Alterations in Vaginal Microbiota among Pregnant Women with COVID-19

Ebru Celik

Koç Üniversitesi Tıp Fakültesi <https://orcid.org/0000-0002-0774-4294>

Gulin Ozcan

Koc Üniversitesi Tıp Fakültesi

Cansel Vatansever

Koc Üniversitesi Tıp Fakültesi

Erxiati Paerhati

Koc Üniversitesi Mühendislik Fakültesi

Mert Ahmet Kuskucu

Cerrahpasa Medical School: Istanbul Üniversitesi-Cerrahpasa Cerrahpasa Tıp Fakültesi

Ozlem Dogan

Koc Üniversitesi Tıp Fakültesi

Sebile Guler Cekic

Koc Üniversitesi Hastanesi

Onder Ergonul

Koc Üniversitesi Tıp Fakültesi

Atilla Gursoy

Koc Üniversitesi Mühendislik Fakültesi

Ozlem Keskin

Koc Üniversitesi Mühendislik Fakültesi

Fusun Can (✉ fucan@ku.edu.tr)

Koc Üniversitesi Tıp Fakültesi <https://orcid.org/0000-0001-9387-2526>

Research

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Abstract

Background: The maintenance of vaginal microbiota is an important factor to achieve ideal pregnancy outcomes. Coronavirus disease (COVID-19) has been shown to have potential adverse effects on pregnancy and neonatal outcomes. Pregnancy itself is a risk factor for the severity of COVID-19, with an increased risk of intensive care unit (ICU) admission, maternal morbidity, and mortality. the role of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection in vaginal microbiome composition in pregnant women with COVID-19 has not yet been investigated. Therefore, we anticipate that COVID-19 may unfavorably affect the composition of the vaginal microbiota, resulting in adverse pregnancy outcomes. We aimed to describe the alterations of the composition of vaginal microbiota in pregnant women with COVID-19.

Results: A prospective case-control study was conducted among 19 pregnant women with COVID-19 and 28 healthy controls matched according to the gestational week and age. The vaginal swabs were collected during the active phase of infection and consecutively, within a month after recovering from infection. In three patients, longitudinal samples before, in the course, and after infection were also obtained. The microbiome alterations were examined by 16S rRNA gene sequencing. We indicated that COVID-19 was associated with vaginal dysbiosis during pregnancy, which was indicated by an increased alpha diversity index. There was a significantly decrease in Firmicutes ($P=0.007$) and *Lactobacillus* ($P=0.019$) with an increase in Bacteroidetes ($P=0.024$) in the COVID-19 group. In the moderate/severe group, increased amounts of *Ureaplasma* and vanishing of *Lactobacillus gasseri* were found in women, compared to the asymptomatic or mild group ($P=0.001$). In longitudinal analysis, elevation of Actinobacteria with reduction of Firmicutes and Bacteroides were indicated during the active phase.

Conclusions: The study revealed vaginal dysbiosis with a low abundance of *Lactobacillus* and an increase in Bacteroidetes in relation to SARS-CoV-2 infection. Vaginal dysbiosis in COVID-19 could be a contributing factor in pregnancy adverse outcomes. Trial registration: clinicaltrials, Registered 15 November 2019, <https://www.clinicaltrials.gov/ct2/show/NCT04165252?cond=microbiota&cntry=TR&draw=3&rank=12>

Background

Coronavirus disease 2019 (COVID-19) has emerged worldwide while a subset of people was infected severely, the others recovered from the disease without symptoms. COVID-19 has been shown to have potential adverse effects on pregnancy and neonatal outcomes. Pregnancy itself is a risk factor for the severity of COVID-19 disease, with an increased risk of ICU admission, maternal morbidity, and mortality [1, 2]. Furthermore, pregnancy complications such as preeclampsia and preterm birth are more likely to occur in women diagnosed with COVID-19 [3, 4].

The predominance of *Lactobacillus* species (spp.) plays a key role in the inhibition of binding non-domestic and potentially harmful microorganisms to epithelial cells [5–7]. *Lactobacillus* maintains the

protective low vaginal pH through secreting of lactic acid [8]. Pregnant women with decreased amounts of *Lactobacillus crispatus*, *Lactobacillus gasseri*, and *Lactobacillus jensenii* in the vaginal microbiota are more likely to deliver preterm [9]. Likewise, the abundance of *Gardnerella vaginalis* increases the risk of preterm birth (PTB) [5, 10].

The mechanism of action of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on pregnancy varies and remains unknown. The SARS-CoV-2 genome has been identified in the vaginal mucosa of a pregnant woman [11]. Indeed, the role of SARS-CoV-2 infection in vaginal microbiome composition in pregnant women with COVID-19 has not yet been investigated. Therefore, we anticipate that COVID-19 may unfavorably affect the composition of the vaginal microbiota, resulting in adverse pregnancy outcomes. We aimed to describe the alterations of the composition of vaginal microbiota in pregnant women with COVID-19.

Methods

Study population:

A prospective study was conducted at Koc University Hospital between August 2020 and August 2021. Pregnant women with active or recently infected within one month with SARS-CoV-2 were included in the study. SARS-CoV-2 infection was confirmed by a positive nasopharyngeal polymerase chain reaction (PCR) test. The signs and symptoms of COVID-19 were evaluated in all women who had positive PCR tests for SARS-CoV-2 infection. COVID-19 was classified according to NIH COVID-19 clinical guidelines [12]. Patients with symptoms including fever, myalgia, or gastrointestinal system symptoms were categorized as a mild disease, those who required oxygen (O₂) supplementation as moderate or severe disease (MSD). Recovery was defined as clinical improvement in combination with a negative nasopharyngeal PCR test. Single vaginal swab was collected from each participant at the time of COVID-19 and within one month after recovery from COVID-19. In three patients, longitudinal microbiota analysis was performed with a collection of vaginal swabs before, during active infection, and two months after recovery. The overview of patients was presented in Figure 1.

The healthy controls (HC) were recruited from the prospective study entitled “Vaginal, Placental and Neonatal Buccal Mycobiota, and Microbiome in Preterm Birth”. The study was initiated in April 2020 (ClinicalTrials.gov Identifier: NCT04165252). Maternal age and gestational week-matched pregnant women were selected for the healthy controls. Inclusion criteria for the healthy controls are as follows: age older than 18 years with a singleton pregnancy. The exclusion criteria were multiple pregnancies, major fetal structural defects and/or chromosomal abnormalities, stillbirth, having used antibiotics and/or antifungal medication within two weeks at the time of sample collection, the presence of vaginal bleeding at the time of sample collection, and sexual intercourse within 72 hours of sample collection.

Maternal characteristics, medical and obstetrical history were recorded for all participants. Maternal height and weight were measured at the same time with vaginal swabs collection. Gestational age was

determined from the last menstrual period and confirmed from the measurement of fetal crown-rump length at the first-trimester scan. The written informed consent was obtained from all participants. Koç University Research Ethics Board approved the study protocol. The study complies with the declaration of Helsinki.

Sample Collection, Processing, And Sequencing

Vaginal samples were collected with REMEL ESswabs. Vaginal swabs were placed into the sterile tube then stored at -80°C until DNA extraction. Frozen vaginal swabs were immersed in sterile PBS then DNA extraction was performed using the Qiagen DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), as described by the manufacturer. DNA concentration was quantified by Qubit (ThermoFisher Scientific, Waltham MA).

Library preparation was performed using QIAseq 16S/ITS Panel Kit (Qiagen, Hilden, Germany) for sequencing the V1–V9 region of the 16S rRNA gene bacterial gene. Library quantification was done using QIAseq Library Quant Assay (Qiagen, Hilden, Germany) kit following the manufacturer's instructions with Applied Biosystems QuantStudio 7 Flex Real-Time PCR (Applied Biosystems Inc., Foster City, California). Sequencing was performed with the Illumina MiSeq platform using the MiSeq v3 Reagent Kit (Illumina, San Diego, CA, United States).

Bioinformatics

FASTQ files were demultiplexed by different regions using the module in the GeneGlobe Data Analysis Center. (<https://geneglobe.qiagen.com/tr/analyze>). The resulting paired-end FASTQ files were used to profile the microbiota of the samples with DADA2 using default parameters. Sequencing of 16S rRNA gene(V1-V2) regions was performed [13]. Amplicon sequence variants (ASV) were picked out and assigned with taxonomic annotations using the naive Bayesian classifier method embedded in DADA2 [14]. The Silva (v138.1) was used as a reference database (v138.1) [15].

Statistical analysis

Continuous variables were expressed as a median and interquartile range (IQR), whereas categorical variables were expressed as percentages. Mann-Whitney U and Fisher exact tests were applied for comparison of maternal demographic and clinical characteristics between COVID-19 patients with healthy controls. Data were analyzed using IBM SPSS Statistics for Windows, version 26.0 (Armonk, NY: IBM Corp.).

The alpha diversity, beta diversity, and vaginal microbiota composition of the COVID-19 group, and healthy control were compared. Alpha-diversity metrics were calculated as the number of phyla, genus, and species. The vaginal microbiota of asymptomatic or mild, moderate, or severe cases and healthy

controls were compared. Beta-diversity was defined by using Bray-Curtis distance. The differences in alpha diversity metrics were performed by Wilcoxon signed-rank test using Python 3.7 (32-bit) and statistical significance was set as P value <.05. Statistical data were visualized with GraphPad Prism 8.0.2.

Results

Study cohort demographics

There were no differences in maternal age, BMI, gestational age at delivery, and birth weight between women with the COVID-19 group with healthy controls. (Table 1). Of 19 women with COVID-19, 13 women had the asymptomatic or mild disease (AMD) (68.4%), and 6 of those had moderate or severe disease (31.6%). The rate of preterm birth (PTB) was 15.3% (n=3) in the COVID-19 group; 2 of 6 women (33.3%) with moderate or severe disease and 1 of 13 women (7.7%) with asymptomatic or mild disease (Table 2).

Table 1
Maternal demographic and clinical characteristics of cohort

Maternal baseline characteristics and clinical presentations	COVID-19 group (n=19)	Healthy controls (n=28)	P value
Maternal age in years, median (IQR)	33.0 (28.0 – 36.0)	31.0 (29.0 – 34.0)	0.61
BMI on the admission in kg/m ² , median (IQR)	26.4 (23.7 – 28.7)	27.0 (24.9 – 29.1)	0.38
Parity			
Nulliparous, n (%)	8 (42.1)	18 (64.3)	0.15
Multiparous, n (%)	10 (52.6)	11 (39.3)	
Gestational age at delivery in weeks, median (IQR)	38.0 (25.5 – 40.0)	39.0 (37.0 – 40.0)	<.001*
Birthweight in grams, median (IQR)	3200 (725 – 3990)	3265 (2800 – 4000)	0.25
Mode of delivery			
Spontaneous vaginal delivery, n (%)	5 (26.3)	9 (32.1)	0.75
Cesarean section, n (%)	14 (73.7)	19 (67.9)	
BMI, body mass index; *P-value is <.05, statistically significant.			

Table 2
Clinical features of pregnant women with COVID-19

Maternal clinical presentations	Total COVID-19 population (n=19)	Asymptomatic or Mild Cases (n=13)	Moderate or Severe Cases (n=6)	P value
Gestational age at COVID-19 in weeks, median (min-max)	26 (19.0 - 35.0)	22.0 (18.0 – 35.0)	30.0 (25.0 – 35.0)	0.32
Second trimester, n (%)	11 (57.9)	8 (61.5)	3 (50)	1.0
Third trimester, n (%)	8 (42.1)	5 (38.5)	3 (50)	1.0
Presenting signs and symptoms				
Fever, n (%)	9 (52.6)	4 (30.8)	6 (100)	0.01*
Cough, n (%)	9 (47.4)	3 (23.1)	6 (100)	0.003*
Dyspnea, n(%)	7 (36.8)	1 (7.7)	6 (100)	<.001*
Myalgia and fatigue, n (%)	14 (73.7)	9 (69.2)	5 (83.3)	1.0
Diarrhea/GI symptoms	5 (26.3)	3 (23.1)	2 (33.3)	1.0
Headache, n (%)	10 (52.6)	6 (46.2)	4 (66.7)	0.33
Antepartum therapy				
Antibiotics, n (%)	2 (10.5)	-	2 (33.3)	N/A
Antiviral, n (%)	3 (15.8)	1 (7.7)	2 (33.3)	0.22
Prednisolone, n (%)	2 (10.5)	-	2 (33.3)	N/A
Low molecular weight heparin, n (%)	7 (36.8)	1 (7.7)	6 (100)	<.001*
Oxygen support without ICU admission, n (%)	4 (21.1)	-	4 (66.7)	N/A
Admission to ICU, n (%)	2 (10.5)	-	2 (33.3)	N/A
Preterm birth, n (%)	3 (15.8)	1 (7.7)	2 (33.3)	0.22
Admission to NICU, n (%)	3 (15.8)	1 (7.7)	2 (33.3)	0.22
ICU, intensive care unit; NICU, neonatal intensive care unit, GI, gastrointestinal symptoms; P-value is given for the comparison of asymptomatic or mild disease with moderate or severe disease groups. *P represents a statistically significant value (<.05).				

In asymptomatic or mild disease, 61.5% of those had infection in the second trimester and 38.5% in the third trimester (Table 2). Two women with moderate or severe disease received both antibiotic and

antiviral medications. Seven patients received low molecular weight heparin during the active period of COVID-19 disease (Table 2).

Vaginal microbiota composition of women with COVID-19 and healthy controls

Alpha diversity was evaluated by using Shannon and Simpson Index. In the COVID-19 group, the Shannon index was significantly elevated, compared to those of the healthy controls (1.09 vs. 0.75; $P=0.007$), and the Simpson index was significantly lower in the COVID-19 group than those of healthy controls (0.4 vs. 0.61; $P=0.006$) (Figure 2A). In pregnant women with asymptomatic/mild and moderate/severe disease, the Shannon index was found to be significantly higher than those of the healthy controls (1.06 and 1.16 vs. 0.75; $P<0.05$), whereas the Simpson index was significantly decreased, compared to the healthy controls (0.42 and 0.49 respectively, vs. 0.61; $P<0.05$) (Figure 2A). Beta diversity (Bray-Curtis) results indicated that there were no compositional differences between COVID-19 status and healthy controls ($P=0.49$) (Figure 2B).

There were no statistically significant differences in the alpha diversity of vaginal microbiota between the second and third trimesters of healthy controls according to Shannon index and Simpson index (0.41 vs. 0.5; $P=0.22$ and, 0.8 vs. 0.75; $P=0.08$, respectively).

The vaginal microbiota composition and relative abundances of the bacterial phylum, genera and species for two groups were summarized in Figure 3. The three phyla; Firmicutes (86.02%), Actinobacteria (12.12%) and Bacteroidata (Bacteroidetes) (0.54%) accounted for 99.8% of the bacterial species in COVID-19 group. In a comparison of COVID-19 group with healthy controls, Firmicutes significantly decreased (85.9% vs. 96.11, $P=0.049$) while the amounts of Bacteroidetes significantly increased (0.53% vs. 0.44%; $P=0.024$) (Figure 3A).

At the genus level, *Lactobacillus sp.* significantly decreased in the COVID-19 group compared to the healthy controls (80.6% vs. 93.98%; $P=0.019$). Particularly, *Lactobacillus delbrueckii* (*L. delbrueckii*) was *not detected* in the COVID-19 group, although its amount was 1.5% in the healthy controls ($P=0.046$) (Figure 3B).

Lactobacillus iners (*L.iners*), *Lactobacillus crispatus* (*L. crispatus*), and *Lactobacillus jensenii* (*L. jensenii*) showed trends towards a decline in COVID-19 group (26.5%, 3.2%, and 7.1%, respectively) when compared to the healthy controls (32.8%, 3.9%, and 7.5%, respectively) but the differences were not statistically significant ($P>0.05$) (Figure 3C).

In the COVID-19 group, among anaerobe taxa, particularly *Prevotella timonensis* (0.04%) and *Dialister propionicifaciens* (0.02%) were identified but none of these were detected in the healthy controls. In addition to these, the COVID-19-infected group showed a higher abundance of *Gardnerella vaginalis* (6.4% vs. 1.98%; $P=0.213$). Also, *Ureaplasma* (0.69% vs. 0.04%; $P=0.054$) was found to be higher in the COVID-19 group compared to the healthy controls.

The variations of vaginal microbiota composition related to the severity of COVID-19

Mycoplasma hominis, *Fusobacterium nucleatum* and *Anaerococcus tetradius* were only identified in patients with moderate or severe disease (8%, 0.03%, and 0.003%, respectively) (Figure 4). In a comparison of the patients with asymptomatic or mild and moderate or severe disease, there was no significant difference in abundance of *Prevotella timonensis* (0.05% and 0.02%, $p=0.379$); however, *Ureaplasma spp.* was significantly higher in the moderate or severe group (2.09%) than those of the asymptomatic or mild (0.04%, $p=0.005$). In addition, *Gardnerella vaginalis* showed a gradual increase in both two groups (1.99% and 5.11%) compared to the healthy controls (9.33%) (Figure 4).

In the longitudinal vaginal microbiota analysis of the three pregnant women, the relative abundance of Actinobacteria increased from 10.8–59.2% during active infection and returned to 10.8% in the post-COVID-19 phase. There is a declining trend in the abundance of Bacteroidata (Bacteroidetes) (from 65.4–17.9%) and Firmicutes (from 59.2–28.4%) during the phase of active COVID-19 with no regrowth in the post-COVID-19 phase (17.9% and 10.8%, respectively) (Figure 5).

Discussions

The findings of our study indicated that the composition of vaginal microbiota was unfavorably affected by COVID-19 disease and there was a prominent dysbiosis during active COVID-19 infection. Intrauterine infection is a well-established reason for preterm birth. The most common and genuine pathway is that microorganisms can access the amniotic cavity by ascending from the vagina and cervix, resulting in the development of intrauterine infection and subsequent inflammatory response in fetoplacental tissues that eventuates preterm birth [16, 17]. Since evidence on the relation of dysbiosis in vaginal microbiota and preterm birth is accumulating, we can speculate that one of the mechanisms for the explanation of increased rate of COVID-19-associated PTB may be vaginal dysbiosis [5, 18, 19].

Vaginal dysbiosis is defined as an increase of alpha diversity in vaginal microbiome communities [20]. The study found that the Shannon index was remarkably high (1.16) in the COVID-19 group compared to the healthy controls (0.75). Recent studies have revealed that vaginal dysbiosis has a negative impact on vaginal protective mechanisms via increasing local pro-inflammatory effectors [21, 22].

We identified diminished *Lactobacillus* communities in women with COVID-19 disease, particularly more profound in those with moderate or severe disease (77%) when compared to the healthy controls (93%; $P=0.04$). *Lactobacillus delbrueckii* ($P=0.046$) significantly decreased among women with COVID-19. Within-subgroup analysis, *Lactobacillus gasseri* (*L. gasseri*) disappeared in patients with moderate or severe disease. It is well-established that pregnant women with low amounts of *L. crispatus*, *L. gasseri*, or *L. jensenii* in their vaginal microbiota are more likely to deliver before term [9, 23]. In a case-control study, the abundance of *L. gasseri* was found to be associated with decreased risk of early spontaneous preterm birth [24].

In the longitudinal study with three patients, we didn't perform analysis at the species level because of the small sample size. There was approximately a 40% decline in the relative abundance of Firmicutes and Bacteroides during the active COVID-19 period, which was sustained after recovery. In addition, the

abundance of Actinobacteria was the highest in the active disease stage compared to the pre and post COVID-19 periods. Ceccarani et al. revealed that the vaginal flora of healthy women was constituted of mainly Firmicutes and Bacteroidetes, albeit with a low abundance of Actinobacteria [25]. In view of our findings, it would be considered that SARS-CoV-2 infection negatively affects the vaginal compositions of pregnant women.

Disruption of balance of vaginal microbiota causes invades of several facultative or strict anaerobes, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella spp.*, *Fusobacterium spp.*, *Ureoplasma spp.*, and *Porphyromonas spp.* and replacement of *Lactobacilli* [26–28]. Aligning with the aforementioned results, we identified a significantly higher abundance of Bacteroidetes in COVID-19 group. In particular, *Prevotella timonensis* was only identified in women with COVID-19 (0.04%). Likewise, *Dialister propionicifaciens* appeared in the COVID-19 group (0.02%). The SARS-CoV-2 infection triggers the production of prostaglandins and pro-inflammatory mediators to confer ischemia resulting in widespread tissues [29, 30]. Notably, we determined an increase of anaerobic species such as *Gardnerella vaginalis*, *Anaerococcus tetradius*, *Fusobacterium nucleatum*, *Prevotella timonensis* abundance in women with severe disease. Based on these results, we postulated that ischemia in genito-urinary compartments could be a predisposing factor for overgrowth of anaerobes in vaginal microbiota.

Pregnant women are more susceptible to SARS-CoV-2 infection due to physiological, mechanical, and immunological changes during pregnancy [2, 31]. Data supported that pregnancy is a risk factor for severe disease related to COVID-19 [4, 32]. Recently, in a large population-based cohort study, fetal death and preterm birth occurred more frequently in women with SARS-CoV-2 infection than non-infected women (adjusted odds ratio (aOR), 2.21; 95% confidence interval (CI), 1.58 – 3.11; $P < 0.001$ and OR, 2.17; 95% CI, 1.96 – 2.4; $P < 0.001$, respectively) [1]. In the study, the prevalence of preterm birth (15.3%) was high, especially in the severe COVID-19 (2 out of 3 preterm birth), as compared to the prevalence reported before COVID-19 (9.6%) [33]. Even with the small sample size of our study, the rate of preterm birth increased in women with severe COVID-19 disease than those with asymptomatic or mild disease, aligning with a recent meta-analysis [34]. The abundance of *Ureaplasma* and *Mycoplasma* species increased the risk of preterm delivery through chorioamnionitis, salpingitis, bacterial vaginosis, and postpartum endometritis [20, 35]. We found that *Mycoplasma hominis* (8% vs. 0%; $P = 0.01$) and *Ureaplasma spp.* (2.09% vs. 0.04%, $p = 0.001$) showed significantly higher abundance in moderate or severe cases compared to those of the healthy controls. Of note, the abundance of *Ureaplasma spp.* was significantly higher in women with moderate or severe disease than those of asymptomatic or mild disease ($p = 0.005$). We claim in light of these findings and previous evidence from microbiota studies that the subsequent preterm birth in women with severe disease could be a consequence of impaired vaginal composition.

There are several limitations. Our sample size was small to detect the statistical significance between women with severe COVID-19 and women with asymptomatic or mild disease, albeit with some significant differences. Secondly, a potential confounding factor that may differ among the groups was the use of antibiotics at the time of sample collection from severe cases during the active stage.

In conclusion, COVID-19 causes dysbiosis in vaginal microbiota with a significant reduction in the abundance of *Lactobacillus* combined with an increase of Bacteroidetes, especially in *Prevotella timonensis*. We detected that the severity of the disease was associated with increased *Ureaplasma spp.* Based on these findings, we suggest that COVID-19 promotes an unfavorable vaginal microenvironment, which may provide insight into the risk of adverse pregnancy outcomes such as preterm birth. These results raise clinically relevant questions regarding the use of microbiome associated biomarkers as a risk assessment tool for preterm birth in pregnant women during COVID-19. The implication of findings would postulate possible targeted therapy, comprising modification of the vaginal microbiota composition in pregnant women with COVID-19.

Abbreviations

Coronavirus disease

COVID-19

Intensive care unit

ICU

Severe acute respiratory syndrome coronavirus-2

SARS-CoV-2

Species

spp.

Polymerase chain reaction

PCR

Preterm birth

PTB

Healthy controls

HC

CT

Total COVID-19 patients

Asymptomatic or mild disease

AMD

Moderate or severe disease

MSD

Lactobacillus delbrueckii

L. delbrueckii

Lactobacillus iners

L.iners

Lactobacillus crispatus

L. crispatus

Lactobacillus jensenii

L. jensenii

Lactobacillus gasseri

L. gasseri

Adjusted Odds Ratio

aOR

Confidence interval

CI

Declarations

Ethical Approval and Consent to participate: The ethical approval was obtained for the healthy controls and pregnant women with COVID-19 from Koc University Research Ethics Board (No:2019.093IRB2.030 and No:2020.138.IRB1.028). The written consent forms were collected from all participants.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: F.C., E.C., G.O. and C.V. designed and conducted the research. F.C., M. K., O.D., E.C., O.E., G.O., C.V., A.G. and O.K. interpreted the results and wrote the manuscript. S.G.C and E.C. collected the samples and clinical outcomes. G.O. and C.V. processed the samples, prepared the DNA for sequencing, and conducted the experiments. E.P, G.O., C.V., E.C. analyzed the data and prepared the figures and tables. E.P. processed the bioinformatic analysis. A.G. and O.K. supervised the bioinformatics analysis.

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Authors' information: E.C is working in Koc University Maternal and Fetal Department as an associate professor.

G.O. is working in Koc University, Department of Medical Microbiology and IsBank Research Center for Infectious Diseases (KUISCID) as a PhD student.

C.V. is working in Koc University, Department of Medical Microbiology and IsBank Research Center for Infectious Diseases (KUISCID) as a PhD student.

E.P. is working in Koç University, College of Engineering as a PhD student.

M.A.K. is working in Cerrahpaşa Medical School of Istanbul University-Cerrahpaşa, Medical Microbiology as an associate professor.

O.D. is working in Koc University, Department of Medical Microbiology and IsBank Research Center for Infectious Diseases (KUISCID) as an assistant professor.

S.G.C. is working in Koc University Hospital, department of Obstetrics and Gynecology as an attending physician.

O.E. is head in Koc University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology and IsBank Research Center for Infectious Diseases (KUISCID). He is a well-known author in the infectious disease.

O.K. is working in Koc University, College of Engineering as a professor.

A.G. is working in Koc University, College of Engineering as a professor.

F.C. is head of Koc University, Department of Medical Microbiology and IsBank Research Center for Infectious Diseases (KUISCID). She is well-known researcher in medical microbiology and had several ongoing investigations, granted by TUBITAK.

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Figures

Figure 1

An overview of study design Figure 1 includes 19 pregnant COVID-19 patients and 28 healthy controls selected from Clinical Trials. NCT04165252 study. Longitudinal study cases were shown in purple. The three cases complicated with preterm birth were shown in black circles.

Figure 2

The comparison of alpha diversity of the vaginal microbiota between healthy controls and pregnant women with COVID-19 Total (CT) (n=19) and healthy control (HC) (n=28). Pregnant women with COVID-19 were divided into two sub-groups according to clinical severity as Asymptomatic or Mild (AMC) (n=13) and Moderate or Severe (MSC) (n=6). Mann Whitney U rank test is performed for comparison of alpha diversity metrics. The P value less than 0.05 is accepted as significant (* $P < 0.01$, ** $P < 0.05$). B. Beta diversity was analyzed with Bray-Curtis distances ($P = 0.49$). The healthy controls and COVID-19 patients were shown in blue and red, respectively.

Figure 3

The vaginal microbiome composition of healthy controls and women with COVID-19 Healthy control (n=28) and women with COVID-19 (n=19); A. The most abundant bacteria at the phylum level are represented for two groups. B. The most abundant bacteria at the genus level are represented for two groups. C. The relative abundance of different bacteria species in two groups. Only phyla and genera present at relative abundances >0.01% are reported. Remaining taxa are grouped as “Others”.

Figure 4

The comparison of abundance of vaginal microbiota species between Asymptomatic or Mild and Moderate or Severe cases with Healthy Control * represent statistical significance $P < 0.05$, ** represent statistical significance $P < 0.01$. P1 represents the comparison of bacteria species between the healthy controls with women with asymptomatic/mild disease; P2 represents the comparison of bacteria species between the healthy controls with women with moderate/severe disease. P3 represents the comparison of bacteria species between women with asymptomatic/mild disease with those of moderate or severe disease.

Figure 5

The longitudinal analysis of vaginal microbiota The abundance rate of Phyla in vaginal microbiota before COVID-19, during acute phase and after recovery in three patients (asymptomatic or mild [n=2] and moderate or severe [n=1])