The Prevalence, Antibiotic Resistance Pattern, and Associated Factors of Bacterial Vaginosis Among Women of the Reproductive Age Group from Felege Hiwot Referral Hospital, Ethiopia

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Background: Bacterial vaginosis is an infection of the vagina, which results due to change in the normal balance of vaginal bacteria. The aim of this study was to assess the magnitude and determinants of bacterial vaginosis among women of reproductive age group from Felege Hiwot Referral Hospital.

Materials and Methods: A cross-sectional study was conducted among women during the reproductive period at Felege Hiwot Referral Hospital from September 1, 2019 to October 2020. About 413 vaginal swab samples were collected and examined using the Nugent scoring system and culture. Data were analyzed by SPSS version 25. The odds ratio (OR) was used as a measure of the strength of association and reported with 95% confidence intervals. P -value ≤ 0.05 was considered to be statistically significant. Bivariate and multivariate logistic regression models were used to identify possible associated factors with bacteria causing bacterial vaginosis.

Results: The overall prevalence of bacteria causing bacterial vaginosis was 39.5%. The predominant bacteria were *S. aureus* (25.4%), *G. vaginalis* (22.7%), *S. agalactiae* (14.1%), and *E. coli* (13.5%). *S. aureus* was resistant to erythromycin (69.8%) and trimethoprim/sulfamethoxazole (53.5%); despite this, it was susceptible to ciprofloxacin (93%), gentamycin (93%), and cefoxitin (90.7%). On the other hand, *E. coli* was resistant to trimethoprim/sulfamethoxazole (91.3%) and ceftriaxone (63.6%), but was susceptible to ciprofloxacin (95.5%) and gentamycin (93%).

Conclusion: The high prevalence of bacterial vaginosis was significantly associated with the pH level of the vagina (≥ 4.5), participant age ≤ 20 , pregnancy, and history of HIV infection. Therefore, early identification of factors leading to bacterial overgrowth on the vagina is very important to protect maternal and child morbidity and mortality.

Keywords: aerobic bacteria, bacterial vaginosis, *Gardnerella vaginalis*, *Lactobacillus*

Background

Bacterial vaginosis is the condition that occurs when the balance of the vaginal flora (normal hydrogen peroxide (H_2O_2) producing *Lactobacillus* species) is dysregulated, and replaced by high concentrations of bad bacteria in the vagina like *Gardnerella vaginalis*, *Prevotella*, *Mobiluncus*, and *Bacteriodes* species,¹ and some aerobic bacteria occasionally *E. coli*, *S. aureus*, *E. faecalis*, and group B streptococcus (GBS) cause aerobic vaginitis¹⁻⁴ and vulvo-vaginal candidiasis.⁵

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Bacterial vaginosis was first reported by Gardner and Dukes in 1955.^{6,7} Bacterial vaginosis is the most common cause of vaginitis and associated with preterm labor, premature rupture of membranes and spontaneous abortion, chorioamnionitis, cervicitis, endometritis, urinary tract infections, cervical intraepithelial neoplasia, pelvic inflammatory disease (PID), and delivery of low-birth-weight infants.^{8,9} About 50–75% of women with BV are asymptomatic;¹⁰ however, some of the symptoms of BV are characterized by thin white discharge, fishy odor noticeable during menstruation and after sex, vulval or vaginal irritation, or soreness.^{11,12}

The lower genital tract microbiota is a dynamic bacterial community that is influenced by many aggravating factors, like age, smoking habit, alcohol use, increased number of sex partners, menopausal period, use of hormonal contraception, and the presence of acute and chronic infections.^{13,14} In a recent study, BV has been significantly associated with HIV infection. BV is increased by 60% if the women are HIV positive; and HIV is increased by 40% if the women are BV infected.¹⁵ Bacterial vaginosis is increased by HSV2 infection, because HSV2 infection promotes a reduction of the normal vaginal lactobacilli.¹⁶ Being pregnant is one of the most significant associated factors to BV.¹⁷ The most significant and recent risk factor in the incidence of BV is the use of multiple and the same-sex partners, which is suggested as a trigger of BV by disruption of the normal vaginal flora through exposure to alkaline semen, tampon use, clothes sharing, herbs, and flowers.¹⁸ This problem worsened in the community by leading different complications like preterm labor, infertility, abortion leading to fetal loss, chorioamnionitis, cervicitis, endometritis, urinary tract infections, and others.¹⁹

Globally, the overall prevalence of BV was over 29.2%, among women aged 14–49 years.²⁰ In white women the prevalence of BV was 23%, whereas in Mexican Americans it was 32%.²¹ In African-American women the prevalence of BV was about 51%, which is too high.¹⁰ In sub-Saharan African, the prevalence of BV was 50%.²² An estimate of 75,000 maternal deaths were reported annually in the world, the majority of them in resource-limited countries.¹⁷ According to the global report, more than 2.6 million antibiotic-resistant bacterial infections and 44,000 deaths were reported annually.^{23,24} The emergence and spread of antibiotic-resistant pathogens are a major public health issue today, which requires a global action in order to control further complications.²⁵

A single study in Addis Ababa showed 48.6% of BV prevalence, because as yet studies are limited in our country.²⁶ The cause and associated risk factors of BV are not well studied in Africa, especially in Ethiopia there are no well-structured data showing the causative agent, its prevalence, antibiotic resistance pattern, and associated factors of BV.²⁷ This is due to limitation of resources to fulfill diagnostic materials for both aerobic and anaerobic culture and treatment centers, and researchers were not rising their interest to do research on BV during the last decade.²⁸ As a result of those factors, the resource-limited countries are suffering a lot of maternal morbidity, disability, and death. Therefore, we were initiated to do this research on BV during women's reproductive age. Early diagnosis and treatment are used for the prevention and control mechanisms of BV. Understanding how and what factors are aggravated BV is essential to set a prevention and control strategy for BV. In order to prevent and control the prevalence and burden of BV, noticeable research findings should be required, because the previous and current finding makes a sound to policy-makers to move the resource towards the area having the problem and adjust preventive mechanisms. Therefore, the aim of the current study was to assess the prevalence, antibiotic resistance pattern, and associated factors of bacterial vaginosis among women of reproductive age, from Felege Hiwot Referral Hospital, Ethiopia.

Materials and Methods

Study Design and Period

A cross-sectional study was conducted from September 1, 2018 through October 2019 at Felege Hiwot Specialized Referral Hospital in Amhara Region Northwest Ethiopia. Felege Hiwot Specialized Referral Hospital is the only specialized hospital in Bahir Dar, which has well-standardized microbiology laboratory for bacterial culture, Gram stain, and molecular laboratory for HIV viral load detection.

Study Participants

The participants were HIV positive and pregnant, were suspected of bacterial vaginosis during the study period, and had no history of antibacterial therapy within two weeks prior to sample collection. Women having genital malignancy, used vaginal cream or ointment product, spermicides and vaginal lubricants or feminine sprays within 72 hours were excluded from this study.

Sample Size

The sample size was calculated using a single population proportion formula and considering the following assumptions such as, prevalence (P) = 50% (since there is no previous study to show the prevalence of BV caused by both *G. vaginalis* and aerobic bacteria, this study was forced to use 50% prevalence), confidence interval²⁹ of 95%, marginal error (d = 0.05%), Z = Z score for the 95% confidence intervals = 1.96, and 10% non-response rate.

$n = Z^2 p(1-p)/d^2 = (1.96)^2 0.5(0.5)/(0.05)^2 = 384$, and by adding 10% non-response rate, $n = 384 + 38$.

The total sample size was 422, of which 413 study participants were participating in the study. Nine patients were excluded based on the exclusion criteria.

The selected study participants were sampled using a convenience sampling technique because, in the study area, we did not find a list of the population and the case was very sensitive.

Data Collection

Data were collected after preparing standardized questionnaires and getting ethical clearance from an ethical review committee of Debre Markos University. Well-trained data collectors were used to collect the data based on socio-demographic, gynecological, and clinical variables. The vaginal swab samples were collected from symptomatic women, who are fulfilling the Amsel's criteria at antenatal care service clinic and maternity ward for bacteriological laboratory analysis.

Specimen Collection

After obtaining informed consent, two vaginal swab samples were collected from the lateral wall or posterior fornix of the vagina in each of the participants by a nursing professional working in the antenatal care service clinic and maternity ward. One sample was used for pH meter detection and Gram stain (Nugent score test) and the other was used for culture. The swab samples were placed into a clean and dry container and deliver to the microbiology laboratory before 24 hours of collection.³⁰

Laboratory Processing for Identification and Isolation of Bacteria

The laboratory processes were done at the Amhara Public Health Institute and Felege Hiwot Referral Hospital Microbiology Laboratory. The reasons to process at two laboratory sites were due to time saving and comparing

inter-laboratory results. The pH meter measurement was performed at the sample collection site. But Gram stain (Nugent score test) for anaerobic bacteria and culture for aerobic bacteria were performed at the microbiology laboratory diagnostic site as soon as the samples were delivered. After colony growth, biochemical tests were performed to identify the specific isolates. The biochemical tests like catalase, coagulase, CAMP test, bacitracin, and optochin were used for Gram-positive bacteria identification, whereas Kliger's iron agar, indole, motility, citrate agar, and urease tests were used to identify Gram-negative bacteria.³¹ The pure isolates were processed to perform antibacterial susceptibility tests.³²

Amsel's Test Criteria

This is a clinical diagnosis using at least 3–4 criteria such as vaginal pH > 4.5, color and consistency of the discharge. Vaginal pH was measured by rolling the swab over a pH meter strip immediately after swabbing and the color change result was recorded. A homogeneous thin white discharge was observed by the naked eye after swabbing. The whiff test was done by mixing of 10% KOH with vaginal discharge and detecting the presence of fishy odor.¹⁰

Gram Stain and Nugent Scoring System

Nugent scoring is a golden standard test for BV and vaginal swabs were used to prepare smears on clean, grease-free slides. The smear was allowed to air-dry and then fixed with 95% acetone alcohol. Then, the smear was Gram stained using Gram stain protocol and read by an experienced microbiologist under a light microscope using the oil immersion objective (×100). Slides were read according to Nugent's score as follows: *Lactobacillus* morphotypes were reported as large Gram-positive bacillus, *Gardnerella* were reported as small Gram variable bacilli. Using the Nugent scoring technique, score ranged from 0–4 for *Lactobacillus*; 0 indicating that ≥ 30 organisms were found and 4 indicated that no organism were found. In contrast, for *Gardnerella* a score 0 indicated that no organism were found and 4 indicated that ≥ 30 organisms were found. A total of numerical score was calculated ranging from 0–10 for each sample by summing the scores for each of the three morphotypes. The scores were classified into one of the three ranges to define a case of BV; a score in the range 0–3 indicated normal flora, 4–6 indicated mixed vaginal flora, which is not constituted BV, and 7–10 was constituted of BV.^{33,34}

Gram-stained slides were also examined for *Candida* species and polymorph nuclear cell (PMN) count for diagnosis of candidiasis and other Gram-positive and Gram-negative bacteria.

Culture

In this study, an aerobic culture technique was performed, since anaerobic culture facility was not found in the facility. Swabs collected for culture were transported and delivered to the laboratory immediately. The swabs were inoculated on chocolate agar and human blood agar and incubated at 37 °C for 72 hours within a 5–10% CO₂ humid atmosphere. The swabs inoculated on MacConkey agar were incubated at 37 °C for 48 hours. Biochemical tests were used to identify specific isolates. For Gram-positive isolates; catalase, coagulase, CAMP test, bacitracin, and optochin disk were used. For Gram-negative bacteria; indole, motility, Kligler iron agar test, simmon citrate agar test, urease test, and oxidase test were used.

Antibacterial Susceptibility Test

Mueller Hinton agar (MHA) media was used for antibacterial susceptibility test. The test was done using a modified Kirby–Bauer disk diffusion method and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guideline (2018). The following antimicrograms, like penicillin (10 µg), cefoxitin (30 µg), trimethoprim/sulfamethoxazole (25/23.75 µg), ceftriaxone (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamycin (10 µg), ciprofloxacin (5 µg), tobramycin (10 µg), and amoxicillin (10 µg), were used. Three to five pure isolates were taken from a pure culture medium and mixed with nutrient broth and wait for 3–5 hours. The mixture was compared with 0.5% McFarland standard for seeing equivalent turbidity standard. Then, the suspension was inoculated to MHA and allowed to dry for 5–15 minutes. Antibiotic disks were added to MHA 15 mm away from the edge and ≥24 mm apart from each other and incubated at 37 °C for 18–24 hours. After incubation, the inhibition zone was measured by caliper and interpreted as sensitive, intermediate, and resistant according to the CLSI guideline. The strains of *E. coli* ATCC 25922 was used as a quality control and was tested each time when susceptibility testing was performed.³⁵

Data Management, Analysis, and Presentation

Data were coded and checked for error and transferred from a questionnaire to a computer file (Epi Data 3.1),

and then exported to SPSS version 25 software for statistical analysis. Descriptive statistics were calculated to examine the socio-demographic characteristics of participants with bacterial infection outcome category. Figures and tables were used to present the findings. Odds ratio (OR) was used as a measure of the strength of association and reported with 95% confidence intervals.⁸ P value ≤ 0.05 was considered to be statistically significant. Categorical variables were compared using the Pearson χ^2 test.² The bivariate logistic regression model was fitted to identify possible associated factors with bacterial profile and the multivariate logistic regression model was fitted to control the backward stepwise process by taking $p \leq 0.2$.

Data Quality Control

Daily collected data were recorded and compiled. All steps in data collection and recording were monitored by the principal investigator. The reagents were checked for expiry date and appropriate storage of temperature and humidity. Positive and negative quality control tests were performed. The laboratory protocol was prepared and strictly followed. The quality assurance was ensured with good practice in preparing and reading Gram stains by competent microbiologists.³⁶

Operational Definition

Reproductive age group: the age group of women from 15–49 who can give birth to a child.

Results

Frequency of Socio-Demographic, Gynecological, and Clinical Variables

A total of 413 study participants was involved in this study. The majority of participants were orthodox Christian (377 (91.3)), urban dwellers (343 (83.1)), married (341 (82.6)), antenatal care service user pregnant (334 (80.9)), and having their age group from 31–35 years (134 (32.4)); of 134 participants, 68 (50.7%) were positive for bacterial vaginosis. Based on the educational and occupational level, the majority of them were illiterate and a housewife (122 (29.5%) and 189 (45.8%) respectively). In this study, some of the clinical variables were assessed for seeing their association with bacterial vaginosis, for instance, 22 (5.3%) diabetes mellitus, 249 (32.2%) human immunodeficiency virus, 24 (5.8%) herpes simplex virus 2, 46 (11.1%) gonorrhea, and 158 (38.3%) thin white with abnormal odor

discharge were assessed, of which 59.3% DM, 35.3% HIV, 45.8% HSV2, 54.2% gonorrhea, and 88.6% thin white with abnormal odor discharge had been associated with their magnitude (Table 1).

Table 1 The Characteristics of Socio-Demographic, Gynecological and Clinical Factors

S. no	Characteristics		Frequency (%)
1	Age	≤20	28 (6.8)
		21–25	31 (7.5)
		26–30	65 (15.7)
		31–35	134 (32.4)
		36–40	67 (16.2)
		≥40	88 (21.3)
2	Residence	Urban	343 (3.1)
		Rural	70 (16.9)
3	Religion	Orthodox	377 (91.3)
		Other	36 (8.7)
4	Educational level	Illiterate	122 (29.5)
		1–8	106 (25.5)
		9–12	114 (27.6)
		Higher	71 (17.2)
5	Occupation type	Housewife	189 (45.8)
		Merchant	32 (7.7)
		Employer	170 (41.2)
		Daily labor	22 (5.3)
6	Marital status	Married	341 (82.6)
		Other	72 (17.4)
7	Pregnancy	Yes	334 (80.9)
		No	79 (19.1)
8	DM	Yes	22 (5.3)
		No	391 (94.7)
9	HIV	Yes	249 (32.2)
		No	164 (67.8)
10	HSV2	Yes	24 (5.8)
		No	389 (94.2)
11	Gonorrhoeae	Yes	46 (11.1)
		No	367 (88.9)
12	Types of discharge	Thin white with abnormal odor	158 (38.3)
		Flocculent with normal odor	255 (61.7)
13	pH level	<4.5	265 (64.2)
		≥4.5	148 (35.8)

Abbreviations: DM, diabetes mellitus; HIV, human immunodeficiency virus; HSV2, herpes simplex virus 2; pH, power of hydrogen.

The Prevalence of Bacteria Causing Bacterial Vaginosis

Bacterial vaginosis is an inflammation of the vagina due to the overgrowth of bad bacteria in the vagina. The overall prevalence of bacteria which causes bacterial vaginosis was 39.5%. In this study, mainly Nugent scoring, culture test, and biochemical test were used to identify the micro-organisms. Indeed, different microbial profile was detected from the vagina during investigation; however, our concern were bacteria, so organisms other than bacteria (like *C. albicans* and *T. vaginalis*) were not included in this document, but they were reported to the hospital for better and timely patient management.

In this study, the total of 163 bacterial isolates were identified, of which 22.7% (37/163) isolates were *G. vaginalis*, which was the only anaerobic bacteria detected by Nugent scoring test. On the other hand, the predominant aerobic bacteria were *S. aureus* (25.4%), *S. agalactiae* (14.1%), and *E. coli* (13.5%), which were detected by culture and species were identified by biochemical test Figure 1.

Drug Susceptibility Pattern of Bacteria

In this study, drug susceptibility pattern was done only to aerobic bacteria, because the hospital laboratory did not have an anaerobic facility that supports the growth of *G. vaginalis*. The criteria of selecting antibiotics were based on the CLSI 2018 guideline recommendation, the availability of selected drugs on the market, and frequency of drugs prescribed in the area. Therefore, the most important drugs we selected and their antibiotic susceptibility pattern are presented in Table 2. Most of the Gram-positive bacteria were resistant to erythromycin (72.2%), trimethoprim/sulfamethoxazole (55.6%), and amoxicillin (46.1%), but susceptible to ciprofloxacin (92.2%), gentamycin (91%), clindamycin (84%), and cefoxitin (76.3%). Among Gram-positive bacterial species, *S. aureus* was highly prevalent bacteria and resistant to erythromycin (69.8%), trimethoprim/sulfamethoxazole (53.5%), and amoxicillin (39.5%), but despite this they were susceptible to ciprofloxacin (93%), gentamycin (93%), cefoxitin (90.7%), tobramycin (83.7%), and clindamycin (81.4%). On the other hand, Gram-negative bacteria were resistant to trimethoprim/sulfamethoxazole (73.4%) and amoxicillin (72.2%), but they were susceptible to ciprofloxacin (93.5%), gentamycin (92.3%), nitrofurantoin (82.6%),

Figure 1 The Prevalence of Vaginosis Causing Bacteria.

cefoxitine (58.4%), and tobramycin (54.8%). From Gram-negative bacteria, *E. coli* were highly prevalent bacteria and resistant to trimethoprim/sulfamethoxazole

(91.3%) and ceftriaxone (63.6%), but susceptible to ciprofloxacin (95.5%), gentamycin (93%), and nitrofurantoin (81.8%) (Table 2).

Table 2 Percentage of Antibigram Susceptibility Pattern

Bacterial Species	Test Result	Types of Antibigrams									
		AMOX	CD	Cefoxitine	CER	SXT	CIP	GEN	ER	TOB	F
<i>S. aureus</i>	R%	39.5	18.6	9.3	20.9	53.5	7	7	69.8	16.3	16.3
	S%	60.5	81.4	90.7	79.1	46.5	93	93	30.2	83.7	83.7
<i>S. agalactiae</i>	R%	8.7	4.3	21.7	8.7	52.2	0	0	56.5	8.7	0
	S%	91.3	95.7	78.3	91.3	47.8	100	100	43.5	91.3	100
CoNS	R%	90	25	40	20	60	16.3	20	91.3	40	35
	S%	10	75	60	80	40	83.7	80	8.7	60	65
Total	R%	46.1	16	23.7	16.5	55.2	7.8	9	72.6	21.7	17.1
	S%	53.9	84	76.3	83.5	44.8	92.2	91	27.4	78.3	82.9
<i>E. coli</i>	R%	44.4	–	50	63.6	91.3	4.5	7	–	68.2	18.2
	S%	55.6	–	50	36.4	8.7	95.5	93	–	31.8	81.8
<i>K. pneumoniae</i>	R%	100	–	33.3	38.9	55.6	8.5	8.5	–	22.2	16.7
	S%	0	–	66.7	61.1	44.4	91.5	91.5	–	77.8	83.3
Total	R%	72.2	–	41.6	51.2	73.4	6.5	7.7	–	45.2	17.4
	S%	27.8	–	58.4	48.8	26.6	93.5	92.3	–	54.8	82.6

Abbreviations: AMOX, amoxicillin; CD, clindamycin; CER, ceftriaxone; SXT, trimethoprim/sulphamoxazole; CIP, ciprofloxacin; GEN, gentamycin; ER, erythromycin; TOB, tobramycin; F, nitrofurantoin; R, resistance; S, susceptible.

Associated Risk Factors

In this study, different associated factors were assessed using bivariate and multivariate logistic analysis. The significant associated factors were pH level, age, pregnancy, HIV infection, DM, and discharge type. The pH level (≥ 4.5) had been 4.3 and 2 times highly increased for bacterial infection than pH level < 4.5 in both bivariate (4.3 (95% CI, 2.790, 6.563)) and multivariate (2 (95% CI, 1.031, 4.317)) analysis. The age of the participant, like age ≤ 20 (7 (95% CI, 1.57, 31.79)) and age from 31–35 (0.5 (95% CI, 0.30, 0.92)), pregnancy (1.7 (1.008, 2.705) and 4.6 (2.179, 9.696)), HIV infection (1.5 (1.031, 2.305) and 1.8 (1.166, 2.720)), types of discharge (0.01 (95% CI, 0.007, 0.024 and 0.2 (95% CI, 0.007, 0.032)) were associated with the overgrowth of bacteria from vagina

causing bacterial vaginosis. But factors like religion and occupational status were not significantly associated with the overgrowth of bacteria from the vagina, in both bivariate and multivariate logistic regression analysis (Table 3).

Discussion

Globally, bacterial vaginosis is a public health concern to women especially during the reproductive period. In this study, the prevalence of bacteria, which causes bacterial vaginosis was associated with different factors. About 413 study participants were investigated for bacterial vaginosis, of which the overall prevalence of bacteria which causes bacterial vaginosis was 39.5%. This finding is comparable to studies done in Addis Ababa (41.5%),²⁶ Kenya

Table 3 Factors Associated with Bacterial Overgrowth in the Vagina

Characteristics		Frequency (%)	Bacterial Vaginosis		COR (95% CI)	AOR (95% CI)
			Yes (%)	No (%)		
Age	≤ 20	28 (6.8)	2 (7.1)	26 (92.9)	7 (1.57, 31.79)	
	21–25	31 (7.5)	10 (32.3)	21 (67.7)	1.1 (0.48, 2.73)	
	26–30	65 (15.7)	25 (38.5)	40 (61.5)	0.9 (0.45, 1.69)	
	31–35	134 (32.4)	68 (50.7)	66 (49.3)	0.5 (0.30, 0.92)	
	36–40	67 (16.2)	27 (40.3)	40 (59.7)	0.8 (0.42, 1.55)	
	≥ 40	88 (21.3)	31 (35.2)	57 (64.8)	1	
Residence	Urban	343 (83.1)	128 (37.3)	215 (62.7)	1.7 (1.00, 2.82)	
	Rural	70 (16.9)	35 (50)	35 (50)	1	
Religion	Orthodox	377 (91.3)	148 (39.3)	229 (60.7)	1.1 (0.552, 2.212)	
	Other	36 (8.7)	15 (41.7)	21 (58.3)	1	
Occupation type	Housewife	189 (45.8)	92 (48.7)	97 (51.3)	0.7 (0.298, 1.789)	
	Merchant	32 (7.7)	10 (31.3)	22 (68.7)	1.5 (0.491, 4.724)	
	Employer	170 (41.2)	52 (30.6)	118 (69.4)	1.6 (0.632, 3.904)	
	Daily labor	22 (5.3)	9 (40.9)	13 (59.1)	1	
Pregnancy	Yes	334 (80.9)	124 (37.1)	210 (62.9)	1.7 (1.008, 2.705)	4.6 (2.179, 9.696)
	No	79 (19.1)	39 (49.4)	40 (50.6)	1	
DM	Yes	22 (5.3)	13 (59.3)	9 (40.7)	0.4 (0.180, 1.033)	0.2 (0.065, 0.911)
	No	391 (94.7)	150 (38.4)	241 (61.6)	1	
HIV	Yes	249 (32.2)	88 (35.3)	161 (64.7)	1.5 (1.031, 2.305)	1.8 (1.166, 2.720)
	No	164 (67.8)	75 (45.7)	89 (54.3)	1	
Gonorrhoeae	Yes	46 (11.1)	25 (54.2)	21 (45.8)	0.5 (0.273, 0.939)	
	No	367 (88.9)	138 (37.6)	229 (62.4)	1	
Types of discharge	Abnormal odor	158 (38.3)	140 (88.6)	18 (11.4)	0.01 (0.007, .024)	0.02 (0.007, 0.032)
	Normal odor	255 (61.7)	23 (9)	232 (91)	1	
Ph level	< 4.5	265 (64.2)	72 (27.2)	193 (72.8)	1	
	≥ 4.5	148 (35.8)	91 (61.5)	57 (38.5)	4.3 (2.790, 6.563)	2 (1.031, 4.317)

Abbreviations: DM, diabetes mellitus; HIV, human immunodeficiency virus.

(43.1%),³⁷ Nigeria (33%),^{24,38} and Cameroon (38%).^{39,40} The findings of the present study together with the previous study conducted in Addis Ababa showed a serious concern for women during the reproductive period in Ethiopia.

From a total of 163 (39.5%) confirmed pathogenic bacterial isolates, 37 (22.7%) were *G. vaginalis* which is anaerobic bacteria and 126 (77.3%) were aerobic bacteria. Among aerobic bacteria, nearly 3/4th of them were Gram positive 86 (68.3%) whereas about 1/4th of them were Gram negative 40 (31.7%), and these study findings were in line with a study conducted in Addis Ababa. The prevalence of Gram-positive bacteria was 105 (69.5%) and the prevalence of Gram-negative bacteria was 46 (30.5%). The predominant Gram-positive bacteria were *S. aureus* (43 (50%)) and *S. agalactiae* (23 (26.7%)); on the other hand, the predominant Gram-negative bacteria were *E. coli* (22 (55%)) and *K. pneumoniae* (18 (45%)). The current study findings were in agreement with studies conducted in India *S. aureus* (41.07%),⁴¹ Addis Ababa *E. coli* 43 (41%) and *K. pneumoniae* 28 (30.5%),²⁶ Belgium⁴² and America.⁴³ In this study, 22.7% (37/163) of *G. vaginalis* was detected using Nugent's scoring technique. This findings was lower than studies conducted in Cameroon (38%)⁴⁴ and Gambia (44.4%),⁴⁵ but in line with a study conducted in Italy (26.62%).⁴⁶

Over the last two decades, multidrug-resistant bacteria has been a dramatic surge and the number of pharmaceutical companies developing new antimicrobial agents has declined.²⁹ As a result, antibiotic-resistant bacteria are now one of the most pressing health problems and an alarming issue for the world health leaders, especially in resource-limited countries.⁴⁷ In the present study, the drug susceptibility pattern of aerobic bacteria was detected using routine culture media. Gram-positive bacteria were resistant to erythromycin (72.5%), trimethoprim/sulfamethoxazole (55.2%), and amoxicillin (46.1%), but susceptible to ciprofloxacin (92.2%), gentamycin (91%), clindamycin (84%), and cefoxitin (76.3%); similar findings were reported in Bahir Dar,⁴⁸ Addis Ababa,²⁶ and Pakistan.⁴⁹ Among Gram-positive bacteria *S. aureus* was resistant to erythromycin (69.8%), trimethoprim/sulfamethoxazole (53.5%), and amoxicillin (39.5%), but despite this, they were susceptible to ciprofloxacin (93%), gentamycin (93%), cefoxitin (90.7%), tobramycin (83.7%), and clindamycin (81.4%). The finding agreed with previous reports in Ethiopia and abroad.⁵⁰ On the other hand, Gram-negative bacteria were resistant to trimethoprim/sulfamethoxazole (73.4%) and amoxicillin

(72.2%), but susceptible to ciprofloxacin (93.5%), gentamycin (92.3%), nitrofurantoin (82.6%), cefoxitin (58.4%), and tobramycin (54.8%). Among Gram-negative bacteria, *E. coli* was the predominant isolated bacteria and resistant to erythromycin (95.5%), trimethoprim/sulfamethoxazole (91.3%), and ceftriaxone (63.6%). The current findings were in agreement with a study conducted at Bahir Dar,⁴⁸ Gondar,^{50–52} Addis Ababa,²⁶ India,⁵³ and Portugal.⁵⁴

Gram-negative bacteria were resistant to most antibiotics more than Gram-positive bacteria, for the reason that the biological structure of Gram-negative bacteria is much harder, which acts as a permeability barrier and is used for drug efflux pumps.⁴⁷ Generally, antibiotic resistance bacteria uses a number of mechanisms like physical removal of the drug from the cell, modification of target site, and modification of the folic acid synthesis pathway to resist the inhibiting effect of drugs.^{52,55} The World Health Organization has a future strategy by focusing on the discovery and development of new antibiotics specifically active against multidrug and extensively drug-resistant Gram-negative bacteria.⁵⁶ So, the local health professionals use the updated antibiotics based on WHO recommendations. In addition, the health professionals must use narrow spectrum drugs based on an antibiotic susceptibility test profile rather than using broad spectrum antibiotics. Furthermore, the health minister of the local country should support this idea and access infrastructures to fulfill the health facility, especially the antenatal care clinic and maternity wards.

In the present study, there was a significant association between bacteria causing BV and associated factors to the overgrowth of bacteria in the vagina. Among different factors, the pH level of the vagina (≥ 4.5), being pregnant, history of HIV infection, and age of the women showed a significant association with bacterial overgrowth in the vagina and causing BV. The vagina pH level ≥ 4.5 had been 4 and 2 times significantly associated to BV than pH level < 4.5 by using bivariate and multivariate logistic regression analysis respectively. Early age (≤ 20 years) women had been associated with the overgrowth of bacteria in the vagina causing BV. Similar studies were conducted in Addis Ababa, Ethiopia²⁶ and a South American population.⁴³ Being pregnant was 2 and 5 times significantly associated with BV in bivariate and multivariate logistic regression analysis respectively. This finding was in agreement with the fact that in the women in physiological change, leading to hormonal change, which is prone

to vaginal infection during pregnancy, the number of vaginal *Lactobacillus* was decreased, and this leads to decrease the level of vaginal lactic acid and H_2O_2 .⁵⁷ However, the fact is contradicted with a study conducted India in 2016, saying that during the pregnancy period, the vaginal microbiota is conditioned by high estrogen levels and has a good supply of glycogen and a high percentage of lactobacillary flora which significantly reduces the multiplication of pathogenic organisms, due to production of defense factors by lactobacilli.⁴¹ The women who had a history of previous HIV infection had been 1.5 and 1.8 times highly risked to bacterial overgrowth from their vagina and exposed to BV (OR = 1.5 (95% CI, 1.031, 2.305) and OR = 1.8 (95% CI, 1.166, 2.720)). This study's findings were different from studies conducted in Nigeria⁵⁸ and America.⁵⁹ This might be due to environmental, socioeconomic, and cultural differences between Ethiopia and abroad.⁶⁰ Based on scientifically proved reason, during HIV infection and pregnancy the immune status of women is decreased, leading to various bacterial infections.^{52,61}

However, types of discharge had a significant association with BV, and the association was protective to BV (OR = 0.01 (95% CI, 0.007, 0.024) and OR = 0.02 (95% CI, 0.007, 0.032)). On the other hand, factors like religion and occupational status of the women were not surprisingly associated with BV in both bivariate and multivariate logistic regression analysis. This is in line with a study conducted in India,⁶² but on the other hand, another study conducted in India had showed religion and occupational status of the women had a significant association to bacterial overgrowth in the vagina and causing BV.⁶³

Limitation

The limitation of this study was the absence of anaerobic culture and molecular test facility.

Conclusion

In this study, the Nugent scoring technique and aerobic culture media were used to detect pathogenic bacteria. The overall prevalence of bacteria causing BV was very high. The majority of the isolated bacteria were *G. vaginalis*, *S. aureus*, GBS, and *E. coli* and most of them were resistant to most of the drugs routinely prescribed in the hospital, like amoxacillin, cefoxitine, erythromycin, trimethoprim/sulfamethoxazole, and gentamycin. Therefore, early identification of factors which leads to the overgrowth of different bacteria in the vagina and selecting

appropriate drugs for the specific bacterial isolate are very important for preventing and controlling the complication of BV from women during reproductive age.

Based on the current research findings, the authors recommend to the health institutions, especially Felege Hiwot Referral Hospital administrative office and all staffs to give especial attention for women during the reproductive age. Moreover, the health professional do early case identification based on laboratory confirmation and give immediate response as much as possible, because improving the health status of the women leads to improve the community health status indirectly, since the women takes the lion share of the community development both in health and economy. Other researchers should do further longitudinal study by using more advanced technique like anaerobic culture and molecular diagnostic tests.

Abbreviations

BV, bacterial vaginosis; CVL, cervicovaginal lavage; H_2O_2 , hydrogen peroxide; IUD, intrauterine device; PID, pelvic inflammatory disease; PMN, polymorphonuclear cell; SPSS, Statistical Package for Social Science; STI, sexually transmitted infection.

Data Sharing Statement

All data generated and analyses during this study were included in the manuscript.

Ethical Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki. Ethical clearance was obtained from DebreMarkos University, Institutional Research Ethics Review Committee (IRERC). The research proposal was submitted to DebreMarkos University community and research coordinating office of the health science college to the IRERC. The committee has nine members. The IRERC scrutinized the proposal for ethical issue and made the research to correct and incorporate essential elements. The IRERC were approved the research by a signing ethical approval number (HSC/R/C/Ser/Co/103/11/11), by the date 18/03/2019. Permission was obtained from Felege Hiwot Referral Hospital. The samples taken from the study participants were only for the sake of this study. A written informed consent was obtained from mothers after explaining the purpose and objective of the study. Participants had a full right to continue or withdraw

from the study. All information was kept confidential by assigned code and assessed only by the principal investigator and supervisors. The laboratory results were communicated with physicians or nurses for the early management of the participants.

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Author Contributions

All authors made a significant contribution to this study reported, whether that is in the conception, study design, execution, acquisition of data, analysis and data interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this study.

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