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Dispersion and alterations of vaginal flora across pregnancy trimesters

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ABSTRACT

Objectives: This study aimed to examine the distribution and variations in vaginal pathogens throughout different trimesters of pregnancy, utilizing both vaginal culture and polymerase chain reaction (PCR) techniques, in order to ascertain the presence and prevalence of microorganisms across the various stages of pregnancy.

Materials and Methods: A total of forty-six healthy pregnant women with no reported discharge complaints were recruited for this study. They were monitored at 6-13 weeks, 20-26 weeks, and 32-38 weeks of gestation until delivery. Vaginal swab samples were collected and subjected to multiplex PCR, microscopic examination, aerobic culture media, and gram staining.

Results: *Candida* species (spp.) emerged as the most frequently isolated microorganisms in vaginal swab samples from each trimester, followed by *Escherichia coli* and *Ureaplasma urealyticum*. The prevalence of *Candida* spp. increased proportionally with advancing trimesters. Notably, the pregnancies of these women showed no complications such as premature labor or premature membrane rupture. *Gardnerella vaginalis* was the predominant microorganism isolated in the first trimester, succeeded by *Atopobium vaginae*, *Bacteroides fragilis*, *Mobiluncus curtisii*, and *Mobiluncus mulieris* in decreasing order. However, the quantity of *Gardnerella vaginalis* did not exhibit a significant increase in the second and third trimesters.

Conclusion: This study suggests *Gardnerella vaginalis* as the most commonly isolated vaginal microorganism during the first trimester, alongside other bacterial species associated with bacterial vaginosis. Importantly, the quantities of these bacteria remained relatively stable in the second and third trimesters. These findings suggest a dynamic shift in vaginal microbiota during pregnancy, including an escalation in *Candida* spp. as pregnancy progresses. Further research is warranted to comprehend the implications of these changes and their potential ramifications for vaginal and feto-maternal health.

Keywords: *Candida;* pregnancy; vaginal flora; vaginal pathogens

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INTRODUCTION

The vaginal flora represents a complex ecosystem comprising a variety of aerobes and anaerobes, with lactobacilli, particularly those producing lactic acid, playing a central role in maintaining a harmonious environment. While lactobacilli are the prevailing species, other bacteria such as *Staphylococci*, *Mobiluncus*, *Bacteroides*, and *Peptostreptococci* also coexist.[1,2] Numerous risk factors can disrupt this natural balance, including systemic diseases, vaginal contraceptives, antibiotic usage, immune-suppressive conditions like diabetes, pregnancy, smoking, a high number of sexual partners, inadequate hygiene, vaginal douching, and menstruation.[1,2] Changes in the vaginal flora due to various factors may cause discharge, which can be infection-related or not. Most common infections of vagina are; bacterial vaginosis (BV) characterized by an overgrowth of *Gardnerella vaginalis* (*G. vaginalis*) or other microbial agents such as *Candida* spp. and *Trichomonas vaginalis* (*T. vaginalis*) respectively.

Previous research has consistently demonstrated that the vaginal microbiota during pregnancy is primarily characterized by a dominant community of *Lactobacilli*.[3] And it is a known fact that untreated BV can even lead to pregnancy complications, postpartum or post abortion infections, pelvic inflammatory disease and even infertility, underscoring the vital importance of accurate diagnosis and timely treatment.[4,5] However, it's worth noting that in routine antenatal care, the culture of anaerobic bacteria in genital discharge samples is not frequently carried out, and treatment typically remains empirical without specifically targeting the underlying cause. Although serological and molecular methods can be employed for identification, a significant number of pregnant women are managed in primary healthcare centers where microbiology laboratory resources might be limited. Consequently, many symptomatic patients end up receiving empirical treatment.

On account of this, we aimed to investigate the presence and dynamics of vaginal pathogens during pregnancy, determine the distribution of colonizing microbial agents, and offer guidance for the appropriate use of antimicrobial agents in primary care settings.

MATERIALS AND METHODS

A total of 138 vaginal swab samples were collected from 46 healthy pregnant women who were under the care of the department of obstetrics and gynecology at a tertiary university hospital. Each woman provided three separate samples, corresponding to each trimester of pregnancy. The study encompassed pregnant women aged between 19 and 40 and was carried out over the course of one year.

Exclusion Criteria

Women with any chronic diseases, active vaginal infection, or vaginal bleeding were excluded from the study. All pregnant participants underwent an investigation to gather data on various factors that could influence their vaginal flora, including examination results, marital status, history of systemic diseases, surgical interventions, method of achieving pregnancy (spontaneous or assisted reproductive techniques), medications used, emergence of conditions during pregnancy, previous culture tests due to discharge complaints, culture test outcomes, and any administered treatments for positive results. Patients with systemic diseases that might impact vaginal flora and cause immune deficiency (such as diabetes, liver disease, kidney disease, heart failure, rheumatic diseases, and dermatological diseases) were also excluded. Additionally, patients referred to high-risk pregnancy clinics and anticipated to receive steroid treatment for fetal lung maturation were initially excluded from our study.

As a result of these criteria, all pregnant women with chronic diseases, vaginal bleeding, or active vaginal infection were ineligible for participation. Initially, 50 patients were invited to take part in the study. However, four patients were unable to continue with the follow-up procedures after the initial samples and were subsequently removed from the study. This led to a final inclusion of 46 patients in the study.

Sample Collection and Examination

Prior to the examination, patients were instructed to empty their bladder and refrain from sexual intercourse, vaginal medications, or hygiene products for a minimum of 24 hours. Patients were positioned in the dorsal lithotomy stance and underwent examination using a sterile, lubricated speculum. Vaginal swabs were meticulously acquired from the posterior fornix or lateral vaginal wall using a sterile cotton-tipped applicator, ensuring no contact with the speculum, vaginal mucosa, or vulva. All samples were promptly placed in a transport medium and transferred to the microbiology laboratory.

One swab from each sample was placed in a tube containing a preservative solution for multiplex polymerase chain reaction (PCR) analysis. One of the other two swabs was directly examined for *T. vaginalis*, and the other was cultured aerobically on nutrient media and stained with Gram stain. From each sample, one swab was placed in a tube with a preservative solution for subsequent multiplex PCR analysis. Of the remaining two swabs, one was directly inspected for the presence of *T. vaginalis*, while the other underwent aerobic culture on nutrient media and was stained using the Gram stain method.

In the process of direct microscopic examination, a droplet of physiological saline was applied to a clean slide. The swab-obtained sample was mixed with this droplet to create a suspension, which was subsequently covered with a clean cover slip. The presence of *T. vaginalis* was assessed using a light microscope at a magnification of 400x.

The second swab was used to inoculate blood, chocolate and MacConkey agars, as well as liquid and solid media for mycoplasma and ureaplasma, and was finally smeared on a clean slide and examined under a microscope after Gram staining. The swab was then used to inoculate the nutrient media in the following order: Solid mycoplasma, liquid mycoplasma, blood, MacConkey, and chocolate agars. The nutrient media were then inoculated using the dilution method with blood, chocolate, and MacConkey agars. The liquid mycoplasma-ureaplasma medium was inoculated by dipping the swab into the liquid medium. During Gram staining, the sample smeared on a clean slide was air-dried, heat-fixed, and stained with Gram stain. The stained preparation was examined at 1000x magnification, and the characteristics of the microorganisms, the number of leukocytes, and the presence of clue cells were recorded.

The swabs used in our study were stored in Earle's salt solution (catalog number L1915) at +4 °C. The samples were transported to the laboratory as soon as possible, and the maximum time from sampling to inoculation was four hours.

Identification of Aerobic and Anaerobic Organisms

The identification of different morphologically diverse microorganisms that grow on blood, chocolate, and MacConkey media for aerobic bacteria is determined by Gram staining and then characterized according to their coccus or rod features. Gram-negative rods that grow on MacConkey media were identified based on their ability to ferment glucose, citrate, and produce indole and mobility in MIO and TSI media. The antibiotic susceptibility of identified rods is also determined.

The identification of yeast was based on their colony morphology and microscopic features. The germ tube formation test was performed to differentiate *Candida* spp., and those that form a germ tube in two hours are identified as *Candida albicans* (*C. albicans*). *Candida* is also tested for chlamydospore formation on cornmeal agar, and different subtypes are identified based on the different colors they produce on chromogenic agar.

For the identification of *Gardnerella vaginalis*, the presence of clue cells in the Gram-stained preparations and the typical morphology of the growing colonies were used for identification. *Ureaplasma urealyticum* (*U. urealyticum*) and *Mycoplasma hominis* (*M. hominis*) were identified by inoculating liquid ureaplasma media and incubating them at 37 °C with 5-10% CO_2. After 24 hours, passages were taken from the reddened media to solid ureaplasma media, and after 2-3 days of incubation at 37 °C with 5-10% CO_2, typical star-shaped colonies on the solid media are identified as ureaplasma. The samples were also inoculated into solid mycoplasma media and incubated for 3 days. Typical colonies with a fried egg appearance were identified as *M. hominis* under the microscopic investigation.

For the identification of anaerobic organisms, the TEEGENE SEEPLEX STI Master ACE Detection Panel 2 kit (SD6512X, South Korea) was used. Multiplex PCR was used to identify *G. vaginalis*, *Atopobium vaginae*, *B. fragilis, Mobiluncus curtisii*, and *Mobiluncus mulieris* in this kit.

DNA Isolation

DNA isolation was performed using the Viral DNA-RNA extraction kit (Viral GENE-SPIN, South Korea) according to the recommended protocol. In the multiple PCR method used in this study, after preparing the PCR master mix, the mixture wass inverted five times and then centrifuged. 17 µL of the PCR master mix was distributed into PCR tubes, and then 3 µL of sample DNA was added to each tube to create a total reaction volume of 20 µL. For the negative control, 3 µL of STI MP negative control wass added instead of nucleic acid, and for the positive control, 3 µL of STI MP positive control was added (Table 1). After centrifuge of the tubes at 12,000 rpm for 1 minute, they were placed in a PCR machine heated to 94 °C. The resulting PCR products were loaded onto a 2% agarose gel with specific markers from the kit and run at 120 volts for 20 minutes, then imaged and analyzed under a ultraviole transilluminator.

Statistical Analysis

Statistical analysis was conducted using standard descriptive statistical methods for continuous quantitative variables (such as age) (mean, standard deviation, median). Categorical variables (frequency of occurrence) were presented with frequencies and percentages of the total. The evaluation of quantitative measurements was performed using the "Student's t-test" or "Wilcoxon Signed-rank test" according to the distribution characteristics of the data. Comparisons of categorical variables were made using the chi-square or Fischer's Exact test depending on the distribution of cases. Cases with a p-value <0.05 were considered statistically significant.

RESULTS

A total of forty-six pregnant women, meeting the inclusion criteria, were monitored as they attended the antenatal clinic during their first trimester. Vaginal swab samples were

collected from each participant in each trimester, and they were followed until delivery. The mean age of the 46 participants was 30.4±8.9 years. Out of the 46 pregnant women, 40 (87.3%) were spontaneous pregnancies, 4 (8.7%) were achieved through in vitro fertilization, and 2 (4.3%) were achieved through in utero insemination.

In the first trimester, out of the vaginal swab samples collected from each participant, *T. vaginalis* was detected in two (4.3%) samples, *M. hominis* in four (8.7%), *U. urealyticum* in five (10.9%), *Escherichia coli* (*E. coli*) in five (10.9%), and *Candida* spp., including *C. albicans*, in 13 (28.3%) samples. *Neisseria gonorrhoeae* (*N. gonorrhoeae*) was not detected in the first trimester vaginal swab samples.

In the second trimester, *T. vaginalis* was detected in four (8.7%) samples, *M.* hominis in eight (17.4%), *U.* urealyticum in seven (15.2%), *E. coli* in five (10.9%), and *Candida* spp., including *C. albicans*, in 17 (37%) samples. No *N. gonorrhoeae* was detected in the second trimester vaginal swab samples.

In the third trimester, out of the vaginal swab samples collected from each participant, *T. vaginalis* was detected in four (8.7%) samples, *M. hominis* in eight (17.4%), *U. urealyticum* in eight (17.4%), *E. coli* in five (10.9%), and *Candida* spp., including *C. albicans*, in 18 (39.2%) samples. No *N. gonorrhoeae* was detected in the third trimester vaginal swab samples. The results of the cultures according to trimesters are shown in Table 2.

Table 1. STI master panel 2 primer features

STI master panel 2	Base length
Control group	981
Gardnerella vaginalis	661
Bacteriodes fragilis	415
Mobiluncus curtisii	320
Atopobium vaginae	240
Mobilincus mulieris	182

STI: sexually transmitted infection

The most frequently isolated microorganisms from the samples were *Candida* spp., followed by *E. coli* and *U. urealyticum*. *N. gonorrhoeae* was not detected in any of the samples from all the three trimesters. There was no statistically significant difference in the incidence of *E. coli* between trimesters (p=1). While there was no difference in the incidence of *Candida* spp., *M. hominis, U. urealyticum*, and *T. vaginalis* among the three trimesters, an increase in the incidence of *Candida* spp. was found to be statistically significant (p=0.01). None of the pregnant women who had *Candida* spp. isolated from their vaginal swab samples during the study period had early delivery or low birth weight.

According to multiplex PCR results, 11 (23.9%) were positive for *G. vaginalis*, three (6.5%) were positive for *B. fragilis*, two (4.3%) were positive for *Mobiluncus curtisii* (*M. curtisii*), 10 (22.1%) were positive for *Atopobium vaginae* (*A. vaginae*), and two (4.3%) were positive for *Mobilunkus mulieris* (*M. mulieris*) in the first trimester. In the second trimester samples, 13 (28.3%) were positive for *G. vaginalis*, four (8.7%) were positive for *B. fragilis,* three (6.5%) were positive for *M. curtisii*, 11 (23.9%) were positive for *A. vaginae*, and two (4.3%) were positive for *M. mulieris*. In the third trimester samples, 13 (28.3%) were positive for *G. vaginalis*, 4 (8.7%) were positive for *B. fragilis*, 3 (6.5%) were positive for *M. curtisii*, 12 (26.1%) were positive for *A. vaginae*, and 2 (4.3%) were positive for *M. mulieris*. Group B streptococcus (GBS) was not detected in any of the samples from all trimesters. There was no statistically significant difference in the PCR results between the second and third trimesters. The results are shown in Table 3.

DISCUSSION

The normal vaginal flora consists of a variety of microorganisms, including lactobacilli, which help restrain the growth of potentially harmful microorganisms. However, pregnancy-related hormonal shifts and immune system modulation can trigger alterations in microorganism composition. This study focused on examining vaginal samples from asymptomatic,

Table 2. Proportion of agents reproducted in culture by trimester of pregnancy

	1. trimester n (%)	2. trimester n (%)	3. trimester n (%)	*p*
T. vaginalis	2 (4.3%)	4 (8.7%)	4 (8.7%)	0.368
M. hominis	4 (8.7%)	8 (17.4%)	8 (17.4%)	0.05
U. urealyticum	5 (10.9%)	7 (15.2%)	8 (17.4%)	0.368
E. coli	5 (10.9%)	5 (10.9%)	5 (10.9%)	1
Candida	13 (28.3%)	17 (37%)	18 (39.2%)	0.01
N. gonorrhoeae	0	0	0	N/A

Friedman test was used; N/A: statistical evaluation was not performed as there was no reproduction

healthy pregnant women across all trimesters, without vaginal infections. Both aerobic and anaerobic cultures were conducted, with PCR being utilized to identify anaerobic organisms.

Throughout the monitoring period, none of the pregnant women showed the presence of GBS or *N. gonorrhoeae*. The most commonly identified cultured organisms were *Candida* spp., followed by *E. coli* and *U. urealyticum*. Notably, only the proliferation of *Candida* spp. exhibited statistical significance. Furthermore, PCR analysis revealed a substantial proportion of pregnant women testing positive for *G. vaginalis* (28.3% in the second and third trimesters). Interestingly, no significant statistical disparity was found in the microorganisms detected by PCR analysis between the second and third trimesters.

The impact of both internal and external factors on vaginal flora during pregnancy has been extensively explored in various studies, employing fundamental culture techniques and advanced methods like PCR, as demonstrated in our own research.[6-8] The mechanisms accountable for potential shifts in flora during pregnancy encompass hormonal fluctuations and immune system suppression. Regardless of the mechanism, the disruption of flora equilibrium holds significance due to the potential risk of vaginal infections, ultimately associated with diverse pregnancy complications.[9,10] Notably, the centers for disease control and prevention in the US has achieved a consensus regarding the evaluation and treatment of vaginal infections in pregnant women.[11]

Among the agents implicated in vaginal infections during pregnancy, *Candida* spp., *G. vaginalis*, *T. vaginalis*, and GBS are the most frequently encountered.[12] In our study, *Candida* spp. proved to be the most common microorganism, isolated from vaginal swab samples taken individually during each trimester from participating pregnant women, followed by *E. coli* and *U. urealyticum*. Strikingly, *N. gonorrhoeae* was not detected in any vaginal swab sample across the three trimesters. We did observe

Table 3. Proportion of anaerobic agents detected in PCR by trimester of pregnancy

	1. trimester n (%)	2. trimester n (%)	3. trimester n (%)	*p*
G. vaginalis	11 (23.9%)	13 (28.3%)	13 (28.3%)	0.607
B. fragilis	3 (6.5%)	4 (8.7%)	4 (8.7%)	N/A
M. curtisii	2 (4.3%)	3 (6.5%)	3 (6.5%)	N/A
A. vaginae	10 (22.1%)	11 (23.9%)	12 (26.1%)	0.607
M. mulleris	2 (4.3%)	2 (4.3%)	2 (4.3%)	N/A
GBS	0	0	0	0

GBS: group B streptococcus, Friedman test was used; N/A: statistical evaluation was not performed as there was no reproduction; PCR: polymerase chain reaction

Table 4. Comparison of culture and PCR results of 100 non-pregnant women without discharge complaints (157) and 46 pregnant women sampled during the 1st, 2nd, and 3rd trimesters of pregnancy of the current study

	Non-pregnant 100 women n (%)	1. trimester n (%)	2. trimester n (%)	3. trimester n (%)
T. vaginalis	0	2 (4.3%)	4 (8.7%)	4 (8.7%)
M. hominis	6 (6%)	4 (8.7%)	8 (17.4%)	8 (17.4%)
U. urealyticum	7 (7%)	5 (10.9%)	7 (15.2%)	8 (17.4%)
E. coli	4 (4%)	5 (10.9%)	5 (10.9%)	5 (10.9%)
Candida spp.	3 (3%)	13 (28.3%)	17 (37%)	18 (39.2%)
N. gonorrhoeae	0	0	0	0
G. vaginalis	68 (68%)	11 (23.9%)	13 (28.3%)	13 (28.3%)
B. fragilis	6 (6%)	3 (6.5%)	4 (8.7%)	4 (8.7%)
M. curtisii	12 (12%)	2 (4.3%)	3 (6.3%)	3 (6.5%)
A. vaginae	25 (25%)	10 (22.1%)	11 (23.9%)	12 (26.1%)
M. mulleris	3 (3%)	2 (4.3%)	2 (4.3%)	2 (4.3%)
Grup B streptoccocus	0	0	0	0

GBS: group B streptococcus; PCR: polymerase chain reaction

a notable rise in *Candida* spp. presence. Remarkably, during the course of our study, no instances of preterm birth or low birth weight were observed in any of the pregnant women who were monitored and exhibited *Candida* spp. in their vaginal swab samples.

Further, we identified *G. vaginalis* as positive in the vaginal swab samples obtained from the first trimester, succeeded by *A. vaginae*, *B. fragilis*, *M. curtisii*, and *M. mulieris* in decreasing frequency. However, no significant increase was noted in the prevalence of these bacteria during the second and third trimesters. Notably, GBS was not isolated in any trimester. In a study conducted by Balaka et al.[13] involving 306 pregnant women, vaginal swab samples were collected from 118 participants in the 29-32nd gestational weeks, 104 participants in the 33-36th gestational weeks, and 84 participants in the 37-40th gestational weeks. The study revealed that *C. albicans* was identified in 33.3% of the cultures from the samples, *E. coli* in 10.9%, *Staphylococcus aureus* (*S. aureus*) in 15.4%, *G. vaginalis* in 13.6%, and *T. vaginalis* in 10.6%. The most striking finding in our study was the increasing frequency of *Candida* spp. as trimesters progress. It has been shown that bacteriocins secreted by bacteria in the normal vaginal flora suppress the growth and germination of yeasts. It has also been determined *in vitro* that certain species of *Lactobacilli* prevent yeast colonization by allowing themselves to bind to the vaginal epithelium instead of yeasts, due to a protein they produce.[14] The increased estrogen hormone levels during pregnancy also increase the adhesion of these types of bacteria to the vaginal epithelium. As in non-pregnant women, vulvovaginal candidiasis (VVC) also causes symptoms such as discharge, itching, burning, and dysuria in pregnant women, and findings such as erythema, edema, and fissures can be detected in the vagina. A pH of less than 4.5 and the absence of odor are typical features of this infection.[14,15] The infection can be detected in 30-40% of women during pregnancy, and it is especially more common in the last trimester. The high estrogen levels during pregnancy cause an increase in the reproduction of *Candida* spp. and an increase in the amount of glycogen in the vaginal epithelium, which serves as a source of nutrition for germination.[16,17] According to the study conducted by Cotch et al.[18], there was no association between *Candida* spp. causing VVC, and early or low birth weight deliveries. None of the pregnant women followed during our study who produced *Candida* spp. in their vaginal swab samples had early or low birth weight deliveries.

Another important finding obtained in our study is that there was no increase in the amount of anaerobes. Although BV is a common infection in pregnant women, only 23.9% of the samples taken from the patients who participated in our study in the first trimester were positive for *G. vaginalis*, 6.5% for *B. fragilis*, 4.3% for *M. curtisii*, 22.1% for *A. vaginae*, and 4.3% for *M. mulieris*. While 28.3% of the second trimester samples were positive for *G. vaginalis*, 8.7% for *B. fragilis*, 6.5% for *M. curtisii*, 23.9% for *A. vaginae*, and 4.3% for *M. mulieris*, 28.3% of the third trimester samples were positive for *G. vaginalis*, 8.7% for *B. fragilis*, 6.5% for *M. curtisii*, 26.1% for *A. vaginae*, and 4.3% for *M. mulieris*, and no significant difference was found between trimesters.

The pathophysiology of BV is complex and not yet fully understood.[14] *G. vaginalis* is one of the bacteria associated with this infection and is listed among the most commonly produced agents in vaginal swab samples taken from pregnant women, with up to 50% of women being asymptomatic. Other bacteria associated with this infection include *Mobiluncus* species, *Bacteroides* species other than *B. fragilis*, and anaerobic Gram-positive cocci such as *Prevotella* and *Peptostreptococcus*.[14] In a study conducted in the first trimester pregnant women, BV was determined at a rate of 17.9%, *C. albicans* at a rate of 15.1%, and *T. vaginalis* at a rate of 3.8%.[19] In our study, *Candida* spp. was the most commonly produced, while BV pathogens were produced in the second place and *T. vaginalis* was produced in even smaller amounts. Balaka et al.[20] detected *C. albicans* in 33.5% of 308 pregnant women in the third trimester, BV in 21.5%, and *T. vaginalis* in 10.6%. Kaźmierczak et al.[21] found *Candida* species in 42% of 450 pregnant women, BV in 19%, and *T. vaginalis* in 4%. Morales et al.[22] detected *Candida* species in 44% of 3.217 women, GBS in 15%, and BV in 11%.

T. vaginalis constitutes one of the most common infections among sexually transmitted diseases.[21] Low socio-economic status and having multiple sexual partners are among the risk factors for this infection. Some studies have found that this infection can occur together with other vaginal infections, and it can be associated with BV in pregnant women at a rate of 22%.[23] *T. vaginalis* was detected in 4.3% of the vaginal swab samples taken from the pregnant women participating in our study in the first trimester and in 8.7% in other trimesters. The pregnant women with positive cultures did not have any symptoms, and no symptoms observed in BV were detected, nor was any association between *T. vaginalis* and BV observed.

GBS is a group of bacteria that are encountered at a rate of 5-40% in the pharynx, vagina, and gastrointestinal system flora.[15,23] The main source of GBS carriage in pregnant women is the intestinal system, and colonization in the vagina and cervix develops from there. GBS infections lead to early rupture of membranes and prematurity by causing desidual infections. Symptomatic GBS infections in early childbirth can cause severe infections in the

baby. Routine screening of GBS in pregnant women has become important to prevent early rupture of membranes, neonatal infections, and maternal morbidity and mortality.

The study of vaginal flora changes during pregnancy was investigated in several studies, and a noteworthy one is by Ross and Needham[24] In this study, vaginal swab samples were taken separately from 131 healthy, asymptomatic pregnant women in each trimester, and the culture results were compared. Twenty different species were identified in the cultures, and as the trimesters progressed, a decrease in Lactobacillus and *Candida* spp. was observed, while an increase in *E. coli* and GBS growth rates was seen. In our study, an increase in *Candida* spp. growth rates was observed, while *E. coli* growth rates remained constant, and no GBS was detected in any sample.

In a study conducted in 2011 at the university's medical faculty[25], 100 sexually active women with vaginal discharge complaints were taken as the study group, and 100 sexually active women without any complaints were taken as the control group. Similarly, aerobic cultures were performed on the samples, and multiplex PCR was done for anaerobic pathogens. In the control group, 7% had *U. urealyticum*, 6% had *M. hominis*, 4% had *E. coli*, 1% had *Klebsiella* spp., 3% had *C. albicans*, and 4% had *Candida* spp. In the control group, PCR showed that 68% were positive for *G. vaginalis*, 25% for *A. vaginae*, 12% for *M. curtisii*, 6% for *B. fragilis*, and 3% for *M. mulieris*. The comparison of these results with our study findings is shown in Table 4, and it can be seen that all other pathogens except *G. vaginalis* had similar percentages in pregnant and non-pregnant cases. The fact that the results obtained in the first trimester, when hormonal effects and immune system suppression are not yet evident, are similar to those obtained in the other trimesters supports our study. However, while *G. vaginalis* was detected in non-pregnant cases at a rate of 68%, it was found to be 23.9%, 28.3%, and 28.3% in the first, second, and third trimesters, respectively. When these values were statistically evaluated, there was a significant difference between non-pregnant cases and rates observed in the first trimester ($p<0.001$), as well as between non-pregnant cases and rates observed in the second or third trimester ($p<0.001$). This difference could be explained by the possibility of different patient populations or the protective effect of pregnancy. However, there is a challenge in terms of study methodology as we would need a large number of cases to take samples from a non-pregnant woman and then follow her throughout pregnancy. Therefore, this comparison needs to be confirmed by larger scale studies.

An important limitation of this study is the absence of antibiogram test results. As a result, the findings lack the comprehensive data required to propose empirical treatments for the identified pathogens. It's essential for future research to encompass antibiogram results in order to furnish more comprehensive insights in this aspect.

CONCLUSION

In conclusion, within the studied population, GBS infection doesn't seem to pose a significant risk during pregnancy in Türkiye. Regarding anaerobic pathogens, *G. vaginalis* emerged as the most prevalent pathogen in the first trimester, succeeded by *A. vaginae, B. fragilis, M. curtisii*, and *M. mulieris* in decreasing order. Throughout all trimesters, *Candida* spp. remained the predominant pathogen, followed by *E. coli* and *U. urealyticum*. There was a notable rise in the occurrence of *Candida* spp. as the trimesters advanced, although this increase wasn't linked with preterm delivery or low birth weight.

ETHICS

Ethics Committee Approval: Cerrahpaşa Medical School Ethical Committee no: 83045809/3959.

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Contributions

Surgical and Medical Practices: H.Ç.K., T.A.; Concept: H.Ç.K., T.A.; Design: H.Ç.K., T.A.; Data Collection or Processing: H.Ç.K., O.G., E.P., K.C.K.; Analysis or Interpretation: H.Ç.K., O.G., E.P., K.C.K., T.A.; Literature Search: H.Ç.K., O.G., İ.C.S; Writing: H.Ç.K., O.G., İ.C.S., T.A.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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