

VAGINAL POSTPARTUM MICROBIOME AFTER C- SECTION AND SPONTANEOUS VAGINAL DELIVERY

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Hormonal changes in pregnancy and early postpartum significantly influence the vaginal microbiome and can lead to overcolonisation of pathogenic microorganisms leading to infections. Endometritis is an inflammation of the lining of the uterus and is a leading cause of postpartal sepsis. The diagnosis of postpartum endometritis involves the described clinical presentation, a positive bacterial culture from the uterus, along with the presence of leukocytosis, an increased number of neutrophils, and a rise in C-reactive protein.

Objective of the study is to determine the frequency of lochial colonization by pathogenic bacteria after C-section and vaginal delivery, to correlate CRP levels with the lochial swab results and mode of delivery. This prospective study included 130 postpartum patients: 100 delivered by C-section (elective or emergency) and 30 by spontaneous vaginal delivery. Anamnestic data, history of infections during pregnancy, CRP levels, and microbiological findings from lochial swabs obtained on the third postpartum day were analyzed. All patients undergoing C-section received antibiotic prophylaxis. Statistical analysis was done using standard statistical software.

Infections during pregnancy were recorded in 48% of patients in the C-section group. Positive lochial swabs were detected in 57 patients after C-section and 13 after vaginal delivery. No statistically significant association was found between infections during pregnancy or delivery mode and lochial swab positivity ($p > 0.05$). However, emergency C-sections showed the highest frequency of positive lochial swabs and the highest mean CRP values. A statistically significant association was observed between the type of delivery and the type of isolated microorganism ($p < 0.05$), with *Enterococcus faecalis* predominating in emergency C-section patients.

To conclude, effective primary prevention measures and appropriate secondary prevention (such as antibiotic prophylaxis), significantly reduce the risk of postpartum endometritis. The lochial swab can be informative in patients after delivery, but the incidence of endometritis is low even in patients with higher risk, therefore routine control of the postpartum swab is unnecessary. Careful clinical monitoring and prompt treatment are essential in preventing severe complications of postpartum endometritis.

Key words: Endometritis, postpartum, C-section, microbiota

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Postpartalni mikrobiom vagine nakon porođaja carskim rezom i vaginalnim putem

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Hormonske promene u trudnoći i ranoj postporođajnoj dobi značajno utiču na vaginalni mikrobiom i mogu dovesti do prekomerne kolonizacije patogenih mikroorganizama. Endometritis je zapaljenje sluzokože materice i vodeći je uzrok postporođajne sepse. Dijagnoza postporođajnog endometritisa se postavlja na osnovu kliničke slike, pozitivne bakterijske kulture iz materice, zajedno sa prisustvom leukocitoze, povećanim brojem neutrofila i porastom C-reaktivnog proteina.

Cilj studije je da se utvrdi učestalost kolonizacije lohija patogenim bakterijama nakon carskog reza i vaginalnog porođaja, da se korelišu nivoi CRPa sa rezultatima lohijalnog brisa i načinom porođaja.

Ova prospektivna studija obuhvatila je 130 pacijentkinja posle porođaja: 100 porođanih carskim rezom (elektivnim ili hitnim) i 30 spontanim vaginalnim porođajem. Analizirani su anamnestički podaci, istorija infekcija tokom trudnoće, nivoi CRPa i mikrobiološki nalazi iz lohijalnih briseva dobijenih trećeg dana nakon porođaja. Sve pacijentkinje koje su podvrgnute carskom rezu primale su antibiotsku profilaksu.

Statistička analiza je urađena korišćenjem standardnog statističkog softvera.

Infekcije tokom trudnoće zabeležene su kod 48% pacijentkinja u grupi carskog reza. Pozitivni lohijalni brisevi otkriveni su kod 57 pacijentkinja nakon carskog reza i 13 nakon vaginalnog porođaja. Nije pronađena statistički značajna veza između infekcija tokom trudnoće ili načina porođaja i pozitivnosti lohijalnog brisa ($p > 0,05$). Međutim, hitni carski rezovi pokazali su najveću učestalost pozitivnih lohijalnih briseva i najviše srednje vrednosti CRP. Statistički značajna veza je primećena između vrste porođaja i vrste izolovanog mikroorganizma ($p < 0,05$), pri čemu je *Enterococcus faecalis* preovladavao

kod pacijentkinja sa hitnim carskim rezom. Zaključno, efikasne mere primarne prevencije i odgovarajuća sekundarna prevencija (kao što je antibiotska profilaksa) značajno smanjuju rizik od postporođajnog endometritisa. Lohijalni bris može biti informativan kod pacijentkinja nakon porođaja, ali je incidenca endometritisa niska, stoga rutinska kontrola postporođajnog brisa nije potrebna.

Ključne reči: Endometritis, postporođajni period, Carski rez, mikrobiom

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Introduction

A balanced vaginal microbiome is a reflection of good reproductive health and strongly influences the reproductive outcome (1). During a normal pregnancy, biodiversity in the vaginal fluid is increased, but the presence of various strains of *Lactobacillus* remains dominant, particularly *Lactobacillus crispatus* (2). Traditionally, it is considered that the dominance of *Lactobacillus spp.*, through the production of bacteriocins and lactic acid, maintains an acidic pH and prevents the colonization of the vagina by other microorganisms (3). The dominance of *Lactobacillus* during pregnancy is associated with high estrogen levels produced by the placenta. After delivery, the estrogen level decreases, and so does the number of *Lactobacilli*. It is highly likely that the production of lactic acid also decreases, the pH rises, and the possibility of inhibiting the growth of microorganisms decreases (4).

At the end of pregnancy, the vaginal microbiome changes radically. Changes in the microbiome and the composition of the vaginal fluid are a consequence of changes in the immune response and the physiology of the host. After delivery, the biodiversity of the vaginal microbiome increases. The estrogen level decreases, as well as the production of lactic acid, the vaginal fluid pH rises, and the capacity for inhibiting microbial growth declines (5). In the postpartum period, Community State Types (CST) III and IV are dominant in the vaginal microbiome (6). The number of *Lactobacillus spp.* decreases and can even completely disappear, while the number of *Streptococcus anginosus* and *Prevotella bivia* increases. CST III is characterized by the dominance of *Lactobacillus iners*. CST IV is characterized by the dominance of anaerobes. The CST III community is unstable and easily transitions into CST IV (5). Evidence exists that the vaginal microbiome also depends on the host's ethnicity and biogeographical position (2, 3). Such a change, and increase in biodiversity, with a dominance of obligate anaerobes and facultative anaerobes, persists for up to 9.5–12 months after delivery in as many as 55% of patients (1).

Endometritis is an inflammation of the lining of the uterus that most commonly occurs via the ascending route. Endometritis can be acute, with symptoms up to 30 days, and chronic if the inflammation lasts longer than 30 days (6). Culture-based methods show that early postpartum endometritis is associated with the presence of bacterial dysbiosis and the presence of *Gardnerella vaginalis*, *Peptococcus spp.*, *Bacteroides spp.*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Ureaplasma urealyticum* (3,7).

Postpartum septic complications are the third leading cause of maternal mortality, after hemorrhage and hypertensive disorders in pregnancy (8). Endometritis in the postpartum can be complicated by myometritis and parametritis. Also, endometritis is the most common cause of puerperal sepsis. Untreated and poorly treated endometritis can result in a fatal outcome. Pathogens can be *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Mycoplasma spp.* Most commonly, it is a polymicrobial infection with aerobic and anaerobic pathogens. Risk factors for the development of postpartum endometritis are prolonged labor, a greater number of examinations,

premature rupture of membranes, chorioamnionitis, and Cesarean section (C-section) delivery. The increase in the incidence of operative deliveries, specifically C-sections, increases the probability of postpartum infections and especially endometritis. Signs of endometritis are: fever, chills, malaise, pain in the lower abdomen, profuse lochia with an unpleasant odor (9). The diagnosis of postpartum endometritis involves the described clinical presentation, a positive bacterial culture from the uterus, along with the presence of leukocytosis, an increased number of neutrophils, and a rise in C-reactive protein (CRP) (10).

The infection is most commonly polymicrobial, so according to the experience of some authors, swabs are not mandatory and will not significantly affect the treatment and outcome. Antibiotic prophylaxis in patients with a C-section reduces the incidence of endometritis by up to 60%. The application of antibiotic prophylaxis in patients with an operative vaginal delivery is controversial and probably unnecessary, given the absolute frequency of infections of 1% (3, 11).

Research Objective: Determining the frequency of lochial colonization by pathogenic bacteria in the group of patients with C- section and vaginal delivery. Correlating CRP values with findings in the lochial swab. Monitoring the investigated parameters in the group of patients with elective and emergency C-section.

Material and Methods: This prospective study was conducted at the Clinic for gynecology and obstetrics University Clinical Center Niš. The study included a total of 130 patients who delivered at our hospital in the course of the one year, from January to December of 2025. This study was conducted according to the Declaration of Helsinki ethical principles, with guaranteed discretion of personal data, and had been approved by the Ethics Committee of the University Clinical Center Niš, protocol number 11233.

Patients were divided into two groups: the experimental group consisted of patients who delivered via C- section (100), and the control group (30) of patients after spontaneous vaginal delivery. Patients were evaluated based on their medical history, clinical examination, laboratory tests (CRP levels), and microbiological analysis (a lochial swab performed on the third postpartum day). Anamnestic data included information on the patient's age, parity, education, body mass index (BMI), present comorbidities, and previous uterine interventions (Table 1). Infections during the course of pregnancy were also noted. Patients who underwent C- section were divided into two subgroups: elective and emergency C- sections. All patients in the C- section study group received antibiotic prophylaxis, whereas no antibiotic therapy was administered to the control group.

Table 1. Characteristics of the experimental group and control group

Parameter	Experimental Group (CS)	Control Group (Vaginal)
Age (years)	28 (19–47)	29 (18–42)
Education	Primary: 5 (5%); High school: 40 (40%); Higher: 30 (30%); University: 25 (25%)	Primary: 3 (10%); High school: 12 (40%); Higher: 7 (23%); University: 8 (27%)
BMI (kg / m ²)	Average: 25.3 (18–37)	Average: 24.8 (19–34)
Body Weight (kg)	Average: 72 (52–103)	Average: 70 (45–95)
Height (cm)	Average: 168 (150–183)	Average: 167 (155–178)
Mid-arm Circumference (cm)	Average: 28.5 (24–34)	Average: 27.9 (23–33)
Waist Circumference (cm)	Average: 82 (68–102)	Average: 80 (65–98)
Parity	I-para: 53; II-para: 28; III or more: 19	I-para: 11; II-para: 13; III or more: 6
Comorbidities	Hypertension: 20; Hypothyroidism: 10; GDM/IR: 15	Tachycardia: 2; Hypertension: 1; Hypothyroidism: 1; IR: 2
Previous Uterine Interventions	Total: 34 (CS I: 19; CS II: 8; CS III: 2; CS IV: 1; Other: 4)	0

Statistical analysis was done using standard statistical software. A p-value of <0.05 was considered statistically significant. Results were presented as absolute frequencies and percentages. The comparison of categorical features was performed using the Chi-square test. Pearson's correlation was also used. All statistical tests were two-tailed.

Research Results

In the study group, which comprises 100 patients, the presence of infections during pregnancy was recorded in 48 patients (48%). Viral respiratory infections, vagino-cervical, and urinary infections were most frequently noted.

Patients from the group with registered cervicovaginal infections during pregnancy had a positive lochial swab (predominantly *Enterococcus faecalis*) in 50% of cases after delivery (Table 2).

Table 2. Patients with positive swabs during pregnancy and lochial swab results after delivery

Lochial Swab Result	Patients with treated cervicovaginal infections in pregnancy	Percentage (%)
Positive lochial swab	27	56.3%
Normal lochial swab	21	43.7%
Total	48	100%

In the control group, consisting of 30 patients (vaginal delivery), 8 of them had a positive vagino-cervical swab (V+C) during pregnancy. Furthermore, respiratory infection was noted in one patient, while another presented with a combined urinary and respiratory infection. Out of these 10 patients, 5 had a normal lochial swab, while 5 had a positive lochial swab.

The distribution of cervicovaginal infections across the study (C- section) and control (vaginal delivery) groups is presented in Table 3. The Chi-square test showed no statistically significant association between infections during pregnancy and lochial swab findings ($p > 0.05$).

Statistical analysis showed no significant association between the type of delivery and a positive lochial swab (χ^2 test, $p > 0.05$). The highest percentage of positive swabs and the highest average CRP values were recorded in patients who underwent an emergency C- section, while vaginal deliveries had the lowest frequency of infections and the lowest CRP values. These findings suggest a potential association between the type of delivery and postpartum infections (Tables 3 and 4).

Table 3. Frequency of cervicovaginal infections and pathological lochial swab findings in the experimental and control groups

Group	Total Patients	With infections during pregnancy	Positive lochial swab	Normal lochial swab
Experimental	100 (100%)	48 (48%)	27 (27%)	73 (73%)
Control	30 (100%)	10 (33%)	5 (17%)	25 (83%)

Table 4. Comparison of lochial swab results and average CRP values in relation to the type of delivery

Category	Elective CS	Emergency CS	Vaginal Delivery
Normal lochial swab	18 (47%)	25 (40%)	17 (56%)
Positive lochial swab	20 (53%)	37 (60%)	13 (44%)
Average CRP (mg/L)	53.2	72.9	12.9

The average CRP values in the selected patients who had a positive lochial swab was 72.5 (0.29–143.5). The average CRP values in subjects with a negative lochial swab, but with a present infection during pregnancy, was 89.7, ranging from (24.4–300). A total of 48 patients had an infection during pregnancy, of which 27 had a positive lochial swab after delivery (56.3%), and 21 had a normal lochial swab (43.7%). The average CRP value in patients with an infection and a positive swab was 72.5 (range 0.29 – 143.5). In those with an infection during pregnancy but a negative lochial swab, the average CRP was 89.7 (range 24.4 – 300).

The association between the type of delivery (C- section and vaginal delivery) and the type of isolated microbiological agents from the lochial swab was investigated (Figure 1). For statistical processing purposes, elective and emergency C- sections were aggregated into a single category (CS). The frequencies of individual pathogens among patients who had a CS delivery were compared to those who had a vaginal delivery. In the group of patients who had an emergency C- section, a significantly higher

frequency of positive lochia swab results was observed compared to planned C- sections, with a particularly dominant presence of the bacterium *Enterococcus faecalis* (23 vs 10 cases). Based on the obtained p-value (< 0.05), it can be concluded that there is a statistically significant association between the type of delivery and the type of isolated pathogen. This indicates that the distribution of microorganisms significantly differs depending on the method of delivery. The incidence of isolated pathogens in lochial swabs was significantly higher among patients who underwent C- section compared to those with spontaneous vaginal delivery.

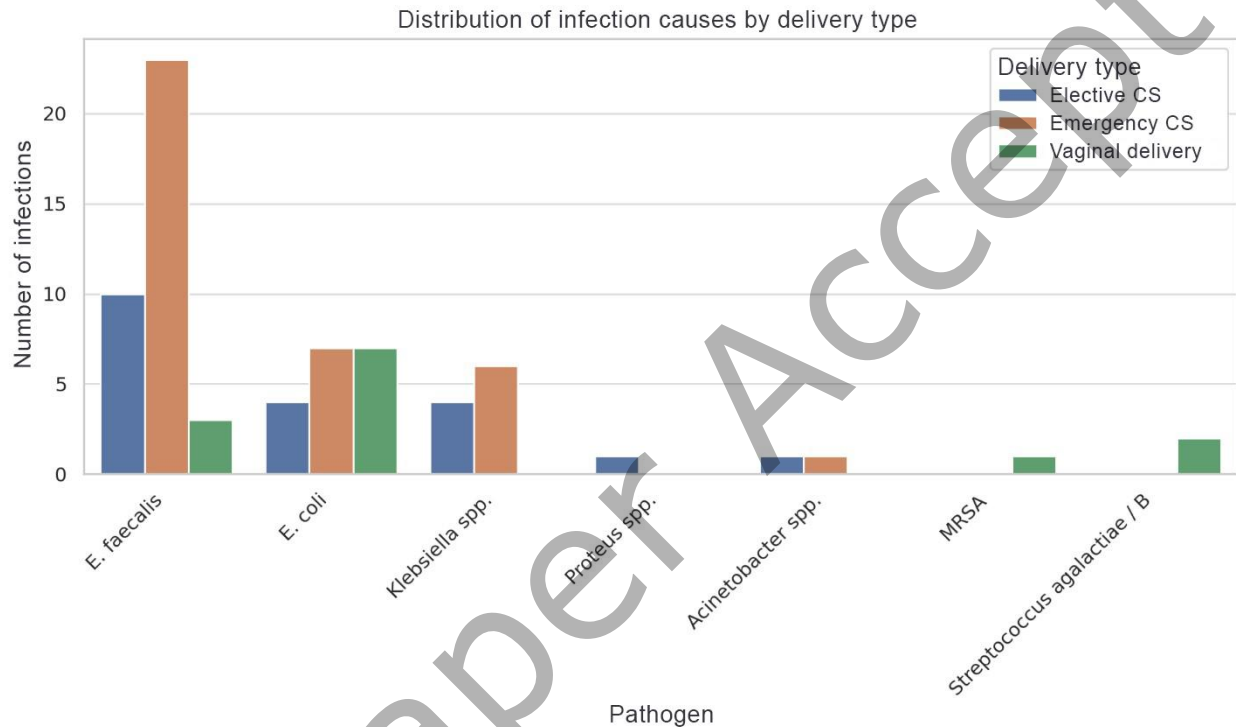


Figure 1. Distribution of pathogens in the elective, emergency C- section, and vaginal delivery groups

Discussion

The vaginal ecosystem is linked to women's health, as well as to the reproductive outcome (12). Pregnancy and childbirth are associated with a pro-inflammatory cytokine response and the loss of *Lactobacillus* dominance. (13) Research shows that the time for achieving re-dominance of *Lactobacillus* is longer after the birth of a live child (1). In the bacterial community, *L. crispatus* usually dominates, and if the bacterial community is of a sub-optimal configuration, other types of *Lactobacillus* dominate (14). The dominance of certain types of *Lactobacillus* also depends on the ethnicity and geographical position of the host (3). In our sample, 50% of patients during pregnancy do not have *Lactobacillus* dominance. After delivery: mechanical trauma, alkaline lochial discharge, breastfeeding, and the return of the cycle are factors that will influence the re-dominance of *Lactobacillus*. The vaginal microbiome

varies both during pregnancy and after delivery depending on the course of pregnancy, hypertensive disorders, the time of rupture of the membranes, the method of delivery, and the mother's age (2,15). In certain communities (women of the Malawi People), *Gardnerella vaginalis* can also be dominant postpartum, and this dominance can last even longer than a year (6).

Postpartum endometritis most commonly occurs within the first 30 days following delivery (16). While the overall incidence is 1–3%, the rate of endometritis is significantly higher (4–6%) among patients delivered via C- section (11). In our sample, there were no patients with signs of acute endometritis. This can be explained by the small sample (130 patients), but also by good antibiotic prophylaxis and good postpartum monitoring. Although we did not have any patients with acute endometritis, the tested lochia swabs still showed the presence of microorganisms in 70 patients, specifically 57 in the study group and 13 in the control group.

Our study showed that 50% of patients with cervicovaginal infection during pregnancy also have a positive lochia smear, especially *Enterococcus spp*, a significant opportunist in the postpartum period (17,18).

The presence of positive swabs alongside absent clinical signs is not sufficient for a diagnosis of endometritis (19). The level of CRP was also monitored in our patients. Patients who had an emergency cesarean section had the highest proportion of positive lochia smears and the highest CRP values, while patients who delivered vaginally had the lowest values of these parameters. Although there was no statistical significance, we can conclude that the mode of delivery has an impact on the risk of postpartum infections. This can be explained by the fact that patients with an emergency C-section underwent operation with reduced prevention measures due to the urgency of the procedure, prolonged labor, ruptured membranes or existing maternal infections (20). Authors state that values around 60 ng/ml are a reliable predictor of postpartum endometritis, with a sensitivity of 79% and a specificity of 93%, which is consistent with our finding of higher CRP values in patients with urgent C-section (10).

Conclusion

The presence of microorganisms in the vaginal and cervical swab can be expected in about half of pregnant patients. Increased biodiversity is the result of complex hormonal and immunological events and does not increase the probability for the occurrence of postpartum endometritis. Effective primary prevention measures and appropriate secondary prevention (such as antibiotic prophylaxis), significantly reduce the risk of postpartum endometritis. The lochial swab can be informative in patients after delivery, but the incidence of endometritis is low even in patients with higher risk, therefore routine control of the postpartum swab is unnecessary. Careful clinical monitoring and prompt treatment are essential in preventing severe complications of postpartum endometritis.

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