Turkish Journal of Immunology - Open Access
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Turk J Immunol 2017; 5(2):51–60

The Effect of *Lactobacillus reuteri* on the Percentage of Th17 Cells and Level of IL-17 in *Staphylococcus aureus*-Induced Puerperal Infection BALB/c Mice

Farelerde *Staphycoccus aureus* ile Oluşturulan Lohusalık Ateşinde *Lactobacillus reuteri*'nin Th17 Hücreleri ve IL-17 Düzeyine Olan Etkisi

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Abstract

Background: Puerperal infection is an infection of the reproductive tract during labor or puerperal period, which is largely caused by *Staphylococcus aureus* infection resulting in Toxic Shock Syndrome (TSS). The prevalence of postpartum infection has increased over the past three years, coupled with an increase in *Staphylococcus aureus* (MRSA) Methicillin Resistant, resulting in high treatment costs and high morbidity and mortality rates.

Objective: This study aimed to determine the effect of *Lactobacillus reuteri* on the percentage of Th17 cells and the level of IL-17 in *Staphylococcus aureus*-induced puerperal BALB/c mice.

Methods: Mice were divided into 4 groups with each group consisting of 4 mice in puerperal period and 4 mice in three days postpartum period; Group I (mice were induced with *Staphylococcus aureus* at 0–12 hours postpartum), Group II (mice were administered orally with *Lactobacillus reuteri*), Group III (mice were treated with *Lactobacillus reuteri* and *Staphylococcus aureus*) and group IV (control). The percentage of Th17 cells was measured by Flow cytometry method, while the level of IL-17 was measured by ELISA method.

Results: The results showed that the administration of *Lactobacillus reuteri* significantly influenced the percentage of Th17 cells and the levels of IL-17 in *Staphylococcus aureus*-induced puerperal mice.

Conclusion: In summary, *Lactobacillus reuteri* may act as a preventive agent of puerperal infections in *Staphylococcus aureus*-induced mice during the puerperal period and three days postpartum.

Keywords: IL-17, *Lactobacillus reuteri*, *Staphylococcus aureus*, Th17 cells

Öz

Giriş: Lohusalık ateşi doğum sırasında veya lohusalık dönem *Staphilococcus aureus* enfeksiyonunun neden olduğu, toksik şok sendromuna (TSS) neden olan bir üreme sistemi enfeksiyonudur. Son 3 yıldır metisiline dirençli *Staphylococcus aureus*un neden olduğu doğum sonrası enfeksiyonlarda artış olmuştur. Bu artış ta tedavi maliyetlerinin morbidite ve mortalite oranında artış olmuştur.

Amaç: Bu çalışmada amaç Balb/c farelerde *Staphylococcus aureus* ile oluşturulmuş lohusalık ateşinde *Lactobacillus reuteri*'nin Th17'nin ve Il-17 seviyelerine olan etkisi araştırmaktır.

Yöntemler: Bu çalışmada, her grupta tam lohusalık döneminde olan 4 fare ile doğumdan 3 gün sonra gruplandırılmış 4 fare bulunduğu 4 fare bulunmakta idi: Grup I (Doğumdan 0-12 saat sonra *Staphylococcus aureus* verilmiş fareler), Grup II (sadece ağızdan *Lactobacillus reuteri* verilmiş fareler), Grup III (*Lactobacillus reuteri* ve *Staphylococcus aureus* verilmiş fareler), Grup IV (Kontrol). Akan hücre ölçer ile Th17 hücrelerinin oranı ve ELISA yöntemi ile IL-17 ölçüldü.

Sunular: Çalışmada, *Staphylococcus aureus* ile oluşturulmuş lohusalık ateşi olan farelerde *Lactobacillus reuteri* verilmesinin Th17 hücrelerinin oranını ve IL-17 seviyesini düşürdüğü saptandı.

Sonuç: *Lactobacillus reuteri, Staphylococcus aureus* ile doğum döneminde ve doğumdan 3 gün sonra oluşturulmuş lohusalık ateşinde koruyucu bir rol oynamaktadır.

Anahtar Kelimeler: IL-17, *Lactobacillus reuteri, Staphilococcus aureus,* Th17 hücreleri

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Received: Feb 08, 2017
Accepted: Jul 12, 2017

doi: 10.25002/tji.2017.539

Introduction

According data from WHO, from 1997 to 2007 the cause of maternal death was haemorrhage (35%), hypertension (18%), sepsis (8%), indirect causes (18%), other

causes (11%), and emboli (1%).[1] While the biggest cause of maternal mortality in Indonesia in 2010 until 2013 is bleeding (30.3%), hypertension (27.1%), and infection (7.3%). Based on Riskesdas (Basic Health Research) data in 2013, 9.12% of childbirth process is assisted by non-health workers, this may resulted in the possibility of birth complications during puerperal periods.[2]

Puerperal infection is a bacterial infection in genital tract during childbirth process or puerperal period.[3] Infectious bacteria are originally endogenous bacteria that become pathogens in tissue damage such as episiotomy lesions and laceration of the vagina or cervix. The bacteria that mostly play a role in puerperal infection is *Staphylococcus aureus*.[3,4,5]

Staphylococcus aureus is an extracellular bacteria which is the main cause of puerperal infections resulting in the presence of Toxic Shock Syndrome (TSS).[6] In this case, Th17 cells play an important role in the protection of hosts against extracellular pathogens and autoimmune diseases. Methicillin Resistant Staphylococcus aureus (MRSA) is a bacterial resistant state of *Staphylococcus aureus* against antibiotics. This becomes a worrisome issue as it results in morbidity and mortality.[7,8]

Some types of *Lactobacillus* are efficient future probiotic medications for the treatment of infections due to antibiotic-resistant of *Staphylococcus aureus*.[9] *Lactobacillus reuteri* is able to inhibit the growth of *Staphylococcus aureus* and clinical isolates of MRSA in vitro. In accordance with the Cochrane database review results, *Lactobacillus reuteri* is commonly used as a therapy for reproductive health problems in woman (vaginosis).[10,11] *Lactobacillus reuteri* (ATCC 6475) is able to regulate Foxp3$^+$ Treg cells level, decrease the level of IL-17A, and restore the balance of Th17 cells (a study in experimental animals, and woman with chronic inflammation).[12] Here, we reported the effect of *Lactobacillus reuteri* on the percentage of Th17 cells and the levels of IL-17 cytokines in *Staphylococcus aureus*-induced puerperal mice.

Methods

Mice

A total of 32 normal BALB/c female pregnant mice (with gestational age of 10 days), between the ages of 10–12 weeks old were used. Mice were obtained from Integrated Research and Testing Laboratory (LPPT) Islamic University Malang and maintained in pathogen-free facility. Mice were divided into 4 groups with each group consisting of 4 mice in puerperal period and 4 mice in three days of postpartum period; Group I/KI (mice were induced with *Staphylococcus aureus* at 0–12 hours postpartum), Group II/KII (mice were administered orally with *Lactobacillus reuteri*), Group III/KIII (mice were treated with *Lactobacillus reuteri* and *Staphylococcus aureus*) and group IV/KIV (control).

Puerperal Infection and *Lactobacillus reuteri* Oral Administration

Mice were intravaginally induced with *Staphylococcus aureus* in 0–12 hours postpartum with a dose of $5x10^7$ to prepare mice with puerperal infection. While *Lactobacillus reuteri* (ATCC 6475) was administered orally from 12 days of gestation to puerperal period and 3 days of postpartum with a dose of $1x10^{10}$ CFU/mice a day.

Lymphocyte Isolation

The mice were anesthetized by using chloroform. Spleen were isolated and pressed clockwise with syringe base. Aggregates were separated by gentle pipetting, and debris was discarded by passaging the suspension through a cell strainer (100-µm Nylon). The suspension in propylene was added with PBS up to 10 mL and were then centrifuged at 2500 rpm, 4°C for 3 minutes. Supernatant was removed and pellet was resuspended with 1 mL of sterile PBS.

Intracellular Staining and Flow Cytometry Analysis

The combinations of antibody for intracellular staining was FITC Anti-Mouse CD4 (Biolegend, USA, No. 100406) and PE Anti-Mouse IL-17A (Biolegend, USA, No. 506904). The cells were incubated with extracellular antibodies for 20 min in the ice box at 4°C. After incubation, the suspension was washed and the pellet was resuspended in cytofix buffer (50 µL) for 20 min in dark conditions at 4°C. Then the suspension was resuspended in 500 µL wash-perm and centrifuged at 2500 rpm, 10°C for 5 min. Supernatant was discarded and pellet was subjected to intracellular staining for 20 min, at 4°C. Each sample was transferred into flow cytometry cuvet and then analyzed with flow cytometer (FACSCalibur; BD Biosciences, New Jersey, USA), and calculated using BD CellQuest PRO software.

ELISA Method

After 1 cc of blood were isolated from the heart of mice and were put into a blood container tube without EDTA. Blood were centrifuged at 35000 rpm for 15 minutes. Pellet

were discarded and the level of IL17A was measured from blood serum by using ELISA Kit Mouse IL-17A method. Overall the IL17A measurement procedure was d based on the standard method of Biolegend (Biolegend, USA, Catalog Percentage 432507 1 Plate and 432508 5 Plate) as the manufacturer of ELISA Kit used in this study.

Statistical Analysis

All data were analyzed statistically with significance level p≤0.05 and confidence level 95% with Minitab 17.0 software. The normality and homogeneity of data were tested with Saphiro-Wilk test and Levena test, respectively and followed by Nested ANOVA and Tukey (Post Hoc Test) parametric test. Data were then subjected to multivariate analysis with MANOVA and some approach methods including Wilks', Lawley-Hotelling, Pillai's, and Roy's.

Results

Lactobacillus reuteri is able to decrease the percentage of Th17 cells and level of IL-17 in *Staphylococcus aureus*-induced BALB/c mice during puerperal period

Th17 cells are subset of naïve CD4 T cells which is primarily present in mucosal tissues (vaginal, uterine, gastrointestinal, respiratory tract) that play an important role in host protection against inflammation due to infection of extracellular pathogens and autoimmune disease.[7,20] Figure 1 shows that the administration of *Lactobacillus reuteri* (KII and KIII) could significantly reduced the percentage of Th17 cells p<0.001 compared with *Staphylococcus aureus*-induced group and control group (KI and KIV). While there is no significant difference between control group (KIV) and *Lactobacillus reuteri* group (KII and KIII) (p>0.05)

IL-17 has an important function in host defense that induces neutrophil-rich inflammation by stimulating the production of chemokines and other cytokines (such as TNF) that carry neutrophils and monocytes to the site of T-cell activation. In addition, IL-17 also stimulates the production of antimicrobial substances (defensin) from various cell types.[14] Figure 2 shows that *Staphylococcus aureus* group (KI) has the highest mean value among the other groups of treatment. Furthermore, the oral administration of *Lactobacillus reuteri* in *Staphylococcus aureus*-induced mice during the puerperal period could significantly decrease the level of IL-17. This indicated that there is a significant influence of *Lactobacillus reuteri* on the percentage of Th17 cells and the level of IL-17 cytokine in *Staphylococcus aureus*-induced mice, during the puerperal period.

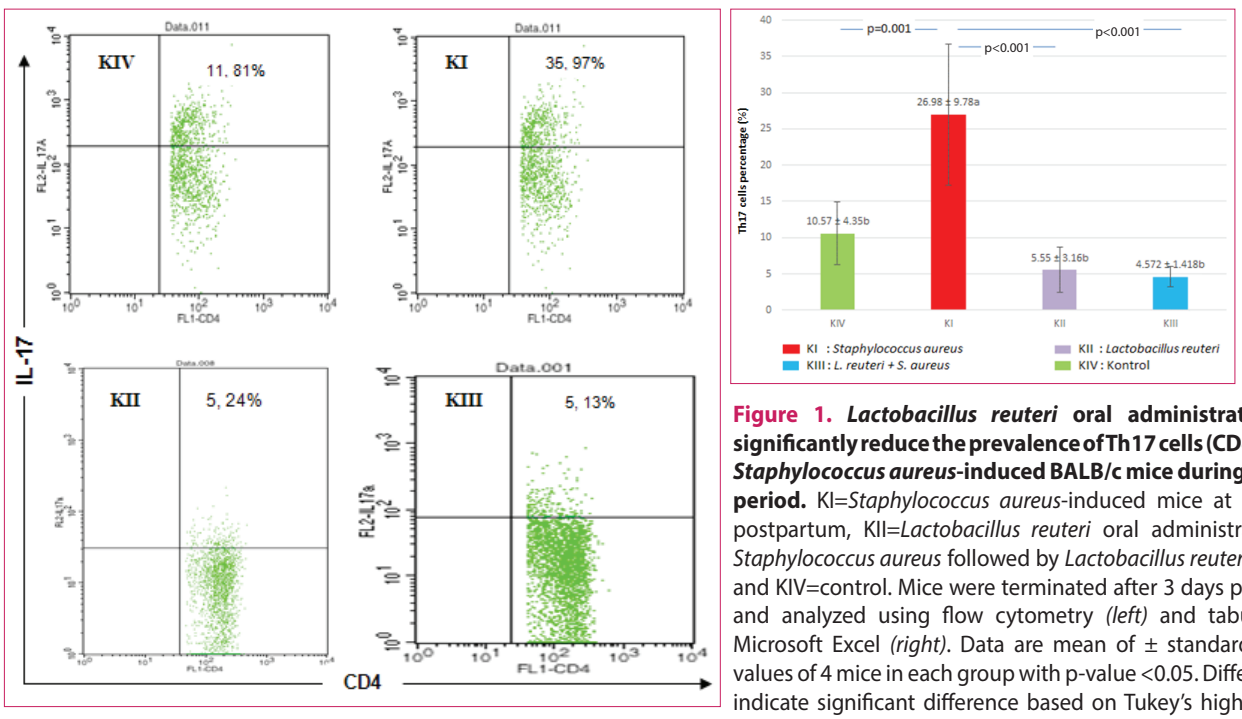

Figure 1. *Lactobacillus reuteri* oral administration could significantly reduce the prevalence of Th17 cells ($CD4^+IL\text{-}17^+$) in *Staphylococcus aureus*-induced BALB/c mice during puerperal period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII=*Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and analyzed using flow cytometry *(left)* and tabulated into Microsoft Excel *(right)*. Data are mean of ± standard deviation values of 4 mice in each group with p-value <0.05. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

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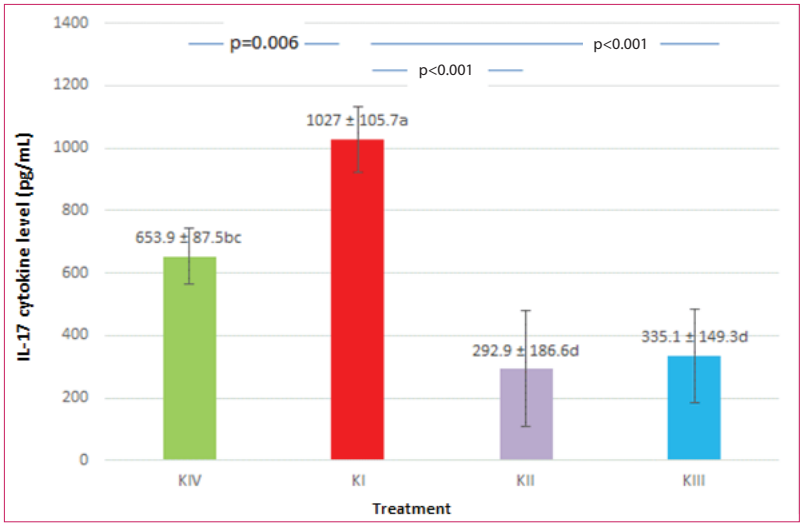


Figure 2. The level of IL-17 is markedly depleted after *Lactobacillus reuteri* treatment in *Staphylococcus aureus*-induced BALB/c mice during puerperal period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII= *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and peripheral blood were analyzed using ELISA method. Data are mean of ± standard deviation values of 4 mice in each group with p-value <0.05. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

Lactobacillus reuteri reduce the percentage of Th17 cells and level of IL-17 cytokine in *Staphylococcus aureus*-induced BALB/c mice in 3 days during post-partum period

We further examined the effect of *Lactobacillus reuteri* oral administration on the percentage of Th17 cells in *Staphylococcus aureus*-induced BALB/c mice in 3 days during post-partum period. Figure 3 shows marked depletion of Th17 cells ($CD4^+IL\text{-}17^+$) in *Lactobacillus reuteri* treatment group (KII and KIII) after *Staphylococcus aureus* induction compared to *Staphylococcus aureus* treatment (KI) (p=0.004 and 0.012 respectively). The percentage of Th17 cells were significantly higher in *Staphylococcus aureus* treatment (KI) group. However, no significant difference was found between KII (*Lactobacillus reuteri* oral administration) and KIII in terms of percentages of Th17 (*Staphylococcus aureus* followed by *Lactobacillus reuteri*) (p>0.05).

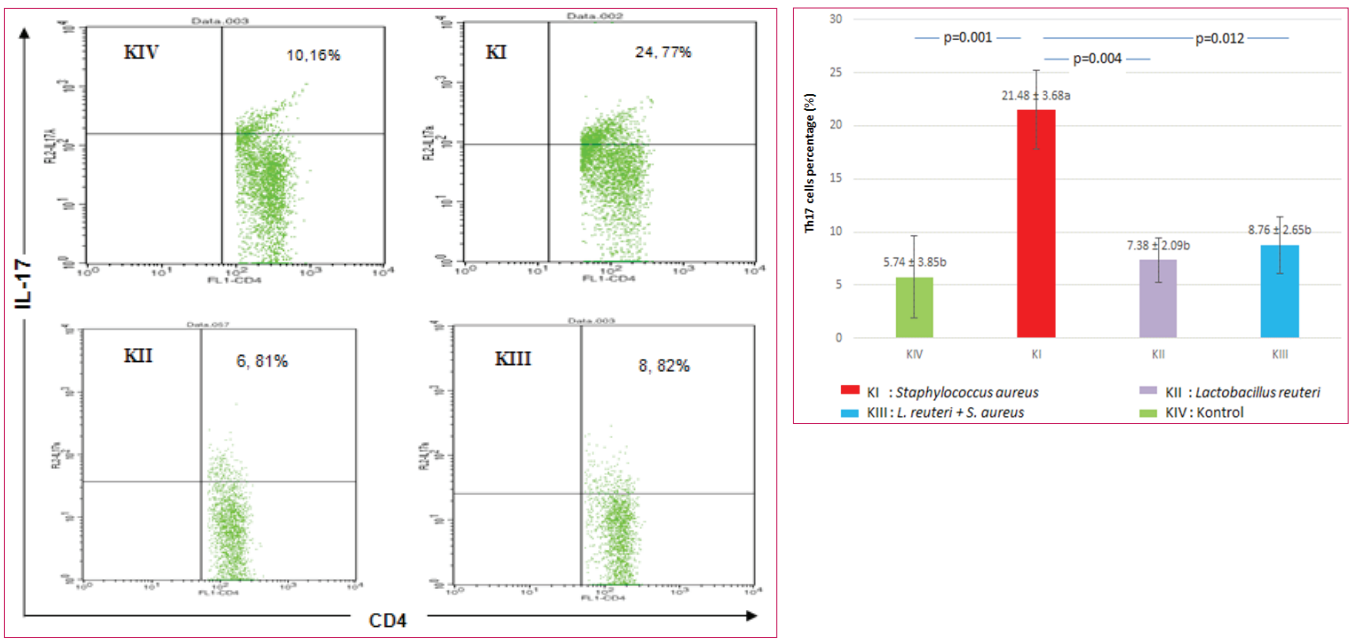


Figure 3. The depletion of Th17 cells ($CD4^+IL\text{-}17^+$) in *Staphylococcus aureus*-induced BALB/c mice after *Lactobacillus reuteri* oral administration in 3 days during post-partum period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII= *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and analyzed using flow cytometry *(left)* shown in graph *(right)*. Data are mean of ± standard deviation values of 4 mice in each group with p-value <0.05. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

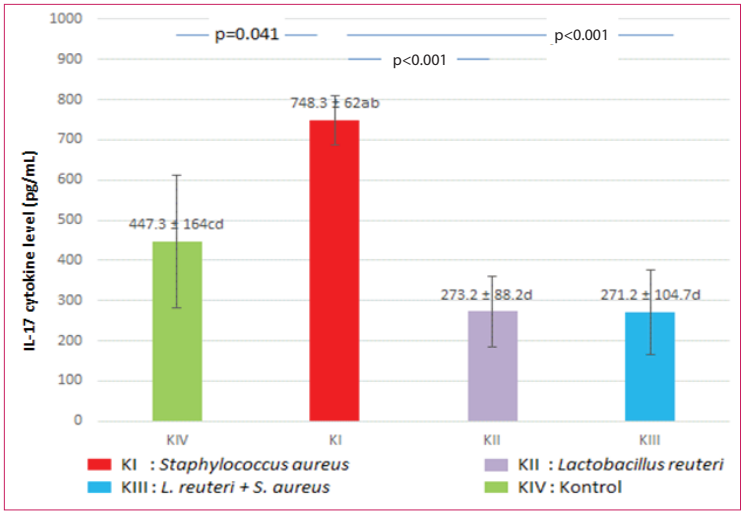
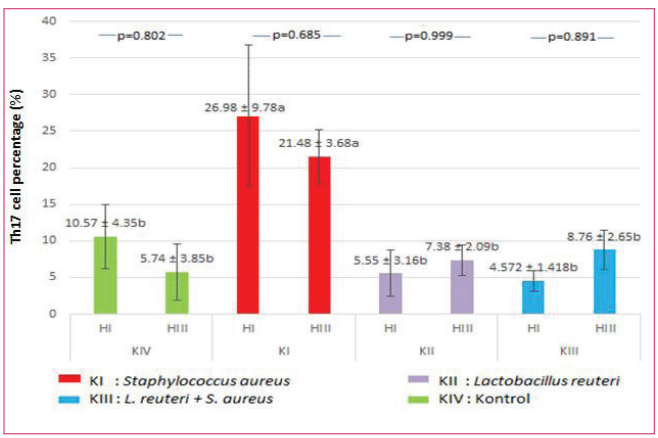


Figure 4. The level of IL-17 is markedly reduced after *Lactobacillus reuteri* treatment in *Staphylococcus aureus*-induced BALB/c mice in 3 days during post-partum period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII= *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and peripheral blood were analyzed using ELISA method. Data are mean of ± standard deviation values of 4 mice in each group with p-value <0.05. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

The level of IL-17 was measured using ELISA method. Based on the result of ELISA analysis, the level of IL-17 was significantly reduced in *Lactobacillus reuteri* treatment (KII and KIII) compared to that of *Staphylococcus aureus*-induced BALB/c mice in 3 days during post-partum period. The highest level of IL-17 was found in *Staphylococcus aureus* treatment group (p<0.001). However, no significant difference was found between KII (*Lactobacillus reuteri* oral administration) and KIII (*Staphylococcus aureus* followed by *Lactobacillus reuteri*) (p>0.05) (Fig. 4).

The difference of Th17 and IL-17 level between puerperal periods and 3 days post-partum of in *Staphylococcus aureus*-induced BALB/c mice

Based on ANOVA nested test and 5% Tukey test on Th17 cell count and IL-17 levels between puerperal period and 3 days post-partum period, p-value was greater than 0.05 (p>0.05) which showed no significant difference. However, the mean ± SD value of Th17 cell count and IL-17 levels in the control group and the *Staphylococcus aureus* group during the puerperal period was higher than 3 days post-partum period. Whereas in the *Lactobacillus reuteri* group and the *Lactobacillus reuteri* + *Staphylococcus aureus* group the mean ± SD value of Th17 cell count in 3 days postpartum period was higher than the puerperal period, but mean ± SD levels of IL-17 during the puerperal period was higher than in 3 days postpartum period (Fig. 5).



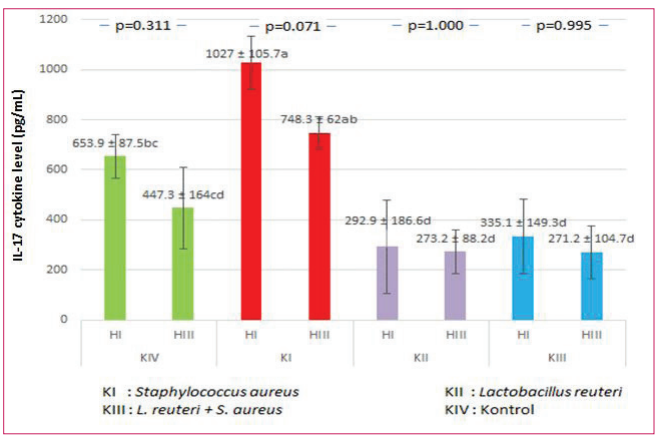


Figure 5. The difference in flow cytometry results of Th17 cells (a) and IL-17 cytokine (b) between puerperal periods and 3 days post-partum period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII= *Staphylococcus aureus* induction followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and peripheral blood were analyzed using flow cytometry and ELISA method. Data are mean of ± standard deviation values. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

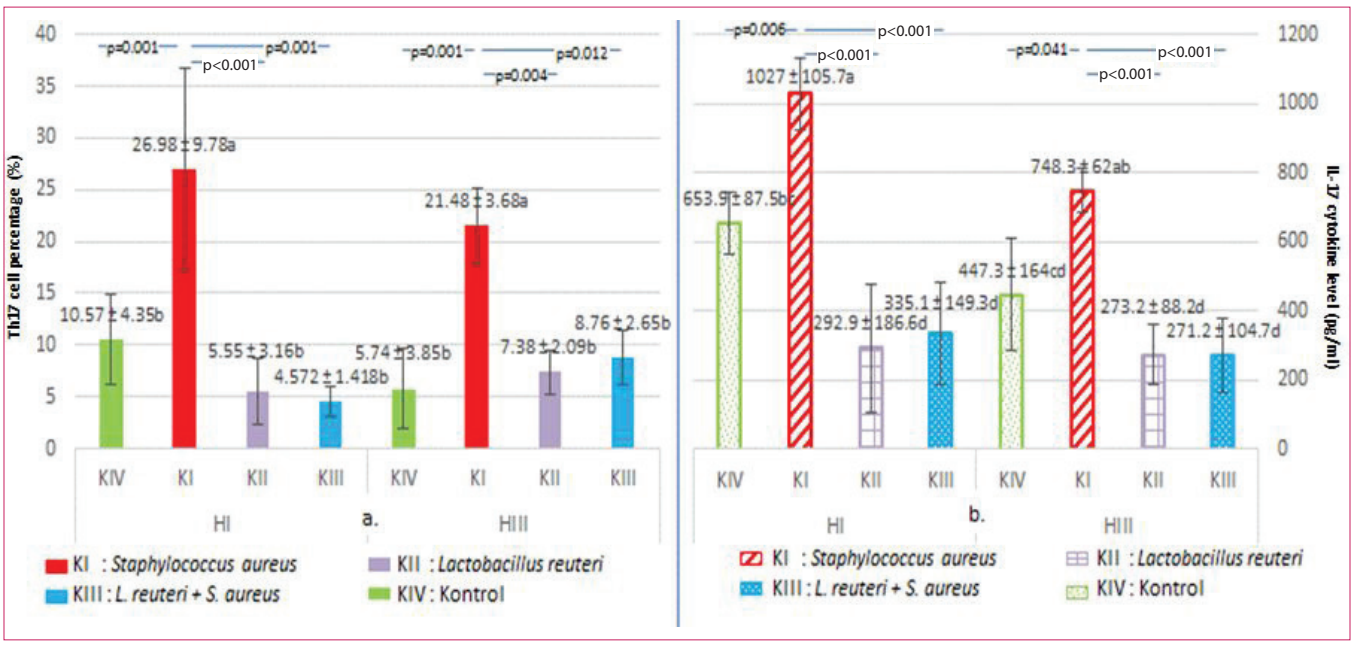


Figure 6. The difference in flow cytometry results of Th17 cells (a) and IL-17 cytokine (b) between puerperal periods and 3 days post-partum period. KI=*Staphylococcus aureus*-induced mice at 0-12 hours postpartum, KII=*Lactobacillus reuteri* oral administration, KIII=*Staphylococcus aureus* induction followed by *Lactobacillus reuteri* treatment and KIV=control. Mice were terminated after 3 days post-partum and peripheral blood were analyzed using ELISA method. Data are mean of ± standard deviation values of 4 mice in each group with p-value<0.05. Different letters indicate significant difference based on Tukey's high significant differences test at a 95% significance level.

Multivariate analysis (MANOVA) revealed that Lactobacillus reuteri affected the percentage of Th17 cells and level of IL-17 cytokine simultaneously in *Staphylococcus aureus*-induced mice (Fig. 6).

In this study, *Staphylococcus aureus* induction caused thrombosis in the puerperal period (Fig. 7).

Some characteristics of *Staphylococcus* lesions are abscess, local tissue damage followed by hyperemia and a strong inflammatory response characterized by a large accumulation of poly-morfonuclear leukocytes. In our study, abscesses in placental implantation and lacerations was occurred during the puerperal period (22 hours after *Staphylococcus aureus* induction), local tissue damage in the inoculation region was occurred during puerperal or

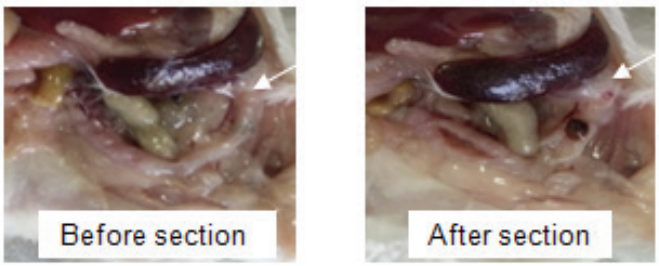


Figure 7. Thrombosis and abscesses in uterus of Staphylococcus aureus-induced mice in puerperal period.

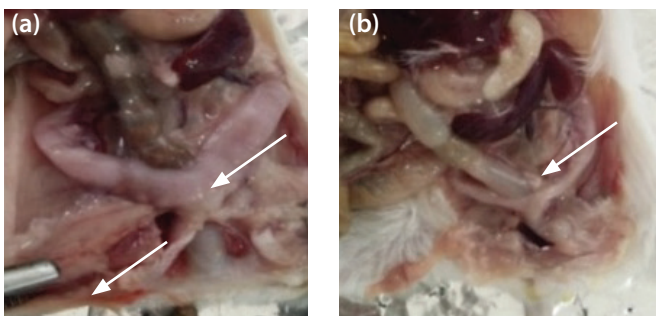


Figure 8. Abscess in tissue after placental implantation and laceration which occurred during puerperal period (a) followed by necrosis of lesions in 3 days post-partum period (b). The white arrow indicates the uterine tissue after placental implantation

3 days post-partum which resulting in complications of puerperal infection (Fig. 8).

Lactobacillus reuteri has a significant effect in repairing uterine tissue of *Staphylococcus aureus*-induced mice in puerperal period (Fig. 9).

The repairing tissue in *Lactobacillus reuteri* group was better compared to other groups (Fig. 10).

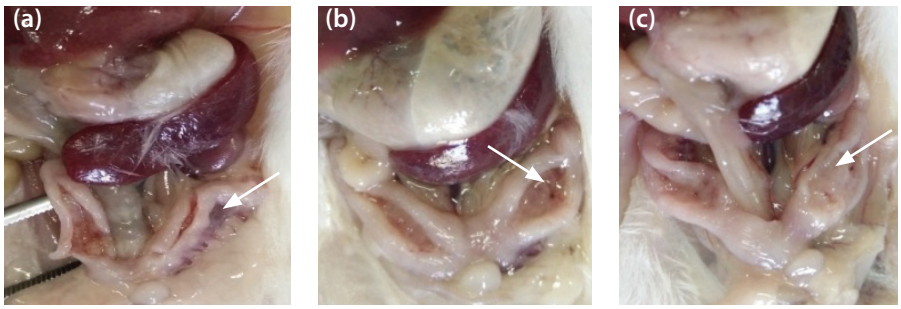


Figure 9. Differences in anatomy repair in uterine tissue after placental mice implantation.
Lactobacillus reuteri is effective for puerperal infections (due to *Staphylococcus aureus* infection) which stimulates repair of damaged tissue due to placental implantation processes (white arrow). (a) Control (K4), (b) *Lactobacillus reuteri* treatment (K2), (c) *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment (K3). The white arrow indicates the uterine tissue after placental implantation.

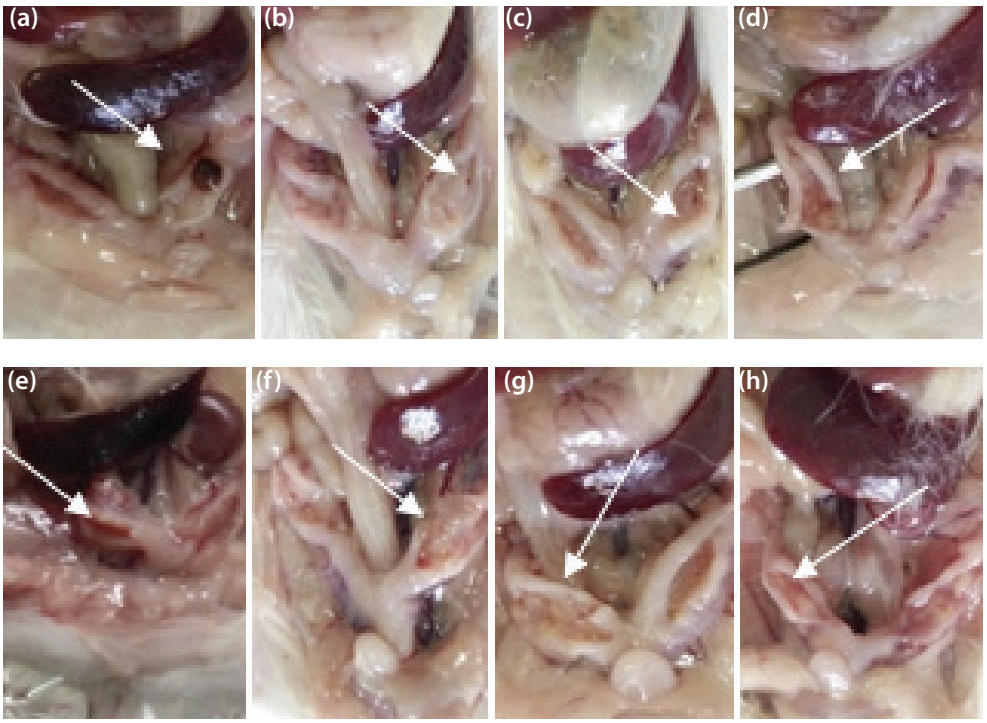


Figure 10. Injured tissue repair after placental implantation and laceration which occurred during puerperal period followed by necrosis of lesions in 3 days post-partum period. (a) *Staphylococcus aureus*-induced mice at 0–12 hours postpartum during puerperal period, (b) *Lactobacillus reuteri* oral administration during puerperal period, (c) *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment during puerperal period (d) control (e) *Staphylococcus aureus*-induced mice at 0–12 hours postpartum during 3 days post-partum period , (f) *Lactobacillus reuteri* oral administration during 3 days post-partum period, (g) *Staphylococcus aureus* followed by *Lactobacillus reuteri* treatment during 3 days post-partum period (h) control. The white arrow indicates the uterine tissue after placental implantation.

Discussion

Puerperal infections may extend along the veins and capable of causing thrombosis.[6] There are some factors affecting the puerperal infection in women, including; various vaginal examinations during labor, amniotomy, imperfect aseptic techniques, inadequate perineal care, vaginal/cervical infection, extensive tissue injuries or open wounds, corticosteroid use, obesity, diabetes, etc.[3,15] Caesarean delivery has a 3x-10x higher risk of uterine infection or a 25x increase in mortality due to infection compared with vaginal delivery which also caused in the increasing of endometritis wound complications.[6,15] Based on a five-year survey of 45.000 mothers who gave birth at Parkland Hospital, Brown, et al. in Cunningham, et al.

Thrombophlebitis Pelvic Septic occurred in 1:9.000 events after vaginal delivery and 1:800 in Caesarean section.

The National Health and Nutrition Examination Survey (NHANES) reported that 17% of pregnant women exhibited colonization in the vagina and increases in late pregnancy (third trimester), especially at the time of postpartum in humans with increased complications on 3 days post-partum.[8,16] *Staphylococcus* is the most common vaginal pathogen and remains one of the most common infectious bacteria. *Staphylococcus aureus* is an endogenous extracellular bacteria and becomes pathogenic if there is a tissue damage. In general, the host (postpartum mother) is infected with this bacteria on the surface of the skin or mucosa of injury or tissue damage due to labor processes such as vaginal mucosal injuries, episiotomy lesions, laceration of both the vagina and the cervix and the wound of Sectio Caesarea.[3,13] Th17 cells provide an important response in mucosal tissue by expressing IL-17. IL-17 is the first cytokine produced which is involved in mucosal infection. IL-17 is protective against certain extracellular pathogens that are bound to the mucosal surface and play an important role in pathology in inflammation.[17,18] Therefore, induction with *Staphylococcus aureus* as an infectious agent in this study led to an increase in the percentage of Th17 cells and IL-17 levels as an immune response to the presence of puerperal infections which leading to inflammation.

$CD4^+$ T helper cells play a major role in initiating and inducing an adaptive immune response through both cellular and humoral effector. T helper cell function is to recognize peptides that combine with class II MHC molecules. Class II MHC molecules of fragments is derived from extracellular proteins (exogenous) which are present in the intracellular compartments.[13,19] Several studies have reported a link between Th17 cells and inflammation. Th17 cells play a critical role in host defense which is the primary effector function of Th17 cells to destroy extracellular bacteria and fungi. This process is mainly by inducing neutrophilic inflammation, which would phagocyte and kill extracellular microbes.[14,20] Immunity mediated by Th17 cells is very important in the mucosal and surface epithelium, as shown by expression patterns of chemokine receptors and effector cytokines, a percentage of pathogens ie extracellular bacteria and fungi primarily induce a Th17 response to express some cytokines and induce other immune cells to eliminate the bacteria which resulting in inflammation.[21] The results in our study is in line with this theory as number of Th17 cells are increased in *Staphylococcus aureus*-induced mice both during puerperal and 3 days post-partum periods.

The proliferation of Th17 cells is stimulated by pro-inflammatory cytokines in response to *Staphylococcus aureus* bacteria and they induce the production of IL-6, IL-1, and IL-23. These cytokines help to promote the differentiation of $CD4^+$ T cells into Th17 cells.[14]

IL-17 is a pro-inflammatory cytokine that induces other cytokines, chemokines and prostaglandins to response due to the presence of antigen. IL-17 has six family members known as "17A-F".[20] IL-17 plays a critical role between adaptive immune-mediated T cells and acute inflammatory responses.[14] Our results indicated an acute inflammatory response with high levels of IL-17 in the *Staphylococcus aureus* group compared with control group. In our study, *Lactobacillus reuteri* lowered IL-17 in all study groups. This showed the possible effective role of *Lactobacillus reuteri* as a therapy of puerperal infection.

Lactobacillus reuteri is a type of probiotic that beneficial for reproductive health and urinary tract infections therapy in postmenopausal women.[22,23] *Lactobacillus reuteri* ATCC 6475 which administered orally is also capable of decreasing vaginal PH as well as stimulating fertility due to vaginal acidity (from *Lactobacillus* colonization) that correlates with peak of fertility age.[24] It may also increase fertility in men by repairing testicular tissue (by increasing the percentage of Leydig cells and increasing testosterone hormone.[12]

Lactobacillus reuteri is naturally found in the digestive tract of humans and animals, also in breast milk. Its benefits in health are to strengthen the immune system and help to maintain the balance of other beneficial microorganisms by releasing an antibacterial substance called "reuterin". *Lactobacillus reuteri* is a promising therapy for the repair of infantile colic, eczema, reduction of infection due to *Helicobacter pylori* and also act as an effective treatment for diarrhea because of rotavirus in children.[25] In addition *Lactobacillus reuteri* is also the most widely documented as a probiotic strain for women's health because of its ability to treat various bacteria in the vagina (Vaginosis), and provide benefits to the intestine and reduce the risk of bladder infections.[24]

Based on the results of the study there was no significant difference in the percentage of Th17 cells between the puerperal period and 3 days post-partum period in control group, but the mean ± SD of Th17 cells percentage during the puerperal period was higher than 3 days post-partum period as the normal conditions of the estrogenous period. This result is consistent with the study of Tyagi et al.[26] that estrogen deficiency causes increased in differentiation of Th17 cells by up-regulating STAT3, ROR-γt and ROR-α as well and down-regulation in Foxp3 which antagonists with Th17 cell differentiation. The decline in the average percentage of Th17 cells 3 days post-partum period was a sign that mice was in the proestrus phase. The proestrus phase is a phase before estrus phase that causes estrogen secretion increase. The increasing percentage of Th17 cell in puerperal period compared to 3 days post-partum period in the control group was the effect of estrogen hormones that decreased dramatically during the puerperal period.

Paul, et al reported that estrogen can reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans*, this is an important factor in the susceptibility of hormones associated with *C. albicans* and vaginitis.[27] Estrogen is particularly strong for inhibiting the lipopolysaccharide / TLR4-induced pro-inflammatory pathway in human cells. Estrogen is shown to increase the levels of IL-4, IL-10, and TGF-β and increase the expression of $CD8^+$ and Foxp3, which increases $CD4^+Tim\text{-}3^+CTLA4^+$ and $CD4^+CD25^+Fox3^+$Treg populations.[28] Similarly, according to Lelu et al.[29] sex hormones, especially estrogen, play an important role in the development of Th17 cells, for example alpha receptor signal in T lymphocytes necessary for inhibition of mediation-estradiol in Th1 and Th17 cell differentiation and protection against Experimental Autoimmune Encephalomyelitis (EAE). Estrogen treatment provides a protective effect on EAE and clinical trials for some sclerosis therapy.[29]

Our study showed that the T17 cell percentage in 3 days post-partum period decreased compared to that of mice in puerperal period. This indicated that the function of estrogen is also able to suppress the inflammation reaction by inhibiting the differentiation of IL-17 through down-regulation of ROR-γt mRNA and the protein expression. [30]

The activation of macrophages play a crucial role in repairing function after various types of tissue injuries. Macrophages could induce fibrosis by secreting growth factors that stimulate fibroblast (Platelet-Derived Growth Factor) proliferation, collagen (IL-13, Transforming Growth Factor-β [TGF-β]) synthesis, and the formation of new blood vessels or angiogenesis (Fibroblast Growth Factor).[14]

In summary, we demonstrated that oral administration of *Lactobacillus reuteri* play an important role in decreasing the percentage of Th17 cells and IL-17 level in *Staphylococcus aureus*-induced mice both during the puerperal period and 3 days post-partum. This study supports other studies that have proven the positive effects of *Lactobacillus reuteri* as probiotics especially related to reproductive health.

References

1. Surveilans, 2014. Epidemiologi Kematian Ibu dan Target MDGs 5. http://www.Indonesian-publichealth.com/surveilanskematianibu. Accessed on May 05, 2015.
2. Direktorat Kesehatan Ibu, 2010-2013 dalam Kementrian Kesehatahan RI, 2014. InfoDatin;Pusat Data Dan Informasi Kementrian Kesehatan RI Mother's Day Situasi Kesehat IBU. http://www.depkes.go.id. Accessed on May 12 2015.
3. Varney H, Kriebs JM, Gegor CL. Buku Ajar Asuhan Kebidanan Edisi 4 Volume 2. Jakarta: EGC; 2008. p.1005-6.
4. Bobak, Lowdermilk, Jensen. Buku Ajar Keperawatan Maternitas Edisi 4. Jakarta: EGC; 2005. p. 683-6.
5. Fraser DM. dan Cooper MA. Myles Buku Ajar Bidan (Myles Textbook For Middwives) Edisi 14. Jakarta: EGC; 2009. p. 626.
6. Cunningham, FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. Obstetri Williams Ed 23 Volume 1. USA: McGraw-Hill; 2010. p. 682, 691-700.
7. Leung S, Liu X, Fang L, Chen X, Guo T, Zhang J. Te cytokine milieu in the interplay of pathogenic th1/th17 cells and regulatory t cells in autoimmune disease. Cell Mol Immunol 2010;7:182-9. doi: 10.1038/cmi.2010.22
8. Guta CD. Microbiological Study of Antepartum and Postpartum Vaginal Flora. Clinical and Laboratory Research and Terapeutical Particularities. Craivo: University of Medicine and Pharmacy of Craiova Faculty of Medicine; 2013.
9. Lazarenko L, Babenko L, Sichel LS, Pidgorskyi V, Mokrozub V, Voronkova O, Mykola S. Antagonistic Action of Lactobacilli and Bifdobacteria in Relation to Staphylococcus aureus and Teir Influence on the Immune Response in Cases of Intravaginal Staphylococcosis in Mice. Probiotics & Antimicro. Prot. 2012;4: 78–89. doi: 10.1007/s12602-012-9093-z
10. Sirkosa H, Smoragiewicz W. Role of Probiotics in the Prevention and Treatment of Meticillin-Resistant Staphylococcus aureus Infections. Int J Antimicrob Agents 2013;42:475-81. doi:10.1016/j.ijantimicag.2013.08.003
11. Chen A, Sears CL. Prebiotics, Probiotics, and Synbiotics.Basic Principles in the Diagnosis and Management of Infectious Diseases. Ncbi. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elsevier Inc.; 2014. p19-25.
12. Poutahidis T, Kleinewietfeld M, Smillie C, Levkovich T, Perrotta A, Bhela S, Varian BJ, et al. Microbial reprogramming inhibits Western diet-associated obesity. PLoS ONE 2013;8:e68596. doi:10.1371/journal.pone.0068596

13. Soedarto O. Mikrobiologi Kedokteran (Medical Microbiology). Jakarta: Sagung Seto; 2015. p. 7-11, 20-2, 172, 198-9.
14. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology: Student Consult, Chapter 1, 2, 4, 9, 10, 15, 16, Appendix IV, 8th ed. Elsevier Health Sciences; 2012.
15. Manuaba IBG. Kapita Selekta Penatalaksanaan Rutin Obstetri Ginekologi dan KB. Jakarta: Penerbit Buku Kedokteran EGC;2001. p.299.
16. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spog CY. Obstetri Williams Ed 23 Vol 2. USA: McGrawHill;2010. p1291
17. Ernst BP. Mucosal Immunity. Chapter 7, In: Bennett JE, Dolin R, Blaser MJ, editors. Elsevier Inc. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Elsevier Inc; 2015. p70-7
18. Stumhofer JS, Silver J, Hunter CA. Negative Regulation of Th17 Responses. Elsevier j.smim. Journal. Semin Immunol. 2007 Dec;19(6):394-9. doi: 10.1016/j.smim.2007.11.006. Epub 2008 Jan 24
19. Hammerich L, Heymann F, Tacke F. Role of IL-17 and T17 Cells in Liver Disease. Clin Dev Immunol 2011; 345803. doi: 10.1155/2011/345803
20. Singh RP, Hasan S, Sharma S, Nagra S, Yamaguchi DT, Wong DT. Hahn BH, Hossain A. T17 cells in inflammation and autoimmunity. Autoimun Rev 2014; 13:1174-81. doi: 10.1016/j.autrev.2014.08.019
21. Miossecc P, Korn T, Kuchroo VK. Interleukin-17 and Type 17 Helper T Cells. N Engl J Med 2009;361:888-98. doi: 10.1056/NEJMra0707449
22. Beerepoot MA, ter Riet G, Nys S, van der Wal WM, de Borgie CA, de Reijke TM, et al. Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women. Arch Intern Med 2012;172:704–12. doi: 10.1001/archinternmed.2012.777.
23. Vujic G, Jajac Knez A, Despot Stefanovic V, Kuzmic Vrbanovic V. Eficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: a double-blind, randomized, placebo-controlled study. Eur J Obstet Gynecol Reprod Biol 2013;168:75–9. doi: 10.1016/j.ejogrb.2012.12.031.
24. Reid G. Probiotics for Women's Health: Te Story of Lactobacillus Rhamnosus GR-1 and Lactobacillus Reuteri RC-14. http://www.jarrow.com/articles/release/id/403/Probiotics_for_Women%27s_Health:_Te_Story_of_Lactobacillus_Rhamnosus_GR-1_and_Lactobacillus_Reuteri_RC-14. Accessed on October, 23. 2015.
25. Deraz SF, Khalil AA, El-Dewany AI. Booming Agents for Prophylaxis and Terapeutic Applications. World Applied Sciences Journal 2011;14:293–323.
26. Tyagi AM, Srivastava K, Mansoori MN, Trivedi R, Chattopadhyay N, Singh D. Estrogen Defciency Induces the Differentiation of IL-17 Secreting T17 Cells: A new candidate in the pathogenesis of osteoporosis. PLoS ONE 2012;7:e44552. doi: 10.1371/ journal.pone.0044552
27. Paul L, Fidel JR, Jessica C, Chad S. Effects of Reproductive Hormones on Experimental Vaginal Candidiasis. Infect. Immun. 2000;68:2: 651-657. doi: 10.1128/IAI.68.2.651-657.2000
28. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease evaluated from a pathological perspective. Am J Pathol 2008;173:600–9. doi: 10.2353/ajpath.2008.071008
29. Lélu K, Laffont S, Delpy L, Paulet PE, Périnat T, Tschanz SA, Pelletier L, Engelhardt B, Guéry JC. Estrogen receptor α signaling in T lymphocytes is required for estradiol-mediated inhibition of T1 and T17 cell differentiation and protection against experimental autoimmune encephalomyelitis. J Immunol 2011;187:2386-93. doi: 10.4049/jimmunol.110157829.
30. Fan YM, Zhang QY, Liu S, Li QL, Ke GL, Li C, et al. Estradiol inhibits Th17 cell differentiation through inhibition of ROR gamma T transcription by recruiting the ER Alpha/REA complex to estrogen response elements of the ROR gamma T oromoter. Journal of Immunology 2015;194(8):4019-28,639.