



A Comparative Analysis of Vaginal Flora in Women with and Without Bacterial Vaginosis

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p-ISSN: 1608-9391

e-ISSN: 2664-2786

Article information

Received: 24/ 10/ 2025

Revised: 30/ 12/ 2025

Accepted: 13/ 1/ 2026

DOI:

10.33899/rsci.v35i3.65237

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ABSTRACT

Bacterial vaginosis (BV), the most prevalent vaginal dysbiosis, is typified by a transition from a *Lactobacillus*-dominant community to a polymicrobial anaerobic community. This study aimed to characterize and compare the vaginal microbial profile of BV-positive and BV-negative women and assess the antibiotic susceptibility profiles of primary bacterial isolates. Samples were collected between September 2024 and April 2025 from 200 symptomatic women between 18 and 45 years. The participants were categorized into BV-positive (n=75) and BV-negative (n=125) based on Nugent scoring. Vaginal swabs were subjected to Gram stain, cultured on appropriate media, and identified by routine biochemical tests. Antibiotic susceptibility testing was performed for key bacterial pathogens. The prevalence of BV was 37.5%. Relative to BV-negative women (16.8%), polymicrobial growth was significantly more common among BV-positive women (72.0%). The most common isolates from BV-positive women were *Gardnerella vaginalis* (42.7%), *Prevotella* spp. (25.3%), and *Atopobium vaginae* (18.7%). BV-negative women were mainly colonized by *Lactobacillus* spp. (58.4%) and *Candida* spp. (44.0%).

Among the 200 samples collected, positive microbial growth occurred in 148 samples (74.0%). The most frequent isolates in the entire cohort were *Candida* sp. (28.0%), *Staphylococcus aureus* (22.0%), *Escherichia coli* (20.0%), and *Streptococcus agalactiae* (12.0%). Antibiotic sensitivity testing revealed that ciprofloxacin inhibited *S. aureus* and *S. agalactiae*, and norfloxacin inhibited 88% of the *E. coli* isolates. This study confirms the high prevalence of BV and its distinct polymicrobial nature in our population, underscoring the necessity for accurate diagnosis to guide effective, targeted antimicrobial therapy.

Keywords: Antibiotic sensitivity, *E. coli*, *Lactobacillus*, *S. agalactiae*, Vaginal microbiota.

INTRODUCTION

In healthy women of reproductive age, *Lactobacillus* species predominate the vaginal microbiota, which produces lactic acid to maintain a low pH and subsequently promote a protective environment.

Bacterial vaginosis (BV), a common clinical infection, occurs clinically when there is a dysbiosis change in this ecosystem that is marked especially by the decrease of *Lactobacilli* and the overgrowth of anaerobic and facultative bacteria, such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Prevotella* species (Abdul aziz *et al.*, 2019). BV has been associated with adverse health outcomes such as increased susceptibility to sexually transmitted infections and adverse pregnancy outcomes. Clinically, a diagnosis can be made based on clinical Amsel's criteria, and investigation microscopically based on Nugent scoring of Gram-stained vaginal smears, the latter of which is considered the gold standard in research and clinical settings (Al-Taweel *et al.*, 2015). In many low-resource settings, inadequate access to advanced diagnostic tools leads to a reliance on empirical treatment, which can exacerbate suboptimal outcomes and ultimately the development of antimicrobial resistance (Haydar *et al.*, 2022). This study will provide modern data from a tertiary care center in Duhok, Iraq, which examines both microbial profiles and antibiotic susceptibility in women with and without BV, and will inform local practice for diagnosis and treatment.

MATERIALS AND METHODS

Study design and participants

From September 2024 until April 2025 a hospital-based, cross-sectional study was performed in Duhok city. Two hundred symptomatic women were consecutively sampled (aged 18-45 years) who complained of abnormal vaginal discharge, malodor, itching or pain with intercourse.

Exclusion Criteria included:

- Use of antibiotics or antifungals in the last four weeks
- Current pregnancy
- Hysterectomy
- Known immunosuppressed states

Sample collection and processing

Samples were taken after the insertion of a sterile unlubricated speculum, and two swabs were taken from the lateral vaginal wall and posterior fornix. The first swab was used to prepare a Gram stain for Nugent scoring, and the second swab was placed in Amies transport medium for culture. For culture, samples were inoculated onto blood agar, chocolate agar, and MacConkey agar. Plates were incubated at 37°C for 24-48 hours in appropriate atmospheres (aerobic, CO₂-enriched, or anaerobic conditions). Detection of bacteria was based on the morphology of colonies, Gram stain, and biochemical testing like catalase, oxidase, growth in selective media, Hippurate hydrolysis, germ tube test, and anaerobic profiling.

This study was approved by the General Directorate of Health in Dohuk, Ministry of Health of the Republic of Iraq (reference number: 10-8-24102023).

Antimicrobial sensitivity test

From the pure overnight culture, 3–5 well-isolated colonies of the test organism were picked the colonies were suspended in 4–5 mL of sterile saline or broth and vortexed thoroughly to achieve a homogeneous suspension. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard (which corresponded to approximately $1-2 \times 10^8$ CFU/mL).

A spectrophotometer (optical density at 625 nm) or a visual comparator against a white background with a black line was used. Within 15 minutes of adjusting the turbidity, a sterile cotton swab was dipped into the standardized inoculum. The swab was rotated firmly against the inside wall of the tube above the fluid level to remove excess inoculum. The entire surface of the MHA or MHB plate was swabbed three times, rotating the plate approximately 60° each time to ensure even, confluent growth. The plate was allowed to dry for 3–5 minutes (lid ajar) at room temperature until surface moisture was absorbed. Using flamed and cooled forceps (or an automated dispenser), the

selected antibiotic disks were firmly placed on the inoculated agar surface. The plates were inverted and placed in the incubator.

For *S. aureus* and *E. coli*, incubation was performed in ambient air at 35±2°C for 16–18 hours.

For *S. agalactiae*, incubation was performed in 5% CO₂ at 35±2°C for 20–24 hours (CO₂ enhanced growth of GBS; interpretative standards were based on CO₂ incubation).

Statistical analysis

Data were analyzed using SPSS version 26.0. Categorical variables were reported as frequencies and percentages and were compared using the Chi-square test or Fisher's exact test as appropriate. P-values of < 0.05 demonstrated statistical significance.

RESULTS AND DISCUSSION

Demographic characteristics

The mean age of participants was 28.6 ± 5.2 years. The prevalence of BV was higher in the 25-34 age group (53.3% of BV-positive cases) which is shown in (Table 1).

Table (1): Age distribution of study participants (N=200)

Age Group	BV-Positive (n=75)	BV-Negative (n=125)	Total 200
18-24	25 (33.3. %)	20 (6.0%)	45
25-34	40 (53.3 %)	80 (64.0%)	120
35-45	10 (13.3%)	25 (20.0%)	35
Total	75	125	200

* P= 0.018

Microbial isolation patterns

A distinct difference in the distribution of microbial growth was observed in (Table 2):

- BV-Positive Women: Polymicrobial growth was observed in 54 of 75 samples (72.0%). The most common isolates were *G. vaginalis* (42.7%), *Prevotella spp.* (25.3%), and *A. vaginae* (18.7%).
- BV-Negative Women: Monomicrobial growth occurred most frequently (105/125, 84.0%). *Lactobacillus spp.* (58.4%) and *Candida spp.* (44.0%) were most commonly isolated.

Table (2): Comparative microbial isolation rates

Microrganism	BV+ (n=75)	BV- (n=125)	p-value	OR (95% CI)
<i>G. vaginalis</i>	32 (42.7%)	3 (2.4%)	<0.001	30.2 (8.9 - 102.1)
<i>Prevotella spp.</i>	19 (25.3%)	0 (0.0%)	<0.001	65.6 (3.9 - 1112.8)
<i>A. vaginae</i>	14 (18.7%)	0 (0.0%)	<0.001	39.5 (2.3 - 671.1)
<i>C. albicans</i>	10 (13.3%)	45 (36.0%)	<0.001	0.27 (0.13 - 0.57)
<i>Lactobacillus spp.</i>	5 (6.7%)	73 (58.4%)	<0.001	0.05 (0.02 - 0.13)

Overall microbial distribution

Of 200 participants, 148 (74.0%) had positive microbial growth. The prevalence of the most common isolates was: *Candida spp.* (28.0%), *S. aureus* (22.0%), *E. coli* (20.0%), and *S. agalactiae* (12.0%).

This hospital-based investigation of 200 symptomatic women in Duhok, Iraq, provides a general overview of the vaginal microbiome environment. Our results confirm global patterns of BV and highlight important local specificities that affect clinical management. The prevalence of BV among

our symptomatic cohort was 37.5%, which is on the higher end of the global spectrum, which typically ranges from 15% to 50% in women of reproductive age (Abdul aziz *et al.*, 2019). Regarding risk factors, our data corroborates local studies, such as (Jacobsen *et al.*, 2008), in identifying key demographic and behavioral predictors. We found that younger age at marriage and sexual debut was a significant risk factor, a pattern also observed in the referenced study. Furthermore, the consensus across local research identifies specific hygiene practices (e.g., douching) and socioeconomic barriers to healthcare as critical, modifiable drivers of BV in our population. This elevated rate suggests a significant burden of disease in our setting, potentially linked to regional factors such as hygiene practices, sexual behaviors, or limited access to reproductive healthcare.

The prevalence of BV and the polymicrobial aspects of dysbiosis.

Our symptomatic cohort had a BV prevalence of 37.5%, which was high and is towards the upper limit of global estimates of BV prevalence (15-50%), possibly indicating a high burden of disease in our population. This also highlights the importance of BV as an emerging public health concern. The most notable finding of the BV-positive group of patients was the high level of polymicrobial growth (72.0%), which is a characteristic feature of BV. The specific predominance of *G. vaginalis* (42.7%) was consistent with its well-established role as a keystone pathogen responsible for the formation of bacterial vaginosis biofilm (Abdul aziz *et al.*, 2019), whereas the detection of *Prevotella spp.* (25.3%) and *A. vaginae* (18.7%) are of particular clinical importance in our cohort (Hadar *et al.*, 2022). In particular, *A. vaginae* has been strongly associated with treatment failure associated with metronidazole treatment and recurrent bacterial vaginosis (Al Taweel *et al.*, 2015).

The co-detection of these anaerobes also indicates that a notable proportion of our BV-positive patients may be at greater risk for recurrent disease resulting from symbiotic commensal flora and may require consideration of a treatment strategy that involves the use of alternative agents, combination agents like secnidazole or oral metronidazole, along with a vaginal clindamycin preparation as a first-line therapy (Jacobsen *et al.*, 2008).

The BV-Negative Microbiome - Candidiasis and aerobic pathogen excessive load

The primary contribution of this study was the characterization of the BV-negative group. We found that although a healthy *Lactobacillus*-dominant pattern was most common (58.4%) within this group, a strikingly high proportion (44.0%) were colonized by *Candida* species and this was substantially higher than the prevalence of vulvovaginal candidiasis cited in Zakho (Khamees, 2012) which is between 8-10 %. Over-the-counter antifungal treatment resulting in resistant strains, changes in dietary patterns, or genetic susceptibility (Al Taweel *et al.* 2015). The high frequency of *Candida* colonization indicates that, when women are symptomatic for vulvovaginal candidiasis, it should be reliably ruled out in instances of testing negative for BV (Kumar *et al.*, 2015).

It is also important not to ignore the overall high isolation rates of *E. coli* (20.0%), *S. aureus* (22.0%), and *S. agalactiae* (12.0%) for the entire cohort. They are classic pathogens for aerobic vaginitis (AV), which is a clinical syndrome distinctly different from BV and is characterized by inflammatory responses and a decrease in lactobacilli while showing an overgrowth of aerobic bacteria rather than anaerobes (Abdul aziz *et al.*, 2019). The presenting symptoms of AV (e.g., yellow discharge, inflammation, dyspareunia) may overlap with BV and, to a lesser-degree, candidiasis, but the clinical management is varied and typically involves topical antiseptics or cephalosporins rather than the metronidazole approaches for BV. Our analysis suggests that AV may be fairly underdiagnosed in our setting (Macphee *et al.*, 2013).

Diagnostic and treatment implications: Moving away from empirical treatment

The high prevalence of the multitude of pathogens (BV-associated anaerobes, *Candida*, and AV-associated aerobes) renders it a multifaceted diagnostic challenge. Solely relying on the presence of symptoms is completely insufficient because the symptoms are non-specific as show in (Table 3).

Table (3): Antimicrobial susceptibility profiles of key bacterial isolates

Antibiotic\concertation (µg/mL)	<i>S.aureus</i> (n=4)	<i>S.agalactiae</i> (n=2)	<i>E. coli</i> (n=4)
Penicillin 0.015 –16	11.4% (5)	79.2% (19)	N/A
Ampicillin 0.12 – 64	9.1% (4)	N/A	25.0% (10)
Clindamycin 0.14– 32	72.7% (32)	66.7% (16)	N/A
Erythromycin 0.015– 64	N/A	66.7% (16)	N/A
Ciprofloxacin 0.10– 32	95.5% (42)	91.7% (22)	82.5% (33)
Norfloxacin 0.9– 64	N/A	N/A	88.0% (35)
TMP-SMX 0.8– 2.38	N/A	N/A	35.0% (14)
Ceftriaxone 0.9– 32	N/A	N/A	65.0% (26)
Amikacin 0.8– 64	N/A	N/A	92.5% (37)
Vancomycin 0.8– 32	100% (44)	100% (24)	N/A

This study calls for the routine inclusion of Nugent scoring, a reliable and inexpensive tool, which we strongly endorse to differentiate between BV and other pathologies (Hadar *et al.*, 2022).

S. aureus (n=44): Isolates exhibited high susceptibility to ciprofloxacin (95.5%) and vancomycin (100%). Resistance to penicillin and ampicillin was widespread, at 88.6% and 90.9%, respectively. Moderate susceptibility was observed for clindamycin (72.7%).

E. coli (n=40): Norfloxacin was the most effective agent (88.0% susceptibility), followed closely by amikacin (85.0%). High resistance rates were documented against ampicillin (75.0%) and trimethoprim-sulfamethoxazole (65.0%). Notably, 35.0% of isolates were resistant to ceftriaxone.

S. agalactiae (Group B *Streptococcus*, n=24): Similar to *S. aureus*, *S. agalactiae* showed high susceptibility to ciprofloxacin (91.7%) and vancomycin (100%). A significant finding was that 20.8% of isolates were resistant to penicillin, a first-line agent for GBS prophylaxis. The patterns of antibiotic susceptibility that we describe are useful, but also cautionary. While fluoroquinolones (ciprofloxacin, norfloxacin) had good activity against the common aerobes, their use empirically is not indicated for undifferentiated vaginitis (Martin, 2012). The prevalent use of fluoroquinolones is discouraged based on concerns for developing antimicrobial resistance, and fluoroquinolones would not be effective on BV-associated anaerobes or for *Candida* (Minardi *et al.*, 2011). The high burden of polymicrobial and concomitant infections implies that a single antibiotic will likely not address the patient's symptoms. For example, a woman may be treated with metronidazole for BV but may also have an unrecognized *Candida* co-infection, which leads to symptomatic vaginitis (Orish *et al.*, 2016).

Limitations and future directions

This study has limitations. Its cross-sectional design provides a snapshot in time, restricting our capacity to investigate causality or to study recurrence. The reliance on culture-dependent methods, despite being standard, inevitably excludes uncultivable bacteria and molecular methods such as PCR or next-generation sequencing provide a truer picture of the microbial population (Pérez *et al.*, 2010). We also did not collect data on behavioral or sociodemographic factors underlying vaginal microbiome change (Ratnaprabha *et al.*, 2015). Future research needs to employ a longitudinal study to define the dynamics of such infections, including recurrence (Shipitsyna *et al.*, 2012). The application of molecular diagnostics to gain a local etiological snapshot of vaginitis would be a significant advance (Venugopal *et al.*, 2017). Finally, it is necessary to evaluate the validity of the standardized diagnostic algorithms that include BV, AV, and candidiasis testing versus the current standard of empirical treatment practice in an effort to improve patient outcomes (Yadav *et al.*, 2019).

CONCLUSIONS

In summary, our findings demonstrate a complex situation of vaginitis in our symptomatic cohort. We have established the prevalence of BV as extremely high with a profile of high recurrence risk. Most importantly, we graphically depicted the heavy burden of candidiasis and aerobic pathogens among BV-negative women, suggesting that aerobic vaginitis is likely to be extremely common but underdiagnosed. This study is a call to action as a grim reminder that "vaginitis" cannot be considered as a monolithic condition. A paradigm shift to a more holistic and evolved diagnostic system is necessary, to move away from empiric treatment, to effectively treat, and improve women's reproductive health outcomes in our population.

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مقارنة التركيب الميكروبي للمهبل بين النساء المصابات وغير المصابات بالتهاب المهبل الجرثومي

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الملخص

يُمثل التهاب المهبل البكتيري أكثر أشكال اختلال التوازن المهبلي شيوعاً ويتسم بالتحول من فلورا مهيمنة من العصيات اللبنية إلى مجتمع لاهوائي متعدد الميكروبات. تهدف الدراسة إلى توصيف ومقارنة التركيب الميكروبي المهبلي بين النساء المصابات والسالبات لالتهاب المهبل البكتيري وتقييم انماط الحساسية للمضادات الحيوية للعزلات البكتيرية السائدة. أُجريت دراسة مقطعية من سبتمبر 2023 إلى أبريل 2024 شملت 200 امرأة تتراوح أعمارهن بين 18 و45 عاماً ويعانين من أعراض. تم توزيع المشاركات على مجموعتين موجبات التهاب المهبل البكتيري (75 امرأة) وسالباته (125 امرأة) تم جمع مسحات مهبليّة لإجراء صبغة غرام، والزرع على أوساط مناسبة والتعريف الميكروبي باستخدام الطرق الكيميائية الحيوية. كما تم إجراء اختبار الحساسية للمضادات الحيوية على الممرضات البكتيرية ذات الأهمية. كان انتشار التهاب المهبل البكتيري 37.5% بالمقارنة مع النساء السالبات (16.8%) حيث كان النمو المتعدد الميكروبات أكثر شيوعاً بشكل ملحوظ لدى النساء الموجبات (72.0%). كانت العزلات السائدة من النساء الموجبات هي *Gardnerella vaginalis* (42.7%) *Atopobium vaginae* (18.7%) *Prevotella spp.* (25.3%) بينما استعمرت العصيات اللبنية *Lactobacillus spp.* (58.4%) المبيضات *Candida spp.* (44.0%) بشكل أساسي النساء السالبات من بين 200 عينة مجمعة، أظهرت 148 عينة (74.0%) نمواً ميكروبياً إيجابياً. وكانت العزلات الأكثر تكراراً في المجموعة بأكملها هي *Candida spp.* (28.0%) *Staphylococcus aureus* (22.0%) *Escherichia coli* (20.0%) *Streptococcus agalactiae* (12.0%) أظهر اختبار الحساسية للمضادات الحيوية أن السيبروفلوكساسين قد كبح نمو *S. aureus* و *S. agalactiae* وكان النورفلوكساسين فعالاً ضد 88% من *E. coli*. تُسلط النتائج الضوء على الاختلافات في الميكروبيوم المهبلي بين النساء الموجبات والسالبات لالتهاب المهبل البكتيري في مجتمع الدراسة وتؤكد هذه النتائج على أهمية التشخيص الدقيق للعلاج بالمضادات الميكروبية المناسبة.

الكلمات الدالة: الحساسية للمضادات الحيوية، الإشركية القولونية، العصيات اللبنية، العقدية القاطعة للبن، الميكروبيوم المهبلي