Vaginal microbiota associated with preterm delivery

Shi, Yutoku
Tanimura, Kenji
Sasagawa, Yuki
Yamada, Hideto

(Citation)
Journal of Infection and Chemotherapy,26(11):1134-1138

(Issue Date)
2020-11

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90007523

Vaginal microbiota associated with preterm delivery

Yutoku Shi, Kenji Tanimura, Yuki Sasagawa, Hideto Yamada

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

Corresponding author: Hideto Yamada M.D., Ph.D., Professor and Chairman

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine,

7-5-1, Kusunoki-Cho, Chuo-ku, Kobe 650-0017, Kobe, Japan

Tel: +81-78-382-6000; fax: +81-78-382-5756

E-mail: yhideto@med.kobe-u.ac.jp

All authors meet the ICMJE authorship criteria.

Running title: Microbiota and preterm delivery

Abstract

Objectives

The aim of this study was to evaluate whether vaginal microbiota is associated with threatened premature labor and preterm delivery.

Methods

This prospective study enrolled 64 pregnant women who underwent vaginal microbiome analyses using 16S ribosomal RNA sequence method with informed consent. The 64 pregnant women consisted of 47 women with threatened premature labor and 17 women with other diseases (non-threatened premature labor) in a case-control study. In a cohort study of threatened premature labor group, 23 pregnancies ended in preterm delivery, and the remaining 24 ended in full-term deliveries. The differences in vaginal microbiota between threatened and non-threatened premature labor groups, and between preterm and full-term delivery groups were evaluated.

Results

There were no differences in vaginal microbiota between threatened and non-threatened premature labor groups. There were significant differences between preterm and full-term delivery groups in Nugent score [median 3 (range 0-7) vs. 0 (0-4), $p<0.05$], percentage of *Lactobacillus* species [88% (0-100) vs. 99.8% (55.4-100), $p<0.01$], the number of bacterial

species [3 (1-13) vs. 2 (1-5), $p<0.05$], and positivity of *Ureaplasma* species (61% vs. 17%, $p<0.01$). Univariate and multivariable analyses revealed that positivity of *Ureaplasma* species was a predictive factor of preterm delivery in women with threatened premature labor (OR, 6.5; 95% CI, 1.3-33.0; $p<0.05$).

Conclusion

Increased positivity of *Ureaplasma* species in vaginal microbiota was a risk factor for preterm delivery among women with threatened premature labor. Vaginal microbiome analysis may identify high risk pregnancies for preterm delivery.

Keywords: *Lactobacillus*, premature labor, preterm delivery, *Ureaplasma*, vaginal microbiome

Introduction

Preterm delivery is a major cause of perinatal mortality and long-term neurologic morbidity. Cervical length measured by ultrasonography and biochemical markers such as fetal fibronectin are helpful for predicting preterm delivery, but precise prediction methods of preterm delivery have not been established [1]. Bacterial infection in the vagina is known to be associated with preterm delivery. The risk of preterm delivery is higher in pregnant women with bacterial vaginosis (BV) [2]. BV results from stabilization or colonization of several vaginal bacteria such as *Gardnerella*, *Ureaplasma*, *Mycoplasma*, and *Bacteroides* species. The Nugent score is a method of diagnosing BV. However, it is somewhat subjective, and its morphologic assessment of bacteria is complex. In recent years, microbiome analysis has become possible with 16s ribosomal RNA (rRNA) analysis using a next-generation sequencer. Human vaginal microbiota currently seem to have a role in causing or defending against BV [3].

The presence of *Gardnerella vaginalis* in the vagina was associated with BV in non-pregnant women [4], while the presence of *Ureaplasma* and *Myocplasma* species in the amniotic fluid increased the risk of preterm delivery and chorioamnionitis [5–9]. A recent study reported that the vaginal microbiome profiles in healthy pregnant women shifted toward *Lactbacillus*-dominant states during early pregnancy [10].

However, the relationship between maternal vaginal microbiota and the risk of preterm delivery remained controversial. Previous studies suggested that a decrease in *Lactobacillus* species was associated with preterm delivery in African-American and European women [11–13]. A cohort study of vaginal microbiota to predict preterm delivery has not been performed in Japan. Therefore, this prospective cohort study aimed to evaluate whether vaginal microbiota including *Lactobacillus*, *Gardnerella*, *Ureaplasma, and Mycoplasma* species during pregnancy is associated with preterm delivery.

Patients and Methods

Study participants

In this prospective study, participants were recruited with written informed consent under a protocol approved by the ethics committee at Kobe University Hospital. Between January 2018 and June 2018, 64 pregnant women during the second or third trimester who were admitted to the university hospital were enrolled. Pregnant women with multiple pregnancies, fetal abnormalities, or placenta previa were excluded. The 64 pregnant women underwent vaginal microbiome analyses using 16S rRNA sequence method as well as examinations of Nugent score for diagnosis of BV, AmnioTest^{TM} nitrazine yellow swabbing test kit, Check PROM IGFBP-1 detection kit in the vagina, and Fagnos Elastase Dip

granulocyte elastase detection kit in the uterine cervix at the time of admission. BV was diagnosed, when Nugent score was 7 or more. Threatened miscarriage and threatened premature labor were defined as conditions causing subjective symptoms of uterine contractions, bleeding, or shortening of uterine cervical length, and therefore requiring bed rest and tocolytic agents.

In a case-control study, the 64 pregnant women consisted of 47 women who were admitted to the hospital with a diagnosis of threatened miscarriage (n=4) and threatened premature labor (n=43), and designated as threatened premature labor group. Thirty-nine of the 47 women received ritodrine hydrochloride intravenously, 2 received magnesium sulfate intravenously, 1 received both ritodrine and magnesium sulfate intravenously, and 3 received ritodrine hydrochloride orally. The other 17 women who were admitted with other diseases including hypertensive disorders of pregnancy (n=8), fetal growth restriction (n=3), systemic lupus erythematosus (n=2), transient osteoporosis (n=1), pneumonia (n=1), uterine leiomyoma (n=1), and placenta accreta (n=1), and designated as non-threatened premature labor group. In a cohort study of threatened premature labor group, 23 pregnancies ended in spontaneous miscarriage or preterm delivery (preterm delivery group), and the remaining 24 ended in full-term deliveries (full-term delivery group).

The differences in the vaginal microbiota between threatened premature labor group and non-threatened premature labor group, and between preterm delivery group and full-term delivery delivery group were evaluated. The 64 pregnant women consisted of 61 Japanese women, one Chinese, one Australian, and one Indonesian woman.

Procedures

The samples for vaginal microbiome analysis and Nugent scoring were simultaneously obtained by swabbing the vaginal walls using different swabs. An OMNIgene VAGINAL for microbiome kit containing swab tips and DNA/RNA stabilizing liquid tubes was used. The swabbing movement involves tracing several full circles along the vaginal walls for 20 seconds. Thereafter, the swab was immediately inserted into the collection tube, which contained stabilizing liquid for microbiome. The apex of swab tip remained in the tube of liquid. These specimens were sent to Varinos Inc, Tokyo, Japan, for vaginal microbiome analysis. The bacterial 16S ribosomal RNA gene was amplified from the specimens, as previously described [14,15]. The 16S rRNA gene sequencing was performed according to the Illumina 16S, Metagenomic Sequencing Library Preparation protocol.

Nugent scoring system evaluates bacterial morphotypes microscopically for a diagnosis of BV. Nugent scores range from 0 to 10, according to the quantitative presence of 3

bacterial morphotypes, which are assessed for Gram-stained vaginal fluid. The quantitative number of *Lactbacillus* morphotype are scored from 0 to 4, where 0 indicates a lowest amount; small Gram-variable rods (*Gardnerella vaginalis* and *Bacteroides* morphotypes) are scored from 0 to 4, where 4 indicates a highest amount; and curved Gram-variable rods (*Mobiluncus* morphotypes) are scored from 0 to 2, where 2 indicates a highest amount. BV was diagnosed, when Nugent score was 7 or more [16, 17].

The number of bacterial species, positivity and percentage of *Lactobacillus, Gardnerella, Mycoplasma,* and *Ureaplasma* species as vaginal microbiome were evaluated. Data on biomarkers for threatened premature labor, including Nugent score, cervical granulocyte elastase, IGFBP-1, and vaginal pH change (AmnioTest) were collected.

Risk factors for preterm delivery in women with threatened premature labor were evaluated. These factors included Nugent score, the positivity of cervical granulocyte elastase, IGFBP-1, and the AmnioTest in biomarker analyses; and the number of bacterial species detected, the percentage of *Lactobacillus* species, and the positivity of *Gardnerella*, *Mycoplasma*, *Ureaplasma* species in microbiome analyses.

Statistical Analysis

Categorical variables were compared by Fisher's exact test or the Mann-Whitney U-test. All P-values were two sided, and P-values of 0.05 or less were considered statistically significant. A stepwise approach analysis was used to determine risk factors for preterm delivery in women with threatened premature labor. The variables with *p*-values <0.05 in univariate logistic regression were analyzed using multivariable logistic regression analyses. Variables with *p*-values <0.05 in multivariable logistic regression analyses were determined to be independent risk factors for preterm delivery in women with threatened premature labor.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of the R commander that was designed to include statistical functions frequently used in biostatistics [18].

Results

Comparison between threatened premature labor group and non-threatened premature labor group

Table 1 shows comparison of clinical characteristics, biomarkers and microbiome between threatened premature labor group and non-threatened premature labor group. Maternal

age [mean 33 (range 19-44) years old] and gestational week (GW) [27 (14-34) GW] at admission in threatened premature labor group were significantly ($p<0.05$) lower than those in non-threatened premature labor group [34 (27-43) years old, and 32 (19-35) GW, respectively]. The biomarkers including Nugent score, results of cervical granulocyte elastase, IGFBP-1, and AminoTest, were not significantly different between threatened premature labor group and non-threatened premature labor group.

The number of bacterial species detected, positivity and percentage of *Lactobacillus*, *Gardnerella, Mycoplasma, or Ureaplasma* species were not significantly different between threatened premature labor group and non-threatened premature labor group (Table 1).

Comparison between preterm delivery group and full-term delivery group

Table 2 shows comparison of clinical characteristics, biomarkers and microbiome between preterm delivery group and full-term delivery group. GW at delivery in preterm delivery group [33 (19-36) GW] was significantly ($p<0.01$) lower than that in full-term delivery group [38 (37-40) GW]. There were no significant differences in maternal age, GW at admission, gravity, or parity between two groups. Tocolytic agents were administrated to 21 of 23 women in preterm delivery group, while all 24 women in full-term delivery group had tocolytic agents. Only one woman in preterm delivery group received vaginal suppository of

metronidazol for BV. There were no significant differences in the proportion of women with administration of tocolytic agents or vaginal suppository between two groups.

Nugent score in preterm delivery group [median 3 (range, 0-7)] was significantly ($p<0.05$) higher than that in full-term delivery group [0 (0-4)]. The biomarkers of cervical granulocyte elastase, IGFBP-1, or AminoTest were not significantly different between preterm delivery group and full-term delivery group.

The number of bacterial species detected in preterm delivery group [3 (1-13)] was significantly ($p<0.05$) higher than that in full-term delivery group [2 (1-5)]. The percentage of *Lactobacillus* species in preterm delivery group [88 (0-100)%] was significantly ($p<0.01$) lower than that in full-term delivery group [99.8 (55.4-100)%]. The positivity (61%) and percentage [0.1 (0-89.7)%] of *Ureaplasma* species in preterm delivery group were significantly ($p<0.01$) higher than those in full-term delivery group [17% and 0 (0-1.7)%, respectively].

Table 3 shows univariate and multivariable logistic regression analyses for risk factors for preterm delivery. Univariate analyses revealed that a Nugent score [odds ratio (OR) 1.6, 95% confidence interval (CI) 1.1-2.3; $p<0.05$], number of bacterial species detected (OR 1.5, 95%CI 1.1-2.2; $p<0.05$), percentage of *Lactobacillus* species (OR 0.96, 95%CI 0.93-0.99; $p<0.05$), and *Ureaplasma* positivity (OR 7.8, 95%CI 2.0-30; $p<0.01$) were associated with preterm delivery in women with threatened premature labor. Multivariable logistic regression

analyses of the 4 factors selected in univariate analyses demonstrated that *Ureaplasma* positivity (OR 6.5, 95%CI 1.3-33; $p<0.05$) was a single factor associated with preterm delivery in women with threatened premature labor.

Discussion

The present study found there were no differences in vaginal biomarkers, microbiota of *Lactobacillus,* and other species during pregnancy between threatened premature labor and non-threatened premature labor groups. The cohort study of threatened premature labor group, however, demonstrated that increases in Nugent score, number of bacterial species, positivity and percentage of *Ureaplasma* species, and decreases in percentage of *Lactobacillus* species during pregnancy were associated with preterm delivery. Multivariable logistic regression analyses determined that positivity of *Ureaplasma* species in vaginal microbiota was a single risk factor for preterm delivery in women with threantened premature labor. These results coincided with results of a previous cohort study that showed that the presence of *Ureaplasma* species in the vagina was associated with preterm delivery [19]. *Lactobacillus* species have the beneficial function of protecting against ascending infections of microorganisms during pregnancy [20]. Decreases in percentage of *Lactobacillus* species and increases in positivity of *Ureaplasma* species in vaginal microbiota may be associated with BV, vaginal infection, and

ascending infections of microorganisms, which may cause intrauterine infections, such as chorioamnionitis. Approximately 30% of premature deliveries are caused by infections in mothers and fetuses [1]. Vaginal microbiome analyses at regular maternity checkups using 16s rRNA sequencing may be clinically useful to identify pregnancies at high risk for preterm delivery. However, a prophylactic strategy of treat abnormal vaginal microbiota to prevent preterm delivery has not been established. A prospective study of treatment interventions for abnormal vaginal microbiota in pregnant women at high risk for preterm delivery is further necessary.

The loss of *Lactobacillus* species in vaginal microbiota during pregnancy does not simply predict preterm delivery [21]. *Lactobacillus* species include five groups according to microbiome community composition and structure, and they are referred to as community state type Ⅰ (*L. crispatus*), Ⅱ (*L. gasseri*), Ⅲ (*L. iners*), Ⅳ *(mixed bacterial species*), and (*L. jensenii*) [21]. Dominance of *L. iners* in the vagina was associated with preterm delivery; in contrast, dominance of *L. crispatus* was associated with full-term delivery, as demonstrated via 16S rRNA gene sequencing [12]. Unfortunately, the community state types of *Lactobacillus* species were not analyzed in the present study.

These results will provide useful information for clinical practitioners to assess risks of preterm delivery. However, this study has limitations. The sample size is small. The

participants were not only Japanese. Human vaginal microbiota changes according to race, diet, living environment, and life cycle stage. In addition to *Lactobacillus* and *Ureaplasma* species, factors of other microbiota must be further elucidated to predict and prevent preterm delivery.

Acknowledgments

We are grateful for participation of the subjects and care provided by staff at Kobe University Hospital. We thank the clinical and laboratory personnel who supported this study at Kobe University Hospital.

This work was supported by the Ministry of Health, Labour and Welfare of Japan (grant no. H23-Jisedai-Ippan-001), and Japan Agency for Medical Research and Development (grant nos. AM55708030, and JP19gk0110047).

Potential conflicts of interest

All authors report no potential conflicts of interest.

References

[1] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75–84. https://doi.org/10.1016/S0140-6736(08)60074-4.

[2] Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs R, Martin DH, et al. "Association between Bacterial Vaginosis and Preterm Delivery of a Low- birth-weight Infant." Stud Fam Plann 2006;27:57. https://doi.org/10.2307/2138082.

[3] Benz HL. Maternal Microbiomes in Preterm Birth: Reecent Progress and Analytical Pipelines 2017;41:1–10. https://doi.org/10.1109/EMBC.2016.7590696.Upper.

[4] Janulaitiene M, Paliulyte V, Grinceviciene S, Zakareviciene J, Vladisauskiene A, Marcinkute A, et al. Prevalence and distribution of Gardnerella vaginalis subgroups in women with and without bacterial vaginosis. BMC Infect Dis 2017;17:1–9. https://doi.org/10.1186/s12879-017-2501-y.

[5] Gravett MG, Novy MJ, Rosenfeld RG, Reddy AP, Jacob T, Turner M, et al. Diagnosis of intra-amniotic infection by proteomic profiling and identification of novel biomarkers. J Am Med Assoc 2004;292:462–9. https://doi.org/10.1001/jama.292.4.462.

[6] Yoon BH, Romero R, Kim M, Kim EC, Kim T, Park JS, et al. Clinical implications of detection of Ureaplasma urealyticum in the amniotic cavity with the polymerase chain

reaction. Am J Obstet Gynecol 2000;183:1130–7. https://doi.org/10.1067/mob.2000.109036.

[7] Yoon BH, Romero R, Lim JH, Shim SS, Hong JS, Shim JY. The clinical significance of detecting Ureaplasma urealyticum by the polymerase chain reaction in the amniotic fluid of patients with preterm labor. Am J Obstet Gynecol 2003;189:919–24. https://doi.org/10.1067/S0002-9378(03)00839-1.

[8] Namba F, Hasegawa T, Nakayama M, Hamanaka T, Yamashita T, Nakahira K, et al. Placental features of chorioamnionitis colonized with ureaplasma species in preterm delivery. Pediatr Res 2010;67:166–72. https://doi.org/10.1203/PDR.0b013e3181c6e58e.

[9] Larsen B, Hwang J. Mycoplasma, Ureaplasma, and adverse pregnancy outcomes: A fresh look. Infect Dis Obstet Gynecol 2010;2010. https://doi.org/10.1155/2010/521921.

[10] Serrano MG, Parikh HI, Brooks JP, Edwards DJ, Tom J, Edupuganti L, et al. Racioethnic diversity in the dynamics of the vaginal microbiome during pregnancy 2019;25:1001–11. https://doi.org/10.1038/s41591-019-0465-8.Racioethnic.

[11] Brown RG, Marchesi JR, Lee YS, Smith A, Lehne B, Kindinger LM, et al. Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is

exacerbated by erythromycin. BMC Med 2018;16:1–15. https://doi.org/10.1186/s12916-017-0999-x.

[12] Kindinger LM, Bennett PR, Lee YS, Marchesi JR, Smith A, Cacciatore S, et al. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. Microbiome 2017;5:1–14. https://doi.org/10.1186/s40168-016-0223-9.

[13] Callahan BJ, DiGiulio DB, Aliaga Goltsman DS, Sun CL, Costello EK, Jeganathan P, et al. Replication and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. Proc Natl Acad Sci U S A 2017;114:9966–71. https://doi.org/10.1073/pnas.1705899114.

[14] Kyono K, Hashimoto T, Kikuchi S, Nagai Y, Sakuraba Y. A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. Reprod Med Biol 2019;18:72–82. https://doi.org/10.1002/rmb2.12250.

[15] Kyono K, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. Reprod Med Biol 2018;17:297–306. https://doi.org/10.1002/rmb2.12105.

[16] Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297–301. https://doi.org/10.1128/jcm.29.2.297-301.1991.

[17] Brookheart RT, Lewis WG, Peipert JF, Lewis AL, Allsworth JE. Association between obesity and bacterial vaginosis as assessed by Nugent score. Am J Obstet Gynecol 2019;220:476.e1-476.e11. https://doi.org/10.1016/j.ajog.2019.01.229.

[18] Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. Bone Marrow Transplant 2013;48:452–8. https://doi.org/10.1038/bmt.2012.244.

[19] Jayaprakash TP, Wagner EC, Van Schalkwyk J, Albert AYK, Hill JE, Money DM, et al. High diversity and variability in the vaginal microbiome in women following Preterm Premature Rupture of Membranes (PPROM): A prospective cohort study. PLoS One 2016;11:1–19. https://doi.org/10.1371/journal.pone.0166794.

[20] Witkin SS. The vaginal microbiome, vaginal anti-microbial defence mechanisms and the clinical challenge of reducing infection-related preterm birth. BJOG An Int J Obstet Gynaecol 2015;122:213–8. https://doi.org/10.1111/1471-0528.13115.

[21] Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci 2011;108:4680–7. https://doi.org/10.1073/pnas.1002611107.

Table 1. Comparison of clinical characteristics, biomarkers and microbiome between threatened premature labor and non-threatened premat

	All n=64	threatened premature labor group n=47	on-threatened premature labor group n=17	p value
Clinical characteristics				
Age, years old	33 (19-44)	33 (19-44)	34 (27-43)	<0.05
Gestational weeks at admission	29 (14-35)	27 (14-34)	32 (19-35)	<0.05
Gravity	2 (1-6)	2 (1-6)	2 (1-6)	0.4
Parity	0 (0-3)	0 (0-3)	0 (0-3)	0.9
Biomarker				
Nugent score	0 (0-9)	0 (0-7)	0 (0-9)	0.2
Cervical granulocyte elastase positive	3 (4.7%)	2 (4.3%)	1 (5.9%)	0.6
IGFBP-1 positive	2 (3.1%)	2 (4.3%)	0	0.6
Amniotest positive	6 (9.4%)	5 (11%)	1 (5.9%)	0.6
Microbiome				
Number of bacterial species detected	2 (1-13)	2 (1-13)	3 (1-13)	0.3
Lactobacillus positive	63 (98%)	46 (98%)	17 (100%)	1
Percentage of *Lactobacillus*	98.6 (0-100)	98.7 (0-100)	93.9 (0.1-99.9)	0.4
Gardnerella positive	24 (38%)	16 (34%)	8 (47%)	0.4
Percentage of *Gardnerella*	0 (0-99.7)	0 (0-98.8)	0 (0-99.7)	0.2
Mycoplasma positive	1 (1.6%)	1 (2.1%)	0	0.7
Percentage of *Mycoplasma*	0 (0-0.7)	0 (0-0.7)	0	1
Ureaplasma positive	25 (39%)	18 (38%)	7 (41%)	0.8
Percentage of *Ureaplasma*	0 (0-89.7)	0.1 (0-89.7)	0 (0-18.1)	0.5

median (range)

IGFBP-1, insulin-like growth factor binding protein-1

Table 2. Comparison of clinical characteristics, biomarkers and microbiome between preterm delivery and full-term delivery groups

	All n=47	preterm delivery group n=23	full-term delivery group n=24	p value
Clinical characteristics				
Age, years old	33 (19-44)	33 (21-44)	31 (19-41)	1
Gestational weeks at admission	27 (14-34)	28 (18-33)	27 (14-34)	0.5
Gravity	2 (1-6)	2 (1-4)	2 (1-6)	0.7
Parity	0 (0-3)	0 (0-3)	0 (0-3)	0.9
Gestational weeks at delivery	37 (19-40)	33 (19-36)	38 (37-40)	<0.01
Treatment for BV	1 (2.1%)	1 (4.3%)	0 (0%)	0.5
Administration of tocolytic agents	45 (96%)	21 (91%)	24 (100%)	0.2
Biomarker				
Nugent score	0 (0-7)	3 (0-7)	0 (0-4)	<0.05
Cervical granulocyte elastase positive	2 (4.3%)	0	2 (8.3%)	0.5
IGFBP-1 positive	2 (4.3%)	2 (8.7%)	0	0.2
Amniotest positive	5 (11%)	4 (17%)	1 (4.2%)	0.2
Microbiome				
Number of bacterial species detected	2 (1-13)	3 (1-13)	2 (1-5)	<0.05
Lactobacillus positive	46 (98%)	22 (96%)	24 (100%)	0.3
Percentage of *Lactobacillus*	98.7 (0-100)	88 (0-100)	99.8 (55.4-100)	<0.01
Gardnerella positive	16 (34%)	9 (39%)	7 (29%)	0.5
Percentage of *Gardnerella*	0 (0-98.8)	0 (0-98.8)	0 (0-43.2)	0.9
Mycoplasma positive	1 (2.1%)	1 (4.3%)	0	0.5
Percentage of *Mycoplasma*	0 (0-0.7)	0 (0-0.7)	0	0.5
Ureaplasma positive	18 (38%)	14 (61%)	4 (17%)	<0.01
Percentage of *Ureaplasma*	0 (0-89.7)	0.1 (0-89.7)	0 (0-1.7)	<0.01

median (range)

BV, bacterial vaginosis; IGFBP-1, insulin-like growth factor binding protein-1

Table 3. Logistic regression analyses of risk factors for preterm delivery

	Univariate analysis		Multivariable analysis	
	Odds ratio (95% confidence interval)	*p* -value	Odds ratio (95% confidence interval)	*p* -value
Biomarker				
Nugent score	1.6 (1.1-2.3)	<0.05	1.4 (0.01-15.0)	0.9
Cervical granulocyte elastase positive	6.4×10^{-8} (0-999)	1.0	-	
IGFBP-1 positive	1.8×10^{-7} (0-999)	1.0	-	
Amniotest positive	4.7 (0.5-46.0)	0.2	-	
Microbiome				
Number of species detected	1.5 (1.1-2.2)	<0.05	1 (0.7-1.5)	0.9
Percentage of *Lactobacillus*	0.96 (0.93-0.99)	<0.05	1 (0.9-1.0)	0.4
Gardnerella positive	1.6 (0.5-5.3)	0.5	-	
Mycoplasma positive	6.3×10^{6} (0-999)	1.0	-	
Ureaplasma positive	7.8 (2.0-30)	<0.01	6.5 (1.3-33.0)	<0.05

median (range)

IGFBP-1, insulin-like growth factor binding protein-1