Original Research Article | Iran J Med Microbiol. 2025; 19(1): 50-61

Category: Medical Bacteriology | Peer Reviewed | Open Access

10.30699/ijmm.19.1.50

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Insights Into Bacterial Vaginosis During Pregnancy and Its Relationship with Preterm Birth: A Comprehensive Study in Shahid Akbarabadi Hospital, Tehran, Iran

Parisa Rahimi[1,2], Shirin Dashtbin[1,2], Maryam Kashanian[3], Shiva Mirkalantari[1,2], Nooshin Eshraghi[4], Faramarz Masjedian Jazi[1,2*]

1. *Microbial Biotechnology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
3. *Shahid Akbarabadi Clinical Research Development Unit (ShACRDU), School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
4. *Department of Obestrics and Gynecology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*

ABSTRACT

Background and Aim: Bacterial Vaginosis (BV) is a common condition affecting women of reproductive age, including pregnant women. It involves a disruption of the microbial balance in the vaginal environment, which can lead to undesirable outcomes such as preterm birth. This study aimed to assess the incidence of BV and its relationship with preterm delivery among pregnant women visiting Shahid Akbarabadi Hospital in Tehran, Iran.

Materials and Methods: A cohort study was conducted between September 2022 and April 2023, involving pregnant women who underwent vaginal swab sampling for BV. Diagnosis of BV was made using Amsel's criteria. Real-time PCR was employed to detect the presence of *Gardnerella vaginalis, Atopobium vaginae, Prevotella bivia, and Lactobacillus crispatus.* Statistical analyses were performed using GraphPad Prism 8.4.3.

Results: Out of the 55 pregnant women who participated in the study, 20 were found to be positive for BV. In our study of pregnant women, we found that the prevalence of bacterial vaginosis is 36.36% based on the Amsel criteria.

Conclusion: Our results highlight significant correlations between the levels of *G. vaginalis, A. vaginae, P. bivia, and L. crispatus* and the clinical signs and symptoms of bacterial vaginosis in this population. However, no significant differences were observed in the levels of studied bacteria in the lower genital tract of patients who experienced preterm delivery compared to those who delivered at term.

Keywords: Bacterial Vaginosis, Preterm Birth, Dysbiosis, Risk Factor, Pregnant Women

Received: 2024/12/12; **Accepted**: 2025/03/15; **Published Online:** 2025/03/30;

Corresponding Information: **Faramarz Masjedian Jazi**, *Microbial Biotechnology Research Center, Iran University of Medical Sciences, Tehran, Iran & Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran &*
Email: masjedian.f@iums.ac.ir

Use a device to scan and read the article online

Rahimi P, Dashtbin S, Kashanian M, Mirkalantari, Eshraghi N, Masjedian Jazi F. Insights Into Bacterial Vaginosis During Pregnancy and Its Relationship with Preterm Birth: A Comprehensive Study in Shahid Akbarabadi Hospital, Tehran, Iran. Iran J Med Microbiol. 2025;19(1):50-61.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks
Send citation to: Mendeley Zotero RefWorks

1. Introduction

Bacterial vaginosis (BV), also known as vaginal dysbiosis, is among the prevalent vaginal conditions linked to abnormal alterations in the vaginal microbiome (VMB) **(1)**. BV frequently reoccurs post-treatment, with 50% of women experiencing the return of symptoms within 12 months **(2, 3)**. Some studies indicate that it might trigger preterm labor and has been linked to the onset of pelvic inflammatory disease (PID) **(3, 4)**. Bacterial vaginosis represents the leading cause of vaginal discharge and odor in women, impacting 29% of the female population in general. Factors contributing to the risk comprise: Black or Hispanic ethnicity, Regular douching Smoking, Multiple sexual partners, and Same-sex activity (typically affecting both individuals) **(2, 5)**.

BV is distinguished by alterations in the composition of the vaginal flora, marked by a significant decrease in *Lactobacilli* and a substantial proliferation of obligate or facultative anaerobes, which were previously a minority in the vagina. These anaerobes include *Gardnerella vaginalis*, *Atopobium vaginae*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, as well as various fastidious and uncultured bacteria, including BV-associated bacteria (BVAB-1 to 3) **(1)**. The cause behind the proliferation of anaerobic bacteria in this context remains unidentified. It is associated with an alkaline vaginal environment resulting from an elevation in vaginal pH subsequent to the diminished protective effects of *Lactobacilli* **(6)**. According to a recent prospective study, a revised conceptual model illustrating the pathogenesis of BV was delineated **(7-10)**. The potential synergistic interaction among *G. vaginalis*, *P. bivia*, and *A. vaginae* was investigated **(11, 12)**. After exposure to virulent strains of *G. vaginalis* through sexual contact, these strains replace the vaginal *Lactobacilli* and trigger the formation of a biofilm linked to bacterial vaginosis on the vaginal epithelium. **(13)**.

Preterm birth (PTB), defined as childbirth occurring before 37 weeks of gestation, poses a significant global health concern **(14)**. Approximately 15 million pregnancies experience PTB each year, presenting a major risk factor for neonatal mortality **(15)**. PTB and various adverse obstetric outcomes have been linked to bacterial vaginosis (BV) in several studies **(15, 16)**.

Studies have shown that high levels of BV-associated microbes, including A. vaginae and G. vaginalis, can be associated with PTB risk **(17, 18)**. Other BV-associated microbes, such as *Sneathia sanguinegens*, *Prevotella*, and *Mobiluncus curtsii/mulieris*, are known risk factors for PTB **(19)**. A recent multi-omic study with a large sample size showed increased levels of BV-associated microbes and a significant decrease in *L. crispatus* in women **(15)**.

BV-associated microbes may contribute to infections during gestation, potentially moving into the uterus before pregnancy **(13)**.

Given the high prevalence of BV, interventions aimed at reducing BV incidence could have a substantial impact on the occurrence of BV-associated diseases. Therefore, accurate and efficient diagnosis and treatment of BV may be crucial in preventing these diseases.

We aimed to investigate the prevalence of *G. vaginalis, A. vaginae, P. bivia* and *L. crispatus*, on pregnant women in the third trimester of pregnancy from September 2022 to April 2023 at Shahid Akbarabadi Clinical Research Development Unit (ShACRDU) by quantitative Real-Time Polymerase Chain Reaction (qPCR). Moreover, the correlation between the occurrence of PTB and the bacteria was examined.

2. Materials and Methods

2.1. Ethical Statement and Participant Enrollment

The study received ethical approval from the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1401.242) and was conducted in accordance with the principles of the Helsinki Declaration. All procedures adhered to the approved guidelines, and written informed consent was obtained from all participants prior to sampling.

A total of 55 pregnant women, aged between 19 and 39 years, with no medical issues or adverse outcomes in previous pregnancies, were enrolled in the longitudinal study. Participants in the third trimester of pregnancy (between 28 and 36 weeks) were recruited at the Shahid Akbarabadi Clinical Research Development Unit from September 2022 to April 2023 and followed until delivery.

Inclusion criteria included self-reporting as Iranian, confirmation of gestational age, reproductive age (18 years or older), absence of intercurrent infections, no complications in previous or current pregnancies, no use of supplemental progesterone, the ability to provide informed consent, and willingness to participate. Exclusion criteria included intercurrent infections requiring antibiotic therapy, vaginal bleeding, recent use of antibiotics, underlying diseases such as diabetes and hypertension, kidney diseases, presence of vaginal herpes lesions, a history of uterine surgery, history of premature birth or miscarriage, and

douching practices aimed at mitigating infection or PTB risks.

The research team collected foundational data, and participants were regularly followed up during antenatal visits, gathering information on maternal and clinical variables until delivery, including both term and PTB.

2.2. Sample Collection and Gram Staining Procedure

Sterile cotton-tipped swabs were used to collect vaginal discharge from the lateral vaginal wall and the posterior fornix of the vagina. These swabs were employed to apply a vaginal sample to a microscope slide, which was then subjected to Gram staining. The analysis of Gram-stained smears involved the classification of vaginal microbiota according to the criteria established by Nugent et al **(20)**. Microscopic evaluations were conducted at up to ×1000 magnification, with scores ranging from 0 to 3 indicating normal microbiota, 4 to 6 indicating dysbiosis, and 7 to 10 indicating bacterial vaginosis (BV).

2.3. Clinical Assessment

The pregnant women underwent a clinical examination, during which a vaginal swab was obtained and assessed for BV using the Amsel criteria, proposed by Amsel et al **(21)** in 1983. A diagnosis of BV is established if three out of the following four criteria are present:

1. Increased homogeneous milky vaginal discharge.

2. A pH of the secretion exceeding 4.5.

3. An amine odor observed when a 10% potassium hydroxide solution is added to a drop of vaginal secretions.

4. The presence of clue cells in wet preparations.

2.4. DNA Extraction from Swab Medium

According to the manufacturer's instructions, genomic DNA from vaginal samples was extracted using the BetaPrep Genomic DNA Extraction Kit (Nürnberg, Germany). To evaluate the quality and concentration of DNA, agarose gel electrophoresis and a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) were employed **(22)**. The verified extracted DNAs were immediately preserved at -20°C.

2.5. Real-Time PCR (qPCR)

A quantitative real-time PCR (qPCR) was performed to assess the presence and relative quantity of microbial DNA. Primers for *G. vaginalis*, *A. vaginae*, *P. bivia*, and *L. crispatus* were used as representatives of vaginal microbial DNA. All primers were synthesized by Pishgam (Tehran, Iran), with details provided in Table 1.

Table 1. Utilized primers in the present study.

Target bacteria	Primer	Oligonucleotide sequence (5′ to 3′)	Product size (bp)	Reference
Lactobacillus crispatus	Forward	5′- TTCGCTGACCTTGATGATGC -3′..	75	This Study
	Reverse	5′- GGGCCATAATCCTTGCTACC -3′		This Study
Gardnerella vaginalis	Forward	5′- TGGCGTTTCAATCGCTAAGG -3′	143	This Study
	Reverse	5′- CCAGAGATTGAGCCAACACG -3′		This Study
Atopobium vaginae	Forward	5′- TCAGTCATGGCCCAGAAGAC -3′..	129	This Study
	Reverse	5′- CCCTATCCGCTCCTGATACC-3′		This Study
Prevotella bivia	Forward	5′- AACCCAGCGAAAGTTGGACT -3′..	97	This Study
	Reverse	5′- AATCAGACGCATCCCCATCC -3′..		This Study

Each Real-Time PCR reaction was performed in a final volume of 20 µl, containing 0.6 µM of each primer, 10 µL of 2X Q-PCR Master Mix (SYBR, ROX) (SMOBIO, Taiwan), and 5.8 µL of sterilized ultra-pure

water. The input DNA was 3 ng/reaction. The cycling conditions were as follows: initial denaturation at 95°C for 5 min; followed by 40 cycles at 95°C for 10 s, and annealing/extension at 59–61°C for 60 s. Reactions were run on the Rotor-Gene 6000 real-time PCR cycler (Qiagen Corbett, Germany). For negative controls, all ingredients of the reaction mixture were used except for template DNA.

To verify primer specificities, melting curves were generated at the end of each PCR reaction. Fluorescent data were acquired during the extension phase. After 40 cycles, a melting curve for each gene was generated by increasing the temperature from 60 to 95°C (1°C per step), while the fluorescence was measured. Samples were run in duplicates.

For the determination of the number of *L. crispatus, A. vaginae, P. bivia, and G. vaginalis* present in each sample, standard curves were constructed corresponding to 10^1 to 10^10 copies/ml **(23)**. These curves were created based on the normalized copy number of the 16S rRNA gene for each species (Biosystems, 2013) and Applied Biosystems tutorials. The bacterial concentrations from each sample were calculated from the threshold cycle values (CT) obtained from the standard curves. According to previous studies **(22, 23)**, bacterial standard strains were selected from the American Type Culture Collection (ATCC).

2.6. Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8.4.3 (GraphPad Software, San Diego, CA, USA). The Shapiro–Wilk test was used to assess the normality of the data. The Mann-Whitney U test or t-test was applied to compare two groups of continuous numerical data. Additionally, Fisher's exact test was utilized to assess the association between two categorical datasets. A p-value of less than 0.05 was considered statistically significant for the analyses.

3. Results

3.1. The Characteristics of Patients with Bacterial Vaginosis and Healthy Controls

In our study, the prevalence of bacterial vaginosis was found to be 36.36%. The mean age at the time of sampling was 32.25 weeks of gestation, with a standard deviation of 2.221 weeks. And the minimum and maximum gestational ages were 28 and 36 years respectively. Our results show that there are no significant differences in delivery and maternal ages between patients with bacterial vaginosis and healthy controls (P-values > 0.05) (Table 2). However, patients with bacterial vaginosis exhibited milky vaginal discharge, positive whiff test results, Clue cells, and higher vaginal pH values compared to healthy mothers (All P-values < 0.0001) (Table 2).

3.2. The Association of Vaginal Bacterial Presence with PTB

We utilized quantitative polymerase chain reaction (qPCR) to assess the presence of the studied bacteria in the included participants. Our study indicated that the presence of *G. vaginalis, L. crispatus, P. bivia,* and *A. vaginae* was not significantly associated with PTB (All P-values > 0.05) (Table 3). In this table, concentrations above the normal limit were reported as positive, while those below were reported as negative.

3.3. The Association of Bacterial Presence with Bacterial Vaginosis Presence and Manifestations

Our results demonstrated that patients with bacterial vaginosis had lower cycle threshold (CT) values for *G. vaginalis, P. bivia*, and *A. vaginae*, and higher CT values for *L. crispatus* (P-values < 0.0001) (Table 4). We also examined the potential association between the CT values of each bacterium and the signs of bacterial vaginosis. It was found that individuals with a positive whiff test, milky vaginal discharge, higher vaginal pH, and Clue cells had lower CT values for *G. vaginalis, P. bivia,* and *A. vaginae*. Conversely, these individuals had higher CT values for *L. crispatus* (All P-values < 0.0001) (Table 4).

3.4. The Prevalence of Bacterial Vaginosis

Our results showed that the prevalence of bacterial vaginosis based on the Amsel criteria is 36.36% (Table 4). The qPCR results demonstrated that the prevalence rates for *G. vaginalis, L. crispatus, P. bivia,* and *A. vaginae* were 40%, 45.45%, 38.18%, and 36.36%, respectively (Table 5 and Figure 1).

3.5. The Sensitivity, Specificity, and Predictive Values of qPCR

We also assessed the sensitivity, specificity, and positive and negative predictive values of qPCR in detecting bacterial vaginosis. Overall, the results indicated that qPCR has substantial sensitivity and specificity in detecting *G. vaginalis, P. bivia,* and *A. vaginae.* The sensitivity, specificity, and both positive and negative predictive values for qPCR in detecting these bacteria were all above 90% (Table 6).

Table 2. The characteristics of pregnant women with vaginosis and healthy pregnant women.

		Vaginosis (n=20)	Healthy (n=35)	P-value
Delivery age		37.80 ± 1.542	37.80 ± 1.982	>0.9999
Mother age		26.35 ± 5.451	29.17 ± 5.451	0.0727
Vaginal pH		4.375 ± 0.3193	3.714 ± 0.2510	<0.0001
Vaginal discharge	Milky	19	0	<0.0001
	Clear	1	35	
Whiff test	Present	20	0	<0.0001
	Absent	0	35	
Clue cells	Present	20	0	<0.0001
	Absent	0	35	
Vaginal pH	≥ 4.5	17	0	<0.0001
	< 4.5	3	35	

Table 3. The association of vaginal bacterial with preterm delivery

		Preterm	Term	P-value	RR	95% CI
Gardnerella vaginalis	Positive	5	17	0.7620	0.8333	0.3225 to 2.039
	Negative	9	24			
Lactobacillus crispatus	Positive	7	23	0.7619	0.8333	0.3460 to 2.021
	Negative	7	18			
Prevotella bivia	Positive	4	17	0.5285	0.6476	0.2333 to 1.663
	Negative	10	24			
Atopobium vaginae	Positive	4	15	0.7486	0.7579	0.2732 to 1.927
	Negative	10	26			

Table 4. The relationship of bacterial presence with the presence and signs of bacterial vaginosis.

		Gardnerella vaginalis (CT)	*Lactobacillus crispatus* (CT)	*Prevotella bivia* (CT)	*Atopobium vaginae* (CT)
Vaginosis	Present	19.76 ± 3.210****	29.21 ± 2.695****	24.06 ± 3.469****	18.79 ± 3.770****
	Absent	25.87 ± 2.569****	23.70 ± 2.565****	28.00 ± 3.080****	23.42 ± 2.276****
Discharge	Milky	19.64 ± 3.254****	29.31 ± 2.734****	23.98 ± 3.573****	18.72 ± 3.858****
	Clear	25.76 ± 2.614****	23.80 ± 2.602****	27.94 ± 3.062****	23.33 ± 2.305****
Whiff test	Positive	19.76 ± 3.210****	29.21 ± 2.695****	24.06 ± 3.469****	18.79 ± 3.770****
	Negative	25.87 ± 2.569****	23.70 ± 2.565****	28.00 ± 3.080****	23.42 ± 2.276****
Vaginal pH	≥ 4.5	20.07 ± 3.144****	29.24 ± 2.869****	24.33 ± 3.578****	19.11 ± 3.696****
	< 4.5	25.24 ± 3.384****	24.12 ± 2.895****	27.57 ± 3.387****	22.91 ± 2.290****
Clue cells	Present	19.76 ± 3.210****	29.21 ± 2.695****	24.06 ± 3.496****	18.79 ± 3.770****
	Absent	25.87 ± 2.569****	23.70 ± 2.565****	28.00 ± 3.080****	23.42 ± 2.276****

****: P-value < 0.0001

Table 5. The prevalence of the bacterial vaginosis.

	Bacterial vaginosis Positive based on Amsel criteria (%)	Positive based on Real-time q-PCR (%)
Gardnerella vaginalis	36.36	40
Lactobacillus crispatus	36.36	45.45
Prevotella bivia	36.36	38.18
Atopobium vaginae	36.36	36.36

Table 6. The sensitivity, specificity, and positive and negative predictive values of qPCR.

	Sensitivity	95% CI	Specificity	95% CI	Positive predictive value	95% CI	Negative predictive value	95% CI
Gardnerella vaginalis	100.00%	83.16% to 100.00%	94.29%	80.84% to 99.30%	90.91%	72.25% to 97.46%	100.00%	89.42% to 100.00%
Lactobacillus crispatus	100.00%	83.16% to 100.00%	85.71%	69.74% to 95.19%	80.00%	63.99% to 90.01%	100.00%	88.43% to 100.00%
Prevotella bivia	90.00%	68.30% to 98.77%	91.43%	76.94% to 98.20%	85.71%	66.82% to 94.70%	94.12%	81.06% to 98.36%
Atopobium vaginae	95.00%	75.13% to 99.87%	97.14%	85.08% to 99.93%	95.00%	73.30% to 99.25%	97.14%	83.41% to 99.57%

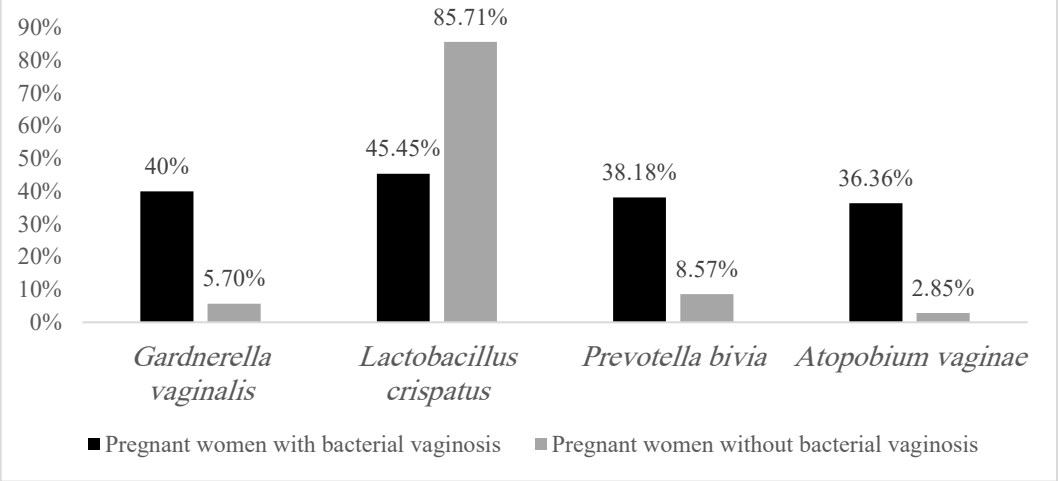

Figure 1. The percentage of bacteria abundance based on the quantitative Real time-PCR method.

4. Discussion

Premature delivery is the primary cause of morbidity and mortality during pregnancy in most countries **(24)**. Bacterial vaginosis has been implicated in developing PTB and subsequent complications **(25)**. *G.vaginalis*, *A.vaginae,* and *P.bivia* have been introduced as the main culprits for developing bacterial vaginosis **(26)**. Since the microbiome has a considerable variety in different geographical areas and races, we studied the impact of those bacteria PTB in Iranian mothers.

Classical diagnostic methods, such as the Amsel criteria and Nugent scoring systems, are the practical and cost-effective options for diagnosing bacterial vaginosis **(27, 28)**. Although culture is considered the standard diagnostic approach for many bacterial

infections, it is not recommended for bacterial vaginosis due to the challenges in isolation and their scarcity in normal vaginal flora **(29)**. As an alternative diagnostic method, polymerase chain reaction (PCR) can identify the type of bacteria **(30)**. Of interest, our results have depicted significant trends among the load of *G.vaginalis*, *A.vaginae, P.bivia*, and *L. Crispatus* with clinical signs and symptoms of bacterial vaginosis in our samples. The present study has shown that the prevalence of bacterial vaginosis based on Amsel's criteria is 36/36% in our 55 included cases. Consistent with this, Ruh Bakhsh et al **(31)** reported that the prevalence of bacterial vaginosis was 31% in the Gilan province of Iran in 2019. The estimated prevalence of the present study was higher than the reports from Ethiopia (19.4%) and India (20.5%) and close to the reports from Kenya (37%) and Zimbabwe (32.5%) **(32-35)**. Thus, the prevalence of bacterial vaginosis is remarkable in different geographical regions.

In this study, we demonstrated the high sensitivity and specificity of the qPCR method. Although qPCR requires specialized equipment, it proves to be more efficient and less labor-intensive than Nugent scoring, which relies on the manual assessment of Gram-stained smears. This makes qPCR a suitable option in settings with limited skilled personnel but access to basic molecular biology tools **(36)**. While Amsel's criteria are straightforward and require minimal equipment, they exhibit lower sensitivity and specificity, increasing the risk of misdiagnosis, particularly in complicated cases. qPCR provides rapid results, which are crucial for timely treatment initiation—an essential factor in low-resource settings where diagnostic delays can worsen health outcomes **(37)**. Furthermore, qPCR's ability to simultaneously detect multiple pathogens, including those responsible for BV, trichomoniasis, and vulvovaginal candidiasis, enables a comprehensive approach to diagnosis. This is particularly beneficial for identifying co-infections, which are common and can complicate treatment strategies **(38)**. Overall, qPCR presents a promising tool for improving diagnostic accuracy and patient care in a variety of clinical settings.

The present study has demonstrated that mothers with bacterial vaginosis have higher levels of *G. vaginalis*, *P. bivia*, and *A. vaginae* bacteria, but these mothers have lower levels of *L. crispatus* bacteria. Our results have not identified any significant relationships between the presence of studied bacteria with PTB; this is in line with the study by Adesiji et al **(39)**.

The vaginal microbiome composition in our study (prevalence of *Lactobacillus* species) is consistent with the prevalence and microbial profile findings from the Kenyan and Zimbabwean studies, but slightly higher than from India and Ethiopia **(40, 41)**. This discrepancy warrants further exploration into the potential influence of ethnic variations, behavioral practices (such as hygiene and dietary habits), and even diagnostic methodologies employed across these diverse populations. Roohbakhsh et al **(42)**'s 2019 study within Iran provides a valuable internal comparison, and the noted differences with other regions underscore the importance of considering the multifaceted factors that can shape the vaginal microbiome **(42)**. Future research could benefit from standardized protocols and larger, multi-center studies to disentangle these complex interactions and provide a more comprehensive understanding of global variations in the vaginal microbiome **(42)**.

Consistent with this, Livani et al **(43)** have reported that there is no considerable difference between the levels of *G. vaginalis* and *A. vaginae* bacteria in mothers with PTB compared to those with term delivery **(43)**. However, Lim et al **(44)** have reported that the presence of *A. vaginae* is higher in patients with preterm delivery or premature rupture of membranes. Also, Keli et al **(45)** have shown that women with preterm delivery have low levels of *Lactobacillus* species and higher levels of *Gardnerella*, *Atopobium*, *Megasphaera*, and *Streptococcus* in the lower genital tract. Besides, Prodan-Barbulescu et al **(46)** have demonstrated that the presence of *G. vaginalis* is substantially associated with PTB. Also, Nguyen et al **(47)** have indicated that bacterial vaginosis, unlike fungal infection, increases the risk of PTB and preterm premature rupture of membranes.

The null association between BV-associated bacteria and PTB observed in our studies presents a paradox with other research findings. This discrepancy may arise from variability in diagnostic criteria, which can contribute to conflicting results **(48)**. Additionally, the timing of diagnosis is crucial: BV detected before 16 weeks' gestation shows a strong correlation with PTB, while diagnoses made later demonstrate weaker associations **(49)**. Moreover, pathogen specificity may play a role; subclinical infections or polymicrobial interactions **(50)**, such as those involving *G. vaginalis* and *Mycoplasma hominis*, could increase risk more than BV alone **(49)**. For instance, one study reported a 2.1-fold increased risk of PTB when both pathogens were present **(49)**. The host immune response is also a key factor, as BV-associated bacteria can trigger inflammatory cytokines (e.g., IL-1β, IL-6), which weaken fetal membranes **(51)**. However, genetic or immunological variability across different populations may modulate the effects of these inflammatory responses, potentially explaining the inconsistencies observed in various studies **(51)**.

Cultural and behavioral factors in Iran may explain these differences. For instance, lower alcohol consumption and smoking rates among Iranian women, compared to their Western counterparts,

could reduce confounding behavioral risks for PTB **(52)**.

The present study suffers from several limitations. First, we only studied bacterial vaginosis in the third trimester. Given the dynamic nature of bacterial vaginosis in pregnant women, longitudinal studies are needed to comprehensively investigate the impact of bacterial vaginosis on preterm delivery throughout all trimesters. Second, Given the cohort of only 55 participants and a prevalence of PTB at 36.36%, this study may lack the statistical power necessary to detect subtle associations, highlighting the need for more substantial sample size in future research.

Third, we utilized real-time PCR to quantify the abundance of specific bacterial groups; however, this method does not provide information about the metabolic activities of each bacterium in bacterial vaginosis. Overall, the current study provides novel insights into the bacterial vaginosis prevalence and the impact of *G. vaginalis*, *L.crispatus*, *P.bivia*, and *A.vaginae* in preterm labor of Iranian pregnant women.

5. Conclusion

In our included pregnant women, the prevalence of bacterial vaginosis is 36.36% according to the Amsel criteria. Our results have shed light on the significant trends between the load of *G.vaginalis*, *A.vaginae, P.bivia*, and *L. Crispatus* with clinical signs and symptoms of bacterial vaginosis in pregnant women. However, there has been no significant difference in the load of *G.vaginalis*, *A.vaginae, P.bivia*, and *L. Crispatus* in the lower genital tract of patients with PTB compared to those with term delivery.

6. Declarations

6.1 Acknowledgment

The authors would like to thank the Shahid Akbarabadi Clinical Research Development Unit (ShACRDU), School Of Medicine Iran University of Medical Sciences(IUMS), Tehran, Iran for their specialists who helped collect vaginal swab samples throughout the preiod of study (IR.IUMS.FMD.REC.1401.242). All the authors have been reading and concurring with this manuscript.

6.2 Ethical Considerations

The study received ethical approval from the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1401.242) and was conducted in accordance with the principles of the Helsinki Declaration.

6.3 Authors' Contributions

Parisa Rahimi: Writing – original draft, Project administration, Investigation. Shirin Dashtbin: Supervision. Shiva Mirkalantari: Writing – review & editing. Maryam Kashanian: Writing – review & editing. Nooshin Eshraghi: Writing – review & editing. Faramarz Masjedian Jazi: Writing – review & editing, Resources, Project administration, Conceptualization. All authors read and approved the final manuscript.

6.4 Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6.5 Financial Support and Sponsorship

This study was financially supported in Iran University of Medical Sciences (Tehran, Iran).

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

References

1. Abbe C, Mitchell CM. Bacterial vaginosis: a review of approaches to treatment and prevention. Front Reprod Health. 2023;5:1100029. [PMID] [PMCID] [DOI:10.3389/frph.2023.1100029]
2. Bradshaw CS, Vodstrcil LA, Hocking JS, Law M, Pirotta M, Garland SM, et al. Recurrence of bacterial vaginosis is significantly associated with posttreatment sexual activities and hormonal contraceptive use. Clin Infect Dis. 2013;56(6):777-86. [DOI:10.1093/cid/cis1030] [PMID]
3. Reiter S, Kellogg Spadt S. Bacterial vaginosis: a primer for clinicians. Postgrad Med. 2019;131(1): 8-18. [DOI:10.1080/00325481.2019.1546534] [PMID]
4. Ravel J, Moreno I, Simón C. Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. Am J Obstet Gynecol. 2021;224(3):251-7. [DOI:10.1016/j.ajog.2020.10.019] [PMID]

5. Coudray MS, Madhivanan P. Bacterial vaginosis-A brief synopsis of the literature. Eur J Obstet Gynecol Reprod Biol. 2020;245:143-8. [PMCID] [DOI:10.1016/j.ejogrb.2019.12.035] [PMID]

6. Abou Chacra L, Fenollar F, Diop K. Bacterial Vaginosis: What Do We Currently Know?. Front Cell Infect Microbiol. 2021;11:672429. [PMCID] [DOI:10.3389/fcimb.2021.672429] [PMID]

7. Alves P, Castro J, Sousa C, Cereija TB, Cerca N. Gardnerella vaginalis outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. J Infect Dis. 2014;210(4):593-6. [DOI:10.1093/infdis/jiu131] [PMID]

8. Schellenberg JJ, Paramel Jayaprakash T, Withana Gamage N, Patterson MH, Vaneechoutte M, Hill JE. Gardnerella vaginalis Subgroups Defined by cpn60 Sequencing and Sialidase Activity in Isolates from Canada, Belgium and Kenya. PLoS One. 2016;11(1):e0146510. [PMID] [PMCID] [DOI:10.1371/journal.pone.0146510]

9. Castro J, França A, Bradwell KR, Serrano MG, Jefferson KK, Cerca N. Comparative transcriptomic analysis of Gardnerella vaginalis biofilms vs. planktonic cultures using RNA-seq. NPJ Biofilms Microbiomes. 2017;3:3. [PMCID] [DOI:10.1038/s41522-017-0012-7] [PMID]

10. Muzny CA, Taylor CM, Swords WE, Tamhane A, Chattopadhyay D, Cerca N, et al. An Updated Conceptual Model on the Pathogenesis of Bacterial Vaginosis. J Infect Dis. 2019;220(9): 1399-405. [DOI:10.1093/infdis/jiz342] [PMID] [PMCID]

11. Muzny CA, Blanchard E, Taylor CM, Aaron KJ, Talluri R, Griswold ME, et al. Identification of Key Bacteria Involved in the Induction of Incident Bacterial Vaginosis: A Prospective Study. J Infect Dis. 2018;218(6):966-78. [DOI:10.1093/infdis/jiy243] [PMID] [PMCID]

12. Gilbert NM, Lewis WG, Li G, Sojka DK, Lubin JB, Lewis AL. Gardnerella vaginalis and Prevotella bivia Trigger Distinct and Overlapping Phenotypes in a Mouse Model of Bacterial Vaginosis. J Infect Dis. 2019;220(7):1099-108. [DOI:10.1093/infdis/jiy704] [PMID] [PMCID]

13. Chen X, Lu Y, Chen T, Li R. The Female Vaginal Microbiome in Health and Bacterial Vaginosis. Front Cell Infect Microbiol. 2021;11:631972. [DOI:10.3389/fcimb.2021.631972] [PMID] [PMCID]

14. Griggs KM, Hrelic DA, Williams N, McEwen-Campbell M, Cypher R. Preterm Labor and Birth: A Clinical Review. MCN Am J Matern Child Nurs. 2020;45(6):328-37. [DOI:10.1097/NMC.0000000000000656] [PMID]

15. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, et al. The vaginal microbiome and preterm birth. Nat Med. 2019; 25(6):1012-21. [PMID] [PMCID] [DOI:10.1038/s41591-019-0450-2]

16. Liu X, Cao Y, Xie X, Qin X, He X, Shi C, et al. Association between vaginal microbiota and risk of early pregnancy miscarriage. Comp Immunol Microbiol Infect Dis. 2021;77:101669. [DOI:10.1016/j.cimid.2021.101669] [PMID]

17. Menard JP, Mazouni C, Salem-Cherif I, Fenollar F, Raoult D, Boubli L, et al. High vaginal concentrations of Atopobium vaginae and Gardnerella vaginalis in women undergoing preterm labor. Obstet Gynecol. 2010;115(1):134-40. [DOI:10.1097/AOG.0b013e3181c391d7] [PMID]

18. Jayaram PM, Mohan MK, Konje J. Bacterial vaginosis in pregnancy - a storm in the cup of tea. Eur J Obstet Gynecol Reprod Biol. 2020;253:220-4. [DOI:10.1016/j.ejogrb.2020.08.009] [PMID]

19. Elovitz MA, Gajer P, Riis V, Brown AG, Humphrys MS, Holm JB, et al. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. Nat Commun. 2019;10(1):1305. [PMID] [PMCID] [DOI:10.1038/s41467-019-09285-9]

20. Delaney ML, Onderdonk AB, Microbiology, Group PS. Nugent score related to vaginal culture in pregnant women. Obstet Gynecol. 2001;98(1):79-84. [DOI:10.1016/S0029-7844(01)01402-8] [DOI:10.1097/00006250-200107000-00015] [PMID]

21. Colonna C, Steelman M. Amsel Criteria. [Updated 2023 Jul 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: [https://www.ncbi.nlm.nih.gov/sites/books/NBK542319/]

22. Cheng X, Hong X, Khayatnezhad M, Ullah F. Genetic diversity and comparative study of genomic DNA extraction protocols in Tamarix L. species. Caryologia. 2021;74(2):131-9. [DOI:10.36253/caryologia-1056]

23. Bakhshi A, Delouyi ZS, Taheri S, Alivandi A, Mohammadzadeh N, Dabiri H. Comparative study of lactobacilli and bifidobacteria in vaginal tract of individual with bacterial vaginosis and healthy control by quantitative PCR. Rev Res Med Microbiol. 2019;30(3):148-54. [DOI:10.1097/MRM.0000000000000186]

24. da Fonseca EB, Damião R, Moreira DA. Preterm birth prevention. Best Pract Res Clin Obstet Gynaecol. 2020;69:40-9. [DOI:10.1016/j.bpobgyn.2020.09.003] [PMID]

25. Witkin SS. The vaginal microbiome, vaginal anti-microbial defence mechanisms and the clinical challenge of reducing infection-related preterm birth. BJOG: Int J Obstet Gynaecol. 2015;122(2): 213-8. [DOI:10.1111/1471-0528.13115] [PMID]

26. France MT, Fu L, Rutt L, Yang H, Humphrys MS, Narina S, et al. Insight into the ecology of vaginal bacteria through integrative analyses of metagenomic and metatranscriptomic data. Genome Biol. 2022;23(1):66. [PMID] [PMCID] [DOI:10.1186/s13059-022-02635-9]

27. Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment. Expert Rev Anti Infect Ther. 2009;7(9):1109-24. [DOI:10.1586/eri.09.87] [PMID]

28. 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(2):297-301. [DOI:10.1128/jcm.29.2.297-301.1991] [PMID] [PMCID]

29. Mengistie Z, Woldeamanuel Y, Asrat D, Yigeremu M. Comparison of clinical and gram stain diagnosis methods of bacterial vaginosis among pregnant women in ethiopia. J Clin Diagn Res. 2013;7(12):2701-3. [PMID] [PMCID] [DOI:10.7860/JCDR/2013/5872.3736]

30. Bhujel R, Mishra SK, Yadav SK, Bista KD, Parajuli K. Comparative study of Amsel's criteria and Nugent scoring for diagnosis of bacterial vaginosis in a tertiary care hospital, Nepal. BMC Infect Dis. 2021;21(1):825. [PMID] [PMCID] [DOI:10.1186/s12879-021-06562-1]

31. Roohbakhsh E, Mojtahedi A, Khavari-Nezhad RA, Amirmozafari N. Za0Using the PCR method for detection of bacterial vaginosis in women suspected of vaginosis in Guilan province. Iran J Obstet Gynecol Infertil. 2019;21(12):29-36.

32. Mengistie Z, Woldeamanuel Y, Asrat D, Adera A. Prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. BMC Res Notes. 2014;7:822. [PMID] [PMCID] [DOI:10.1186/1756-0500-7-822]

33. Lata I, Pradeep Y, Sujata, Jain A. Estimation of the Incidence of Bacterial Vaginosis and other Vaginal Infections and its Consequences on Maternal/Fetal Outcome in Pregnant Women Attending an Antenatal Clinic in a Tertiary Care Hospital in North India. Indian J Community Med. 2010;35(2):285-9. [PMID] [PMCID] [DOI:10.4103/0970-0218.66855]

34. Romoren M, Velauthapillai M, Rahman M, Sundby J, Klouman E, Hjortdahl P. Trichomoniasis and bacterial vaginosis in pregnancy: inadequately managed with the syndromic approach. Bull World Health Organ. 2007;85(4):297-304. [DOI:10.2471/BLT.06.031922] [PMID] [PMCID]

35. Kurewa NE, Mapingure MP, Munjoma MW, Chirenje MZ, Rusakaniko S, Stray-Pedersen B. The burden and risk factors of Sexually Transmitted Infections and Reproductive Tract Infections among pregnant women in Zimbabwe. BMC Infect Dis. 2010;10:127. [PMID] [PMCID] [DOI:10.1186/1471-2334-10-127]

36. Amor I, Alberola A, De Salazar A, Viñuela L, Úbeda-Portugués S, Galán MI, et al. Evaluation of the Vaginal Panel Realtime PCR kit (Vircell, SL) for diagnosing vaginitis: A comparative study with routinely used diagnostics. PloS One. 2024; 19(11):e0313414. [PMID] [PMCID] [DOI:10.1371/journal.pone.0313414]

37. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. J Clin Microbiol. 2007;45(10):3270-6. [DOI:10.1128/JCM.01272-07] [PMID] [PMCID]

38. Kusters J, Reuland E, Bouter S, Koenig P, Dorigo-Zetsma J. A multiplex real-time PCR assay for routine diagnosis of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015;34:1779-85. [PMCID] [DOI:10.1007/s10096-015-2412-z] [PMID]

39. Adesiji Y, Taiwo S, Adekanle D, Oboro V, Fayemiwo S, Opaleye O. Bacterial vaginosis and pregnancy outcome in Osogbo, Nigeria. Res J Med Sci. 2007;1(4):195-8.

40. Lata I, Pradeep Y, Jain A. Estimation of the incidence of bacterial vaginosis and other vaginal infections and its consequences on maternal/fetal outcome in pregnant women attending an antenatal clinic in a tertiary care hospital in North India. Indian J Community Med. 2010;35(2):285-9. [DOI:10.4103/0970-0218.66855] [PMID] [PMCID]

41. Mengistie Z, Woldeamanuel Y, Asrat D, Adera A. Prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. BMC Res Notes. 2014;7:1-5. [PMID] [PMCID] [DOI:10.1186/1756-0500-7-822]

42. Roohbakhsh E, Mojtahedi A, Roohbakhsh Z, Khavari-Nejad RA, Amirmozafari N. Identification

of gardnerella vaginalis and atopobium vaginae in women with bacterial vaginosis in Northern Iran. Infect Dis Clin Pract. 2019;27(2):81-4. [DOI:10.1097/IPC.0000000000000691]

43. Livani S, Nosrat SB, Alhosseini MN, Sheykholeslami AS, Vakili MA, Ghaemi EA. The role of Gardnerella vaginalis, Autopobium vaginae and Mobiloncus spp in preterm delivery: A case-control study. Iran J Obstet Gynecol Infertil. 2023; 26(6):50-9.

44. Lim KH, Brooks H, McDougal R, Burton J, Devenish C, De Silva T. Is there a correlation between bacterial vaginosis and preterm labour in women in the Otago region of New Zealand?. Aust N Z J Obstet Gynaecol. 2010;50(3):226-9. [DOI:10.1111/j.1479-828X.2010.01149.x] [PMID]

45. Hočevar K, Maver A, Vidmar Šimic M, Hodžić A, Haslberger A, Premru Seršen T, et al. Vaginal Microbiome Signature Is Associated With Spontaneous Preterm Delivery. Front Med. 2019; 6:201. [DOI:10.3389/fmed.2019.00201] [PMID] [PMCID]

46. Prodan-Barbulescu C, Bratosin F, Folescu R, Boeriu E, Popa ZL, Citu C, et al. Analysis of Vaginal Microbiota Variations in the Third Trimester of Pregnancy and Their Correlation with Preterm Birth: A Case-Control Study. Microorganisms. 2024;12(2):417. [PMID] [PMCID] [DOI:10.3390/microorganisms12020417]

47. Nguyen QHV, Le HN, Ton Nu VA, Nguyen ND, Le MT. Lower genital tract infections in preterm premature rupture of membranes and preterm labor: a case-control study from Vietnam. J Infect Dev Ctries. 2021;15(6):805-11. [DOI:10.3855/jidc.13244] [PMID]

48. Mohanty T, Doke PP, Khuroo SR. Effect of bacterial vaginosis on preterm birth: a meta-analysis. Arch Gynecol Obstet. 2023;308(4):1247-55. [DOI:10.1007/s00404-022-06817-5] [PMID]

49. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. N Engl J Med. 1995;333(26): 1737-42. [DOI:10.1056/NEJM199512283332604] [PMID]

50. Shimaoka M, Yo Y, Doh K, Kotani Y, Suzuki A, Tsuji I, et al. Association between preterm delivery and bacterial vaginosis with or without treatment. Sci Rep. 2019;9(1):509. [PMID] [PMCID] [DOI:10.1038/s41598-018-36964-2]

51. Daskalakis G, Psarris A, Koutras A, Fasoulakis Z, Prokopakis I, Varthaliti A, et al. Maternal infection and preterm birth: from molecular basis to clinical implications. Children. 2023;10(5):907. [DOI:10.3390/children10050907] [PMID] [PMCID]

52. Esmaeili ED, Kalankesh LR, Zeinalzadeh AH, Ghaffari A, Dastgiri S. Development, Validation, and Cross Cultural Adoption of Persian Version of Behavioral Risk Factor Tool. Med J Islam Repub Iran. 2024;38:21.

Appendix

Lactobacillus crispatus

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTCGCTGACCTTGATGATGC	20	58.63	50.00	3.00	2.00
Reverse primer	GGGCCATAATCCTTGCTACC	20	58.09	55.00	4.00	2.00

Products on target templates

>CP047142.1 Lactobacillus crispatus strain C25 chromosome, complete genome

```
product length = 75
Forward primer  1       TTCGCTGACCTTGATGATGC  20
Template        253232  ....................  253251

Reverse primer  1       GGGCCATAATCCTTGCTACC  20
Template        253306  ....................  253287
```

>CP180627.1 Lactobacillus crispatus strain T31e chromosome, complete genome

Gardnerella vaginalis

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGCGTTTCAATCGCTAAGG	20	58.92	50.00	7.00	1.00
Reverse primer	CCAGAGATTGAGCCAACAC	19	55.91	52.63	4.00	2.00

Products on target templates

>LT629773.1 Gardnerella vaginalis strain DSM 4944 genome assembly, chromosome: I

```
product length = 143
Forward primer  1        TGGCGTTTCAATCGCTAAGG  20
Template        1679592  ....................  1679611

Reverse primer  1        CCAGAGATTGAGCCAACAC  19
Template        1679734  ...................  1679716
```

Atopobium vaginae

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCAGTCATGGCCCAGAAGAC	20	59.38	55.00	5.00	2.00
Reverse primer	CCCTATCCGCTCCTGATACC	20	58.81	60.00	5.00	2.00

Products on target templates

>MF048526.1 Uncultured bacterium clone A1944 16S ribosomal RNA gene, partial sequence

```
product length = 129
Forward primer  1    TCAGTCATGGCCCAGAAGAC  20
Template        377  ....................  358

Reverse primer  1    CCCTATCCGCTCCTGATACC  20
Template        249  ....................  268
```

>CP184248.1 Fannyhessea vaginae strain Q9002 chromosome, complete genome

```
product length = 129
Forward primer  1       TCAGTCATGGCCCAGAAGAC  20
Template        232648  ....................  232629

Reverse primer  1       CCCTATCCGCTCCTGATACC  20
Template        232520  ....................  232539


product length = 129
Forward primer  1       TCAGTCATGGCCCAGAAGAC  20
Template        111441  ....................  111422

Reverse primer  1       CCCTATCCGCTCCTGATACC  20
Template        111313  ....................  111332
```

>MN165521.1 Uncultured Atopobium sp. clone IQB.A.No.2 16S ribosomal RNA gene, partial sequence

Prevotella bivia

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AACCCAGCGAAAGTTGGAC	19	58.28	52.63	3.00	1.00
Reverse primer	AATCAGACGCATCCCCATCC	20	59.89	55.00	4.00	0.00

Products on target templates

>AP038911.1 Prevotella bivia GTC18476 DNA, chromosome 1, complete sequence

```
product length = 97
Forward primer  1        AACCCAGCGAAAGTTGGAC  19
Template        1214271  ...................  1214289

Reverse primer  1        AATCAGACGCATCCCCATCC  20
Template        1214367  ...................  1214348


product length = 97
Forward primer  1       AACCCAGCGAAAGTTGGAC  19
Template        704041  ...................  704023

Reverse primer  1       AATCAGACGCATCCCCATCC  20
Template        703945  ...................  703964
```

>AP029376.1 Prevotella bivia TOH-2715 DNA, chromosome 2, complete sequence