

Composition and dynamics of bladder, vaginal and bowel microbiota during three trimesters in healthy pregnant women

Yulia Naboka¹

Natalia V. Vorobyeva²

Irina A. Gudima³

Tatiana G. Sinyavskaya⁴

Ruslan S. Ismailov³

Kurt G. Naber⁵

Michael KOGAN⁶

1 Department of Microbiology, Rostov State Medical University, Rostov on Don, Russia

2 Rostov State Medical University, Rostov-on-Don, Russian Federation

3 Rostov State Medical University, Rostov-on-Don, Russian Federation

4 Rostov State University of Economics (RINH), Rostov-on-Don, Russian Federation

5 Technical University of Munich, Munich, Germany

6 Department of Urology, Rostov State Medical University, Rostov-on-Don, Russia

Abstract

Introduction: The microbiota dynamics of the core biotopes during pregnancy are hardly studied, although changes in these compartments have an important role in both the functioning of the female organism and foetal development.

Objective: To study the dynamics and interactions of changes in bladder, vaginal and bowel microbiota in healthy pregnant women over three trimesters (TRI-1, 2, 3).

Materials & Methods: Study design: a single-centre comparative observational longitudinal study. Thirty out of first-time 220 pregnant women were selected for screening at the antenatal clinic from 2021 to 2022. All pregnant women underwent sampling at T-1, 2, 3: mid-stream bladder urine samples, posterior vaginal swabs and faecal masses were collected for culture study. After a special pre-culture preparation, samples were examined on an expanded set of nutrient media (n=13) using special cultivation (aerobic-anaerobic) conditions. Based on the research results, identification frequencies (IDFs), microbial load values (MLVs) and microbial co-occurrence coefficients between the different biotopes were estimated.

Results: Culture study revealed various bacteria in each biotope investigated during all TRIs. In the urine, aerobes and anaerobes were observed from TRI-1 to TRI-3 with different IDFs, but no taxa showed a stable IDFs. In the vagina, IDFs of bacteria were similar to urinary. The bowel microbiota was the most stable biotope and remained almost unchanged during pregnancy. In the urine and vagina, mean MLVs of most aerobes and anaerobes did not change significantly throughout pregnancy. In the bowel, MLVs were consistently higher than in the urine and vaginal swabs. According to the co-occurrence analysis bladder-vagina and bladder-bowel biotopes showed significantly more interconnections between microorganisms in all TRIs.

Conclusion: The observed microbiota structure during all TRIs is associated with uncomplicated gestation. These results will be valuable for studying changes of microbiota in complicated pregnancies.

Keywords: pregnancy, vaginal microbiota, bladder microbiota, bowel microbiota

1 Introduction

Pregnancy involves a series of metabolic changes in the female physiology, primarily in the hormonal and immune systems. Recent studies have identified important rearrangements in the bowel, vaginal, urinary, and oral microbiota and microbiome in pregnant women playing a definite role in pregnancy course and complications [1], [2].

There are plenty of microorganisms, i.e. bacteria, viruses, and fungi that have an impact on human host health. Undoubtedly, the maternal microbiota has a predominant influence on the formation, maturation, and development of microbial communities in foetal and neonatal organs and systems. From this perspective, it is essential to have an insight regarding the microbiota in key biotopes of healthy pregnant women (HPW) during first, second and third trimesters (TRI-1, TRI-2, TRI-3) [2], [3].

By now it is known, the TRI-1 bowel microbiota resembles that of healthy non-pregnant women. However, evidence for changes in the bowel microbiota from TRI-1 up to delivery is limited [3] and strongly associated with individual heterogeneity [1], [3].

It has been determined that bowel microbiota shift during pregnancy aims to cover foetal energy and metabolic needs [1], [4]. Diet during TRI-1 and TRI-2 affects the bowel microbiota composition in the TRI-3 [5], and the formation of neonatal microbiota [6]. Several studies indicate that bowel microbiota shift is associated with an increased risk of gestational arterial hypertension, diabetes mellitus, obesity, early preeclampsia, and pregnancy loss [7], [8], [9], [10], [11], [12].

It is well recognised how the vaginal microbiota changes throughout a female lifecycle and also related to their menstrual calendar [13]. But it is now also clear that vaginal dysbiosis in pregnancy increases the risk of adverse obstetric outcomes, including spontaneous preterm delivery [14], [15], [16]. In a healthy woman, the lactobacilli that dominate the vaginal discharge contribute to maintaining a low pH, which protects this biotope from bacterial and fungal infections [17]. In turn, altered proportions between lactobacilli and other aerobes/anaerobes in the vaginal microbiota of HPW can occur, increasing the risk of dysbiosis and elevating the risk of dysbiosis and premature delivery chance [18]. There are also changes in lactobacilli composition, so the most found *L. crispatus* in European women is replaced by *L. iners* [18], which is specifically associated with premature pregnancy loss [19], [20]. In HPW, some studies have shown that the TRI-3 vaginal microbiota is analogous to the pre-pregnancy microbiota, but significant changes occur in the vaginal microbiota after childbirth [21]. On contrary, several studies have refuted the resemblance of the vaginal microbiota during pregnancy with that of non-pregnant women, citing both the low taxa diversity and the absence or presence of unique bacteria [22], [23].

Five types of vaginal microbiome in asymptomatic non-pregnant North American women [24], [25], along with differences in the microbiome of white and black women and its ethnicity patterns during pregnancy have been established in several studies [26], [27], [28], [29], [30]. Simultaneously, there are very limited concurrent studies available on the vaginal and bowel microbiota in pregnant women [31], [32], [33]. High interindividual variability and dynamic composition of microbial communities depending on gestational age in physique biotopes have been demonstrated [32]. Bowel lactobacilli decline sharply after TRI-1 while vaginal lactobacilli are stable throughout the three TRIs [33]. Regarding research on the bladder microbiota during an uncomplicated pregnancy in HPW, these studies are extremely valuable in assessing the so-called asymptomatic bacteriuria. Researchers consider it a high-risk factor for gestational pyelonephritis, maternal sepsis, and preterm labour [34], [35]. Therefore, screening for asymptomatic bacteriuria and its treatment during pregnancy is recommended [36], [37], [38], [39]. But is it the right way?

In the last decade, the belief that the urinary system is sterile has been challenged [40], [41]. The bladder urine microbiota in healthy women has been studied quite fully throughout their lives [42], [43], [44], [45], [46].

The bladder urine microbiota in HPW throughout all trimesters has also been studied, and a diverse range of urinary aerobes and anaerobes has been identified. However, no comprehensive study of the three biotopes (bladder, vagina, bowel) has been conducted in HPW to date.

2 Objective

To study the dynamics and interactions of changes in bladder, vaginal and bowel microbiota in healthy pregnant women over three trimesters (TRI-1, 2, 3).

3 Materials and methods

3.1 Ethical statement

The study was registered by the Ethics Committee of Rostov State Medical University (Protocol №15/20, dated 08/10/2020) [47]. Study design: a single-centre comparative observational longitudinal study .

3.2. Patient brief demographics

From 2021 to 2022, 30 HPW were sequentially included according to criteria planned (Table 1) in a single-center prospective cohort observational study out of 280 who applied to antenatal clinics for the diagnosis of pregnancy during the TRI-1. All participants have read and signed a voluntary informed consent.

Table 1: Criteria for selection of healthy pregnant women

| Inclusion criteria | Exclusion criteria |
|--|--|
| <ul style="list-style-type: none">• healthy pregnant women aged 20–35 years;• singleton primary pregnancy verified before 12 weeks;• absence of any comorbidities;• history of acute lower urinary tract infections (UTI) but no neurogenic lower urinary tract symptoms or infection signs within 3 months prior to pregnancy. | <ul style="list-style-type: none">• pregnancy due to assisted reproductive technologies;• kidney and urinary tract anomalies and diseases;• vaginal dysbiosis;• use of antibiotics since conception;• history, and current presence of sexually transmitted infections,• HIV positive;• drug addiction & abuse;• smoking. |

Demographic data, sexual life patterns, urogenital symptoms were registered for all pregnant women.

3.3 Diagnostic work-up

Pregnant women underwent obstetric examination at gestational ages of 8–12 weeks (TRI-1), 22–24 weeks (TRI-2) and 32–36 weeks (TRI-3). Midstream urine samples, posterior vaginal discharge and faecal masses were collected from all pregnant women for culture study at times under research.

All women completed their pregnancies at 37 to 38 weeks by term vaginal labour (22.0–73.3%) or C-section (8.0–26.7%) and delivered healthy neonates.

• Sampling

Midstream urine samples were collected by trained medical staff after appropriate hygienic procedure when pregnant women urinated independently. Urine was collected in a sterile disposable container (Sterile Uricol™ – “HiMedia Laboratories Pvt. Ltd.”, Mumbai, India). Vaginal samples were collected using a disposable gynaecological mirror and pH was measured using pH strips (colpo-pH test). Vaginal discharge was collected using two sterile swabs with unique identification numbers. One swab was used to prepare a smear followed by Gram staining (Gram Stains-Kit – “HiMedia Laboratories Pvt. Ltd.”, Mumbai, India) to assess the vaginal microbiota according to the Nugent R.P. (1–10 scale) [48]. Other swabs with transport medium (Hiculture Transport Swabs w/Alternative Thioglycollate Medium – “HiMedia Laboratories Pvt. Ltd.”, Mumbai, India) were used for culture study.

Faecal samples were collected in a sterile container (Sterile Clinicol™ – “HiMedia Laboratories Pvt. Ltd.”, Mumbai, India) with a unique identification number.

• Culture study

Urine culture was performed by streaking to quantitatively count the grown colonies on suitable nutrient media [49]. Tampons with vaginal discharge were placed in a tube with 1.0 mL of 0.9% PBS and gently shaken. Further serial dilutions were performed with transfer of 100 µl each into the next tubes with 0.9% PBS. By this way dilutions up to 10⁷ were obtained [33]. From each dilution, seeding (100 µl) were performed on appropriate nutrient media to count the quantity of microorganisms.

Faeces (1,000 mg) were homogenised in 9.0 mL of pre-reduced PBS. Subsequently, nine-fold dilutions were performed, and each sample was cultured on the appropriate nutrient media to count the quantity of microorganisms [50].

In our study we used an advanced nutrient media set for the cultivation of aerobes and anaerobes: MacConkey Agar, HiCrome Klebsiella Selective Agar Base, HiCrome Candida Differential Agar, HiCrome Enterococci Agar, HiCrome Aureus Agar Base, Blood Agar Base, Streptococcus Selection Agar, Rogosa SL Agar, Bifidobacterium Agar, MRS Agar, Anaerobic Agar, Shaedler Agar, Bacteroides Bile Esculinum Agar (“HiMedia Laboratories Pvt. Ltd.”, Mumbai, India). The samples were cultivated under aerobic (24–48 h) and anaerobic (48–72 h) conditions using an AnaeroHiGas Pak (“HiMedia Laboratories Pvt. Ltd.”, Mumbai, India). Microorganisms were identified based on their morphological and haemolytic properties. Smears were prepared from colonies grown in culture medium and heat-fixed for Gram staining according to standard protocol using an oil-immersion microscope (magn. x 900, 10–15 FoV). Final identification of microorganisms was carried out according to their biochemical properties using entero-, staphylo-, and anaerotests (“Erba Lachema s.r.o.”, Brno, Czech Republic).

3.4 Statistics methods

The statistical software IBM SPSS Statistics 26.0 (“SPSS: An IBM Company”, IBM SPSS Corp., Armonk, NY, USA) was used to perform the calculations. The microbial identification frequency (IDF, %) and average microbial load values (MLV, CFU/mL) in the biotopes of HPW were calculated. The mean values permitted the inclusion of low-frequency high levels of MLVs and were used for comparative characterisation rather than as a central measure of distribution. The Mann-Whitney U test was used to compare IDFs and MLVs. Relationships between microbes in different biotopes was investigated using Pearson’s coefficient of reciprocal conjugation (co-occurrence coefficient). Standard statistical significance levels of 1% and 5% were used for hypothesis testing.

4 Results

In HPW, culture study during TRI-1, TRI-2, and TRI-3 revealed various aerobes and anaerobes in each biotope investigated. IDFs of selected bacteria throughout the three TRIs are shown in Figure 1 and Figure 2.

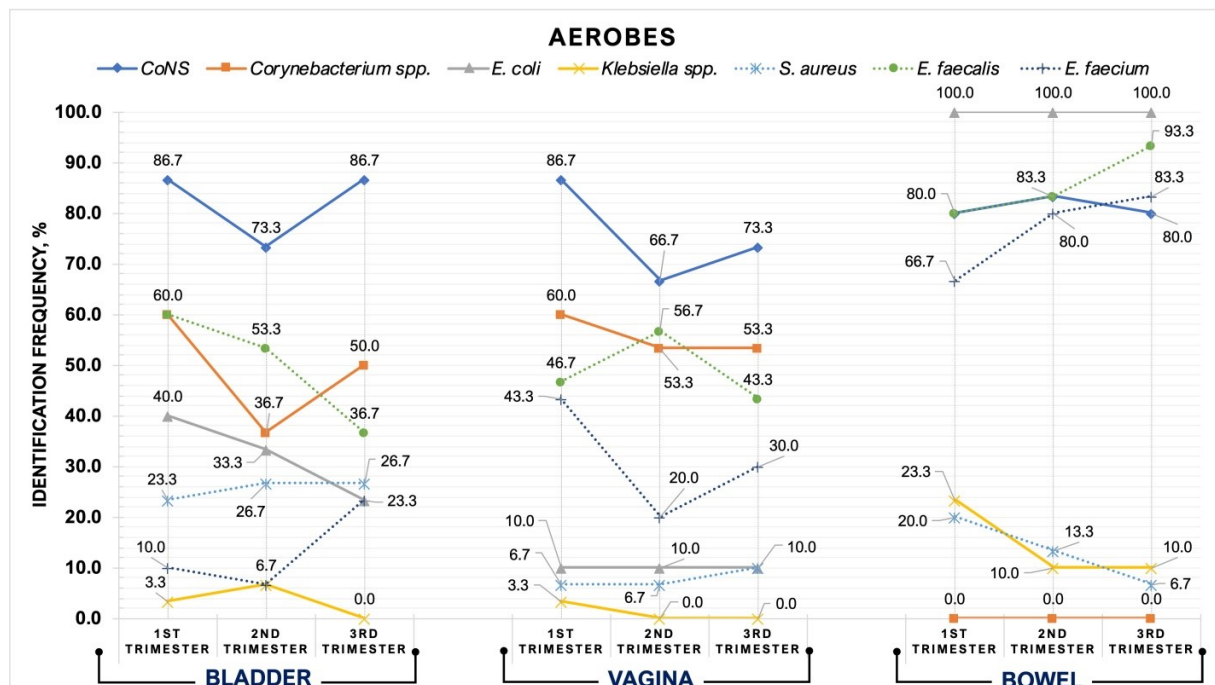


Figure 1: Identification frequency trends of aerobes in three biotopes during pregnancy

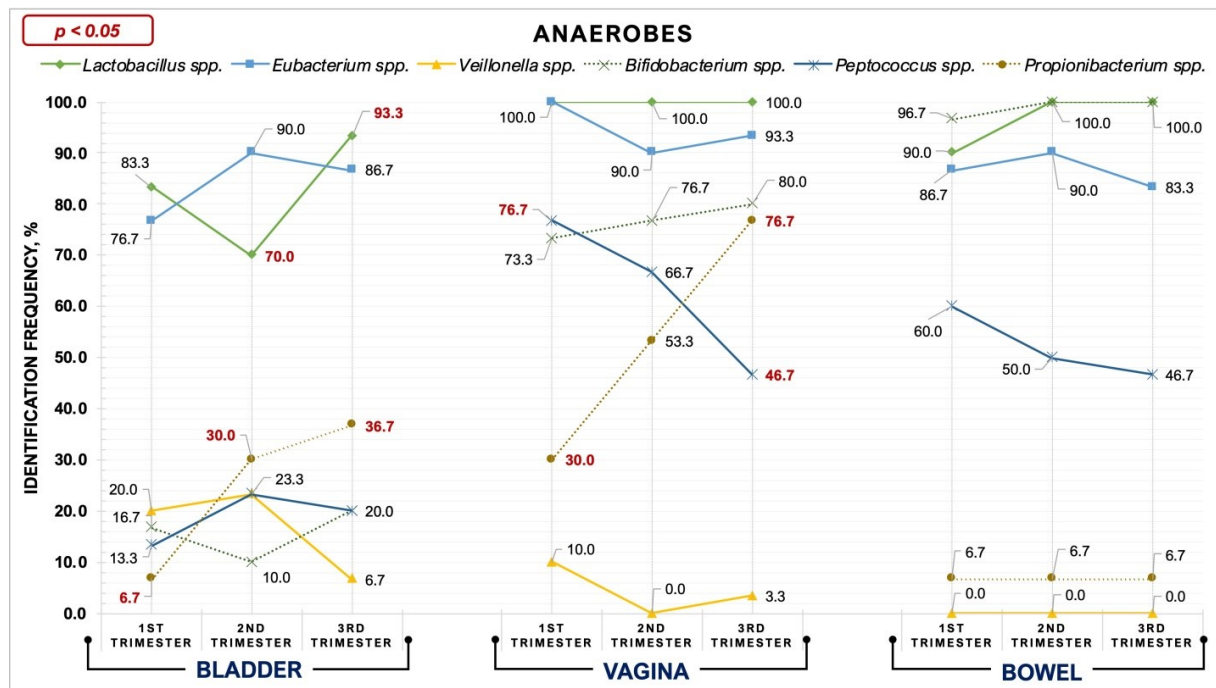


Figure 2: Identification frequency trends of anaerobes in three biotopes during pregnancy

4.1 Evaluation of identification frequency

- **Bladder urine microbiota**

In bladder urine samples, aerobes and anaerobes were detected from TRI-1 to TRI-3 with different IDFs. However, multidirectional trends were found in the urine microbiota regarding IDFs. Thus, IDFs for CoNS, *Corynebacterium* spp., *E. faecium*, *Lactobacillus* spp. and *Bifidobacterium* spp. decreased during TRI-2. In contrast, detection rates for *Klebsiella* spp., *Eubacterium* spp., *Peptococcus* spp., and *Veillonella* spp. were highest throughout TRI-2. Only two taxa (*E. faecalis*, *E. coli*) showed a decrease in IDFs from TRI-1 to TRI-3. Generally, *E. faecium*, *Lactobacillus* spp. (TRI-1 vs TRI-3, $p < 0.05$), *Propionibacterium* spp. (TRI-1 vs TRI-3, $p < 0.05$) and *Bifidobacterium* spp. had the highest urinary detection rates during TRI-3. Only *Klebsiella* spp. were eliminated from the urine during TRI-3.

- **Vaginal microbiota**

Vaginal bacteria detection frequency trends were similar to those for urine. Stable vaginal inhabitants of HPW throughout all TRIs were *Lactobacillus* spp. (100.0%), and *E. coli* isolated with markedly reduced frequency (10.0%). By TRI-3, five aerobes and three anaerobes demonstrated decreasing IDFs. In contrast, *S. aureus*, *Bifidobacterium* spp. and *Propionibacterium* spp. marked an increase in MLV (TRI-1 vs TRI-3, $p < 0.05$). *Klebsiella* spp. were eliminated from vaginal secretions during TRI-2 and TRI-3, and *Veillonella* spp. exceptionally during TRI-2.

- **Bowel microbiota**

Bowel microbiota showed to be the most stable biotope. Thus, *E. coli*, *Propionibacterium* spp. and CoNS remained essentially unchanged in their IDFs. *E. faecalis*, *E. faecium*, *Lactobacillus* spp. revealed the baseline high IDFs during TRI-1 increased throughout TRI-3, but *Eubacterium* spp. and *Peptococcus* spp. revealed a decrease in IDFs. *Klebsiella* spp. and *S. aureus* also diminished towards the TRI-3. *Corynebacterium* spp. and *Veillonella* spp. were not detected in the bowel microbiota throughout pregnancy.

4.2 Evaluation of microbial load values

The average MLVs of bacteria in the studied biotopes are shown in Figure 3 and Figure 4.

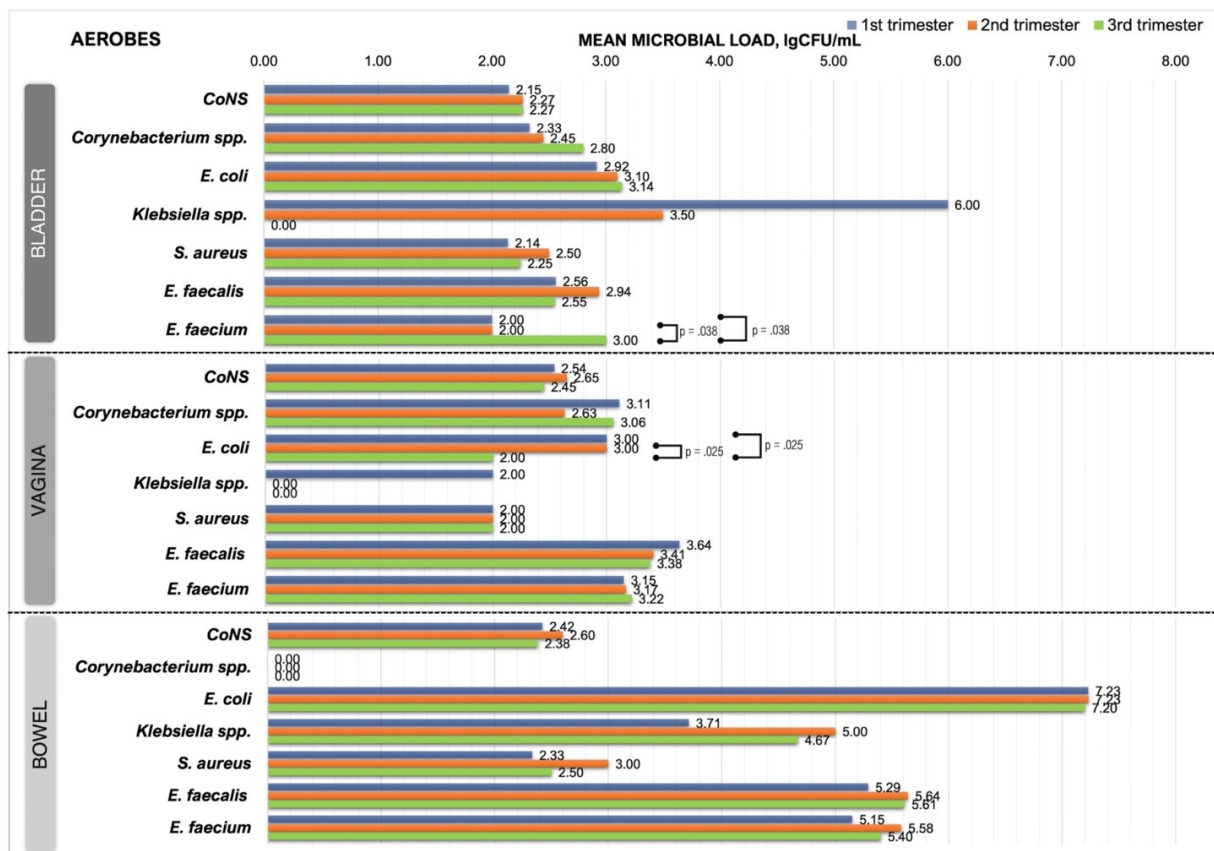


Figure 3: Microbial load dynamics of aerobes from three biotopes during pregnancy (red dotted line – detection limit, 0.00 – below detection limit)

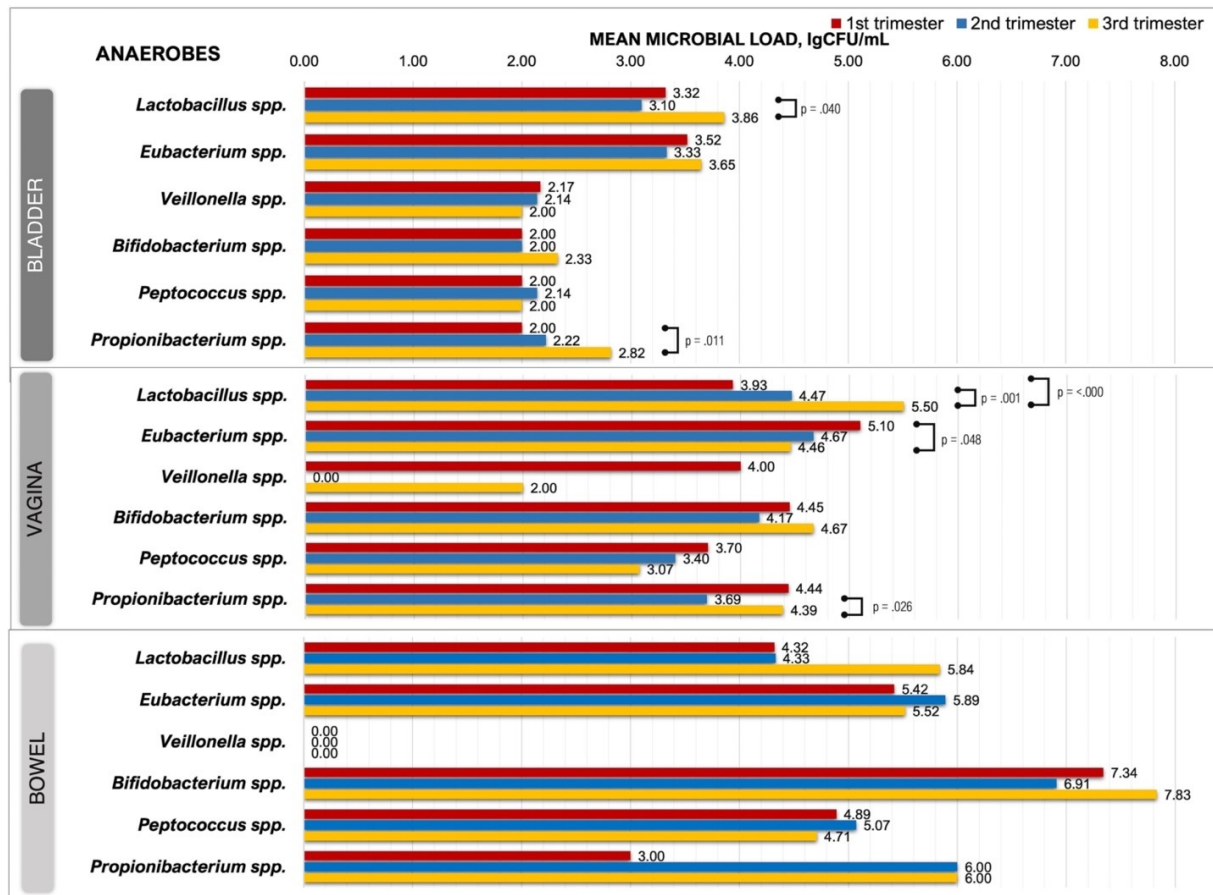


Figure 4: Microbial load dynamics of anaerobes from three biotopes during pregnancy (green dotted line – detection limit, 0.00 – below detection limit)

- **Bladder microbiota**

During TRI-1, mean baseline MLVs for most bacteria were $<10^3$ CFU/ml. Only *Klebsiella spp.*, *Lactobacillus spp.* and *Eubacterium spp.* had mean bacteriuria levels of $10^{6.00}$, $10^{3.32}$ and $10^{3.52}$ CFU/ml respectively.

By TRI-3, mean MLV did not change significantly for most aerobes and anaerobes, only *E. faecium* (TRI-1 vs TRI-3, $p=0.038$; TRI-2 vs TRI-3, $p=0.038$), *Lactobacillus spp.* (TRI-2 vs TRI-3, $p=0.040$) and *Propionibacterium spp.* (TRI-1 vs TRI-3, $p=0.011$) showed a significant increase in bacteriuria. Among aerobes, only *Klebsiella spp.* was non-trend-specific, having a MLV of $10^{6.00}$ CFU/ml in the T1 that completely reduced through the TRI-2 to zero-level in TRI-3.

- **Vaginal microbiota**

Most vaginal aerobes showed that initial (TRI-1) MLVs slightly higher than urinary, excluding *Klebsiella spp.* and *S. aureus*. Fluctuations in aerobic MLVs were insignificant during pregnancy. The differences in MLVs between TRI-1 and TRI-3 were significant only for *E. coli* (TRI-1 vs TRI-3, $p=0.025$; TRI-1 vs TRI-3, $p=0.025$).

In TRI-1, vaginal anaerobes showed MLV generally higher than urinary. The overall MLV of vaginal discharge throughout pregnancy was elevated compared to urinary. *Lactobacillus spp.* showed a significant MLV increase from TRI-1 to TRI-3 (TRI-1 vs TRI-3, $p<0.001$; TRI-2 vs TRI-3, $p=0.001$), and *Eubacterium spp.* demonstrated its decrease (TRI-1 vs TRI-3, $p=0.011$).

- **Bowel microbiota**

Bowel microbiota was characterized by consistently high MLVs ($>10^5$ CFU/ml), specified for *E. coli*, *E. faecalis*, *E. faecium*, *Eubacterium spp.*, *Bifidobacterium spp.*, and *Propionibacterium spp.* However, it should be noted that deviations of MLV for faecal samples with aerobes and anaerobes were unreliable between TRIs.

4.3 Evaluation of biotope interactions

Indices of co-occurrence (contiguity) coefficient between taxa of the three biotopes based on verification frequency during TRIs are presented consecutively in Tables 2, 3, and 4.

Table 2: Co-occurrence coefficients for identification frequencies between taxa of the three biotopes in first trimester

| Microorganism | Urine – Vagina | | Urine – Bowel | | Vagina – Bowel | |
|-----------------------------|----------------|---------------------|---------------|---------------------|----------------|---------------------|
| | COC* | P | COC* | P | COC* | P |
| <i>Corynebacterium</i> spp. | 0.504 | 0.001** | – | – | – | – |
| CoNS | 0.356 | 0.037*** | 0.426 | 0.01*** | 0.387 | 0.25 |
| <i>S. haemolyticus</i> | 0.431 | 0.488 | 0.147 | 0.414 | 0.378 | 0.025** |
| <i>S. saprophyticus</i> | 0.659 | <0.001*** | 0.513 | 0.001*** | 0.563 | <0.001*** |
| <i>S. lentus</i> | 0.484 | 0.002*** | 0.545 | <0.001*** | 0.463 | 0.004*** |
| <i>S. warneri</i> | 0.63 | <0.001*** | 0.579 | <0.001*** | 0.523 | 0.001*** |
| <i>S. epidermidis</i> | 0.392 | 0.02** | 0.522 | 0.001*** | 0.554 | <0.001*** |
| <i>Enterococcus</i> spp. | 0.537 | <0.001*** | 0.14 | 0.439 | 0.13 | 0.472 |
| <i>E. faecalis</i> | 0.531 | 0.001*** | 0.263 | 0.136 | 0.288 | 0.1 |

Note. CoNS – coagulase-negative staphylococci; *COC – co-occurrence coefficient; ** – differences are significant at 5% level; *** – at 1% level; "–" – coefficients are not calculated if values are constants

Table 3: Co-occurrence coefficients for identification frequencies between taxa of the three biotopes in second trimester

| Microorganism | Urine – Vagina | | Urine – Bowel | | Vagina – Bowel | |
|---------------------------|----------------|---------------------|---------------|---------------------|----------------|---------------------|
| | COC* | P | COC* | P | COC* | P |
| CoNS | 0.35 | 0.041** | 0.596 | <0.001*** | 0.245 | 0.166 |
| <i>S. haemolyticus</i> | 0.311 | 0.043** | 0.323 | 0.061 | 0.258 | 0.144 |
| <i>S. saprophyticus</i> | 0.336 | 0.051 | 0.533 | 0.001*** | 0.336 | 0.051 |
| <i>S. lentus</i> | 0.471 | 0.351 | 0.617 | <0.001*** | 0.563 | <0.001*** |
| <i>S. warneri</i> | 0.555 | 0.467 | 0.362 | 0.033** | 0.251 | 0.156 |
| <i>S. epidermidis</i> | 0.542 | 0.584 | 0.442 | 0.007*** | 0.441 | 0.007*** |
| <i>E. faecalis</i> | 0.252 | 0.031** | 0.119 | 0.513 | 0.149 | 0.41 |
| <i>Eubacterium</i> spp. | 0.111 | 0.042** | 0.251 | 0.156 | 0.11 | 0.543 |
| <i>Peptococcus</i> spp. | 0.363 | 0.433 | 0.079 | 0.666 | 0.272 | 0.121 |
| <i>Fusobacterium</i> spp. | 0.707 | <0.001*** | 0.707 | <0.001*** | 0.707 | <0.001*** |

Note. CoNS – coagulase-negative staphylococci; *COC – co-occurrence coefficient; ** – differences are significant at 5% level; *** – at 1% level

Table 4: Co-occurrence coefficients for identification frequencies between taxa of the three biotopes in third trimester

| Microorganism | Urine – Vagina | | Urine – Bowel | | Vagina – Bowel | |
|--------------------------------|----------------|---------------------|---------------|---------------------|----------------|---------------------|
| | COC* | P | COC* | P | COC* | P |
| <i>Corynebacterium</i> spp. | 0.372 | 0.028** | 0.183 | 0.309 | 0.195 | 0.277 |
| <i>S. haemolyticus</i> | 0.504 | 0.001*** | 0.471 | 0.003*** | 0.471 | 0.003*** |
| <i>S. saprophyticus</i> | 0.626 | <0.001*** | 0.336 | 0.051 | 0.071 | 0.696 |
| <i>S. lentus</i> | 0.15 | 0.935 | 0.607 | <0.001*** | 0.617 | <0.001*** |
| <i>S. warneri</i> | 0.383 | 0.087 | 0.513 | 0.001*** | 0.571 | <0.001*** |
| <i>S. epidermidis</i> | 0.289 | 0.099 | 0.36 | 0.035** | 0.067 | 0.713 |
| <i>Eubacterium</i> spp. | 0.253 | 0.041** | 0.523 | 0.001*** | 0.119 | 0.513 |
| <i>Peptococcus</i> spp. | 0.281 | 0.651 | 0.345 | 0.044** | 0.193 | 0.282 |
| <i>Peptostreptococcus</i> spp. | – | – | – | – | 0.707 | <0.001*** |

Note. CoNS – coagulase-negative staphylococci; *COC – co-occurrence coefficient; ** – differences are significant at 5% level; *** – at 1% level; "–" – coefficients are not calculated if values are constants

As observed in TRI-1, high-significant association strengths were found between microorganisms verified in biotopes.

In TRI-2, reduction of such high-significant association strengths characterising the tightness of taxa interrelationships in biotopes was observed. In TRI-3, the probability of such associations becomes higher again.

Bladder and bowel biotopes were more strongly associated in all TRIs, but such associations between vaginal and bowel biotopes occurred less in TRI-2 and TRI-3. Notably, the sought co-occurrence coefficients were highly significant in all TRIs and were greatly marked for aerobes, specifically for CoNS cluster species. By contrast, the estimated coefficients for anaerobes between biotopes were observed only for certain taxa.

5 Discussion

Pregnancy is a period of profound changes in the female physique, both to adapt for the new conditions of functioning and to support and develop the foetus. The female microbiota plays a fundamental role in these processes, although insights into the structural and qualitative microbiota features of different physique sites of pregnant women are still clearly limited. This applies both to cross-biotopic relationships and their impact on the course of healthy and complicated pregnancies. The vaginal microbiota has been studied better than other biotopes in non-pregnant and pregnant women [23], [24], [30]. Studies have shown greater stability of the vaginal microbiota during pregnancy than in the non-pregnant state [23] and the impact on its variation from gestational age [1]. In fact, it has been observed that pregnancy progression does not result in significant taxonomic changes in the vaginal microbiota [31].

The results of our analysis based on microbiota evaluation of exclusively HPW over three TRIs, reveal precisely reliable changes in detection frequencies of most taxa from TRI to TRI, while the IDF of only *Lactobacillus* spp. and *E. coli* remained stable. Furthermore, no significant changes were observed in the

IDFs of vaginal aerobes and anaerobes during pregnancy. However, TRI-3 showed a remarkable increase in MLV for *Lactobacillus* spp., which was accompanied by a significant decrease in MLVs by *E. coli* and *Eubacterium* spp.

Previous studies of the bowel microbiota have revealed an increasing diversity of taxa from TRI-1 to TRI-3 [1]. Conversely, our study demonstrated just stability for taxa of this biotope both in IDFs and MLVs. But the variations of some microorganisms in the upward or downward trend by TRI-3 turned out to be insignificant. In the last 15 years, no studies have investigated the bladder urine of pregnant women along with vaginal and bowel biotopes, although isolated studies on urine in pregnant women have certainly been conducted [34], [35].

None of the 25 urinary microorganisms studied showed stability in IDFs and MLVs. Trends for IDFs of urinary microbes from TRI-1 to TRI-3 were multidirectional. The greatest changes were seen in most bacteria during TRI-2. This was especially true for anaerobes. The average bacteriuria for most aerobes and anaerobes did not exceed 10^3 CFU/ml. However, some anaerobes exhibited a significant increase in bacteriuria by TRI-3. Among the all aerobes, only *Klebsiella* spp. showed high bacteriuria values (10^6 CFU/mL) in TRI-1 and no bacteriuria at all during TRI-3.

In bladder and vaginal microbiota, *CoNS*, *Corynebacterium* spp., *E. coli*, and *E. faecalis* were dominant aerobes, while *Lactobacillus* spp., *Eubacterium* spp. were dominant among the anaerobes. In addition, *Bifidobacterium* spp., *Propionibacterium* spp., and *Peptococcus* spp. were often found in vaginal discharge. In bowel microbiota, *E. coli* was a common microorganism, as are *E. faecalis* and *E. faecium*, and *CoNS* were also high-frequency prevalent in all TRIs. The dominant anaerobic bacteria in this biotope included *Bifidobacterium* spp., *Lactobacillus* spp., and *Eubacterium* spp.

Thus, we determined that *CoNS* and *Lactobacillus* spp. were the dominant in biotopes at all TRIs. Our data on the IDFs of *Lactobacillus* spp. in biotopes are not complementary to the results of other studies, especially regarding to the bowel *Lactobacillus* spp. [31], [32]. The differences may be related to the heterogeneity of the pregnant women cohort where premature births occurred, with the specific dietary and nutritional habits, use of progesterone treatments [52], as well as with the predominant use of sequencing techniques to identify bacteria in other studies, etc.

How can we explain that the same types of bacteria are found in three different biotopes of HPW? One possible explanation is that the bacteria were contaminated during the sampling process or during preparation for examination. However, it is more likely that we are dealing with distinct strains of the same bacteria that may be unidentical in different biotopes [32]. However, colonization can also occur in the uterus, during passage through the genital tract and natural feeding. In addition, it has previously been shown that various *Lactobacillus* spp. are present in the vagina and bowel in diverse combinations and that their diversity depends on the TRI [33].

In this regard, it is important to understand the relationships between the various taxa defined in all biotopes. Our material shows that a reliable consistency exists, and it varies depending on the several co-occurrences from TRI to TRI. It has been established that the co-occurrence between the bladder-vaginal and bladder-bowel biotopes is more pronounced than the relationships of the MLVs in the vaginal–bowel biotopes. Also, the links between aerobes are higher than those between anaerobic ones.

Our results enhance existing knowledge regarding the composition of the microbiota in three biotopes and demonstrate the dynamics and interrelated behaviour of the bladder, vaginal and bowel microbiota throughout pregnancy in first-time HPW. Biotopes are characterised by a stable dominance of certain aerobes and anaerobes. Furthermore, some of these taxa are marked by an unchanged IDFs in given biotopes.

Limitations. The strength of our work lies in the first-present study of the bladder microbiota jointly with the vaginal and bowel microbiota. However, we observe the limitations of our study being the small cohort of HPW, the insufficient breadth of species composition identification inherent in an extended culture-based study compared to gene sequencing approach.

6 Conclusion

The microbiota of the pregnant woman is essential for her health and foetal well-being. It is likely that our discovered microbiota composition at gestational ages observes a crucial role in its straightforward course. It is natural to assume that variations in microbiota composition from the studied one may be involved in the development of several pregnancy complications. Extended culture allows the detection

and observation of these changes in microbial composition without resorting to the complex expensive investigative techniques available in all antenatal clinics worldwide. In this regard, it is essential to pay special attention to the evaluation of the urinary microbiome to prevent acute kidney and urinary tract infections. Consequently, it will be crucial in the future to determine which abnormalities in the microbial composition of the urinary tract convey a potential risk of pregnancy-related complications, such as acute pyelonephritis.

7 Financing

The study was not sponsored.

8 Conflict of interest

The authors declare no conflicts of interest.

9 Ethical statement

The study was designed according to the prescriptions of the Declaration of Helsinki (revised in Fortaleza, Brazil, October 2013).

10 Ethical approval

The study was approved by the Ethical Committee of Rostov State Medical University (Protocol #15/20, certified on 08/10/2020).

11 Authors' contribution

- **Yu.L. Naboka** – study design, supervision, project administration, data analysis, drafting the manuscript, scientific editing;
- **N.V. Vorobyeva** – study design, data acquisition and analysis, research methodology, drafting the manuscript;
- **I.A. Gudima** – data acquisition and analysis, research methodology;
- **T.G. Sinyavskaya** – data analysis, statistical data processing;
- **R.S. Ismailov** – data analysis, scientific editing, software support, data visualisation;
- **K.G. Naber** – data analysis, scientific editing, critical review;
- **M.I. Kogan** – study design, supervision, data analysis, drafting the manuscript, scientific editing, critical review.

Note

This chapter was first published in the journal "Urology Herald" ("Вестник урологии") in 2024 [53]. The article was adapted for book release and re-issued with kind permission of the publisher.

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Corresponding authors: Prof. Yulia Naboka, Rostov State Medical University, Department of Microbiology, - Rostov on Don, Russia, Phone: -, E-mail: Nagu22@mail.ru

Citation note: Naboka Y, Vorobyeva NV, Gudima IA, Sinyavskaya TG, Ismailov RS, Naber KG, KOGAN M. Composition and dynamics of bladder, vaginal and bowel microbiota during three trimesters in healthy pregnant women. Version 2026 Apr 22. In: Bjerklund Johansen TE, Wagenlehner FME, Matsumoto T, Cho YH, Krieger JN, Shoskes D, Naber KG, editors. *Urogenital Infections and Inflammations. Living Handbook*. Berlin: German Medical Science GMS Publishing House; 2017- [2026 Apr 22]. DOI: [10.5680/lhiii000075](https://doi.org/10.5680/lhiii000075)

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