51 \pm 2.92 pg/mL), there is a significant difference (p -value = 0.000 <) the average level of IL-23 in puerperium model mice between the negative control group (K-) (49.45 \pm 5.07 pg/mL) with the group giving *L. reuteri* probiotics (P1) (100.70 \pm 7.73 pg/mL) means that the treatment of *L. reuteri* probiotics in puerperal mice can increase IL-23 levels.

b. Comparison test results between groups of observation on day 1: Based on the results of the free sample t test (independent sample t test) data IL-23 levels in the puerperal model mice are displayed in full in the table below

Table 2: Comparison between groups of observations day 1

Observation group	IL Level-23 (pg/mL)	
	Mean ± SD	p - value
K+	76.51±2.92	-
P1	100.70±7.73	0.001
P2	129.33±15.14	0.000

Information:

K + = group given S.aureus treatment

P1 = group given L.reuteri treatment

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

Showed that there was a significant difference (p-value = 0.001 <) the average IL-23 level in puerperium mice between positive control groups (K +) (76.51 ± 2.92pg/mL) with the group giving L. reuteri probiotics (P1) (100.70 ± 7.73 pg/mL), there is a significant difference (p-value = 0.000 <) the average IL-23 level in puerperal mice between positive control groups (K +) (76.51 ± 2.92pg/mL) with the group giving Probiotic L. bacteria reuteri + S. aureus (P2) (129.33 ± 15.14 pg/mL) means that the administration of L. reuteri + S. aureus bacteria is more able to increase IL-23 levels than only the administration of S. aureus bacteria in puerperium mice.

c. Comparison test results between groups of L.reuteri probiotics with giving of L.reuteri + S.aureus probiotics on day 1: Based on the results of the free sample t test (independent sample t test) data levels of IL-23 and IL-22 levels in the puerperium model mice are displayed in full in the table below

Table 3 Comparison between groups giving L. reuteri probiotics with L. reuteri + S. aureus Probiotics on day 1

Observation groups	IL Level-23 (pg/mL)	
	Mean ± SD	p - value
P1	100.70±7.73	-
P2	129.33±15.14	0.015

Information:

P1 = group given L.reuteri treatment

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

Showed that there was a significant difference (p-value = 0.015 <) the average levels of IL-23 in puerperal mice between the groups giving probiotic L. reuteri (P1) (100.70 ± 7.73 pg/mL) with the group giving Probiotic L. reuteri + S. aureus (P2) (129.33 ± 15.14 pg/mL), means that the administration of L. reuteri + S. aureus Probiotics is more able to increase IL-23 levels than the administration of L. reuteri probiotics alone in puerperium mice

Discussion

In the negative control group there was absolutely no treatment whatsoever but it appeared that on the third day observation the IL-23 level was greater than the first day observation according to Figure 5.3. The puerperal period Mus musculus Balb/c strain occurs within 24 hours where on the third day there has been a change in the reproduction of mice from the puerperium to the estrus period so that the immune system changes in the body of mice increased levels of IL 23 on the third day in the control group This is because the mice are in the estrous period. (5)

Complications of genetal infection due to S. aureus in puerperal mothers mostly occur on the third day, S. aureus is the most common vaginal pathogen which is one of the most frequently involved bacteria in infection, S. aureus colonization of the vaginal mucous membranes can cause Toxic Shock Syndrome which has an impact on maternal mortality. (6)

Local and systemic immune cell response mechanisms for S colonization. nasal aureus as a prevention of disease entry does not fully explain how this bacterium can die, although there are some clues in emphasizing local proinflammatory cytokines to provide signals that facilitate colonization of settled bacteria(7). S. aureus bacteria that are in dendritic cells are able to stimulate the production of cytokines IL 6, IL 1 and IL 23 which will all encourage the differentiation of CD4 + T cells towards Th 17 cells (5)

Th17 cells are a different strain from CD4 + T cells characterized by IL 17 release. In addition Th 17 also excludes IL 22 as a member of IL 10. The expression of IL 22 begins with changes in signaling in growth factors β in IL 6 and other proinflammatory cytokines. IL 22 production will depend on IL 23 production(8)

Normal flora have a role in developing protective host mechanisms. Resident bacteria that are not

pathogenic require ecological needs for their lives so that pathogenic bacteria (non-residents) that will proliferate in locations inhabited by normal flora will have difficulty competing with resident bacteria⁽⁹⁾. But if the normal flora is depressed, the growth of pathogenic bacteria will be more rapid and can cause disease, *S. aureus* is one of the most pathogenic bacteria found in the vagina and is one of the bacteria that causes most infections. This bacterial colonization of the vaginal mucosa can be a cause of toxicoseptic shock⁽¹⁰⁾

Conclusion

There an effect probiotics *Lactobacillus reuteri* on changes in the nature of all that and the keeping up of 23 on mice parturition of being exposed to the staphylococcus bacteria aureus

Ethical Clearance: Ethical clearance of this study was taken from Ethical Committee of Poltekkes Kemenkes Malang, Indonesia.

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Conflict of Interest: There is no conflict of interest in this study

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