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Prevalence of vaginal microorganisms among pregnant women according to trimester and association with preterm birth

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Objective
The aim of this study was to investigate the prevalence of abnormal vaginal microorganisms in pregnant women according to trimester, and to determine whether the presence of abnormal vaginal colonization is associated with higher risk of miscarriage or preterm delivery. Furthermore, we analyzed delivery outcomes according to individual microorganism species.

Methods
We included pregnant women who underwent vaginal culture during routine prenatal check-up between January 2011 and June 2016. We compared delivery outcomes according to the presence or absence of abnormal vaginal flora grouped by trimester.

Results
This study included 593 singleton pregnancies. We classified participants into 3 groups, according to the trimester in which vaginal culture was performed; 1st trimester (n=221), 2nd trimester (n=138), and 3rd trimester (n=234). Abnormal vaginal colonization rate significantly decreased with advancing trimester of pregnancy (21.7% for 1st, 21.0% for 2nd, 14.5% for 3rd; *P*=0.048). Abnormal vaginal colonization detected in the 2nd trimester but not in 1st trimester was associated with a significant increase in preterm delivery before 28 weeks of gestation (6.9% vs. 0%; *P*=0.006). Among abnormal vaginal flora isolated in the 2nd trimester, the presence of *Klebsiella pneumonia* was identified as significant microorganism associated with preterm delivery before 28 weeks of gestation (50% vs. 0.7% for *K. pneumonia*; *P*=0.029).

Conclusion
There is an association between abnormal vaginal colonization detected in the 2nd trimester and preterm delivery before 28 weeks. *K. pneumonia* has been identified as the likely causative microorganisms.

Keywords: Microbiota; Pregnancy; Premature birth; Klebsiella

Introduction

Vaginal microbiome composition changes when women become pregnant. Pregnancy is accompanied by a shift in the community vaginal bacteria to a composition that is typically dominated by *Lactobacillus* [1]. This change is believed to inhibit pathogen growth through secretion of antibacterial bacteriocins, such as lactic acid that can maintain an acid pH [2,3]. Disturbed vaginal environment is associated with complications of pregnancy, particularly an increased risk of preterm birth [4].

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Abnormal vaginal colonization, which replaces normal lactobacilli during pregnancy, includes bacterial vaginosis or aerobic vaginitis. Whereas bacterial vaginosis is dominated by anaerobic overgrowth, aerobic vaginitis is characterized by microorganisms such as *Escherichia coli, group B streptococci*, and *Enterococci* [5,6].

The association between bacterial vaginosis and preterm delivery has been extensively studied, while there is limited research on the clinical significance of abnormal vaginal colonization by aerobic bacteria, particularly in relation to preterm delivery. The exact prevalence of each vaginal microorganism throughout gestation has rarely been reported. Ascending infection by abnormal vaginal microorganisms from the lower genital tract is a well-known route of intra-amniotic infection associated with preterm birth. Furthermore, microorganisms isolated from infected amniotic fluid are mostly aerobic bacteria, rather than anaerobe causing bacterial vaginosis, a detailed analysis of the change in vaginal microorganism during pregnancy is critically important [4,7,8].

The aim of this study is to investigate the prevalence of abnormal vaginal microorganisms, detected by Gram staining and culture in pregnant women, classified by trimester, and to determine if the presence of abnormal vaginal colonization is associated with a higher risk of miscarriage or preterm delivery. Furthermore, we tried to analyze delivery outcomes according to individual microorganism species.

Materials and methods

We included pregnant women who underwent vaginal culture during their routine prenatal check-up in our institution between January 2011 and June 2016. Those included were patients who visited our institution for prenatal check-up for the first time. Women with multiple pregnancies were excluded. All vaginal sampling for Gram staining and culture was consecutively done regardless of symptoms by a single physician who performed routine vaginal screening upon his principle. After insertion of a water-lubricated sterile speculum, a smear was taken from vaginal posterior fornix using a sterile cotton swab. All vaginal culture was incubated primarily under an aerobic condition.

By retrospective review of medical records, we collected data on the maternal baseline characteristics including age, parity, pre-pregnancy body mass index (BMI), smoking, history of spontaneous preterm delivery, and comorbidity. Maternal hypertension, diabetes, and thyroid disease were classified as comorbidities. Gestational age at vaginal culture examination and the results were also examined. We divided the study population into 3 groups according to the trimester in which vaginal culture was performed. Delivery outcomes including gestational age at delivery, mode of delivery, birth weight, sex, Apgar score, neonatal intensive care unit (NICU) admission and early neonatal sepsis were investigated. Miscarriage was divided into early miscarriage (<14 weeks) and late miscarriage (14.0–21.6 weeks) as previously indicated [9]. Preterm delivery was further divided into 3 groups (22.0–27.6 weeks; 28.0–33.6 weeks; 34.0–36.6 weeks). Early neonatal sepsis was defined when microorganisms were isolated from the blood of neonates within 7 days of birth. Patients delivering newborns with major anomalies or twins were excluded from the study.

Statistical analysis was performed with SPSS 18.0 (IBM Corp., Armonk, NY, USA). The Mann-Whitney *U* test and the Kruskal-Wallis test were used for numeric data and Fisher's exact test was used for categorical data among groups. Linear by linear association analysis was also used to check trends in each trimester. A *P*-value <0.05 was considered statistically significant.

Results

This study included 593 singleton pregnancies. We classified the study population into 3 groups, according to the trimester in which vaginal culture was performed; 1st trimester (n=221), 2nd trimester (n=138), and 3rd trimester (n=234) group. Table 1 shows the clinical characteristics of the study population grouped by trimester. The median gestational age at vaginal culture examination for each trimester group was 8.6 weeks, 20.6 weeks, and 33.6 weeks, in the 1st, 2nd, and 3rd trimester, respectively. Pre-pregnancy BMI, nationality, smoking history, and maternal comorbidity were similar among groups. Most participants were of South Korean nationality, with other nationalities including Russian, North American, and Vietnamese. There were no differences between the groups in the rates of nulliparity and history of spontaneous preterm birth.

In Table 2, abnormal vaginal colonization rate was 21.7%, 21.0%, and 14.5% in 1st, 2nd, and 3rd trimester respectively, showing a significant decrease with advancing trimester of

pregnancy (*P*=0.048, linear-by-linear association). Gram-negative bacteria significantly decreased with advancing trimester (*P*=0.008). When analyzed by microorganism species, the prevalence of *E. coli* colonization significantly decreased as the pregnancy progressed: 1st trimester (6.3%), 2nd trimester (3.6%), and 3rd trimester (1.7%). Although the overall prevalence of Gram-positive cocci also significantly decreased with advancing trimester of pregnancy (*P*=0.012), there were no significant changes in the individual species over the trimesters of pregnancy. There were no differences in the prevalence rate of Candida across the trimesters.

To analyze the association of miscarriage or preterm delivery

Table 1. Clinical characteristics of pregnant women in each trimester

Characteristics	1st trimester (n=221)	2nd trimester (n=138)	3rd trimester (n=234)	*P*-value
Maternal age	33.8 (24–45)	33.3 (22–43)	32.1 (19–44)	**<0.001**
Body mass index before pregnancy (kg/m^2)	21.3 (16.1–37.5)	21.1 (15.6–37.9)	21.1 (15.6–44.9)	0.859
Nulliparity	100 (45.3)	75 (54.3)	117 (50.0)	0.234
History of spontaneous preterm birth	10 (4.5)	6 (4.3)	10 (4.3)	0.991
Smoking				0.588
Never smoker	220 (99.5)	138 (100.0)	231 (98.8)	
Previous smoker	1 (0.5)	0 (0.0)	2 (0.8)	
Current smoker	0 (0.0)	0 (0.0)	1 (0.4)	
Comorbidity				0.761
No	184 (83.3)	111 (80.4)	189 (80.8)	
Yes	37 (16.7)	27 (19.6)	45 (19.2)	
Nationality				0.454
South Korea	200 (90.5)	130 (94.2)	214 (91.4)	
Others	21 (9.5)	8 (5.8)	20 (8.6)	
Gestational age at examination (wk)	8.6 (4.1–13.5)	20.6 (14.0–27.6)	33.6 (28.0–40.6)	**<0.001**

Values are presented as number (range) or number (%). *P*-values less than 0.05 are shown in bold.

Table 2. The prevalence of vaginal microorganisms in each trimester

Microorganisms	1st trimester (n=221)	2nd trimester (n=138)	3rd trimester (n=234)	*P*-value[a]	*P*-value[b]
Abnormal vaginal colonization	48 (21.7)	29 (21.0)	34 (14.5)	0.130	**0.048**
Gram-negative bacteria	18 (8.1)	7 (5.1)	6[c] (2.6)	**0.028**	**0.008**
Escherichia coli	14 (6.3)	5 (3.6)	4 (1.7)	**0.038**	**0.011**
Enterobacteriae	1 (0.5)	0 (0.0)	0 (0.0)	0.605	0.243
Klebsiella pneumonia	2 (0.9)	2 (1.4)	1 (0.4)	0.646	0.570
Pseudomonas aeruginosa	1 (0.5)	0 (0.0)	0 (0.0)	0.605	0.243
Gram-positive cocci	26 (11.8)	13 (9.4)	12 (5.1)	**0.038**	**0.012**
Staphylococcus aureus	4 (1.8)	2 (1.4)	2 (0.9)	0.665	0.377
Streptococcus agalactiae	10 (4.5)	7 (5.1)	7 (3.0)	0.556	0.402
Enterococcus faecalis	1 (0.5)	0 (0.0)	0 (0.0)	0.605	0.243
Other gram-positive cocci	11 (5.0)	4 (2.9)	3 (1.3)	0.071	**0.022**
Candida	16 (7.2)	13 (9.4)	20 (8.5)	0.759	0.619

Values are presented as number (%). *P*-values less than 0.05 are shown in bold.
[a]Fisher's exact test was used to compare the prevalence of vaginal microorganisms in each trimester; [b]Linear by linear association analysis was used to check trend according to each trimester; [c]In one case, the identification of the specific microorganism was failed even with the presence of Gram-negative bacteria.

according to maternal vaginal microorganisms, we divided patients into a bacterial colonization group and a no bacterial colonization group in each trimester and compared delivery outcomes (Table 3). In the 1st and 3rd trimester, there was no difference in gestational age at delivery or in the rate of miscarriage or preterm delivery between the bacterial colonization group and no bacterial colonization groups. However, of note, the presence of abnormal vaginal colonization detected in the 2nd trimester was significantly associated with adverse delivery outcome. The rate of preterm delivery before 28 weeks in the bacterial colonization group was significantly higher than the rate in the no bacteria colonization group (6.9% vs. 0%; *P*=0.006). Gestational age at delivery itself failed to reach statistical significance (*P*=0.051), late miscarriage rate in the bacterial colonization group tends to become higher than in the no bacterial colonization group (*P*=0.050). Consequently, abnormal vaginal colonization detected in the 2nd trimester was associated with a lower rate of live births compared with the no bacterial colonization group (89.7% vs. 99.1%; *P*=0.007). Birth weight, Apgar score, NICU admission, and neonatal sepsis rates were similar between the 2 groups.

Table 4 shows the delivery outcomes according to maternal vaginal bacterial colonization, focusing on bacterial species grouped by trimester. Overall, the presence of microorganisms including *E. coli*, *Enterobacter, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae*, and *Enterococcus faecalis* in the 1st and 3rd trimester was not associated with adverse delivery outcome, miscarriage or preterm delivery. However, bacterial colonization by *K. pneumonia* in the 2nd trimester was associated with the increased risk of preterm delivery before 28 weeks of gestation, compared to the no *K. pneumonia* group (50% vs. 0.7%, *P*=0.029). *S. agalactiae* colonization in the 2nd trimester demonstrated higher late miscarriage rate (28.6% vs. 0.8%, *P*=0.007) compared to no colonization group.

To examine whether antibiotics treatment affect delivery outcomes including preterm delivery, birth weight, NICU admission, and neonatal sepsis, we grouped pregnant women according to antibiotics treatment in each trimester. As a result, antibiotics treatment was not related to improved delivery outcome, miscarriage or preterm delivery in bacterial colonization group at each trimester (Supplementary Table 1).

Discussion

Our data showed several important clinical findings regarding the vaginal microbiome in pregnant women. First, we found that the prevalence of abnormal vaginal colonization in pregnant women in each trimester was 21.7%, 21.0%, and 14.5% in the 1st, 2nd, and 3rd trimester, respectively. Second, abnormal vaginal colonization in the 2nd trimester but not in 1st trimester was associated with late miscarriage or preterm delivery less than 28 weeks of gestation. Finally, among individual microbiome species in the 2nd trimester, *S. agalactiae* was found to be related with late miscarriage and *K. pneumonia* isolated was associated with preterm delivery before 28 weeks of gestation.

The composition of the vaginal microbiome during normal pregnancy changes as pregnancy advances, vaginal microorganisms become progressively more benign, until term [10]. As pregnancy progresses, the increased estrogen level has a positive effect on lactobacillary activity and proliferation by increasing glycogen availability [5]. Increased *Lactobacillus* rates are thought to inhibit pathogen growth through secretion of antibacterial bacteriocins, as well as the production of metabolite, such as lactic acid which helps to maintain a low, hostile pH [11]. In our study, we also confirmed that abnormal bacterial colonization decreased significantly as the pregnancy progressed. It should be noted that our study population does not represent a longitudinal study, limiting information of the real change in vaginal microorganisms over gestation. However, a longitudinal study by Romero et al. [12] already demonstrated that the incidence of anaerobe or strict-anaerobe microbial species were found to decrease with advancing gestational age, which is in line with our cross-sectional study derived from each trimester. Our study also demonstrated that a decreasing trend was observed in both Gram-negative and Gram-positive bacteria. Comparable results were reported in another study reporting that vaginal colonization changed from 24% in the 1st trimester to 17% in the 3rd trimester [13].

Although decreased lactobacilli characterizing bacterial vaginosis are associated with preterm delivery [14], particularly among high risk women, the impact of intermediate flora or aerobic vaginitis in pregnancy are not fully understood, with some studies suggesting that it may cause poor pregnancy outcomes, comparable to bacterial vaginosis [8].

The "intermediate flora" state means a transitional stage between normal vaginal flora and bacterial vaginosis [5]. The

Table 3. Delivery outcomes according to maternal vaginal bacteria colonization

Delivery outcomes	1st trimester			2nd trimester			3rd trimester		
	No bacterial colonization (n=173)	Bacterial colonization (n=48)	*P*-value	No bacterial colonization (n=109)	Bacterial colonization (n=29)	*P*-value	No bacterial colonization (n=200)	Bacterial colonization (n=34)	*P*-value
Gestational age at examination (wk)	9.0 (6.6–11.1)	8.1 (5.6–10.2)	**0.030**	21.0 (16.6–25.1)	20.1 (16.1–24.1)	0.326	33.6 (30.6–36.6)	33.2 (30.2–36.2)	0.257
Gestational age at delivery (wk)	37.3 (5.3–42.0)	38.3 (18.1–41.4)	0.137	38.2 (16.5–42.0)	36.0 (19.2–41.4)	0.051	38.3 (30.5–41.6)	39.2 (37.0–42.0)	0.234
Early miscarriage	7 (4.0)	0 (0.0)	0.157	-	-	-	-	-	-
Late miscarriage	1 (0.6)	1 (2.1)	0.330	1 (0.9)	2 (6.9)	0.050	-	-	-
Preterm delivery (wk)									
<28.0	5 (2.9)	0 (0.0)	0.227	0 (0.0)	2 (6.9)	**0.006**	-	-	-
28.0–33.6	1 (0.6)	1 (2.0)	0.341	4 (3.7)	3 (10.3)	0.145	5 (2.5)	0 (0.0)	0.351
34.0–37.0	9 (0.6)	1 (2.1)	0.358	12 (11.0)	2 (6.9)	0.514	17 (8.5)	0 (0.0)	0.078
Full term delivery	150 (86.7)	45 (93.8)	0.180	92 (84.4)	20 (69.0)	0.059	178 (89.0)	34 (100.0)	**0.042**
Abortus	8 (4.6)	1 (2.1)	0.431	1 (0.9)	2 (6.9)	0.050	-	-	-
Stillbirth	0 (0.0)	0 (0.0)		0 (0.0)	1 (3.4)	0.052	1 (0.5)	0 (0.0)	0.679
Live birth	165 (95.4)	47 (97.9)	0.431	108 (99.1)	26 (89.7)	**0.007**	199 (99.5)	34 (100.0)	0.679
Sex (male)	89 (53.9)	24 (51.1)	0.727	62 (57.4)	14 (51.9)	0.603	111 (55.8)	14 (41.2)	0.115
Birth weight (kg)	3.15 (0.37–4.32)	3.40 (1.14–4.27)	**0.021**	3.08 (0.92–4.28)	3.02 (0.85–4.24)	0.653	3.11 (1.30–4.02)	3.17 (2.22–4.34)	0.555
Apgar score at 1 min (<4)	0 (0.0)	1 (2.1)	0.064	2 (1.9)	0 (0.0)	0.476	1 (0.5)	0 (0.0)	0.679
Apgar score at 5 min (<7)	1 (0.6)	0 (0.0)	0.588	2 (1.9)	0 (0.0)	0.476	1 (0.5)	0 (0.0)	0.679
NICU admission	7 (4.2)	2 (4.3)	0.997	11 (10.2)	3 (11.1)	0.888	20 (10.1)	1 (2.9)	0.181
Neonatal sepsis	4 (2.4)	1 (2.1)	0.906	2 (1.9)	1 (3.7)	0.559	6 (3.0)	1 (2.9)	0.981

Values are presented as median (range) or number (%). *P*-values less than 0.05 are shown in bold.
NICU, neonatal intensive care unit.

Table 4. Delivery outcomes according to the presence of vaginal microorganisms in each trimester

Trimesters/ delivery outcomes	*Escherichia coli*			*Enterobacter*			*Klebsiella pneumonia*			*Pseudomonas aeruginosa*			*Staphylococcus aureus*			*Streptococcus agalactiae*			*Enterococcus faecalis*		
	(–)	(+)	*P*-value	(–)	(+)	*P*-value	(–)	(+)	*P*-value	(–)	(+)	*P*-value	(–)	(+)	*P*-value	(–)	(+)	*P*-value	(–)	(+)	*P*-value
1st trimester	(n=207)	(n=14)		(n=220)	(n=1)		(n=219)	(n=2)		(n=220)	(n=1)		(n=217)	(n=4)		(n=211)	(n=10)		(n=220)	(n=1)	
Early miscarriage	7 (3.4)	0 (0.0)	0.628	7 (3.2)	0 (0.0)	0.968	7 (3.2)	0 (0.0)	0.938	7 (3.2)	0 (0.0)	0.968	7 (3.2)	0 (0.0)	0.878	7 (3.3)	0 (0.0)	0.720	7 (3.2)	0 (0.0)	0.968
Late miscarriage	2 (1.0)	0 (0.0)	0.877	2 (0.9)	0 (0.0)	0.991	2 (0.9)	0 (0.0)	0.982	2 (0.9)	0 (0.0)	0.991	2 (0.9)	0 (0.0)	0.964	2 (0.9)	0 (0.0)	0.911	2 (0.9)	0 (0.0)	0.991
Preterm delivery (wk)																					
<28.0	5 (2.4)	0 (0.0)	0.719	5 (2.3)	0 (0.0)	0.977	5 (2.3)	0 (0.0)	0.955	5 (2.3)	0 (0.0)	0.977	5 (2.3)	0 (0.0)	0.912	5 (2.4)	0 (0.0)	0.792	5 (2.3)	0 (0.0)	0.977
<34.0	2 (1.0)	0 (0.0)	0.877	2 (0.9)	0 (0.0)	0.991	2 (0.9)	0 (0.0)	0.982	2 (0.9)	0 (0.0)	0.991	2 (0.9)	0 (0.0)	0.964	2 (0.9)	0 (0.0)	0.911	2 (0.9)	0 (0.0)	0.991
<37.0	10 (4.8)	0 (0.0)	0.512	10 (4.5)	0 (0.0)	0.955	10 (4.6)	0 (0.0)	0.911	10 (4.5)	0 (0.0)	0.955	10 (4.6)	0 (0.0)	0.830	10 (4.7)	0 (0.0)	0.623	10 (4.5)	0 (0.0)	0.955
Full term delivery	181 (87.4)	14 (100.0)	0.164	194 (88.2)	1 (100.0)	0.882	193 (88.1)	2 (100.0)	0.778	194 (88.2)	1 (100.0)	0.882	191 (88.0)	4 (100.0)	0.604	185 (87.7)	10 (100.0)	0.278	194 (88.2)	1 (100.0)	0.882
2nd trimester	(n=133)	(n=5)		(n=138)	(n=0)		(n=136)	(n=2)		(n=138)	(n=0)		(n=137)	(n=1)		(n=131)	(n=7)		(n=138)	(n=0)	
Late miscarriage	3 (2.3)	0 (0.0)	0.894	3 (2.2)	0 (0.0)	-	3 (2.2)	0 (0.0)	0.957	3 (2.2)	0 (0.0)	-	3 (2.2)	0 (0.0)	0.957	1 (0.8)	2 (28.6)	**0.010**	3 (2.2)	0 (0.0)	-
Preterm delivery (wk)																					
<28.0	1 (0.8)	1 (20.0)	0.071	2 (1.4)	0 (0.0)	-	1 (0.7)	1 (50.0)	**0.030**	2 (1.4)	0 (0.0)	-	2 (1.5)	0 (0.0)	0.971	1 (0.8)	1 (14.3)	0.099	2 (1.4)	0 (0.0)	-
<34.0	7 (5.3)	0 (0.0)	0.768	7 (5.1)	0 (0.0)	-	7 (5.1)	0 (0.0)	0.901	7 (5.1)	0 (0.0)	-	7 (5.1)	0 (0.0)	0.901	6 (4.6)	1 (14.3)	0.311	7 (5.1)	0 (0.0)	-
<37.0	14 (10.5)	0 (0.0)	0.581	14 (10.1)	0 (0.0)	-	14 (10.3)	0 (0.0)	0.807	14 (10.1)	0 (0.0)	-	13 (9.6)	0 (0.0)	0.193	14 (10.7)	0 (0.0)	0.465	14 (10.1)	0 (0.0)	-
Full term delivery	108 (81.2)	4 (80.0)	0.654	112 (81.2)	0 (0.0)	-	111 (81.6)	1 (50.0)	0.342	112 (81.2)	0 (0.0)	-	111 (81.6)	1 (50.0)	0.342	109 (83.2)	3 (42.9)	**0.020**	112 (81.2)	0 (0.0)	-
3rd trimester	(n=230)	(n=4)		(n=234)	(n=0)		(n=233)	(n=1)		(n=234)	(n=0)		(n=232)	(n=2)		(n=227)	(n=7)		(n=234)	(n=0)	
Gestational age at delivery																					
<34.0	5 (2.2)	0 (0.0)	0.917	5 (2.1)	0 (0.0)	-	5 (2.1)	0 (0.0)	0.979	5 (2.1)	0 (0.0)	-	5 (2.2)	0 (0.0)	0.958	5 (2.2)	0 (0.0)	0.858	5 (2.1)	0 (0.0)	-
<37.0	17 (7.4)	0 (0.0)	0.738	17 (7.3)	0 (0.0)	-	17 (7.3)	0 (0.0)	0.927	17 (7.3)	0 (0.0)	-	17 (7.3)	0 (0.0)	0.860	17 (7.5)	0 (0.0)	0.586	17 (7.3)	0 (0.0)	-
Full term delivery	208 (90.4)	4 (100.0)	0.672	212 (90.6)	0 (0.0)	-	211 (90.6)	1 (100.0)	0.906	212 (90.6)	0 (0.0)	-	210 (90.5)	2 (100.0)	0.820	205 (90.3)	7 (100.0)	0.496	212 (90.6)	0 (0.0)	-

Values are presented as number (%). P-values less than 0.05 are shown in bold.

term aerobic vaginitis, as opposed to bacterial vaginosis, was coined by Donders et al. [15] and has recently received considerable attention. This condition is another type of disturbed vaginal microorganisms, in which the lactobacilli are replaced by aerobic facultative pathogens such as *Klebsiella, E. coli, Enterococci, Staphylococcus* spp., and *group B streptococci* and is characterized by vaginal leukocytosis and parabasal cells with an increased inflammatory reaction [15].

Recent evidence suggested that aerobic abnormal flora in early pregnancy confer a significant risk factor for preterm labor and is associated with inflammation of the placenta, including chorioamnionitis and funisitis [16]. We found that abnormal bacterial colonization by aerobic bacteria detected in the 2nd trimester was associated with a higher rate of delivery before 28 weeks compared to the no bacterial colonization group. However, this association was not observed in cases of abnormal bacterial colonization isolated in the 1st trimester. Our finding is consistent with an earlier report by Honda et al. [17] who suggested that the mid to late second trimester period is the optimal screening period to identify abnormal vaginal flora, as there is a shift in vaginal flora from normal to intermediate. In this study, we could not elucidate the exact mechanism of trimester specific impacts on abnormal pregnancy outcome. There are several reports which may be related to this phenomenon. In fact, it was previously indicated that immune modulation by the change of helper T cells subtype occurred during 2nd trimester [18]. Mid-pregnancy plasma cytokines including interleukin (IL)-2, IL-4, IL-6, interferon-γ, and transforming growth factor-β were also known to be associated with preterm delivery [19]. Similarly, high levels of immunoglobulin M, ferritin, and C-reactive protein measured in mid-pregnancy, have been linked to increased risk of spontaneous preterm delivery [20-22]. However, the association between abnormal vaginal colonization and the change by immune modulation or cytokine needs to be studied further.

Brocklehurst et al. [23] demonstrated that treatment of bacterial vaginosis before 20 weeks of gestation may be effective for the prevention of preterm birth and Lamont et al. [24] suggested that treatment with oral clindamycin administered to pregnant women with asymptomatic vaginal flora within 22 weeks of gestation reduced the preterm delivery rate.

Our study population was generated from consecutive singleton pregnant women undergoing vaginal culture by a single physician. Due to limitation of retrospective reviews of the medical records, the precise symptoms of vaginitis at the time of vaginal culture could not be retrieved. In fact, symptoms of vaginitis are often nonspecific and do not correlate with bacterial load, therefore differentiation between symptomatic and asymptomatic vaginal colonization is often difficult. This study only includes the Gram staining and culture results, therefore, it has a limited function in the evaluation of the vaginal environment, such as vaginal pH and microscopic diagnosis. A further limitation to our study is the relative small number of cases with abnormal vaginal colonization when the participants were divided by trimester. However, our study could be a basis for further studies on abnormal vaginal colonization in association with preterm delivery by suggesting that the 2nd trimester may be the most critical time to perform vaginal cultures.

It is noteworthy that in this study we focused on individual vaginal microorganisms and their association with adverse pregnancy outcome, including miscarriage and preterm delivery. We found that the rate of preterm delivery before 28 weeks of gestation in *K. pneumonia* colonization group was higher than that in the no colonization group. Among aerobic microorganisms, *Klebsiella* spp. was known to be associated with an increased risk for preterm birth [25]. The association between Klebsiella spp. and preterm birth is stronger than the association between bacterial vaginosis and preterm birth. Our data corroborates the findings of Carey and Klebanoff [26] who demonstrated that *Klebsiella* spp. was linked to preterm birth. We also showed that the late miscarriage rate in *S. agalactiae* colonization group in the 2nd trimester was higher than that in the no colonization group. *Group B streptococcus* is one of the main causes of infection in pregnant women with chorioamnionitis and is related to microbial invasion of the amniotic cavity [27,28]. Vaginal colonization with *group B streptococcus* is associated with preterm labor, as evidenced by our study [27,29].

The most frequently observed microorganisms in the female genital tract are *group B streptococcus, E. coli*, and *Ureaplasma urealyticum. E. coli* is one of the most frequent microorganisms cultured in aerobic vaginitis [15]. In our study, the most common microbiome was *group B streptococcus* (4.0%) followed by *E. coli* (3.8%). We recently demonstrated that *E. coli* was the most frequent microorganism isolated from the vagina in high risk pregnant women with preterm premature rupture of membranes and preterm labor, and correlated with as causative microorganism of early neonatal sepsis [30]. Of note, *E.*

coli and *group B streptococcus* are cited as the most frequent bacteria isolated from neonates with sepsis and meningitis [31,32]. It has been shown that high density colonization with vaginal *E. coli* is more likely among women with a history of preterm birth [33].

At present, the performance of vaginal culture as a routine antenatal test is controversial and is not generally recommended [34]. A Cochrane review demonstrated that antibiotics administered to pregnant women reduced overgrowth of bacteria but did not reduce preterm birth [23]. However, a recent meta-analysis suggested that infection screening and treatment programs in pregnant women may reduce preterm birth and late miscarriage [24]. Interestingly, it has been previously shown that macrolides and clindamycin administered during the 2nd trimester were associated with a lower rate of preterm birth, while metronidazole alone was associated with a greater risk of preterm delivery in a high-risk population [35]. It has also been suggested that clindamycin, unlike metronidazole, has a broader spectrum and is effective against aerobic microorganisms [36]. Considering that metronidazole only has antibacterial effect on anaerobes, the superior efficacy of broad spectrum antibiotics, such as clindamycin, in reducing the risk of preterm birth may indirectly reflect the importance of aerobic vaginitis. In our study, there were no differences in delivery outcomes between treatment group and no treatment group among the groups presenting with abnormal vaginal flora according to trimester of pregnancy. However, due to the small number of the pregnant women with abnormal vaginal flora and the diversity of antibiotics used, we could not show whether a specific antibiotic regimen could reduce the risk of preterm birth.

In conclusion, our study suggested that the association between abnormal vaginal flora and adverse pregnancy outcome may be related to culture time and to the specific microorganism. Colonization in the 2nd trimester colonization by *K. pneumonia* resulted in an increased risk of late miscarriage and colonization by *S. agalactiae* was associated with an increased risk of preterm birth before 28 weeks. Understanding vaginal microbiome may be the first step to introduce a preventive strategy for preterm birth. Therefore, prospective studies on abnormal vaginal flora targeting the specific study population, such as a history of preterm delivery or short cervical length, in relation to abnormal vaginal flora are required.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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