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Original Article

Lack of association between unhealthy vaginal pH and bacterial vaginosis positive status

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Abstract

Introduction: Bacterial vaginosis (BV) remains highly prevalent worldwide. Although vaginal pH has been suggested to be a good predictor of BV, findings are conflicting. This study investigated the prevalence and risk factors associated with BV and the association between BV and vaginal pH in women from Durban, South Africa.

Methods: This was a cross-sectional study that included 150 pregnant and non-pregnant women. BV was diagnosed using Nugent scoring and vaginal pH was measured using Lovibond® pH strips. Vaginal pH results were compared with Nugent scores. Statistical analyses were performed in STATA 17.1 using 95% confidence intervals and a significance level of 0.05.

Results: According to Nugent's criteria, BV prevalence was 29.33%, while an additional 29.33% of the women had intermediate BV. Women aged 31–39 years were more likely to have BV than younger women (odds ratio [OR]: 7.27; 95% CI 1.40- 37.85; $p = 0.018$). Women with intermediate BV had a 2.4-fold higher likelihood of vaginal dysbiosis (OR: 2.42; 95% CI: 1.07-5.45; $p = 0.033$). Significant associations were observed between BV status and the presence of BVAB-1 ($p = 0.016$), BVAB-2 ($p = 0.009$), BVAB-3 ($p = 0.048$), *Atopobium vaginae* ($p = 0.029$), and *Sneathia sanguinegens* ($p < 0.001$). However, vaginal pH was not significantly associated with the prevalence of BV-associated microorganisms ($p > 0.05$).

Conclusions: BV was common among women in Durban, South Africa; however, vaginal pH was not associated with BV or BV-associated bacteria, indicating that it was a poor predictor of BV in this population.

Key words: bacterial vaginosis; vaginal pH; Nugent score; BV-associated bacteria.

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Introduction

Bacterial vaginosis (BV) is the most common dysbiosis among reproductive-aged women [1-3]. BV is characterized by the significant reduction of *Lactobacilli* in the vagina, leading to an overabundance of obligate or facultative anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Mycoplasma hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, and BV-associated bacteria (BVAB-1 to 3) [4,5]. Approximately 50% of BV cases are asymptomatic. However, when symptoms arise, women experience abnormal vaginal discharge, increased vaginal pH, vaginal itching, and vaginal malodor [3]. BV has been associated with adverse gynecologic and pregnancy complications such as pelvic inflammatory disease (PID), preterm delivery [1,5], and postpartum and post-abortion endometritis [6]. Furthermore, previous studies have reported a positive association between BV and increased transmission of sexually transmitted infections (STIs) such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, human immunodeficiency virus (HIV), and human papillomavirus (HPV) [7].

The prevalence of BV varies greatly between and among countries globally, with African countries reporting higher rates [8]. A systematic review conducted by Peebles *et al.*, reported a general population prevalence ranging from 23% to 29% among reproductive-aged women. Within sub-Saharan Africa, BV prevalence rates were higher in Southern and Eastern Africa (33.3%) [2]. South African studies have reported prevalence rates of 31.0% [1] and 42.1% [9]. Higher prevalence rates have also been reported among black women (46.5%) when compared to non-black women (21.3%) and in women living with HIV (35.6%) when compared to their counterparts (25.6%) [2]. Several risk factors have been linked to BV such as contraceptive use, race, intravaginal practices [3,8,10], antibiotic use, age, level of education and menstrual cycle [3].

There are two main diagnostic techniques for BV, the Amsel Criteria, and the Nugent Score. The Amsel's criteria is based on the detection of the following four criteria: (1) a grey-whitish vaginal discharge, (2) a vaginal pH > 4.5, (3) a positive KOH whiff test, and (4) the presence of clue cells during microscopic examination [8,11]. A patient is diagnosed BV positive,

if three out of these four symptoms are present [8]. The Nugent score is a microscopy-based method that calculates the number of bacterial morphotypes present in Gram-stained vaginal discharge smears [11,12]. According to the number of certain bacteria, smears are categorized into three groups: BV negative if the score is 0 – 3, BV intermediate if the score is 4 – 6 and BV positive if the score is 7 or higher [12].

Metronidazole is the recommended first line treatment for BV, with cure rates of 85% [2]. The current recommended treatment guidelines for BV are metronidazole 500 mg orally twice daily for 7 days or metronidazole gel 0.75% (5 g) intravaginally, once daily for 5 days or clindamycin cream 2% (5 g) intravaginally at bedtime for 7 days. Treatment for BV is recommended for symptomatic women only [13]. Recurrent BV occurs in ~58% of treated cases within one year [2,3]. Treatment failure in BV has been associated with reinfection by sexual partner and the presence of some BV-associated bacteria such as BVAB-1 to 3, *Megasphaera* type 2 and *Peptoniphilus lacrimalis* at the beginning of treatment [4].

Changes in vaginal pH has been of interest in understanding the vaginal physiology, gynecological diseases, and drug development [14]. The normal vaginal pH for reproductive-aged women is moderately acidic, ranging from 3.8 to 5.0 [11,14]. The healthy vaginal pH is maintained by *Lactobacillus* species through the production of lactic acid [15]. The acidic vaginal pH provides protection against vaginitis, urinary tract infections (UTIs), and inhibits the overgrowth of pathogens [14]. Several factors such as vaginal infections, sexual activity, age, and vaginal douching, can cause variations in the vaginal pH [11]. Furthermore, previous studies have shown that an increase in vaginal pH may result in BV [11].

The vaginal environment is predominantly regulated by hormonal changes during a woman's reproductive stages, including puberty, menarche, fertility, pregnancy, and menopause [16]. Oestrogen plays a crucial role in promoting a healthy vaginal microbiome by maintaining an acidic environment. Menopause causes a decrease in vaginal *Lactobacilli* due to low oestrogen levels [14] thus leads to high vaginal pH and growth of pathogenic bacteria [16]. The vaginal microbiota has been shown to vary by race and ethnicity. African American and Hispanic women have been reported to be more likely to have a vaginal pH greater than 4.5. This is due to lower abundance of *Lactobacilli* and higher abundance of *Mobiluncus* and Gram-variable rods [4,14].

Studies have shown that the prevalence of BV remains high, globally. This warrants the need for routine BV prevalence studies to better understand its epidemiology and provide awareness. This study aimed to investigate the prevalence and risk factors associated with BV and the association between BV and vaginal pH in women from Durban, South Africa.

Methods

Ethics Consideration

Approval for this study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN) with reference number (BREC/00003674/21).

Study population

This study was a cross-sectional study and involved the diagnosis of BV in pregnant and non-pregnant women. For this study, n = 150 women were recruited from the King Edward VIII hospital in Durban, KwaZulu-Natal, South Africa. Women from the antenatal clinic and outpatient's clinic were invited to participate. The screening to enrolment ratio for the study was 3:1. The women enrolled in the study were aged 18 and above, were willing to provide written informed consent and were willing to provide self-collected vaginal swabs (four swabs). Since the one of the objectives of the study was to determine if vaginal pH was a good predictor of BV, women who reported douching, i.e., washing inside their vagina with substances other than soap and water; women who were menstruating, women who were on antibiotics and women taking any medication for urinary tract infections were excluded from the study since any of the above were likely to alter the vaginal pH.

Instructions on the sample collection were provided by the study team. Data on the woman's sexual behaviour, clinical history and socio-demographic information was obtained from all enrolled women using a structured questionnaire administered by the study team. The study population was recruited from January to August 2022.

Diagnosis of BV

Smears were prepared from the collected vaginal swab samples. The slides were air-dried, heat-fixed, and Gram-stained. Briefly, the fixed smear was covered with crystal violet for 1 minute, washed with water, flooded with Gram's iodine for 1 minute, washed with water, and then decolorized with acetone for 2-3 seconds. The smears were rinsed quickly under running water to stop the decolourisation and then counterstained with safranin for 1 minute. The smear

was rinsed with running water and blot dried. All smears were assessed according to Nugent's method [12].

Vaginal pH assessments

The value on the pH strip (Lovibond®: Tintometer® Group, United Kingdom) was compared to the diagnosis of BV by Nugent scoring. The samples were categorized as follows; Nugent scores 0-3 which indicated the absence of BV, should have a vaginal pH of ≤ 4.5 and Nugent scores 4-6 (intermediate BV) and 7-10 (BV positive), should have a vaginal pH of > 4.5.

Detection of BV associated microorganisms.

DNA isolation from vaginal swabs

DNA was extracted from a second vaginal swab using the PureLink Microbiome Kit (ThermoFisher Scientific, United States) according to the manufacturer's instructions. The purified DNA was stored at -20ºC. The concentration and purity of the DNA was assessed using a Nanodrop spectrophotometer (ThermoFisher Scientific, United States).

Detection assay

Qualitative PCR was used for the detection of *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*, *Megasphaera* type 1, *Sneathia sanguineges*, *BV-associated-bacteria-2* (BVAB2), *BV-associated-bacteria-1* (BVAB1), and *BV-associated-bacteria-3* (BVAB3). The primer sequences used are shown in Table 1. All PCR reactions were carried out in a final volume of 10µL consisting of 5µL PowerUp™ SYBR Green Master Mix (2x) (ThermoFisher Scientific, United Sates), 0.3µM of the forward primer and 0.3µM of the reverse primer, 0.5µL of template DNA and 3.9µL of sterile water. The reactions were then run on a Quant Studio 5 PCR instrument (ThermoFisher Scientific, United States). The following cycling conditions were employed, UDG activation stage for 2 minutes at 50ºC, initial denaturation for 2 minutes at 95ºC, denaturation for 15 seconds at 95ºC, annealing 15 seconds at 55-60ºC with extension for 1 minute at 72ºC. To control for contamination and to assess efficiency of the PCR, a negative control was included in all PCR runs.

Data analysis

The primary objective of this study was to estimate the prevalence rate BV in a sample of women from Durban, South Africa. We also examined risk factors associated with infection. Vaginal pH was the main risk factor we looked at, by examining the association of infection with normal vaginal pH (< 4.5) compared to abnormal vaginal pH (≥ 4.5).

Multinomial regression analyses were used to examine the association of BV (negative, positive, or intermediate) with vaginal pH adjusted for age group, education, marital status smoking, drinking, HIV status, current abnormal vaginal discharge, condom use at last sex, past abnormal vaginal discharge, and past treatment for STIs. A subgroup logistic regression analysis was then conducted after excluding those who were BV negative to determine the association between BV (positive or intermediate) with vaginal pH. This subgroup analysis model was also adjusted for the other vaginal pathogens investigated in this study. Model fit was checked using standard statistical tests, and influential variables removed were detected. All analyses were conducted with 95% confidence intervals and a significance level of 0.05. All analyses were conducted in STATA 17.1.

Table 1. Primer sequences used for the detection of the microorganisms investigated in this study [16-18].

Microorganism	Primer	Sequence (5'-3')	Amplicon size
BVAB-1	Forward	GGAGTGTAGGCGGCACTA	90bp
	Reverse	CTCTCCGATACTCCAGCTCTA	
BVAB-2	Forward	TTAACCTTGGGGTTCATTACAA	260bp
	Reverse	GAATACTTATTGTGTTAACTGCGC	
BVAB-3	Forward	CATTTAGTTGGGCACTCAGGC	160bp
	Reverse	ACATTTGGGGATTTGCTTCGCC	
Prevotella bivia	Forward	CCAGCCAAGTAGCGTGCA	170bp
	Reverse	TGGACCTTCCGTATTACCGC	
Gardnerella vaginalis	Forward	TTACTGGTGTATCACTGTAA	238bp
	Reverse	CCGTCACAGGCTGAACAGT	
Atopobium vaginae	Forward	TAGGTCAGGAGTTAAATCTG	155bp
	Reverse	TCATGGCCCAGAAGACCGCC	
Sneathia sanguineges	Forward	AATTATTGGGCTTAAAGGGCATC	102bp
	Reverse	AGTACTCTAGTTATACAGTTTTGTAG	
Megasphaera type 1	Forward	GATGCCAACAGTATCCGTCCG	211bp
	Reverse	CCTCTCCGACACTCAAGTTCGA	

Table 2. Baseline characteristics for women screened for bacterial vaginosis (BV).

Variables	BV Negative *n (%)*	BV Positive *n (%)*	BV Intermediate *n (%)*	Total *n (%)*	*p*
Overall	62 (41.3)	44 (29.3)	44 (29.3)	150 (100)	
SOCIODEMOGRAPHIC STATUS					
Age group					0.637
18-24	14 (22.5)	5 (11.3)	7 (15.9)	26 (17.3)	
25-30	15 (24.1)	11 (25)	10 (22.7)	36 (24)	
31-39	11 (17.7)	14 (31.8)	11 (25)	36 (24)	
40+	22(35.4)	14 (31.8)	16 (36.3)	52 (34.6)	
Level of education					0.156
College	49 (79.0)	31 (70.4)	34 (77.2)	114 (76)	
High school	1 (1.61)	5 (11.3)	1 (2.27)	7 (4.66)	
Primary school	1 (1.61)	0 (0)	1 (2.27)	2 (1.33)	
Marital status					0.126
No	47 (75.8)	40 (90.9)	38 (86.3)	125 (83.3)	
Yes	15 (24.1)	4 (9.09)	6 (13.6)	25 (16.6)	
RISK BEHAVIOUR					
Has a regular sex partner					0.286
No	11 (17.7)	8 (18.1)	13 (29.5)	32 (21.3)	
Yes	51 (82.2)	36 (81.8)	31 (70.4)	118 (78.6)	
Living with regular sexual partner					0.315
No	38 (61.2)	28 (63.63)	33 (75)	99 (66)	
Yes	24 (38.7)	16 (36.3)	11 (25)	51 (34)	
Age of sexual debut					0.828
15-20	50 (80.6)	35 (79.5)	33 (75)	118 (78.6)	
21-25	11 (17.7)	7 (15.9)	9 (20.4)	27 (18)	
< 15	1 (1.61)	2 (4.5)	2 (4.5)	5 (3.3)	
Lifetime number of sexual partners					0.804
1	9 (14.5)	6 (13.6)	3 (6.81)	18 (12)	
2 to 4	37 (59.6)	26 (59.0)	29 (65.9)	92 (61.3)	
> 4	16 (25.8)	12 (27.2)	12 (27.2)	40 (26.6)	
Partner has other partners					**0.032**
No	14 (22.5)	19 (43.1)	10 (22.7)	43 (28.6)	
Yes	7 (11.2)	7 (15.9)	6 (13.6)	20 (13.3)	
Don't know	38 (61.2)	16 (36.3)	20 (45.4)	74 (49.3)	
Condom use during last sexual act					0.926
No	40 (64.5)	30 (68.1)	29 (65.9)	99 (66)	
Yes	22 (35.4)	14 (31.8)	15 (34.0)	51 (34)	
Smokes					0.854
No	55 (88.7)	38 (86.3)	40 (90.9)	133 (88.6)	
Yes	7 (11.2)	6 (13.6)	4 (9.0)	17 (11.3)	
Drinks alcohol					**0.034**
No	52 (83.8)	29 (65.9)	28 (63.6)	109 (72.6)	
Yes	10 (16.1)	15 (34.0)	16 (36.3)	41 (27.3)	
CLINICAL HISTORY					
Current abnormal vaginal discharge					0.126
No	52 (83.8)	36 (81.8)	30 (68.1)	118 (78.6)	
Yes	10 (16.1)	8 (18.1)	14 (31.8)	32 (21.3)	
Contraception method					0.292
None	49 (79.0)	31 (70.4)	34 (77.2)	114 (76)	
IUCD/Implant	1 (1.61)	5 (11.3)	1 (2.2)	7 (4.6)	
Tubal ligation	4 (6.4)	2 (4.5)	5 (11.3)	11 (7.3)	
Injectables	3 (4.8)	4 (9.1)	3 (6.8)	10 (6.6)	
Pills	4 (6.4)	2 (4.5)	0 (0)	6 (4)	
Other	1 (1.6)	0 (0)	1 (2.2)	2 (1.3)	
Past abnormal vaginal discharge					**0.781**
No	26 (41.9)	19 (43.1)	16 (36.3)	61 (40.6)	
Yes	36 (58.0)	25 (56.8)	28 (63.6)	89 (59.3)	
Treated for past STI					0.460
No	27 (43.5)	24 (54.5)	19 (43.1)	70 (46.6)	
Yes	35 (56.4)	20 (45.4)	25 (56.8)	80 (53.3)	
Pregnancy status					0.297
Not pregnant	37 (59.6)	32 (72.7)	37 (84.0)	106 (70.6)	
Pregnant	25 (40.3)	12 (27.2)	7 (15.9)	44 (29.3)	
HIV status					0.117
Negative	24 (38.7)	9 (20.4)	16 (36.3)	49 (32.6)	
Positive	38 (61.2)	35 (79.5)	28 (63.6)	101 (67.3)	

Results

Baseline characteristics and prevalence of bacterial vaginosis (BV)

A total of 150 women were screened for BV. Overall, most of the study women were above the age of 40 (34.6%), reported college as their highest level of education (76%), and were unmarried (83.3%). Of the 150 women included in this study, 106 (70.6%) were not pregnant and 101 women (67.3%) were living with HIV. A total of 44/150 (29.3%) BV women were diagnosed as BV positive. Similarly, 44/150 (29.3%) women were diagnosed as intermediate. Of the 44 women who BV positive, 36 (81.8%) did not report having current abnormal discharge (thin white or grey, foul-smelling vaginal discharge). Among the women who were BV intermediate, 30/44 (68.1%) did not report abnormal vaginal discharge. Baseline characteristics stratified by BV status are summarised in Table 2.

Socio-demographic status

For the women who were BV positive and intermediate, the highest proportions fell in the age groups 31-39 (31.8% and 25.0%, respectively) and above 40 (31.8% and 36.3%, respectively). However, age was not significantly associated with infection (p = 0.637). The level of education and marital status had no statistically significant association with a positive or intermediate BV status ($p > 0.05$).

Risk behavior

Women whose partners had other partners and women who consumed alcohol were likely to be BV positive and intermediate, p = 0.032 and p = 0.034; respectively. For the women who were BV positive, 43.1% reported that their partners did not have other partners and 36.3% reported that they did not know if their partners had other partners. In addition, 34% of these women reported that they consumed alcohol. Among the BV intermediate group, 45.4% of the women did know if their partners had other partners and 22.7% reported that their partners did not have other partners. Moreover, 36.3% of the BV intermediate women reported alcohol consumption. All the other behavioural factors were not significantly associated with BV status ($p > 0.05$).

Clinical history

For the women who were BV positive, 81.8% did not report having current abnormal vaginal discharge, 70.4% did not use contraceptives, 56.8% had previous abnormal vaginal discharge, 54.5% had not been previously treated for STIs, 72.2% were not pregnant and 79.5% were living with HIV. Among the BV intermediate women, 68.1% did not report current abnormal vaginal discharge, 77.2% did not use contraceptives, 63.6% had previous abnormal vaginal discharge, 56.8% had been previously treated for STIs, 84% were not pregnant and 63.6% were living with HIV. However, none of these clinical factors were significantly associated with BV status ($p > 0.05$).

Risk factors associated with BV

Women who were in the age group 31-39 years were 7.3 times more likely to test positive for BV compared to younger women [(odds ratio [OR]: 7.27; 95% CI 1.40–37.85; p = 0.018). Women who did not know whether their partners had other partners were less likely to test positive for BV compared to women who reported that their partners that did not have other partners (OR: 0.17; 95% CI 0.05–0.61; p = 0.007). None of the clinical factors were significantly associated with a positive BV status ($p > 0.05$) (Table 3).

Prevalence of BV-associated microorganisms across BV intermediate and positive women

BVAB-1 was present in 38.5% and 61.5% of the BV intermediate and BV positive women, respectively. There was a significant association between BV status and the presence of BVAB-1, p = 0.016. BVAB-2 was more prevalent in BV positive women (66.7%) compared to BV intermediate women (33.3%) and this was significant, p = 0.009. A higher proportion of BV positive women (63.6%) tested positive for the presence of BVAB-3 compared to 36.4% of BV intermediate women. BVAB-3 presence was significantly associated with BV status, p = 0.048. Similarly, a higher prevalence of *A. vaginae* (59.3%) was observed among BV positive women when compared to BV intermediate women (40.7%). *A. vaginae* presence was significantly associated with BV status, p = 0.029. *S. sanguinegens* presence was significantly associated with BV status, $p < 0.001$. *S. sanguinegens* was present in 75% and 25% of the BV positive and BV intermediate women, respectively. A higher prevalence of *G. vaginalis, Megasphaera* type 1, and *P. bivia* was shown to be present in BV positive women when compared to BV intermediate women, however, this was not significant, $p > 0.05$ (Table 4).

Clinical factors associated with vaginal pH

For the analysis described in Table 5, vaginal pH was stratified into two groups, pH ≤ 4.5 and pH > 4.5.

Table 3. Risk factors associated with being BV positive compared to BV negative.

BV Status – Positive	Adjusted Odds Ratio	95% Confidence Interval	*p*
Age			
18-24	1.00		
25-30	4.32	0.83-22.42	0.081
31-39	7.27	1.40-37.85	**0.018**
40+	4.97	0.87-28.53	0.072
Current abnormal vaginal discharge			
No	1.00		
Yes	1.50	0.39-5.81	0.555
Education			
Primary school	1.00		
High school	8.68	0.52-145.64	0.133
College	11.96	0.64-224.15	0.097
Marital status			
No	1.00		
Yes	0.16	0.02-1.14	0.067
Regular sex partner			
No	1.00		
Yes	1.31	0.19-8.82	0.784
Cohabiting with regular partner			
No	1.00		
Yes	1.21	0.33-4.36	0.775
Age first sex			
15-20	1.00		
21-25	0.90	0.25-3.23	0.868
< 15	4.22	0.24-75.34	0.327
Lifetime number of sex partners			
1			
2-4	0.36	0.07-1.91	0.230
> 4	0.19	0.03-1.23	0.082
Partner has other partners			
No	1.00		
Yes	0.34	0.06-1.87	0.215
Don't know	0.17	0.05-0.61	**0.007**
No data	0.20	0.02-2.38	0.204
Condom used last sex			
No	1.00		
Yes	0.89	0.30-2.61	0.834
Smoking			
No	1.00		
Yes	0.46	0.09-2.23	0.335
Drinking			
No	1.00		
Yes	2.65	0.72-9.77	0.142
Contraceptive method			
None	1.00		
IUCD/Implant	3.57	0.22-58.20	0.372
Tubal ligation	0.38	0.04-3.73	0.407
Injectable	2.90	0.32-26.66	0.347
Pills	0.26	0.03-2.25	0.219
Other	0.00	0.00	0.995
Past abnormal discharge			
No	1,00		
Yes	2.64	0.29-23.64	0.386
Treated for past STI			
No	1.00		
Yes	0.17	0.02-1.35	0.093
Pregnancy status			
Not pregnant	1.00		
Pregnant	0.58	0.13-2.59	0.476
HIV status			
Negative	1.00		
Positive	2.70	0.71-10.27	0.144

Table 4. Prevalence of BV-associated microorganisms across BV intermediate and positive women.

Variables	Bacterial vaginosis status		*p*
	Positive *n (%)*	Intermediate *n (%)*	
Overall	44 (50)	44 (50)	
BVAB-1			0.016
Negative	28 (45.2)	34 (54.8)	
Positive	16 (61.5)	10 (38.5)	
BVAB-2			0.009
Negative	20 (38.5)	32 (61.5)	
Positive	24 (66.7)	12 (33.3)	
BVAB-3			0.048
Negative	23 (41.8)	32 (58.2)	
Positive	21 (63.6)	12 (36.4)	
Atopobium vaginae			0.029
Negative	12 (35.3)	22 (64.7)	
Positive	32 (59.3)	22 (40.7)	
Prevotella bivia			0.112
Negative	11 (37.9)	18 (62.1)	
Positive	33 (55.9)	26 (44.1)	
Gardnerella vaginalis			0.257
Negative	12 (41.4)	17 (58.6)	
Positive	32 (54.2)	27 (45.8)	
***Megasphaera* type 1**			0.088
Negative	19 (41.3)	27 (58.7)	
Positive	25 (59.5)	17 (40.5)	
Sneathia sanguinegens			< 0.001
Negative	20 (35.7)	36 (64.3)	
Positive	24 (75)	8 (25)	

A healthy vaginal environment is associated with a pH of ≤ 4.5 and a disturbed flora is associated with a pH > 4.5.

According to the analysis, within the group of women who had a vaginal pH of ≤ 4.5, the majority (45.5%) were diagnosed as BV negative, followed by 31.3% who were BV positive and 23.3% who were BV intermediate. However, in the group of women who had vaginal pH readings > 4.5, the majority (41.2%) were diagnosed as BV intermediate, followed by 33.3% who were BV negative and 25.5% who were BV positive. There was no significant association between BV status and vaginal pH, $p = 0.071$. Of the women who had a healthy vaginal pH, 70.7% were HIV positive when compared to 29.3% who were HIV negative. Of the women who had a vaginal pH > 4.5, 60.8% were HIV positive when compared to 39.2% who were HIV negative. There was no significant association between HIV status and vaginal pH, $p = 0.220$ (Table 5).

Of the women who had a healthy vaginal pH, the majority, 80.8% did not report having symptoms of abnormal vaginal discharge when compared to 19.2%

Table 5. Clinical characteristics of the study population stratified by vaginal pH.

Variables	Vaginal pH ≤ 4.5	Vaginal pH > 4.5	Total	*p*
	n (%)	*n (%)*	*n (%)*	
Overall	99 (66)	51 (34)	150 (100)	
Current abnormal vaginal discharge				0.372
No	80 (80.8)	38 (74.5)	118 (78.7)	
Yes	19 (19.2)	13 (25.5)	32 (21.3)	
Contraception method				**0.019**
None	78 (78.8)	36 (70.6)	114 (76.0)	
IUCD/Implant	7 (7.1)	0 (0)	7 (4.7)	
Loop/Sterilization	5 (5.1)	6 (11.8)	11 (7.3)	
Injectables	3 (3.0)	7 (13.7)	10 (6.7)	
Pills	4 (4.0)	2 (3.9)	6 (4.0)	
Other	2 (2.0)	0 (0)	2 (1.3)	
Pregnancy status				**0.003**
Not pregnant	62 (62.6)	44 (86.3)	106 (70.7)	
Pregnant	37 (37.4)	7 (13.7)	44 (29.3)	
HIV status				0.220
Negative	29 (29.3)	20 (39.2)	49 (32.7)	
Positive	70 (70.7)	31 (60.8)	101 (67.3)	
BV Status				0.071
Negative	45 (45.5)	17 (33.3)	62 (41.3)	
Positive	31 (31.3)	13 (25.5)	44 (29.3)	
Intermediate	23 (23.2)	21 (41.2)	44 (29.3)	

who reported having discharge. Within the group of women who had vaginal pH readings > 4.5, similarly, the majority (74.5%) did not report having any symptoms of discharge compared to 25.5% who reported having symptoms of discharge. The association between symptoms of abnormal vaginal discharge and vaginal pH was not significant, $p = 0.372$ (Table 5).

Of the women, who were classified as having a healthy vaginal pH (≤ 4.5), the majority had been sterilized (5.1%) followed by 4% who were on oral pills and 3% who were on injectable contraceptives. On the contrary, of the women who had vaginal pH > 4.5, the majority were on injectable contraceptives (13.7%), followed by 11.8% who had been sterilized and 3.9% who were taking oral pills. There was a significant association between contraception method used and vaginal pH, $p = 0.019$. Within the group of women who had a vaginal pH of ≤ 4.5, the majority (62.6%) were not pregnant when compared to 37.4% who were pregnant. Similarly, within the group of women who had a vaginal pH of > 4.5, the majority (86.3%) were not pregnant when compared to 13.7% who were pregnant. There was a significant association between pregnancy status and vaginal pH, $p = 0.003$ (Table 5).

Factors significantly contributing to an increased vaginal pH

According to the univariate analysis, being BV positive slightly increased the odds (Odds Ratio [OR]: 1.11, 95% Confidence Interval [95% CI]: 0.47-2.61) of vaginal dysbiosis, however, this was not significant, $p = 0.811$. Similarly, after performing further adjustment, being BV positive slightly increased the odds (OR: 1.29, 95% CI: 0.50-3.37) of vaginal dysbiosis, this remained not significant, $p = 0.811$ (Table 5).

However, in the univariate analysis, being BV intermediate increased the odds of vaginal dysbiosis by 2.4 times (OR: 2.42, 95% CI: 1.07-5.45) and this was statistically significant, $p = 0.033$. After performing further adjustments, being BV intermediate was still associated with vaginal dysbiosis, in that being diagnosed as BV intermediate increased the odds of developing vaginal dysbiosis by 2.66 times (OR: 2.66, 95% CI: 1.06-6.64) and this association was significant, $p = 0.036$ (Table 6).

Prevalence of BV-associated microorganisms stratified by vaginal pH

There was no significant association between vaginal pH and the prevalence of the individual microorganisms, $p > 0.05$. Overall, a higher prevalence of the individual microorganisms was seen in women with a healthy vaginal pH (< 4.5) when compared to women with a vaginal pH > 4.5 (Table 7). This leads to the suggestion that vaginal pH was a poor predictor of BV.

Discussion

In this study, we describe the prevalence, risk factors for bacterial vaginosis (BV) and the association between BV and vaginal pH among women from Durban, South Africa. Previous studies on the prevalence of BV in the sub-Saharan African region have reported rates ranging from 31.0% to 42.1% [1,2,9]. Consistent with these reported rates, this study observed a high prevalence of 29.3% for positive BV and 29.3% for intermediate BV. A study conducted among Ugandan women aged 18 – 50 years old, reported a prevalence of 24.9% [17]. Lennard *et al.* [18] conducted a study among black South African females aged 16-22 years and reported a higher prevalence of 44% for positive BV and a lower prevalence of 13% for intermediate BV. In our study, the highest prevalence was observed in the older age groups (31-39 and 40+) with a prevalence of 31.8% for both groups. The age group 18-24 had a positive and intermediate BV prevalence of 11.3% and 15.9%, respectively.

Among the pregnant women, 27.2% were BV positive and 15.9% were BV intermediate. Joyisa *et al.* [19] conducted a BV prevalence study among HIV uninfected South African pregnant women. Using the Nugent score, 37.3% of the women were BV positive and 16.1% were BV intermediate. Another study amongst pregnant women from Ghana, reported a BV prevalence of 30.9% [20]. These high prevalence

Table 6. Factors significantly associated with an unhealthy vaginal pH.

Univariate analysis	**Odds Ratio**	**95% Confidence Interval**	***p***
BV Status			
Negative	1.00		
Positive	1.11	0.47-2.61	0.811
Intermediate	2.42	1.07-5.45	0.033
Multivariate analysis	**Odds Ratio**	**95% Confidence Interval**	***p***
BV Status			
Negative	1.00		
Positive	1.29	0.50-3.37	0.597
Intermediate	2.66	1.06-6.64	0.036

Table 7. Prevalence of BV-associated microorganisms in women with a healthy and unhealthy vaginal pH.

Variables	Vaginal pH		*p*
	Healthy (≤ 4.5) *n (%)*	Unhealthy (> 4.5) *n (%)*	
Overall	48 (54.5)	40 (45.5)	
BVAB1			0.394
Negative	32 (51.6)	30 (48.4)	
Positive	16 (61.5)	10 (38.5)	
BVAB2			0.782
Negative	29 (55.8)	23 (44.2)	
Positive	19 (52.8)	17 (47.2)	
BVAB3			1.000
Negative	30 (54.5)	25 (45.5)	
Positive	18 (54.5)	15 (45.5)	
Atopobium vaginae			0.522
Negative	20 (58.8)	14 (41.2)	
Positive	28 (51.8)	26 (48.2)	
Prevotella bivia			0.709
Negative	15 (51.7)	14 (48.3)	
Positive	33 (55.9)	26 (44.1)	
Gardnerella vaginalis			0.934
Negative	16 (55.2)	13 (44.8)	
Positive	32 (54.2)	27 (45.8)	
***Megasphaera* type 1**			0.370
Negative	23 (50)	23 (50)	
Positive	25 (59.5)	17 (40.5)	
Sneathia sanguinegens			0.517
Negative	32 (57.1)	24 (42.9)	
Positive	16 (50)	16 (50)	

reports of BV among pregnant women warrants the need for routine screening and effective BV management strategies among the pregnant populations.

It has been reported that BV has a higher prevalence and is more persistent among women living with HIV compared to the uninfected women [21]. In the current study, the prevalence of positive and intermediate BV was high (79.5% and 63.6%, respectively) among women living with HIV compared to the uninfected women (20.4% and 36.3%, respectively). Similarly, Apalata *et al.* [22] reported a high prevalence of BV among South African women living HIV compared to their counterparts. In their study, 80.3% of the women were BV positive and 19.7% were BV negative in the HIV infected group, whereas 53.8% were BV positive and 46.2% BV negative in the HIV uninfected group. A case-control study by Foessleitner *et al.* [21] reported a high prevalence of BV among women living with HIV (29.9%) compared to uninfected women (17.6%).

Several behavioral, sociodemographic, and socio-economic factors have been associated with BV. In our study cohort, older women in the age group 31 to 40 years old were approximately 7 times more likely to test positive for BV. Similar to our findings, Apalata *et al.* [22] reported that women older than 35 years were more likely to develop BV. Interestingly, women who did not know whether their partners had other partners were less likely to test positive for BV. This may indicate that these women are more cautious about engaging in unprotected sexual acts. Other studies have reported being unmarried, partner infidelity [23], vaginal douching [24] and *Trichomonas vaginalis* infection [1,23] as risk factors for BV.

Bacterial vaginosis has been described as polymicrobial because no single bacterial species has been shown to be solely responsible for this condition [10]. Previous studies have identified several bacterial species involved in the development of BV, with each species having a unique role and/or characteristics [5]. Therefore, we performed an analysis of the microbial profile between the BV positive and BV intermediate women to determine if there is a difference in the prevalence of BV-associated bacteria in these two groups. Although all the BV-associated bacteria had a higher prevalence in the BV positive women, only BVAB-1, BVAB-2, BVAB-3, *A. vaginae* and *S. sanguinegens* were significantly higher.

BVAB1, BVAB2, and BVAB3 belong to the phylum *Clostridium* [5]. BVAB1-3 have been observed in vaginal smears of BV positive women attached to the vaginal epithelial cells. The attachment of these bacteria to vaginal epithelial cells resembles clue cells, that are a distinctive characteristic of BV [5,25]. Therefore, it is not surprising that these bacteria were more prevalent in the BV positive group in this study. In our study cohort, *A. vaginae* was present in 59.3% (vs 40.7%) of the BV positive women. Similar to our findings, Janulaitiene *et al.* [26] also observed a higher prevalence of *A. vaginae* (89.7%) in BV positive

women compared to women with intermediate BV (51.9%). Other studies that have compared the prevalence of this bacterium between BV positive and negative women, reported prevalence rates of 98% vs 18% [27], 94% vs 43% [28], and 67.5% vs 14.4% [29]. However, in the current study the prevalence of this bacterium was not determined in the BV negative women.

The prevalence of *S. sanguinegens* was also significantly higher in the BV positive group (75%) compared to the BV intermediate group (25%). Similarly, Janulaitiene *et al.* [26] observed a higher prevalence of *Leptotrichia*/*Sneathia* in BV positive women (~70%). However, in their study the prevalence of *Leptotrichia*/*Sneathia* in women with intermediate BV was higher (48.2%) compared to the current study. The prevalence of *P. bivia*, *G. vaginalis,* and *Megasphaera* type 1 were not significantly different between the two groups. Naicker *et al.* [30] also observed no significant difference in the abundance of *P. bivia* across the BV states. In contrast to our findings, they reported a significantly high abundance of *G. vaginalis* in BV positive women. With regards to *Megasphaera* type 1, Hilbert *et al.* [28] reported a higher prevalence (68%) in the BV positive specimens compared to BV negative specimens (2%).

Vaginal pH value has a critical role in determining the health state of the vaginal environment [11]. A healthy vaginal pH ranges from 3.8 to 5.0 for reproductive-aged women [11, 14]. We then performed an analysis of clinical factors associated with an increased vaginal pH. In this study, an increased vaginal pH was defined as pH > 4.5. The method of contraception and pregnancy status were the only clinical factors significantly associated with vaginal pH. The majority of the women who had an increased vaginal pH (70.6%) did not use any contraceptive method. Studies by Ocviyanti *et al.* [31] as well as Donders *et al.* [17] reported no association between contraceptive use and vaginal pH [17].

Previous studies have shown that hormonal contraceptive methods tend to have a protective effect against increased vaginal pH [31] and BV [32]. Brooks *et al.* [32] showed that women using combined oral contraceptives (COCs) and depot medroxyprogesterone acetate (DMPA) were less likely to be colonized by BV-associated bacteria compared to women who used condoms. Furthermore, their study showed that women using COCs were more likely to be colonized by beneficial H_2O_2-producing *Lactobacillus* species compared with women using condoms. In this study, a lower percentage of pregnant women (13.72%) had an increased vaginal pH. Like our findings, Donders *et al.* [17] also observed a low proportion of women attending antenatal clinics with abnormal vaginal pH. The lower rates of increased vaginal pH among pregnant women may be because BV becomes less frequent as the stages of pregnancy advance and subsequently, the vaginal pH decreases [17].

In addition to the above-mentioned factors, we then assessed if BV status is associated with an increased vaginal pH in our study population. A positive BV status was not associated with an increased vaginal pH. Interestingly, an intermediate BV status was significantly associated with an increased vaginal pH. Women who had intermediate BV had ~2.5 increased odds of having an increased vaginal pH. This is also evident in the clinical factors associated with an increased vaginal pH analysis. Although the difference was not statistically significant, a higher proportion (41.2%) of the of women with an increased vaginal pH were BV intermediate.

Previous studies have assessed the role of increased vaginal pH as a predictor or marker for BV. Donders *et al.* [17] assessed the use of self-measured vaginal pH as a marker for BV in Ugandan women. In contrast to our findings, they observed a strong association between self-measured pH and the severity score of BV. Krauss-Silva *et al.* [33] also observed an association between BV status and vaginal pH. In their study, the percentage of BV positive women increased with pH levels. It is important to note that other factors not limited to recent sexual intercourse, presence of sperm, and aerobic vaginitis may increase vaginal pH [17]. The findings of the current study suggest that vaginal pH is a poor predictor for BV status.

Furthermore, we assessed whether the prevalence of BV-associated microorganisms was associated with an increased vaginal pH. To our surprise, the prevalence of BV-associated microorganisms was slightly higher in the women with a normal vaginal pH. This finding adds to the observation that vaginal pH is a poor predictor of BV. To the best of our knowledge, no other published studies have investigated the association between the abundance of BV-associated bacteria with vaginal pH. Therefore, our basis for comparison is limited.

This study had the following limitations: race/ethnicity of the study participants was not recorded during recruitment. Since race has been reported as a risk factor for BV, this could have provided some insight if race acts as a risk factor in the studied population. Another limitation is that the prevalence of BV-associated bacteria was not determined in the BV

negative women. This could have provided a true reflection on the abundance of these bacteria across the different BV states. Nonetheless, this study fills a gap in the literature on the prevalence of BV and BV-associated bacteria and their association with vaginal pH among women in Durban, South Africa.

Conclusions

The current study provides evidence that the prevalence of BV among women from Durban, South Africa remains high. The high percentage of women with an intermediate BV status warrants the need for routine screening and management for BV to reduce the burden of this dysbiosis on the women's sexual health. In this study, vaginal pH was not associated with BV, therefore it was a poor predictor for BV in our study cohort.

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Conflict of interest

No conflict of interest is declared.

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