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ORIGINAL RESEARCH

Vulvovaginitis Prevalence Among Women in Gondar, Northwest Ethiopia: Special Emphasis on Aerobic Vaginitis Causing Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors

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Background: Genital tract infections are posing a series of public health challenges for women in both developed and developing countries. Microbial infections of the vagina can lead to serious medical complications such as preterm labor, amniotic fluid infection, premature rupture of the fetal membranes, and low birth weight of the neonate, leading to high perinatal morbidity and mortality. In Ethiopia, limited information is found on the burden, antimicrobial susceptibility profile and associated factors for aerobic vaginitis. Thus, this study was aimed to determine the burden of AV, antimicrobial susceptibility profile of aerobic bacterial isolates and associated factors among women attending Gondar town health facilities, northwest Ethiopia.

Methods: A health facility-based cross-sectional study was conducted on 214 study participants from February 1 to May 31, 2019. For all consecutive women, demographic variables were collected using a structured questionnaire and two vaginal swabs for each were collected. The diagnosis of AV and BV was based on the composite score of Donders and Nugent criteria, respectively. All bacteria were isolated and characterized by conventional culture techniques. The antimicrobial susceptibility pattern was performed using the disc diffusion technique. Logistic regression, univariate and multivariate analysis were carried out. A p-value ≤ 0.05 at 95% CI was considered as statistically significant.

Results: The overall prevalence of vulvovaginitis among women was 50%. The identified aetiologies of vulvovaginitis were bacterial vaginosis (35.5%), candidiasis (23.8%), aerobic vaginitis (22.9%) and trichomoniasis (3.3%). Aerobic bacteria, especially *Enterococcus faecalis* and *Escherichia coli*, were predominantly isolated in the vaginal samples. The prevalence of the multidrug resistance rate was 38.98%. The isolated Gram positive bacteria were sensitive to antibiotics like vancomycin, cefoxitin, ciprofloxacin, clindamycin, and gentamicin, whereas the Gram negative bacteria isolates were sensitive to ciprofloxacin, gentamicin and meropenem.

Conclusion: The high burden of bacterial vaginosis and aerobic vaginitis was reported. Therefore, regular screening of women using microbiological diagnosis should be promoted. The common bacteria isolated were *Enterococcus faecalis* and *Escherichia coli*. Additionally, antibiotics like vancomycin, cefoxitin, ciprofloxacin, clindamycin, gentamicin, and meropenem were shown to have good action against the majority of bacteria isolates.

Keywords: aerobic vaginitis, bacterial vaginosis, candidiasis, antimicrobial susceptibility

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Background

A number of protective Lactobacillus species dominate the healthy vaginal microbiota in most reproductive-age women. However, any imbalance in the naturally occurring bacterial microbiota may result in infections and/or clinical symptoms such as vulvovaginal candidiasis (VVC), bacterial vaginitis (BV), *Trichomonas vaginalis* (*T. vaginalis*), cytolytic vaginosis (CV) or aerobic vaginitis (AV), abnormal leucorrhea, increased discharge, vulval itching and burning pain.[1–3] Vaginal discharge may originate from various causes; physiological, infective, inflammatory, neoplastic or iatrogenic.[2]

The term "aerobic vaginitis (AV)" was coined in 2002 to meet the need to describe "bacterial vaginosis (BV)", another condition of vaginal dysbiosis.[4] According to Donders et al, aerobic vaginitis is a state of abnormal vaginal microbiota and disruption in Lactobacillus dominance accompanied by more extreme inflammatory changes than BV and the presence of mainly aerobic enteric commensals or pathogens (*Escherichia coli, Staphylococcus aureus*, group B streptococcus (GBS), and enterococci).[5–7] Aerobic vaginitis (AV), also known as desquamative inflammatory vaginitis in its severest manifestation, is still not widely known and, so, it is underdiagnosed by many clinicians, and may even have been mistaken as BV.[2,8] Aerobic vaginitis, being present in 2.9–23.7% of women,[2] has been connoted as a possible cause of serious adverse gynecological and obstetric outcomes such as an increased risk of acquiring sexually transmitted infections (STIs), premature rupture of membranes, preterm labour, and ascending chorioamnionitis.[9] Although data concerning the prevalence of AV in the general population is still scarce, there are studies done among pregnant and non-pregnant women. The prevalence of AV among pregnant women ranges from 2 to 51%,[10–12] and among non-pregnant women it ranges from 8.5 to 48.3%.[8,12] The paucity of studies relative to AV emphasizes the need for a better understanding of the epidemiology, etiology, and pathogenesis of AV in both pregnant and non-pregnant women, particularly in Africa, where AV has been poorly investigated.[7] Specific to Ethiopia, AV was poorly investigated. Of course, there is a study done on women of reproductive age attending Felege Hiwot referral Hospital. The study highlighted the common causes of vaginal infections, excluding AV.[13]

Bacterial vaginosis (BV) is a modification of the vaginal microbiota characterized by a diminished or absent microbiota and a significantly increased colonization of several anaerobic or facultative microorganisms, mainly *Gardnerella vaginalis, Prevotella spp, Bacteroides spp, Mobiluncus spp*, gram positive cocci, and genital mycoplasma (*Mycoplasma hominis* and *Ureaplasma urealyticum*).[14] Bacterial vaginosis (BV) is one of the most common diseases in women of reproductive age.[15] The prevalence of BV in China ranged from 5.9% to 15.4%.[16] However, in the United States, this number was 16.3% to 29.2%,[17] in South America 5.6%,[18] and it reached up to 50% in Africa.[19] Even though the number is very few, there are reports that show the prevalence of BV, which ranges from 0.5% to 19.4% in Ethiopia.[13,20–22]

Vulvovaginal candidiasis (VVC), despite the fact that it is debatable whether the symptomatic presence of fungi from the genus Candida in the vagina should be referred to as vulvovaginal "candidiasis" or "candidosis" (VVC), since inflammation is not always present,[15] is considered the second most frequent cause of "vulvovaginitis" symptoms, after BV. Candida can be responsible for the symptoms of vulvovaginitis in up to 30% of cases.[23] Vaginal Candida colonization is found in at least 10 to 20% of healthy, reproductive age, asymptomatic women.[24] It is higher in pregnant women (20 to 40%), especially in the third trimester and in immunocompromised women. Up to 75% of women will experience at least one episode during their lifetime.[25] Half of these will suffer at least one additional episode and 5–10% will have recurrent VVC.[26]

Trichomonas vaginalis (TV) is one more important pathogen that causes trichomoniasis. It is a protozoan parasite which is considered to be sexually transmittable It is known that trichomoniasis can lead to inflammatory pelvic disease, reproductive dysfunction, and an increased risk of premature rupture of fetal membranes and low birth weight.[27,28] Despite a difference in the diagnosis sensitivity of methods for the diagnosis of *T. vaginalis*, the prevalence of *T. vaginalis* varies from 6.6% to 73%.[28,29] As it is known, 90% of trichomoniasis are from resource-limited settings, specifically sub-Saharan countries.[30] Although population-based studies were lacking in Ethiopia, different studies reported different prevalences of *T. vaginalis*; 5.3% from Asella Teaching Hospital, central Ethiopia,[31] 3.1% from Hawassa comprehensive and specialized hospital, Southern Ethiopia,[32] 4.9% from Jimma University Specialized Hospital, Southwest Ethiopia[33] and 2.1% from Felege Hiwot referral Hospital, Ethiopia: a cross sectional study.[13]

Though the association of vaginitis with genital tract infection is a major problem globally, several studies on

the prevalence of bacterial vaginosis have been conducted, but no study on AV has been conducted. Therefore, this study was done to fill the information gap on the prevalence of AV, aerobic bacterial profiles, antimicrobial susceptibility patterns, and associated factors.

Materials and Methods

The health facility-based cross-sectional study was conducted from February 1 to May 31, 2019, at the University of Gondar comprehensive specialized Hospital and Dr. Getachew private gynecology and obstetrics specialty clinic, the gynecologic referral facilities in the town. The facilities are located in Gondar town, which is located 737 km from Addis Ababa, the capital city of Ethiopia. Women presenting with vaginal discharge, no history of antibiotic therapy within the past two weeks prior to their attendance were included, while those with vaginal bleeding and those who were unable to provide vaginal specimens were excluded from the study. A single population proportion was used to estimate the sample size based on the prevalence of 15.4% from the previous study conducted by Mulu et al[13] with a confidence level of 95%, an error margin of 0.05 and considering a 10% non-response rate. Accordingly, the final sample size was 220.

Data and Sample Collection

After getting permission from study participants, using a pre-tested structured questionnaire, socio-demographic characteristics, associated factors and relevant clinical information were collected. The data was collected by interviewing study participants by experienced midwifery BSC nurses. After physical and obstetrical examination by the attending gynecologist, two vaginal samples were collected from the lateral wall of the vagina using sterile cotton tipped applicator swabs. The first sample was spread onto three slides and was mixed with a drop of saline on one slide and a drop of 10% potassium hydroxide (KOH) on a second slide; the third slide was Gram-stained. A second vaginal swab sample was used for aerobic culturing aimed at detection of aerobic bacterial growth. All vaginal samples collected at Dr. Getachew private gynecology and obstetrics specialty clinic were transported immediately to the Microbiology laboratory of the University of Gondar comprehensive specialized hospital by using a vaccine carrier at a temperature of 2–8 degree Celsius.

Laboratory Identification Techniques

Direct Microscopy: Wet-Smear Preparation and Gram Staining

Trichomonas vaginalis was identified by its typical morphology and motility on saline wet mount microscopy examination under bright field microscopy at 40× objective. Simultaneously, pseudo-hyphae or budding yeast cells were observed for confirmation of *Candida* spp on a 10% KOH preparation.

The diagnosis of AV was done based on the diagnostic criteria on the basis of a composite AV score recommended by Donders et al[4] which combine information about bacterial flora, epithelial disruption and inflammation, derived from phase contrast microscopy at 400x magnification of fresh or rehydrated wet mount. Hence, a composite AV score of < 3 corresponds to "no signs of AV", 3–4 to "mild AV", 5–6 to moderate AV, and any score >6 to "severe AV".[4]

Bacterial vaginosis was diagnosed by Gram staining using 100x oil immersion objective under bright field microscopy, and scoring was determined using Nugent criteria scoring method. This approach results in an overall score in which 0–3 indicates a "normal" lactobacilli-dominated microbiota, the score of 4–6 refers to an "intermediate microbiota" and the score of 7–10 indicates BV.[34]

Culture Techniques

A second vaginal swab sample was inoculated on Blood Agar (Oxoid Ltd. England), MacConkey agar (Oxoid Ltd. England) and Chocolate Agar (Oxoid Ltd. England). Blood agar plates and MacConkey agar were incubated at 35–37 degree Celsius for 18 to 24 hours aerobically. Chocolate Agar was incubated with 5% carbon dioxide in a candle jar for micro-aerophillic environment. Plates with no growth after 24 hours were re-incubated for a further 24 hours.

Bacterial Identification

Pure isolates of bacterial pathogen were preliminarily characterized by colony morphology, Gram stain, hemolytic reactions on blood agar plates, color change of media around the colony, odor, shape and texture on agar plates. For Gram positive bacteria biochemical tests like catalase, coagulase, Christie-Atkinson-Munch-Peterson (CAMP) test, bile esculin hydrolysis test, mannitol fermentation test and Bacitracin disc tests were done. Identification of Gram negative bacteria at species level was done by employing an array of routine biochemical tests such as

Indole, Urea, Triple Sugar Iron Agar, Lysine Decarboxylase, Citrate Utilization, Hydrogen Sulfide (H_2S) production, gas formation and Motility.[35]

Antimicrobial Susceptibility Testing

A suspension of pure colony from each confirmed culture isolate was done in sterile normal saline by taking 2–3 colonies and incubated at 37°C for at least 30 minutes. The suspension was adjusted by using 0.5% McFarland standard. A sterile cotton tipped applicator stick was used for uniform distribution of the suspension on Muller Hinton agar (Oxoid Ltd. England). Then, Modified Kirby-Bauer disk diffusion technique was implemented.

For antimicrobial susceptibility patterns, the drug discs were selected as per the recommendations of CLSI 2019; hence, antibiotics such as amox/clavulanic acid (AUG 30 µg), ampicillin (AMP 25µg), cefoxitin (FOX 30 µg), ceftriaxone (CRO 30 µg), ciprofloxacin (CPR 5 µg), clindamycin (DA 2 µg), chloramphenicol (CHL 30 µg), doxycycline (DOX 30 µg), erythromycin (ERY 15 µg), gentamycin (CN 10 µg), meropenem (MER 10 µg), penicillin (PEN 10 µg), tetracycline (TE 30 µg), Sulfamethoxazole-trimethoprim (SXT 25 µg) and vancomycin (VAN 30 µg) were used. After incubation at 37 degree Celsius for 24hrs, the zone of inhibition was measured by a caliper. Finally, the results were interpreted as susceptible, intermediate and resistant using clinical and laboratory standards institute (CLSI) 2019 performance standards for antimicrobial susceptibility testing interpretation chart.[36]

Quality Control

The questionnaire was pre-tested before the actual study begins so as to make sure whether the questionnaire was appropriate and understandable among women other than the ones in the actual study area. The collected data were checked and supervised daily for consistency, completeness and accuracy by the investigators.

Every activity in the laboratory was done by strictly adhering to the standard operation procedures (SOPs) of the laboratory. Sterility of cotton swabs, test tubes, culture plates and other important equipment was done by autoclaving at 121 degree Celsius for 15 minutes. Contamination was avoided by using proper aseptic techniques, using Biosafety cabinet level 2 for sample processing.

Culture media were tested for sterility by randomly selecting prepared culture media and incubating theme overnight to check for growth. Also, ability of culture media to grow and differentiate was done by inoculating ATCC control strains of *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923). Furthermore, gram staining quality was checked by preparing smears from known *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) strains, then examined under the microscope. For Antimicrobial susceptibility test, bacterial suspensions were checked by comparing with 0.5 McFarland standards.

Data Analysis

Data entry was done using EPI-Info version-7.2 and analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version- 21 software. Data quality was ensured by double data entry. Analysis of variables was made using descriptive statistics. The Chi-square test was used to evaluate associations between pregnant, non-pregnant, married, and single women in each type of vulvovaginitis in this study. Logistic regression, univariate and multivariate analysis were used to assess the association between dependent and independent variables. The strength of associations was analyzed using the odds ratio (OR) with 95% confidence interval (95% CI). Then, independent associations between potential factors, with p-value < 0.2 in the univariate analysis, and AV were further evaluated by multivariate logistic regression analysis. A p-value < 0.05 was considered as statistically significant.

Results

Socio-Demographic Characteristics of Study Participants

A total of 214 women were examined for vulvovaginitis in this study. From these, 117 were from Dr. Getachew private gynecology and obstetrics specialty clinic and 97 participants from University of Gondar Comprehensive Specialized hospital. The median age of the participants was 26 years (range: 18–62 years). The majority of the study participants (169 (79.0%)) were from urban areas (Table 1).

Prevalence Vaginitis Among Women

Among 214 women study participants, the overall abnormal vaginal ecosystems was 120 (56%). The prevalence of AV was 49 (22.9%) (95% CI; 17.3–29.0) and vulvovaginitis caused by *Candida spp*. was 51 (23.8%) (95% CI; 17.8–29.9). The prevalence of BV was 76 (35.5%) (95% CI; 29.4–41.6) and only 7 study participants (3.3%) (95% CI; 0.9–5.6) had genital trichomoniasis (Table 2). Of the 49 (22.9%) AV positive women, 16.8% and 6.1% of them had mild and moderate AV respectively (Figure 1).

Table 1 Socio-Demographic, Behavioral Variables Among Women Attending Health Facilities with Healthy Microbiota, Abnormal Microbiota, Bacterial Vaginosis, Aerobic Vaginitis, Candidiasis, and Trichomoniasis at Gondar Town, Northwest Ethiopia, February 1 to May 31, 2019

Variables		Total N (%)	Healthy Microbiota N (%)	Abnormal Microbiota N (%)	Bacterial Vaginosis N (%)	Aerobic Vaginitis N (%	Candidiasis N (%)	Trichomoniasis N (%)
Age group	18–24	72 (33.6)	36 (37.5)	36 (30.0)	22 (28.9)	12 (24.5)	15 (29.4)	2 (28.6)
	25–34	104 (48.6)	44 (45.8)	60 (50.0)	41 (53.9)	25 (51.0)	27 (52.9)	5 (71.4)
	35–44	28 (13.1)	13 (13.5)	15 (12.5)	8 (10.5)	6 (12.2)	7 (13.7)	0 (0.0)
	> 45	10 (4.7)	1 (1.0)	9 (7.5)	5 (6.6)	6 (12.2)	2 (3.9)	0 (0.0)
Marital status	Married	145 (67.8)	61 (64.9)	84 (70.0)	55 (72.4)	37 (75.5)	37 (72.5)	4 (57.1)
	Single	45 (21.0)	20 (21.3)	25 (20.8)	15 (19.7)	8 (16.3)	11 (21.6)	1 (14.3)
	Divorced	24 (11.2)	13 (13.8)	11 (9.2)	6 (7.9)	4 (8.2)	3 (5.9)	2 (28.6)
Educational Status	Unable to read and write	36 (16.8)	14 (14.9)	22 (18.3)	16 (21.1)	11 (22.4)	8 (15.7)	2 (28.6)
	Reading and Writing	19 (8.9)	8 (8.5)	11 (9.2)	5 (6.6)	5 (10.2)	8 (15.7)	0 (0.0)
	Elementary school	46 (21.5)	18 (19.1)	28 (23.3)	16 (21.1)	13 (26.5)	9 (17.6)	3 (42.9)
	Secondary school	48 (22.4)	23 (24.5)	25 (20.8)	15 (19.7)	11 (22.4)	6 (11.8)	0 (0.0)
	College and above	65 (30.4)	31 (33.0)	34 (28.3)	24 (31.6)	9 (18.4)	20 (39.2)	2 (28.6)
Job status	Self Employed	40 (18.7)	17 (18.1)	23 (19.2)	13 (17.1)	11 (22.4)	9 (17.6)	1 (14.3)
	House wife	8 (37.4)	29 (30.9)	51 (42.5)	35 (46.1)	24 (49.0)	17 (33.3)	2 (28.6)
	Civil servant	50 (23.4)	21 (22.3)	29 (24.2)	21 (27.6)	11 (22.4)	14 (27.5)	3 (42.9)
	Unemployed	44 (20.6)	27 (28.7)	17 (14.2)	7 (9.2)	3 (6.1)	11 (21.6)	1 (14.3)
Residence	Rural	45 (21.0)	19 (20.2)	26 (21.7)	17 (22.4)	9 (18.4)	9 (17.6)	2 (28.6)
	Urban	169 (79.0)	75 (79.8)	94 (78.3)	59 (77.6)	40 (81.6)	42 (82.4)	5 (71.4)
Pregnancy status	Pregnant	39 (18.2)	14 (14.9)	25 (20.8)	18 (23.7)	7 (14.3)	16 (31.4)	2 (28.6)
	Non pregnant	175 (81.8)	80 (85.1)	95 (79.2)	58 (76.3)	42 (85.7)	35 (68.6)	5 (71.4)
Douching frequency per day	Once	32 (15.0)	8 (8.5)	24 (20.0)	14 (18.4)	12 (24.5)	9 (17.6)	1 (14.3)
	Twice	67 (31.3)	31 (33.0)	36 (30.0)	19 (25.0)	14 (28.6)	13 (25.5)	4 (57.1)
	Three and above	115 (53.7)	55 (58.5)	60 (50.0)	43 (56.6)	23 (46.9)	29 (56.9)	2 (28.6)
Number of life time sexual partner	One	113 (52.8)	53 (56.4)	60 (50.0)	38 (50.0)	18 (36.7)	29 (56.9)	2 (28.6)
	Two	82 (38.3)	33 (35.1)	49 (40.8)	29 (38.2)	26 (53.1)	19 (37.3)	5 (71.4)
	Three and above	19 (8.9)	8 (8.5)	11 (9.2)	9 (11.8)	5 (10.2)	3 (5.9)	0 (0.0)
Have you used family planning	Yes	98 (45.8)	37 (39.4)	61 (50.8)	37 (48.7)	28 (57.1)	25 (49.0)	4 (57.1)
	No	116 (54.2)	57 (60.6)	59 (49.2)	39 (51.3)	21 (42.9)	26 (51.0)	3 (42.9)
Reused napkin during menstruation	Yes	87 (40.7)	40 (42.6)	47 (39.2)	30 (39.5)	21 (42.9)	20 (39.2)	3 (42.9)
	No	127 (59.3)	54 (57.4)	73 (60.8)	46 (60.5)	28 (57.1)	31 (60.8)	4 (57.1)

(*Continued*)

Table 1 (Continued).

Variables		Total N (%)	Healthy Microbiota N (%)	Abnormal Microbiota N (%)	Bacterial Vaginosis N (%)	Aerobic Vaginitis N (%	Candidiasis N (%)	Trichomoniasis N (%)
Previous similar disease condition	Yes No	100 (46.7) 114 (53.3)	49 (52.1) 45 (47.9)	51 (42.5) 69 (57.5)	30 (39.5) 46 (60.5)	23 (46.9) 26 (53.1)	25 (49.0) 26 (51.0)	2 (28.6) 5 (71.4)
Have you ever aborted	Yes No	41 (19.2) 173 (80.8)	19 (20.2) 75 (79.8)	22 (18.3) 98 (81.7)	12 (15.8) 64 (84.2)	8 (16.3) 41 (83.7)	12 (23.5) 39 (76.5)	1 (14.3) 6 (85.7)
Previous uterine surgery	Yes No	9 (4.2) 205 (95.8)	5 (5.3) 89(94.7)	4 (3.3) 116 (96.7)	3 (3.9) 73 (96.1)	1 (2.0) 48 (98.0)	1 (2.0) 50 (98.0)	0 (0.0) 7 (100.0)
Vaginal itching	Yes No	119 (55.6) 95 (44.4)	44 (46.8) 50 (53.2)	75 (62.5) 45 (37.5)	48 (63.2) 28 (36.8)	32 (65.3) 17 (34.7)	36 (70.6) 15 (29.4)	6 (85.7) 1 (14.3)
Vaginal burning sensation	Yes No	158 (73.8) 56 (26.2)	64 (68.1) 30 (31.9)	94 (78.3) 26 (21.7)	60 (78.9) 16 (21.1)	41 (83.7) 8 (16.3)	39 (76.5) 12 (23.5)	5 (71.4) 2 (28.6)
Pant exchange frequency per week	Once Twice Three times and above	84 (39.2) 81 (37.9) 49 (22.9)	33 (35.1) 41 (43.6) 20 (21.3)	51 (42.5) 40 (33.3) 29 (24.2)	29 (38.2) 26 (34.2) 21 (27.6)	27 (55.1) 13 (26.5) 9 (18.4)	19 (37.3) 17 (33.3) 15 (29.4)	4 (57.1) 1 (14.3) 2 (28.6)

The proportion of AV among married women was higher 37 (75.5%), than single women 12 (24.5) (p=0.186). The proportion of AV was higher among non-pregnant women 42 (85.7%) as compared to pregnant women 7 (14.3%) (p=0.416). The proportion of *Candida spp*. among married women was higher 37 (72.5%), than single women 14 (27.5%) (p=0.402). The proportion of *Candida spp*. was higher among non-pregnant women 35 (68.6%) as compared to pregnant women 16 (31.4%) (p=0.005) (Table 2).

Bacterial Isolation by Culture Media

Culture was done in 49 (22.9%) AV confirmed cases; from these, 44 of them were culture positive while five samples were culture negative. A total of 59 bacterial isolates were recovered, of which 19 (32.2%) of the isolates were Gram-negative and 40 (67.8%) were Gram-positive bacteria. Among Gram-positive bacteria, *E. faecalis* was the predominant pathogen with 19 (32.2%) isolates whereas *E. coli*, with 12 (20.4%) was the commonest among Gram negative organisms (Figure 2).

Antimicrobial Susceptibility Patterns of Isolated Bacteria

For all 59 bacterial isolates, antimicrobial susceptibility test was done as per the CLSI 2019 recommendations and interpretations. The identified Gram positive bacteria were 100% resistant to penicillin, except *E. faecalis*, which showed 10 (52.6%) resistance. All of *S. aureus* and CONS isolates were susceptible to cefoxitin. Drugs like vancomycin, cefoxitin, ciprofloxacin, clindamycin and gentamicin were found to be more effective against the Gram positive isolates with 100% sensitivity while Gram negative isolates were 100% resistant to ampicillin (Table 3).

Table 2 Etiology of Vulvovaginitis Among Women Attending Health Facilities with Pregnancy and Marital Status at Gondar Town, Northwest Ethiopia, February 1 to May 31, 2019

	Pregnant N (%)	Non-Pregnant N (%)	p-value	Married N (%)	Single N (%)	p-value
Bacterial vaginosis (n=76)	18 (23.7)	58 (76.3)	0.125	55 (72.4)	21 (27.6)	0.284
Aerobic vaginitis (n=49)	7 (14.3)	42 (85.7)	0.416	37 (75.5)	12 (24.5)	0.186
Candida Spp. (n=51)	16 (31.4)	35 (68.6)	0.005*	37 (72.5)	14 (27.5)	0.402
Trichomonas vaginalis (n=7)	2 (28.6)	5 (71.4)	0.471	4 (57.1)	3 (42.9)	0.541

Note: *P-value < 0.05.

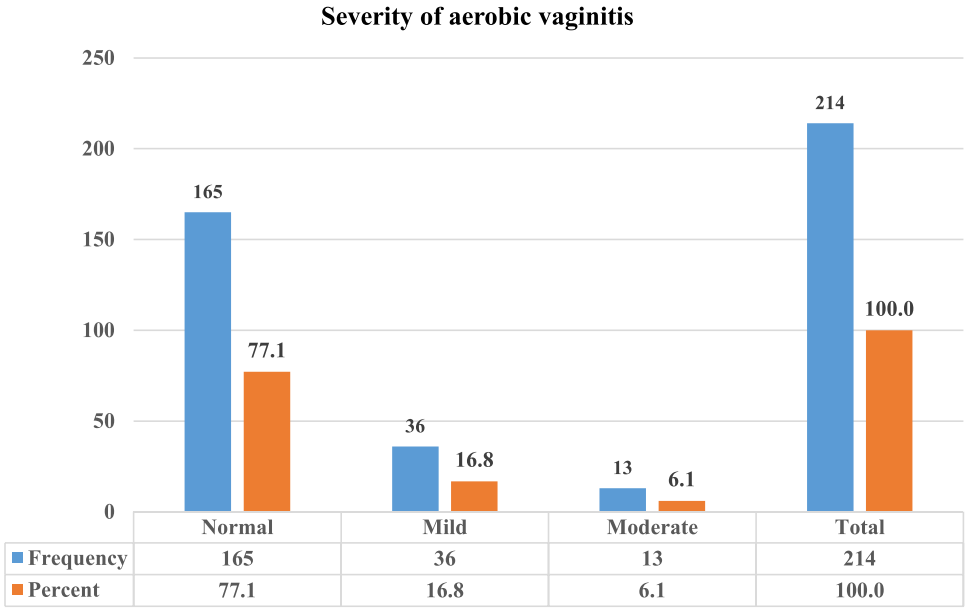

Figure 1 Severity of aerobic vaginitis among women attending at Gondar town health facilities, northwest Ethiopia, February 1 to May 31, 2019.

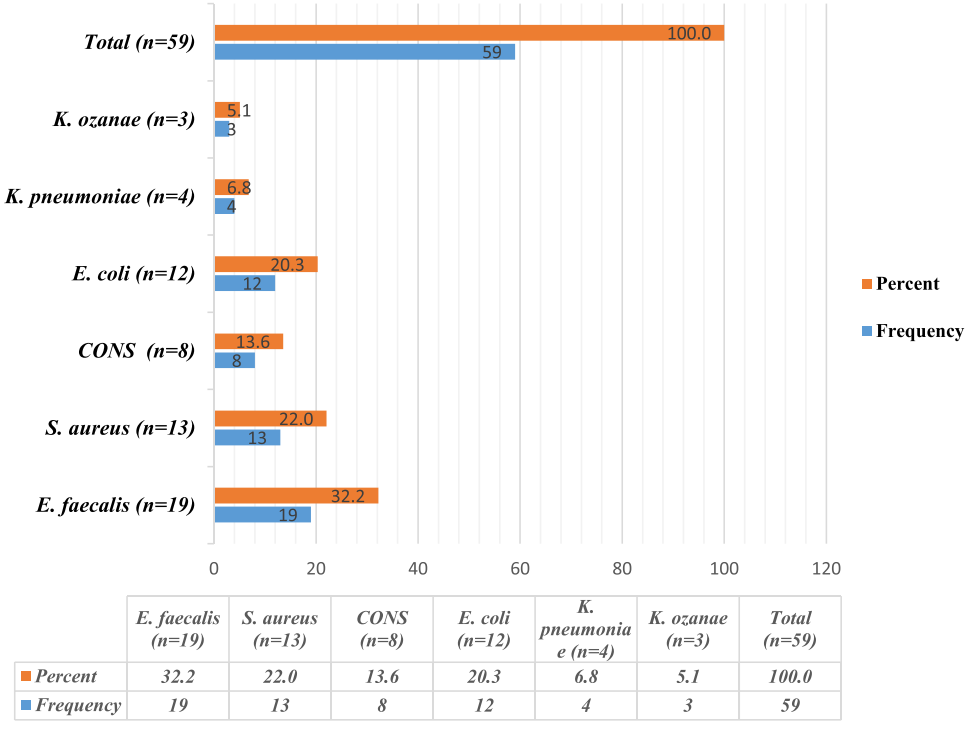

Figure 2 Distribution of bacteria isolated from aerobic vaginitis cases among women attending at Gondar town health facilities, northwest Ethiopia, February 1 to May 31, 2019.

Multidrug Resistance Patterns of Bacterial Isolates Among Women

The overall proportion of multidrug resistant bacterial isolates for three or more classes of antibiotics rate in this study was 30 (50.85%). Among Gram positive bacteria, 10 (77%) isolates of *S. aureus*, 3 (37.5%) isolates of *CONS* and 1 (5.26%) isolate of *E. fecalis* were multidrug resistant. Whereas among Gram negative bacteria, 11 (91.67%) isolates of *E. coli*, 2 (50%) isolates of *K. pneumoniae* and 3 (100%) isolates of *K. ozanae* were multidrug resistant (Table 4).

Table 3 Antimicrobial Susceptibility Patterns of Bacterial Isolates Among Women Attending at Gondar Town Health Facilities, Northwest Ethiopia, February 1 to May 31, 2019

Antibiotics	Isolated Bacteria (n=59)											
	E. fecalis (n=19)		*S. aureus* (n=13)		CONS (n=8)		*E. coli* (n=12)		*K. pneumoniae* (n=4)		*K. ozanae* (n=3)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amox/clav (30 µg)	N/D	N/D	N/D	N/D	N/D	N/D	3(25)	9(75)	1(25)	3(75)	0(0)	3(100)
Ampicillin (25 µg)	N/D	N/D	N/D	N/D	N/D	N/D	0(0)	12(100)	N/D	N/D	N/D	N/D
Cefoxitin (30 µg)	N/D	N/D	13(100)	0(0)	8(100)	0(0)	N/D	N/D	N/D	N/D	N/D	N/D
Ceftriaxone (30 µg)	N/D	N/D	N/D	N/D	N/D	N/D	12(100)	0(0)	3(75)	1(25)	1(33.3)	2(66.7)
Chloramphenicol(30µg)	10(52.63)	9(47.37)	5(38.5)	8(61.5)	6(75)	2(25)	10(83.3)	2(16.7)	2(50)	2(50)	3(100)	0(0)
Ciprofloxacin (5 µg)	N/D	N/D	13(100)	0(0)	8(100)	0(0)	12(100)	0(0)	4(100)	0(0)	3(100)	0(0)
Clindamycin (2 µg)	N/D	N/D	13(100)	0(0)	8(100)	0(0)	N/D	N/D	N/D	N/D	N/D	N/D
Doxycycline (30 µg)	8(42.11)	11(57.89)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Erythromycin (15 µg)	16(84.2)	3(15.8)	12(92.3)	1(7.7)	6(75)	2(25)	N/D	N/D	N/D	N/D	N/D	N/D
Gentamicin (10 µg)	N/D	N/D	13(100)	0(0)	8(100)	0(0)	12(100)	0(0)	4(100)	0(0)	3(100)	0(0)
Meropenem(10 µg)	N/D	N/D	N/D	N/D	N/D	N/D	12(100)	0(0)	4(100)	0(0)	3(100)	0(0)
Penicillin (10 µg)	9(47.37)	10(52.63)	0(0)	13(100)	0(0)	8(100)	N/D	N/D	N/D	N/D	N/D	N/D
Tetracycline (30 µg)	N/D	N/D	0(0)	13(100)	4(50)	4(50)	0(0)	12(100)	1(25)	3(75)	1(33.3)	2(66.7)
TMP/SMX(1.25/23.75 µg)	N/D	N/D	6(46.15)	7(53.85)	5(62.5)	3(37.5)	4(33.3)	8(66.7)	1(25)	3(75)	1(33.3)	2(66.7)
Vancomycin (30 µg).	19(100)	0(0)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Abbreviation: N/D, not done.

Table 4 Multiple Drug Resistance Patterns of Isolated Bacteria Among Women Attending at Gondar Town Health Facilities, Northwest Ethiopia, February 1 to May 31, 2019

Isolated Bacteria	Resistant Antibiotics (%)		
	R3	R4	R5
E. faecalis (n=19)	1(5.26%)	0	0
S. aureus (n=13)	4(30.8%)	6(46.15%)	0
CONS (n=8)	1(12.5%)	1(12.5%)	1(12.5%)
E. coli (n=12)	2(16.67%)	1(8.33%)	1(8.33%)
K. pneumoniae (n=4)	1(25%)	0	1(25%)
K. ozanae (n=3)	0	3(100%)	0
Total (n=59)	9(15.25%)	11(18.64%)	3(5.1%)

Abbreviations: R3, resistant for 3 classes of antibiotics; R4, resistant for 4 different classes of antibiotics; R5, resistant for 5 different classes of antibiotics.

Factors Associated with Aerobic Vaginitis Among Study Participants

Bivariate analysis of the factors showed that participants: with age greater than or equal to 45 years old (COR=7.50, 95% CI=1.83–30.68, (P=0.01), who read and write (COR=2.22, 95% CI=0.64–7.68, P=0.20), who attended elementary school (COR=2.45, 95% CI=0.95–6.35, P=0.06), who were self-employed (COR=5.18, 95% CI=1.33–20.24, P=0.02), who were housewives (COR=5.85 95% CI=1.65–20.77, P=0.01) and civil servant (COR: 3.85 95% C.I: (1.00, 14.86), P=0.05), and those who had once per day douching (COR=2.40, 95% CI=1.03–5.61, P=0.04), who had two lifetime sexual partners (COR=2.45, 95% CI=1.23–4.86, P=0.01), using birth control (COR=1.81, 95% CI=0.95–3.44, P=0.07), with vaginal itching (COR=1.69, 95% CI=0.87–3.27, P=0.12), having vaginal burning sensation (COR=2.10, 95% CI=0.92–4.82, P=0.08) and those who had once per week pant exchange (COR=2.10, 95% CI=0.89–4.95, P=0.08) were found to have p-value ≤ 0.2. Moreover, multivariate analysis of variables was done for those which were significant in the bivariate analysis with p-value of ≤ 0.2. Among socio-demographic and associated factors, age group greater or equal to 45 years with p-value=0.02, (AOR=8.09, 95% CI=1.26–52.06), self-employed with p-value=0.03, (AOR=5.44, 95% CI=1.14–25.98), housewife with p-value=0.01, (AOR=7.66, 95% CI=1.72–34.13) and civil servant with p-value=0.04, (AOR=4.79, 95% CI=1.03–22.10) and two lifetime sexual partners with p-value=0.00, (AOR=3.50, 95% CI=1.54–7.97) were found to be significantly associated with AV (Table 5).

Discussion

Vaginal infections are common medical problems in women and are associated with substantial discomfort,

Table 5 Bivariate and Multivariate Analysis to Aerobic Vaginitis Among Women Attending at Gondar Town Health Facilities, Northwest Ethiopia, February 1 to May 31, 2019

Variables		Aerobic Vaginitis		COR(95% CI)	P-value	AOR(95% CI)	P-value
		Positive	Negative				
		N (%)	N (%)				
Age group	18–24	12(24.6)	60(36.4)	1.00		1.00	
	25–34	25(51.0)	79(47.9)	1.58(0.74, 3.40)	0.24	1.34(0.55, 3.21)	0.51
	35–44	6(12.2)	22(13.3)	1.36(0.46, 4.07)	0.58	0.72(0.19, 2.64)	0.62
	> 45	6(12.2)	4(2.4)	7.50(1.83, 30.68)	0.01	8.09(1.26, 52.06)	0.02
Marital status	Married	37(75.5)	108(65.5)	1.00			
	Single	8(16.3)	37(22.4)	0.63(0.27, 1.48)	0.29		
	Divorced	4(8.2)	20(12.1)	0.58(0.18, 1.82)	0.35		
Educational Status	Unable to read & write	11(22.4)	25(15.2)	2.74(1.01, 7.44)	0.48	1.12(0.24, 5.16)	0.88
	Reading & Writing	5(10.2)	14(8.5)	2.22(0.64, 7.68)	0.20	1.31(0.24, 7.18)	0.75
	Elementary school	13(26.5)	33(20.0)	2.45(0.95, 6.35)	0.06	2.17(0.57, 8.26)	0.25
	Secondary school	11(22.4)	37(22.4)	1.85(0.70, 4.80)	0.21	1.55(0.43, 5.51)	0.49
	College and above	9(18.4)	56(33.9)	1.00		1.00	
Job Status	Self Employed	11(22.4)	29(17.6)	5.18(1.33, 20.24)	0.02	5.44(1.14, 25.98)	0.03
	House wife	24(49.0)	56(33.9)	5.85(1.65, 20.77)	0.01	7.66(1.72, 34.13)	0.01
	Civil servant	11(22.4)	39(23.6)	3.85(1.00, 14.86)	0.05	4.79(1.03, 22.10)	0.04
	Unemployed	3(6.1)	41(24.8)	1.00		1.00	
Residence	Rural	9(18.4)	36(21.8)	1.00			
	Urban	40(81.6)	129(78.2)	1.24(0.55, 2.79)	0.60		
Pregnancy status	Pregnant	7(14.3)	32(19.4)	1.00			
	Non pregnant	42(85.7)	133(80.6)	1.44(0.59, 3.51)	0.41		
Vaginal bathing Frequency per day	Once	12(24.5)	20(12.1)	2.40(1.03, 5.61)	0.04	1.27(0.42, 3.83)	0.66
	Twice	14(28.6)	53(32.1)	1.06(0.05, 2.22)	0.88	0.82(0.33, 2.04)	0.67
	Three and above	23(46.9)	92(55.8)	1.00		1.00	
Number of life time Sexual partner	One	18(36.7)	95(57.6)	1.00		1.00	
	Two	26(53.1)	56(33.9)	2.45(1.23, 4.86)	0.01	3.50(1.54, 7.97)	0.00
	Three and above	5(10.2)	14(8.5)	1.88(0.60, 5.88)	0.27	2.63(0.66, 10.53)	0.17
Have you used birth Control methods	Yes	28(57.1)	70(42.4)	1.81(0.95, 3.44)	0.07	1.61(0.74, 3.50)	0.22
	No	21(42.9)	95(57.6)	1.00		1.00	
Have you used Reused napkin during menstruation	Yes	21(42.9)	66(40.0)	1.12(0.59, 2.15)	0.72		
	No	28(57.1)	99(60.0)	1.00			
Previous similar Disease condition	Yes	23(46.9)	77(46.7)	1.01(0.53, 1.91)	0.97		
	No	26(53.1)	88(53.3)	1.00			
Have you ever Aborted	Yes	8(16.3)	33(20.0)	0.78(0.33, 1.82)	0.56		
	No	41(83.7)	132(80.0)	1.00			
Previous uterine Surgery	Yes	1(2.0)	8(4.8)	0.40(0.05, 3.35)	0.40		
	No	48(98.0)	157(95.2)	1.00			
Vaginal Itching	Yes	32(65.3)	87(52.7)	1.69(0.87, 3.27)	0.12	1.69(0.77, 3.70)	0.18
	No	17(34.7)	78(47.3)	1.00		1.00	

(*Continued*)

Table 5 (Continued).

Variables		Aerobic Vaginitis		COR(95% CI)	P-value	AOR(95% CI)	P-value
		Positive	Negative				
		N (%)	N (%)				
Vaginal burning	Yes No	41(83.7) 8(16.3)	117(70.9) 48(29.1)	2.10(0.92, 4.82) 1.00	0.08	1.76(0.65, 4.74) 1.00	0.25
Pant exchange Frequency/week	Once Twice Three times and above	27(55.1) 13((26.5) 9(18.4)	57(34.5) 68(41.2) 40(24.2)	2.10(0.89, 4.95) 0.85(0.33, 2.16) 1.00	0.08 0.73	1.31(0.46, 3.70) 0.52(0.17, 1.60) 1.00	0.60 0.25

significant morbidity, and, hence, frequent medical visits.[28] In this study, the overall prevalence of vulvovaginitis was 50%. The most common identified aetiologies of vulvovaginitis were BV (35.5%), candidiasis (23.8%), AV (22.9%) and trichomoniasis (3.3%). In the opposite of our findings Salinas et al reported AV as the most common identified aetiology followed by BV, and candidiasis.[18]

The prevalence of AV was 22.9%, which correlates with the score of study conducted by Fan et al (23.74%).[37] Whereas, the prevalence of AV in the present study was greater than the prevalence in the study conducted by Salinas et al (5.6%),[18] Donders et al (15.53%),[4] and Donders et al in 2009, which reported a prevalence of 8.3% AV.[38] On the other hand, these findings were less than the studies conducted by Cheng L et al. (80%) in 2009 and by Razzak et al. (95.45%) in 2011 .[3738] The reason for this variation might be due to the difference in sexual habits of the participants. In those studies, participants had multiple sexual partners compared to ours. Besides, the difference could also be explained in terms of population density, and health status of the participants.

The prevalence of vulvovaginal candidiasis in the present study was 23.8%. This finding was higher than the finding of a study conducted in Bahir Dar, Ethiopia, with 8.3%.[13] Conversely, this study finding was lower than studies conducted in Iran at 35.76%,[41] Nigeria at 47.7%,[42] Kenya at 60%[43] and Ethiopia at 41.4%.[44] Disparity in the prevalence rates of vulvovaginal candidiasis in different studies could result from differences in immunity, pregnancy status and diagnostic methodology. In our study, the proportion of vulvovaginal candidiasis was higher in non-pregnant women than pregnant women (p=0.005). This is inconsistent with the study done by Mulu et al[13] in which the proportion of vulvovaginal candidiasis was higher among pregnant women than non-pregnant women. The reason for this variation may be due to women's gestational age difference, in which most of the women's in Mulu et al's study were at the second and third trimester of gestational age.

The prevalence of trichomoniasis in this study was 3.3%, which was comparable to studies carried out in Kirkuk-Iraq (2.9%) and Ethiopia (2.1%).[13,45] However, it was higher than a study conducted in Sudan (0.5%).[46] In contrast, it was lower than the study carried out in India (18.8%).[47] The observed difference could be due to the variation in the number of sexual partners, personal hygiene practices, socioeconomic and cultural conditions of the study participants. Moreover, the detection of trichomoniasis by a conventional wet mount method in the present study might have reduced the actual prevalence.

In the present study, a total of 59 bacterial isolates were recovered, of which 40 (67.8%) of the isolates were Gram positive and 19 (32.2%) isolates were Gram negative bacteria. *E. faecalis* was the commonest pathogen isolated, covering 32.2%, followed by *S. aureus* with 22%, *E. coli* with 20.4%, CONS with 13.6% and *Klebsiella* spp with 11.8%. This finding was similar to the findings of Zarbo et al[48] and Sangeetha et al.[49]

Currently, there is no generally accepted clinical strategy for treating women with AV. Therapeutic effects and prognoses vary widely for different pathogenic bacteria. Drugs targeting aerobic bacteria such as *E. coli, Enterococcus aerobes, S. aureus*, corynebacteria and haemolytic streptococci may be used for the treatment of AV. Studies have supported the use of systemic and topical kanamycin or clindamycin to treat AV.[50] In this study, the antimicrobial susceptibility patterns were done for all bacterial isolates. The overall antimicrobial susceptibility patterns of Gram positive bacterial isolates ranged from the most sensitive drug, vancomycin, to the most resistant,

penicillin. Additionally, Gram-positive isolates like *S. aureus* and CONS showed maximum resistance to tetracycline and trimethoprim/sulphamethoxazole respectively. However, antibiotics like vancomycin, cefoxitin, ciprofloxacin, clindamycin, and gentamicin were found to be 100% effective against Gram-positive isolates. This finding was similar to a study conducted by Pal, et al.[51] and Singh et al.[52]

In our study, the overall antimicrobial susceptibility patterns of Gram negative isolates were the least sensitive to ampicillin but highly sensitive to ciprofloxacin, gentamicin, and meropenem. *E. coli* was 100% resistant to tetracycline and ampicillin. In addition to our findings, there were 75% and 66.7% of resistance to tetracycline for *K. pneumoniae* and *K. ozanae* respectively. Similarly, Zarbo et al reported that *E. coli* was sensitive to Ciprofloxacin.[48] Additionally Mumtaz et al showed that the most effective chemotherapeutic agents against Gram-negative rods (*E. coli* and *K. pneumoniae*) were imipenem (96.0%, 100%).[53]

Multidrug resistance was seen in 50.85% of the identified bacteria. The cause of this high multidrug resistance rate in this study might be due to irrational and unnecessary use of antibacterial agents. The consequence of this could lead to the emergence of multidrug resistant bacterial strains. This reflected the fact that ampicillin, tetracycline, and cotrimoxazole were the most easily available drugs on the market without a doctor's prescription. The widespread use and, more often, the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria.[54] Nowadays, carbapenem-resistance bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have become common in hospital settings.[55,56] Recently, Donadu et al reported that out of 62 *Acinetobacter baumannii* isolates from various Hungarian and Italian hospitals, 40 (64.5%) of the isolates were resistant to meropenem.[55] Although drug resistance in anti-fungal therapy specifically for immunocompromised patients has become challenging, some evidence indicates natural oil shows prominent anti-fungal activities against clinical isolates of *Candida spp*.[57,58]

In this study, the associations of AV with independent variables were assessed. Of these variables, age groups greater than or equal to 45 years were 8 times more likely to develop AV than those in the age group of 15 to 24 years (p=0.02). This may be due to physiological changes during menopause, and decreased estrogen production after 45 years may make women more susceptible to infection. Additionally, the low incidence of AV among reproductive age groups in this study might be due to the fact that vaginal microbiota maintained by high estrogen levels as a good supply of glycogen and a high percentage of Lactobacillary microbiota significantly reduce the multiplication of pathogenic organisms due to the production of defence factors by Lactobacilli.[48] Study participants who had two sexual partners were 3.5 times more likely to develop AV (p=0.00) than those who had a single partner in their lifetime. This might be due to the fact that having multiple sexual partners increases the chance of acquiring AV due to frequent sexual activity may extensively damage the epithelial cells of the vagina, which may change in the normal vaginal microbiota and lead to vulvovaginitis including AV.

Conclusions

In the present study, the overall prevalence of bacterial vaginosis and aerobic vaginitis was high. Aerobic vaginitis was higher among non-pregnant women as compared to pregnant women, and it was a common female genital tract infection, more prevalent among sexually active females. The high yield of culture positivity was also reported, Gram-positive bacteria were the most frequently isolated bacteria. Of those, *E. faecalis* was the predominant pathogen while *E. coli* was the commonest among Gram negative bacteria. Moreover, except for *E. faecalis*, all the isolated Gram positive and Gram negative bacteria were 100% resistant to penicillin and ampicillin, respectively. Participants whose age group is greater than or equal to 45 years, participants who are self-employed, participants who are housewives or civil servants, and participants who have two lifetime sexual partners were found to be significantly associated with AV. Therefore, regular screening of women for vaginal infection should be promoted at health care facilities, and specifically routine screening of AV is needs to be introduced. Due to lack of reagents and chemicals for molecular technique (PCR) and selective culture media preparation, the diagnosis and identification of specific bacterial species were not done, this is one of the limitations of the study.

Abbreviations

AOR, Adjusted Odds Ratio; AST, Antimicrobial Sensitivity Test; ATCC, American Type Culture Collection; AV, Aerobic Vaginitis; BV, Bacterial Vaginosis; CAMP, Christie Atkins Munch Peterson;

CLSI, Clinical and Laboratory Standards Institute; CONS, Coagulase Negative *Staphylococcus* species; COR, Crude Odds Ratio; GBS, Group B *Streptococcus*; SPSS, Statistical Package Software for Social science; VVC, Vulvo Vaginal Candidiasis.

Data Sharing Statement

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

Ethical Approval and Consent to Participate

Ethical approval was obtained from the ethical review committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar with a reference number SBMLS/2923/11. Likewise, the study was conducted in accordance with the ethical principles of the declaration of Helsinki on human subjects. All study participants were informed concerning the study verbally and a written consent was obtained from each participant.

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Author Contributions

All authors have made a significant contribution to this study, all the way through the conception, study design, execution, acquisition of data, data analysis and interpretation to drafting, revising or critically reviewing stages of the article. The authors also gave final approval of the version to be published, agreed on the journal to which the article has been submitted, and agreed to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest for this work.

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