



Combined Assessment of Vaginal Dysbiosis, Pro-inflammatory Cytokines, and Oxidative Stress Biomarkers in Pregnancy

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Abstract

Background: While the relationship among microbial imbalance, inflammation, and oxidative stress with vaginal dysbiosis and adverse maternal and neonatal outcomes are established, they are not fully characterized. **Aims of the study:** Investigate and assess the presence of bacterial vaginal dysbiosis in pregnant women, oxidative stress biomarkers, inflammatory cytokines and their antimicrobial susceptibility patterns and determine independent predictors of bacterial vaginal dysbiosis. **Methodology:** This study was a case-control design that recruited 100 pregnant women with vaginal dysbiosis and 50 healthy pregnant controls in Thi-Qar, Iraq between July 10, 2025, and June 5, 2026. Amsel criteria and Nugent scoring were used to diagnose vaginal dysbiosis. High vaginal swabs were cultured on standard microbiological media and the bacterial isolates were identified by conventional biochemical methods and antimicrobial susceptibility was determined by the Kirby-Bauer method according to CLSI 2025. Commercial ELISA kits were used to measure serum inflammatory cytokines and Oxidative Stress Biomarkers. **Result:** 100 women with vaginal dysbiosis were recruited along with 50 healthy controls. Baseline characteristics were similar except that cases were more likely to be rural residents, low SES, have previous vaginal infection, and to douche. Abnormal clinical and microbiological findings were significantly more common in women with vaginal dysbiosis and *Gardnerella vaginalis* was the predominant vaginal isolate. All inflammatory cytokines and oxidative stress markers were significantly increased, while antioxidant biomarkers were decreased (all $P < 0.001$). Multivariable analyses revealed *Gardnerella vaginalis*, decreased levels of the *Lactobacillus* flora, and increased levels of malondialdehyde and of the inflammatory cytokine IL-6, as well as vaginal douching, to be independent risk factors for vaginal dysbiosis. **Conclusions:** Vaginal dysbiosis in pregnancy is defined as an imbalance of microbes, with *Gardnerella vaginalis* predominating, less protective bacterial species of the *Lactobacillus* genus, greater systemic inflammation and higher oxidative stress. Combined microbiological and biomarker evaluation can enhance the early diagnosis, risk stratification and personalized treatment of pregnancy for reduced adverse pregnancy outcomes.

Keyword: Vaginal dysbiosis, *Gardnerella vaginalis*, pregnancy, inflammatory cytokines, oxidative stress.

Introduction:

Fetal growth is accompanied by coordinated adaptations of the endocrine, metabolic, immunological and microbial function that allows for fetal growth while simultaneously preventing ascending genital infections. The vaginal microbiome is a key part of this protective system, and typically is more stable in healthy pregnancy, losing diversity and becoming dominated by the species of *Lactobacillus* [1]. The bacteria ferment into lactic acid, antimicrobial metabolites, and bacteriocins, thus keeping the vagina's pH low, preventing the growth of pathogenic bacteria, strengthening the vagina's mucosal barrier, and regulating local immune responses. Of those, *Lactobacillus crispatus* is usually considered to have a protective and stable vagina [2].

Vaginal dysbiosis is the decrease of protective lactobacilli and the overgrowth and variety of *Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia* and *Mobiluncus* species, which are anaerobic organisms. This microbial "set-up" often coincides with Bacterial Vaginosis, but sometimes also exists without any clinical symptoms. A recent meta-analysis showed that decrease in lactobacillus load was correlated with risk of preterm delivery as compared to communities comprised of *L. crispatus* [3]. Likewise, more diverse vaginal microbiomes and the presence of more taxa associated with vaginal dysbiosis have been shown to be correlated with poor pregnancy outcomes [4]. Changes in cervicovaginal microbiota could also have implications for the organization and permeability of the mucus, which would reduce the mechanical barrier from the ascent of microbes [5].

Vaginal Dysbiosis' goes beyond the symptoms it causes. Infertility, implantation failure, premature rupture of the membranes, chorioamnionitis, spontaneous preterm labor, and neonatal infection are all example of dysbiotic communities. An increased likelihood of spontaneous preterm birth was seen in pregnancies after cervical conization, and particularly related to an increase in microbial diversity and community state type IV [6]. *Lactobacillus* depleted communities with *Gardnerella* also have been seen to be correlated with decreased continued pregnancy rates after embryo transfer [7]. In addition, the relationship between group B *Streptococcus* colonization and the surrounding vaginal microbiome could potentially affect colonization density of the mother and her risk of exposing her newborn.

One of the key pathways involved with the dysbiosis and poor pregnancy outcomes is the activation of mucosal inflammation. Dysbiosis associated organisms produce microbial products that can activate the epithelial toll-like receptors and inflammasomes leading to the production of interleukin-1 (IL-1), IL-6, IL-8, and tumour necrosis factor alpha (TNF- α). These mediators will recruit neutrophils, change the integrity of the epithelia, induce prostaglandin synthesis, cervical remodeling, membrane weakening and contractility of the uterus. There is clinical evidence that dysbiotic changes and an increase in local inflammatory activity is linked to preterm delivery [9]. Experimental research also shows that microbial products and IL-1 signaling may impact the cervicovaginal microbiome at the same time as triggering pathways linked to premature labor [10].

However, inflammation-induced preterm labor remains a heterogeneous condition, with none of the single cytokines being a good predictor of all pregnancies at risk [11]. Immune tolerance during gestation is important while inappropriate activation of innate and adaptive immune system may trigger pathological labor [12]. The maternal IL-6, TNF- α , C-reactive protein and other inflammatory markers have been found to have potential as markers of infection, but have shown varying performances depending on the site of sampling, gestational age, and clinical context (13). Genital-tract inflammation may be more directly reflected by the levels of cervicovaginal cytokines, which have been found to be elevated in women with reproductive-tract infections, including clinically silent infections [14] that contain elevated levels of cytokines such as IL-1 β , IL-6, and IL-8.

A second mechanism between dysbiosis, inflammation, and pregnancy complications could be oxidative stress. Reactive oxygen species are physiologically produced in placentation and cellular metabolism, however, a higher production of ROS or a reduced antioxidant capacity can cause damage to lipids, proteins, mitochondrial components and DNA [15]. Epithelial barrier disruption due to dysbiosis could lead to an increase in ROS and thus further impair epithelial barriers, and ROS could activate inflammatory signaling and further recruit inflammatory cells, thereby forming a vicious circle. Lipid peroxidation may be reflected by the malondialdehyde while the other antioxidants measured (superoxide dismutase, catalase, glutathione, total antioxidant capacity) represent complementary aspects of antioxidant defence. Malondialdehyde has been shown to be elevated but superoxide dismutase and catalase are decreased in women who are having a preterm birth [16].

Microbial disruptions are also associated with other pregnancy disorders that are associated with systemic inflammation and endothelial dysfunction, such as preeclampsia [17]. But these areas of investigation (vaginal microbiota, cytokines and oxidative stress) are frequently studied independently of each other. This piecemeal approach may fail to account for biointeractions and a part of the failure of microbial or biochemical measures to predict. The incorporation of microbial and inflammatory/oxidative biomarkers may give a more comprehensive view of the environment of the vaginal host–microbiome. Microbial composition, together with its inflammatory and biochemical effects, was also explored, with the aid of advanced functional approaches, such as metabolomics and metaproteomics [18].

Thus, the purpose of the present study was to simultaneously evaluate vaginal dysbiosis, pro-inflammatory cytokines and oxidative-stress biomarkers in pregnant women, to explore interrelationships among the three and their associations with some clinical parameters of pregnancy and adverse pregnancy outcomes.

Methodology:

A case–control study in hospital setting was conducted from 10th of July 2025 to 5th of June 2026 at Al-Nasiriyah General Hospital and five selected private obstetrics and gynecology clinics in Thi-Qar Governorate, Iraq. This study involved 150 women, 100 of whom had a diagnosis of vaginal dysbiosis (bacterial vaginosis) and 50 were otherwise healthy pregnant women. Data on demographics and obstetrics were collected with a standard questionnaire and medical chart review. Vaginal dysbiosis was diagnosed based on the clinical criteria of Amsel and was confirmed by the Gram-stained vaginal smears based on the Nugent scoring system. Aseptically, two high vaginal swabs were obtained from each participant with sterile cotton swabs. The first swab was used for direct microscopy, Gram staining, vaginal pH and whiff test, whilst the second swab was inoculated onto 5% sheep blood agar, MacConkey agar, Chocolate agar and Mannitol Salt agar and incubated aerobically at 37°C for 24–48 hours. Bacterial isolates were identified by colony morphology, gram staining characteristics, and standard biochemical tests such as catalase, coagulase, oxidase, indole, citrate utilization, urease, triple sugar iron (TSI), motility and API identification system as per requirement. The Mueller–Hinton agar was used for the antimicrobial susceptibility testing by Kirby–Bauer disk diffusion method per the guidelines of Clinical and Laboratory Standards Institute (CLSI 2025). The antibiotics that were sent to the panel were ampicillin, amoxicillin–clavulanic acid, ceftriaxone, ciprofloxacin, gentamicin, nitrofurantoin, trimethoprim–sulfamethoxazole, erythromycin, clindamycin, cefoxitin, vancomycin and linezolid. Reference strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used for quality control. Venous blood samples were taken under sterile condition (5 mL), centrifuged at 3000 rpm for 10 minutes and the serum aliquots were stored at –80°C before analysis. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum levels of inflammatory markers, such as IL-1 β , IL-6, TNF- α , high sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx), following the manufacturer's instructions.

Statistical analysis:

IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA) was used to perform statistical analyses. The normality of data was tested with the Shapiro–Wilk test. Continuous variables were reported as mean $\pm$ standard deviation (SD), categorical variables as frequencies and percentages. Independent-samples Student's t-test or Mann–Whitney U test was used to compare women with vaginal dysbiosis with healthy control women, depending on the distribution of the data. The Pearson chi-square test or Fisher's exact test was used for categorical variables. Pearson's or Spearman's correlation was used to determine correlations between inflammatory cytokines, oxidative stress biomarkers and microbiological findings. Variables with a P value < 0.20 from univariable analysis, and clinically relevant variables, were entered in a multivariable logistic regression model to determine the independent predictors of vaginal dysbiosis. Odds ratios (ORs) with their 95% confidence intervals (95% CIs) were computed. The P value was computed using a two-tailed test and defined as statistically significant at < 0.05 .

Ethical approval:

Before the start of the study, a study protocol was reviewed and approved by the Research Ethics Committee, College of Science, University of Thi-Qar, Iraq. Also, official consent was obtained from the administration of Al-Nasiriyah General Hospital and the clinics which participated in the study. The study was carried out in compliance with the principles of the Declaration of Helsinki (2013 revised). All participants gave written informed consent before sample collection, including explanation of the goals and procedures of the study. All personal information and laboratory data were anonymized by coded identification numbers for privacy of participants and all biological specimens were collected and used for scientific research only.

Results

Comparison of demographic characteristics, obstetric history, lifestyle factors, and potential risk factors between pregnant women with vaginal dysbiosis and healthy controls.

The baseline sociodemographic, obstetric and clinical parameters of the pregnant women based on vaginal dysbiosis status are summarized in Table 1. Both groups were similar in age, gestational age, BMI, gravidity, previous miscarriage, previous preterm birth, and recent antibiotic use, and none of these variables had a significant difference between groups (all $P > 0.05$). Women with vaginal dysbiosis, on the other hand, were significantly more likely to be rural residents, have low SES, have had a previous vaginal infection, and to have douched their vagina ($P < 0.05$ for all). The results indicate that environmental factors, socioeconomic status and infections at the genital mucosa prior to pregnancy can be considered as key risk factors of vaginal dysbiosis.

Table 1. Baseline Sociodemographic, Obstetric, and Clinical Characteristics of Pregnant Women According to Vaginal Dysbiosis Status

Variable	Vaginal dysbiosis (n=100)	Controls (n=50)	P value
Age (years), mean $\pm$ SD	28.7 $\pm$ 5.4	28.0 $\pm$ 5.1	0.447
Gestational age (weeks), mean $\pm$ SD	24.1 $\pm$ 6.8	23.4 $\pm$ 6.5	0.548
BMI (kg/m ²), mean $\pm$ SD	27.5 $\pm$ 4.2	26.8 $\pm$ 4.0	0.329
Primigravida, n (%)	36 (36.0)	20 (40.0)	0.633
Multiparity, n (%)	64 (64.0)	30 (60.0)	—
Rural residence, n (%)	58 (58.0)	20 (40.0)	0.038

Low socioeconomic status, n (%)	51 (51.0)	16 (32.0)	0.028
Previous miscarriage, n (%)	25 (25.0)	8 (16.0)	0.211
Previous preterm birth, n (%)	18 (18.0)	4 (8.0)	0.104
Previous vaginal infection, n (%)	34 (34.0)	7 (14.0)	0.010
Antibiotic use during previous 3 months, n (%)	14 (14.0)	5 (10.0)	0.484
Vaginal douching, n (%)	39 (39.0)	10 (20.0)	0.019

Comparison of clinical manifestations, microbiological findings, and standard diagnostic criteria between pregnant women with vaginal dysbiosis and healthy controls.

The clinical findings and microbiological features of the pregnant women who had vaginal dysbiosis compared with healthy controls are reported in Table 2. Dysbiosis occurred in women with abnormal and malodorous vaginal discharge ($P = 0.001$), vaginal itching or irritation ($P = 0.001$), elevated vaginal pH ($P = 0.001$), positive whiff test ($P = 0.001$) and clue cells ($P = 0.001$) than controls. Likewise, positive Amsel, Nugent scores of 7-10, presence of bacterial growth and mixed bacterial growth were significantly higher in the dysbiosis group, demonstrating dysregulated vaginal microbiota (all $P \leq 0.001$). On the contrary, Lactobacillus depletion was significantly more common in women with vaginal dysbiosis while the predominant Lactobacillus flora was significantly associated with healthy controls (both $P < 0.001$), suggesting that lactobacilli play a key role in maintaining normal vaginal microbial homeostasis.

Table 2. Clinical Presentation, Vaginal Microbiological Findings, and Diagnostic Characteristics of Pregnant Women According to Vaginal Dysbiosis Status

Variable	Vaginal dysbiosis (n=100)	Controls (n=50)	P value
Abnormal vaginal discharge, n (%)	78 (78.0)	6 (12.0)	<0.001
Malodorous discharge, n (%)	63 (63.0)	3 (6.0)	<0.001
Vaginal itching or irritation, n (%)	41 (41.0)	7 (14.0)	0.001
Vaginal pH >4.5, n (%)	82 (82.0)	7 (14.0)	<0.001
Positive whiff test, n (%)	76 (76.0)	2 (4.0)	<0.001
Clue cells $\geq 20\%$, n (%)	71 (71.0)	2 (4.0)	<0.001
Positive Amsel criteria, n (%)	79 (79.0)	3 (6.0)	<0.001
Nugent score 7–10, n (%)	84 (84.0)	3 (6.0)	<0.001
Positive bacterial culture, n (%)	88 (88.0)	10 (20.0)	<0.001

Mixed bacterial growth, n (%)	33 (33.0)	4 (8.0)	0.001
Reduced <i>Lactobacillus</i> spp., n (%)	76 (76.0)	8 (16.0)	<0.001
Predominant <i>Lactobacillus</i> flora, n (%)	20 (20.0)	43 (86.0)	<0.001

Frequency of clinically significant bacterial pathogens isolated from vaginal cultures according to vaginal dysbiosis status during pregnancy.

Table 3 shows that most bacterial isolates from vaginal specimens from pregnant women with vaginal dysbiosis were associated with pregnancy. Table 3 shows the distribution of bacterial isolates obtained from vaginal specimens of pregnant women with vaginal dysbiosis and healthy controls, with the majority of bacterial isolates being associated with pregnancy. The predominant pathogen was *Gardnerella vaginalis*, which was found in 46.0% of isolates from patients with dysbiosis in contrast to 6.0% of isolates in the control group ($P < 0.001$). The presence of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* was observed more often in women with vaginal dysbiosis; however, these differences were not statistically significant ($P > 0.05$). Pathogenic bacterial growth was found to be present in significantly less number of bacterial isolates from the dysbiosis group ($P < 0.001$) than the absence of pathogenic bacterial growth was found in healthy controls ($P = 0.001$). These results suggest that *G. vaginalis* is the major bacterium involved in vaginal dysbiosis and that polymicrobial colonization is another key microbiological factor of the disorder.

Table 3. Distribution of Aerobic Bacterial Isolates Recovered from Vaginal Specimens of Pregnant Women with Vaginal Dysbiosis and Healthy Controls

Bacterial isolate	Vaginal dysbiosis (n=100)	Controls (n=50)	P value
<i>Gardnerella vaginalis</i>	46 (46.0)	3 (6.0)	<0.001
<i>Escherichia coli</i>	22 (22.0)	5 (10.0)	0.071
<i>Staphylococcus aureus</i>	16 (16.0)	4 (8.0)	0.177
<i>Streptococcus agalactiae</i>	14 (14.0)	4 (8.0)	0.292
<i>Enterococcus faecalis</i>	12 (12.0)	3 (6.0)	0.258
<i>Klebsiella pneumoniae</i>	9 (9.0)	2 (4.0)	0.337
<i>Proteus mirabilis</i>	6 (6.0)	1 (2.0)	0.429
<i>Pseudomonas aeruginosa</i>	4 (4.0)	0 (0.0)	0.301
Mixed bacterial isolates	33 (33.0)	4 (8.0)	0.001
No pathogenic bacterial growth	12 (12.0)	40 (80.0)	<0.001

In vitro antimicrobial susceptibility patterns of the predominant bacterial pathogens isolated from vaginal cultures according to CLSI guidelines.

The antimicrobial susceptibility pattern of the dominant bacterial isolates obtained from the pregnant women with vaginal dysbiosis is summarised in Table 4. *Escherichia coli* and *Klebsiella pneumoniae* showed the highest susceptibility frequencies to gentamicin, nitrofurantoin and ceftriaxone, while the resistance frequencies to ampicillin and trimethoprim–sulfamethoxazole were comparatively more. The Gram-positive organisms *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis* were very susceptible to vancomycin and linezolid (100% susceptibility); clindamycin and ceftioxin had good activity against *S. aureus*. Gram-positive isolates were moderately susceptible to erythromycin and ciprofloxacin. These overall results suggest that bacteria isolated from the vagina are generally sensitive to aminoglycosides and glycopeptides; however, resistance to some of the frequently used β -lactam antibiotics has not changed, stressing the need for antimicrobial susceptibility testing to ensure proper therapy during pregnancy.

Table 4. Antimicrobial Susceptibility Profiles of Clinically Significant Bacterial Isolates Recovered from Pregnant Women with Vaginal Dysbiosis

Antibiotic	<i>E. coli</i> (n=27)	<i>K. pneumoniae</i> (n=11)	<i>S. aureus</i> (n=20)	<i>S. agalactiae</i> (n=18)	<i>E. faecalis</i> (n=15)
Ampicillin	9 (33.3)	2 (18.2)	N/A	15 (83.3)	11 (73.3)
Amoxicillin–clavulanate	17 (63.0)	6 (54.5)	N/A	16 (88.9)	12 (80.0)
Ceftriaxone	20 (74.1)	7 (63.6)	N/A	17 (94.4)	—
Ciprofloxacin	18 (66.7)	7 (63.6)	14 (70.0)	N/A	9 (60.0)
Gentamicin	22 (81.5)	9 (81.8)	16 (80.0)	N/A	10 (66.7)
Nitrofurantoin	23 (85.2)	8 (72.7)	N/A	N/A	12 (80.0)
Trimethoprim–sulfamethoxazole	13 (48.1)	5 (45.5)	12 (60.0)	N/A	N/A
Erythromycin	N/A	N/A	11 (55.0)	13 (72.2)	8 (53.3)
Clindamycin	N/A	N/A	15 (75.0)	14 (77.8)	N/A
Ceftioxin	N/A	N/A	16 (80.0)	N/A	N/A
Vancomycin	N/A	N/A	20 (100.0)	18 (100.0)	15 (100.0)
Linezolid	N/A	N/A	20 (100.0)	18 (100.0)	15 (100.0)

Evaluation of systemic inflammatory mediators and oxidative stress biomarkers associated with vaginal dysbiosis during pregnancy.

Table 5 presents the systemic inflammatory cytokines and oxidative stress biomarkers for pregnant women with vaginal dysbiosis and healthy controls. Vaginal dysbiosis women also had significant higher serum concentrations of IL-1 β , IL-6, TNF- α and high-sensitivity C-reactive protein compared to healthy controls (all $p < 0.001$) suggestive of a higher systemic inflammatory response. Moreover, malondialdehyde was found to be significantly increased in the dysbiosis group while total antioxidant capacity, superoxide dismutase activity, and glutathione peroxidase concentration were significantly decreased (all $P < 0.001$) reflecting that the antioxidant defense mechanisms were impaired. Together, these results suggest that there is significant activation of inflammatory pathways and oxidative stress in vaginal dysbiosis during pregnancy, potentially associated with poor pregnancy outcomes.

Table 5. Comparison of Pro-inflammatory Cytokines and Oxidative Stress Biomarkers Between Pregnant Women with Vaginal Dysbiosis and Healthy Controls

Biomarker	Vaginal dysbiosis (n=100)	Controls (n=50)	P value
IL-1 β (pg/mL)	24.6 $\pm$ 8.1	15.2 $\pm$ 5.7	<0.001
IL-6 (pg/mL)	14.9 $\pm$ 5.6	9.1 $\pm$ 3.9	<0.001
TNF- α (pg/mL)	20.7 $\pm$ 6.8	13.6 $\pm$ 4.7	<0.001
High-sensitivity CRP (mg/L)	5.6 $\pm$ 2.5	3.4 $\pm$ 1.7	<0.001
Malondialdehyde (nmol/mL)	4.72 $\pm$ 1.18	3.42 $\pm$ 0.91	<0.001
Total antioxidant capacity (mmol/L)	0.91 $\pm$ 0.21	1.14 $\pm$ 0.23	<0.001
Superoxide dismutase (U/mL)	7.61 $\pm$ 1.72	9.28 $\pm$ 1.89	<0.001
Glutathione peroxidase (U/L)	46.8 $\pm$ 9.2	53.9 $\pm$ 9.6	<0.001

Adjusted logistic regression model identifying microbiological, inflammatory, oxidative stress, and clinical factors independently associated with vaginal dysbiosis during pregnancy.

The results of multivariable logistic regression analyses for factors independently associated with vaginal dysbiosis during pregnancy are presented in Table 6. *Gardnerella vaginalis* and the decreased amount of Lactobacilli bacteria were the most independent microbiologic factors predicting vaginal dysbiosis, with an estimated multiplication of risk factors of 4.9 times and 4.3 times, respectively, (both $P < 0.001$). Mixed bacterial growth, high serum IL-6, high malondialdehyde concentration and douching were also significant independent predictors, even after adjusting for potential confounding factors (all $P < 0.05$). However, the previous vaginal infection, rural living, low socio-economic status and recent antibiotic use were not independently associated with vaginal dysbiosis in the final regression model ($P > 0.05$). Thus, alterations in the normal vaginal flora, such as the overgrowth of *G. vaginalis* and a decrease in the beneficial vaginal flora (*Lactobacillus* spp.), and increased inflammation and oxidative stress responses are the major factors driving vaginal dysbiosis in pregnancy.

Table 6. Multivariable Logistic Regression Analysis of Independent Predictors Associated with Vaginal Dysbiosis in Pregnant Women

Variable	Adjusted OR	95% CI	P value
<i>Gardnerella vaginalis</i> isolation	4.86	2.03–11.64	<0.001
Mixed bacterial growth	2.74	1.17–6.42	0.020
Reduced <i>Lactobacillus</i> flora	4.31	1.91–9.74	<0.001
IL-6, per 1 pg/mL increase	1.10	1.03–1.18	0.006
Malondialdehyde, per 1 nmol/mL increase	1.58	1.11–2.26	0.011
Vaginal douching	2.08	1.01–4.28	0.047
Previous vaginal infection	1.94	0.92–4.10	0.082

Rural residence	1.61	0.81–3.21	0.174
Low socioeconomic status	1.49	0.74–3.01	0.263
Recent antibiotic use	1.18	0.46–3.02	0.733

Discussion:

The current study showed that the socioeconomic and behavioral characteristics, clinical and microbiological alterations, systemic inflammatory response and disturbed oxidant–antioxidant system are associated with vaginal dysbiosis during pregnancy. The age, gestational age, BMI, parity, and miscarriage history, and previous preterm birth and recent antibiotic use were not significantly different, suggesting good baseline comparability. However, because it is a cross-sectional study, the relationships reported are associations, and not cause-and-effect.

Women with dysbiosis were significantly more likely to reside in rural areas and are significantly more likely to have low social-economic status. These associations could be due to differences in sanitation, access to health care, health literacy, nutrition or early diagnosis of genital infections. The effects of both variables were, however, nonsignificant after multivariable adjustments, indicating that their effects might be more distal in terms of behavior and exposures to microbes. Vaginal douching was found to be independently associated with dysbiosis which is biologically plausible given that intravaginal cleansing may lead to the removal of protective lactobacilli from the vagina, the change of vaginal pH, and the introduction of microorganisms and irritants. The behavioral and social factors have been recognized as important modifiers of the vaginal microbial environment by the present-day diagnostic literature [19].

The clinical findings, especially the foul-smelling discharge, the vaginal pH greater than 4.5, the positive whiff test and the presence of clue cells, are characteristic of a bacterial vaginosis like phenotype. Amsel and Nugent scores provide some agreement to aid the classification of the diagnosis but neither fully depicts the vaginal microbial ecosystem. However, the traditional microscopic methods might not be reliable in distinguishing dysbiotic communities, biofilm formation, and a different species of bacteria [20] has been demonstrated by molecular investigations. Therefore, quantitative PCR or 16S rRNA sequencing would be beneficial to support the current findings especially in women with discordant clinical and microscopic findings.

Vaginal dysbiosis is polymicrobial, with *Gardnerella vaginalis* being the predominant species, and a decrease in the number of *Lactobacillus* species, with a corresponding increase in the number of other bacteria. This is in line with Shvartsman et al. [21] highlighting the role of *Gardnerella* species in the adhesion to mucosal surfaces, the development of biofilms, epithelial injury, and immune activation. However, the presence of *Gardnerella* does not necessarily mean that there is disease since there are genetically and functionally different *Gardnerella* species that can be present in asymptomatic female. During pregnancy, *Gardnerella* clades were shown to be highly variable with several different variants often being found in the same microbial community [22]. Therefore, the taxonomic identification of “*G. vaginalis*” by culture could underestimate the ecological and pathogenic role of this organism.

The higher prevalence of multimicrobial communities aligns with metagenome studies indicating that dysbiosis is a shift in the community level and not the result of an infection with a single bacterium. Serrano et al. [23] found that species and strain level interactions with pregnancy outcomes might differ and across populations. This may account for the variation in the findings of the different investigations regarding strong associations between *Gardnerella*-dominant communities and adverse outcomes, and less or no associations. Microbial patterns can significantly change due to geographic variation, ethnicity, sexual practices, gestational age at sampling, regions sequenced, or definition of lactobacillus dominance.

The presence of *E. coli*, *K. pneumoniae*, *S. aureus*, *S. agalactiae* and *E. faecalis* indicate that some of the participants may have also suffered from bacterial/vaginosis and aerobic or mixed vaginitis. They can cause a greater inflammatory response than classical anaerobic dysbiosis. Armstrong [24] characterized BV as a pro-inflammatory state that is characterized by an increase in the diversity of microbes and decrease in lactobacilli. The enteric bacteria results were positive in the controls, and few of the individual results of aerobic species were statistically different, however, suggesting that the results from culture positivity are not necessarily the result of clinically meaningful infections. The culture-based paradigms were also found to be inadequate to represent the ecological complexity of vaginitis as suggested by Guang and Adashi [25].

The lower levels of lactobacillus is especially relevant since these bacteria regulate the acidity, bacteriocin production, binding to the epithelia and inhibiting the formation of pathogenic biofilms. Lactobacilli have been shown to be beneficial in probiotic studies, with some studies demonstrating a reduction in Nugent scores and inflammatory mediators, such as IL-1 β and IL-6, but effects were not consistent [26]. In *Gardnerella*-inoculated human cervical cells, experimental studies by Miquel et al. [27] demonstrated that certain lactobacilli strains are able to promote vaginal epithelial defenses when exposed to the pathogen. Variances among studies can be due to the different probiotic effects of the strains, the different composition of the microbiome at baseline, adherence, co-treatment with antibiotics, and the length of follow-up.

The results of antimicrobial susceptibility revealed low susceptibility in-vitro for *E. coli* and *K. pneumoniae* against ampicillin while gentamicin and nitrofurantoin exhibited good activity. This overall trend is consistent with an upward trend in β -lactam resistance of maternal Gram-negative isolates. However, results of susceptibility testing should not be taken as a direct treatment recommendation. Nitrofurantoin has a poor ability to reach the vaginal tissue's levels of activity needed to treat vaginitis and fluoroquinolones and aminoglycosides should be carefully evaluated for pregnancy-specific risk. Treatment should then be based on the clinical syndrome, organism, gestational age and the authority guidelines of obstetrics and not on the highest susceptibility percentage.

Moderate levels of ESBL were observed in *E. coli* and *K. pneumoniae* and were not as high as those that have been reported in some resource-limited antenatal populations. Getie et al. [28] reported significant level of resistance of Gram-negative isolates to ESBL among pregnant women. Variations are due to the availability of the antibiotics, hospitalization, prior exposure and laboratory methods, as well as regional antimicrobial stewardship practices. In a similar vein, Matalka and colleagues [29] reported the resistance of the bacterial isolates of pregnancy-associated infections to ampicillin and overall corroborated the current trend.

100% of Gram-positive isolates were susceptible to vancomycin and linezolid while susceptibility was lower for erythromycin and clindamycin. Both Zakerifar et al. [30] and Gizachew et al. [31] reported high susceptibility of GBS to β -lactams and vancomycin with variable levels of resistance to macrolides and lincosamides. The 20% MRSA rate is also possible since recent evidence exists that maternal staphylococcal population may contain MRSA in association with *mecA* [32]. For ESBL detection and for detection of methicillin resistance, there must be a confirmatory test, but for disk-diffusion methods, breakpoints, quality-control strains must be specified.

The high levels of IL-1 β , IL-6, TNF- α and hs-CRP suggest concomitant local or systemic immune activation. Dysbiosis related organisms may stimulate Toll-like receptors and stimulate the production of cytokines that recruit leukocytes, disrupt epithelial junction and stimulate PG and MMP production. Experimental studies have demonstrated TLR2-dependent signaling can lead to induction of epithelial matrix metalloproteinases by *G. vaginalis* [33]. This gives a plausible biological pathway between the dysbiosis and the cervical remodeling and membrane weakening—although not tested in the current tables, the pregnancy outcomes.

Concurrently, elevated MDA and decreased TAC, SOD and GPx levels suggest oxidative imbalance. Microbial stimulation can trigger the activation of neutrophils and macrophages, and result in more reactive oxygen species, while the damage induced by the ROS can enhance the inflammatory signaling. When the fetal membranes were stimulated by bacteria, Park et al. [34] showed that this induced oxidative and inflammatory pathways; conversely, treatment with antioxidants inhibited release of IL-6, IL-8, TNF- α and prostaglandins. Other pregnancy studies also have reported increased lipid peroxidation with decreased antioxidant defenses [35]. However, the extent of oxidative changes could vary based on gestational age, dietary antioxidant consumption, obesity, smoking, preeclampsia and sample storage. Independent correlates determined by the regression model included *Gardnerella* isolation, mixed growth, decreased lactobacilli, IL-6, malondialdehyde and douching. These inflammatory and oxidative links persisted after adjustment, suggesting that there is an integrated microbial-immune-oxidative pathway. The addition of *Gardnerella*, reduced lactobacilli, and mixed growth, however, could result in incorporation bias since these microbiological tests may have been part of the initial case definition. Molecular assays also failed to agree with Nugent scoring well for intermediate microbial states [36–38]. Thus, a better model should include cytokines and external risk factors as predictors, and separate the definition of dysbiosis. Recently, treatment of bacterial vaginosis (BV) during pregnancy has also been recommended to be based on a valid diagnosis and pregnancy-safe treatments [39].

Conclusion:

The results of the present study indicate that vaginal dysbiosis occurs in pregnant women, with overgrowth of *Gardnerella vaginalis* and loss of protective *Lactobacilli* species from the vagina. This dysbiosis was associated with a rise in systemic inflammation, which was reflected in higher levels of IL-1 β , IL-6, TNF- α , and hs-CRP, as well as a decrease in antioxidant capacity and an increase in oxidative stress. The antimicrobial susceptibility pattern results show that the majority of the bacterial isolates are still sensitive to multiple commonly used antimicrobials, however there is evidence that resistance to some antimicrobials is present, showing the need for culture directed therapy. Moreover, the microbiological profile, in addition to inflammatory and oxidative stress markers, was a standalone predictor of vaginal dysbiosis, proving the importance of a multi-omics approach to diagnosis. The timely diagnosis and treatment of vaginal dysbiosis can help minimise pregnancy complications and enhance outcomes of pregnancy.

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