

Risk factors associated with bacterial vaginosis in pregnant women in Senegal.

Abstract

Background: Bacterial vaginosis (BV) is associated with a high risk of complications, particularly during pregnancy.

Objectives: The aims of this study were to determine the prevalence of BV in pregnant women in Dakar, Senegal, and to identify its associated risk factors.

Patients and Methods: This prospective cross-sectional study was conducted from July 2020 to March 2021 in pregnant women with 34 to 38 weeks of gestation and seen for their routine prenatal consultation at the Nabil Choucair health center in Dakar, Senegal. Vaginal swabs were taken and examined using the Nugent scoring system for the diagnosis of the BV. Data analysis on SPSS (version 25) was done using the chi-square test to measure the strength of association. A value of $p \leq 0.05$ was considered statistically significant.

Results: BV was found in 28.0% (112/400) of the screened women with a median age 24 (21-29) years. A pH >4.5 predictive of BV was found in 80.4% (90/112) of samples. BV was associated with vaginal candidiasis in 49.1% (55/112) while *Trichomonas vaginitis* was found in 1.8% (02/112). In 80.4% (90/112) of affected women, BV was caused by *Gardnerella vaginalis*. *Mobiluncus* spp. was found in 19.6% (22/112) pregnant women in association with *G. vaginalis*. Symptoms characterized by episodes of pruritus, pelvic pain, burning and/or dyspareunia were seen among 59.8% (67/112) of these women with BV while 40.1% (45/112) of them reported no symptoms.

Conclusion: In view of these results and in order to reduce gestational complications and adverse outcomes in the newborn, screening for BV in pregnant women should be favored in developing countries.

Keywords: Bacterial vaginosis, Pregnant women, Risk factors, Senegal.

Introduction

Bacterial vaginosis (BV) is a poly-microbial syndrome characterized by the loss of normal vaginal flora. This results in a change in the balance of the vaginal microflora with a reduction in lactobacilli, and an increase in facultative and anaerobic bacteria in number and/or type and consequently an increase in vaginal pH [1].

It is manifested by changes in the characteristics of the vaginal fluid such as the thickness, color and odor of the secretions [2].

Globally, the overall prevalence of BV was greater than 29.2% (21.2 million) among women aged 14-49 years [3]. Among white women, the prevalence of BV was 23%, while among Mexican Americans, it was 32% [4]. Among African American women, the prevalence of BV was approximately 51% [5]. In sub-Saharan Africa, the prevalence of BV appears to be high and is estimated at 50% [6].

It appears that the prevalence of BV differs considerably from one country to another, within the same region and even within similar population groups. It is estimated to be between 25-50% in sub-Saharan African countries [7]. It can occur in any age group, but in particular, it is more prevalent among women of childbearing age [8].

In pregnant women, BV is associated with adverse gynecological and obstetric outcomes, such as sexually transmitted infections (STIs), pelvic inflammatory disease, premature rupture of membranes, preterm delivery, miscarriage, and low birth weight [9]. Women with BV have a nine fold increased risk of spontaneous abortion and a twofold increased risk of preterm delivery [10].

Worldwide, 15 million babies are born prematurely each year (more than 1 in 10), of which 1.1 million premature babies die. Premature births represent 11.1% of live births worldwide, 60% of which are in South Asia and sub-Saharan Africa [11].

In Senegal, 55,000 babies are born prematurely each year. Prematurity is the 1st cause of death in newborns with a significant proportion of 29% of neonatal mortality. About 25% of spontaneous preterm births are of infectious origin [12].

Essential neonatal care is provided to preterm infants at birth to optimize their survival. Additional efforts are needed to identify women who are at risk of preterm labor during pregnancy by screening and treating infections, including BV.

However, there is no global consensus on the screening or management of BV in the general population of pregnant women to prevent adverse outcomes.

Recommendations for screening and management of BV are targeted toward symptomatic pregnant women who should be treated and, if there is a history of preterm delivery, an asymptomatic infection should be sought. Pregnant women with recurrent symptoms will be re-treated. Screening of asymptomatic pregnant women with no history of preterm delivery is not recommended by World Health Organization [13].

The cause and risk factors associated with BV are not well studied in Africa, particularly in Senegal. There has been little interest in research on BV in recent years. There are few well-structured data showing the liability, prevalence and determinants associated with BV in pregnant women. Thus, countries with limited resources, such as Senegal, suffer from high maternal morbidity, prematurity and neonatal mortality. For this reason, we initiated this research on BV with the objective of determining the prevalence and risk factors associated with BV among pregnant women attending the maternity ward of the Nabil Choucair health center in Dakar, Senegal.

1. Methodology

1.1. Study design, period and setting

This was a prospective, cross-sectional study with descriptive and analytical aims that took place over a period of 9 months, between July 2020 and March 2021, at the Nabil Choucair health center in Dakar.

1.2. Inclusion and non-inclusion criteria

The inclusion criteria were any pregnant women between 34 and 38 weeks' gestation, without any associated pathology, who came for a routine prenatal consultation at the maternity ward of the Nabil Choucair health center and who were willing to participate in the study.

Women with premature rupture of membranes or in labor and those who had received antibiotic therapy within 15 days prior to the consultation were not included in the study.

1.3. Data collection

Sociodemographic characteristics, such as age, marital status, education level, and reproductive health characteristics such as obstetric history, history of genital infections, and history of current pregnancy namely age of pregnancy, associated pathologies, and local symptoms were collected through face-to-face interviews using a structured questionnaire.

All findings were recorded in the coded questionnaire for each participant.

Sample size

Selected participants were sampled using a systematic random sampling technique.

The sample size was calculated using a single population proportion formula and taking into account the following assumptions such as prevalence (P) = 39.5% (in reference to a recent bacterial vaginosis data obtained in the same city. [14]), 95% confidence interval, marginal error (d = 0.05%), Z for 95% confidence intervals = 1.96 and 10% non-response rate.

$n = Z^2 p(1-p)/d^2 = (1.96)^2 \times 0.395(1-0.395)/(0.05)^2 = 367$, and adding a 10% nonresponse rate, $n = 367+36$.

Three volunteers were excluded on the basis of exclusion criteria.

The total final sample size was 400 pregnant women.

1.4. Specimen collection and transport

Each pregnant woman was placed in the gynecological position and underwent a physical examination including vulva, vagina and cervix. The physicians assessed and recorded the progress of the pregnancy. Subsequently, vaginal sampling was performed at the level of the posterior vaginal cul-de-sac with a sterile swab, kept in one milliliter of sterile normal saline after the placement of a speculum. The pH measurement of the sample was determined with the pH indicator paper. Other macroscopic characteristics were noted including: color, appearance, and odor of the discharge.

The vaginal swabs were then sent as soon as possible (less than 3 hours) to the bacteriology laboratory at Aristide Le Dantec University Teaching Hospital in Dakar.

The study was approved by National Ethics Committee for Health Research (CNERS) of Cheikh Anta Diop University (Dakar, Senegal). All study subjects gave written informed consent prior to enrolment.

1.5. Treatment in the laboratory

In the laboratory, a saline wet preparation of a drop of vaginal secretion was used to count leukocytes, red blood cells, epithelial cells, and clues cells, as well as to assess the presence of *Trichomonas vaginalis* and yeast.

The diagnosis of BV was made using the Amsel criteria [15], which encompass three of the following four criteria: presence of homogeneous vaginal discharge, pH > 4.5, positive amine odor test, and presence of clue cells on the vaginal smear. Gram stain smears were read for morphology typing and scoring according to Nugent criteria [16].

Culture and incubation at 37°C for 18 to 24 hours on Sabouraud-chloramphenicol medium was used to test for yeast. *Escherichia coli* was tested on Eosin Methylene Blue (EBM) agar (Merck, Darmstadt, Germany). Group B Streptococcus (GBS) was tested in parallel on fresh blood agar supplemented with nalidixic acid (Bio-Rad, Marnes-la-Coquette, France) and Granada medium (Becton Dickinson GmbH, Heidelberg, Germany) incubated in anaerobic conditions.

Identification

Positive germ cultures were identified by conventional methods, including Gram stain, colony morphology, filamentation test, classical gallery, and by biochemical tests.

The Nugent score has been adopted to diagnose BV (Nugent score 7-10: BV; 4-6: intermediate BV; 1-3: normal).

The diagnosis of vulvovaginal candidiasis (VVC) was retained when yeast or spores were found. Observation of *T. vaginalis* under a wet preparation was the control for *T. vaginalis* vaginitis (TVV).

Identification of GBS strains was confirmed on the Vitek 2 automated compact system (bio-Merieux, Craponne, France) with GP (Gram positive) cards.

1.6. Statistical analysis

All data from the survey were coded and entered into the FileMaker Pro (version 16) software. Analysis was done using Excel and SPSS version 25 (Statistical Package for Social Sciences Chicago, IL, USA). Categorical variables were presented as frequency and percentage, while continuous variables were expressed as median. Tests of association were performed using chi two square. The level of significance for all statistical tests was set at $p < 0.05$.

2. Results

A total of 400 pregnant women between 34 and 38 days' gestation were included in the study. BV was found in 112 pregnant women, a prevalence of 28% (112/400).

2.1. Socio-demographic and obstetrical characteristics

2.1.1 Age

The median age of women with BV was 24 years (21-29), which was lower than that of women without BV, who were 27 years (22.5-30). Women in the age group [16-24] had a higher prevalence of BV estimated at 53.6% (n = 60). This prevalence was lower, estimated at 42.4% (52/112) in the group of women older than 25 years. The age of the women thus distributed was statistically associated with the occurrence of BV (p = 0.02) as shown in Table 1.

2.1.2. Marital status and education level

In our series, the majority of women with BV were married, with a prevalence of 96.4% (n = 108). Among them, 16.1% (n = 18) were polygamous and 83.9% (n = 94) were monogamous. BV was more prevalent among uneducated women with 36.62% (26/71) of cases and among women educated in Arabic (34.9%; 15/44). However, there was no correlation between BV and marital status on the one hand, and between BV and education level on the other hand (table 1).

2.1.3. Number of pregnancies and stage of pregnancy

In our study, the median gestational age was 2 pregnancies per woman (1-2). BV was more observed in first gestation women with 30.9% against 25.8% in multi gestations women.

The median gestational age was the same for women with and without BV, and was 36 weeks of gestation. BV was more observed at 34 and 37 week' gestation with 39.3% and 19.6% of cases, respectively. There was no association between the number, the stage of pregnancy and BV as shown in Table 1.

Table 1: Socio-demographic and obstetrical characteristics of the study population.

Pregnant women		N (%)			
Parameters	Classification	BV (+)	BV (-)	PP	P
		(n = 112)	(n = 288)		
Age (years)	Median (IQR)	24 (21-29)	27 (22.5-30)		
Age group (years)	16-24	60 (53.6)	106 (36.8)	36.1	0.002

	24-46	52 (46.4)	182 (63.2)	22.2	
Marital status					0.510
	Married	108 (96.4)	281 (97.6)	27.8	
	Single	04 (3.6)	7 (2.4)	36.4	
marital regime					0.526
	Monogamy	94 (83.9)	249 (86.5)	31.6	
	Polygamy	18 (16.1)	39 (13.5)	27.4	
Educational level					0.107
	Not educated	26 (23.2)	45 (15.6)	36.6	
	Primary	21 (18.7)	82 (28.5)	20.4	
	Secondary	40 (35.7)	96 (33.3)	29.4	
	Higher	10 (8.9)	36 (12.5)	21.7	
	Arab	15 (13.4)	29 (10.1)	34.9	
Gestational age (WG)	Median (IQR)	36 (34-37)	36 (34-37)		0.707
	34	44 (39.3)	113 (39.2)	28	
	35	11 (9.8)	20 (6.9)	35.5	
	36	14 (12.5)	31 (10.8)	31.1	
	37	22 (19.6)	53 (18.4)	29.3	
	38	21 (18.8)	71 (24.7)	22.8	
Number of pregnancies	Median (IQR)	2 (1-2)	2 (2-1)		0.262
	First gestures	54 (48.2)	121 (42.0)	30.9	
	Multiple gestations	58 (51.8)	167 (58.0)	25.8	

BV (+): women with BV

BV (-): women without VB

IQR: interquartile range;

PP: partial prevalence

P: P-value

WG: week of gestation

2.2 Clinical and microbiological characteristics

2.2.1. Clinical symptoms

During this study, 59.8% (n = 67) of women had experienced symptoms related to the presence of BV. The clinical manifestations most frequently mentioned were irritation (42%), followed by burning sensations (38.4%), pelvic pain and dyspareunia (25 and 20.5% respectively).

However, the BV was present in 40.2% (45/112) of women who had no clinical symptoms, and 51.11% (23/45) of them were primigravida, as shown in table 2.

Table 2: Clinical symptoms and bacterial vaginosis.

Parameters	Characteristics	Pregnant women N (%)		PP	P
		BV (+) (n = 112)	BV (-) (n = 288)		
Symptoms	None	45 (40.2)	133 (46.2)	25.3	0.278
	Present	67 (59.8)	155 (53.8)	30.2	
Dyspareunia	Present	23 (20.5)	38 (13.2)	37.7	0.067
	Absent	89 (79.5)	250 (86.8)	26.3	
Pelvic pain	Present	28 (25)	75 (26)	27.2	0.831
	Absent	84 (75)	213 (74)	28.3	
Burns	Present	43 (38.4)	86 (29.9)	33.3	0.101
	Absent	69 (61.6)	202 (70.1)	25.5	
Irritation	Present	47 (42)	102 (35.4)	31.5	0.250
	Absent	65 (58)	182 (64.6)	25.9	

BV (+): women with BV; BV (-): women without VB; IQR: interquartile range;

PP: partial prevalence; P: P-value

2.2.2. Stillbirths

The prevalence of stillbirths among pregnant women with BV was 15.18% (17/112).

Spontaneous abortions accounted for 41.41% (n = 7) of these cases, dead egg retention (DER) for 17.65% (n = 3), extra uterine pregnancy (EUP) for 5.88% (n = 1) and in utero fetal death for 35.29% (n = 6). Adverse pregnancy outcomes were more common in women with 5 pregnancies with BV.

However, there was no significant association between the occurrence of adverse pregnancy outcomes and the presence of a BV (p = 0.546).

2.2.3. Isolation rate of microorganisms

Out of the 112 BV diagnosed, only 32.14% (n = 36) were due to a monomorphic flora of *Gardnerella vaginalis*.

Mixed infections were found in 67.88% (76/112) of cases. They were due to a polymorphic flora consisting of *G. vaginalis* associated with other microorganisms. The association with *Candida spp.* represented the main part of the co-infections (55/112) or 49.10%, followed by *Mobiluncus spp.* in 19.64%. Group B Streptococcus (GBS) was found 15 times (13.39%) in women with BV, including one case with both *Candida albicans* and *Mobiluncus spp.* Two cases (1.78%) of trichomoniasis associated with *G. vaginalis* had been detected *E. coli* vaginitis (0.89%) was found in one case of BV. No statistically significant association was found between BV and the occurrence of other vaginal infections as shown in Table 3.

Table 3: Distribution of micro-organisms.

Microorganisms Isolated		Number	Percentage (%)	p
Simple infection	<i>G. vaginalis</i>	36	32.14	

<i>G. vaginalis</i> co-infection	76	67.87	
<i>Candida</i> spp	54	48.2	0.089
<i>Mobiluncus</i> spp.	22	19.64	
Group B Streptococcus (GBS)	15	13.39	0.287
<i>Trichomonas vaginalis</i>	02	1.78	0.548
<i>Escherichia coli</i>	01	0.89	0.688

2.2.5. Appearance of the exo-col

The inspection of the vaginal mucosa showed: a normal appearance of the exo-col in 64.25% of the cases (n = 72), an inflammatory appearance in 22.32% (25/112) of the cases (of which 03 with the presence of Naboth's egg and 13 with bleeding at the cervical contact). In 07 women (6.25%) there was bleeding on contact only. In 8% of the cases, or in 07 women, the cervix had not been visualized. There was also no correlation between the appearance of the ectocervix and the presence of a BV.

2.3 Associated risk factors

In this study, the association of BV with different factors was evaluated using bivariate and multivariate logistic analysis. Factors such as age group and color of vaginal secretions were significantly associated with the occurrence of BV.

But sociodemographic factors such as education level, marital status, marital regime mainly polygamy were not significantly associated with the proliferation of bacteria causing BV.

There was also no statistically significant relationship between the occurrence of vulvovaginal candidiasis, GBS carriage, *T. vaginalis* or *E. coli* vaginitis and the distribution of BV.

3. Discussion

The overall prevalence of BV in pregnant women in the present study as determined by Nugent scoring was 28%.

This obtained prevalence of BV is within the expected range for African women, that's 25-50% [17]. This prevalence rate was higher than those reported by previous studies in Senegal, with rates ranging from 21% [18] to 18.6% [19].

Our results were similar to those of some studies conducted in African countries, namely Tanzania (28.5%) [20], Nigeria (26%) [21] and Cameroon (26.2%) [22].

However, they were at odds with data from Ethiopia and South Africa, which reported lower prevalence rates of 19.4 [23] and 17.6% [24], respectively. Similarly, very high prevalence rates were found in Sudan (49.8%) [25] and Nigeria (60%) [26].

These differences in prevalence rates could be attributed to socio-demographic characteristics, sexual activity, information on reproductive health, and behavioral and genital hygiene of the participants.

In our study, women in the [16-24] age group had a higher prevalence of BV and this had been reported in the work of Ibrahim SM *et al.*, in 2014, in Maiduguri in North East Nigeria [27]. Women in this age group were mostly primigravida. In our study, age distribution was a risk factor significantly associated with the occurrence of BV.

In our series, primigravida participants (31.25%) had a higher rate of BV than multiple gestures (25.45%), although the difference was not significant, corroborating the results of previous studies conducted by Achondou AE *et al.*, in 2016 in Cameroon and by Gyasi KD *et al* [28]., in 2015 in Ghana [29]. This finding could be due to the fact that some multiple gestations participants had been exposed to antenatal care at maternal health care centers where they received health education on vaginal hygiene practices and good health behaviors while primigravidas were still inexperienced.

However, our results were not in agreement with the findings of Ibrahim SM *et al.*, in 2014 [26] and Aduloju OP *et al.*, in 2019 [30] who reported in their work that the prevalence of BV was higher among multiple gestational women and women over 30 years of age. These studies also revealed that the high prevalence of BV in women of childbearing age was related to their sexual

activities. Individuals in these age groups were more sexually active and therefore more at risk of acquiring BV and STIs.

There was no significant association between the occurrence of BV and the level of education, although the highest prevalence was observed in women who had never attended school.

In the present study, no significant association was found between BV and marital status, which is similar to the findings of Shayo PA *et al.*, in 2012 [31] although the prevalence was higher among married women. The explanation for this observation was related to the fact that in Senegal, marriage is the legal framework for sexual relations, but also, the majority of participants in our study were married (392/400, 98%).

Mixed infections of BV and CVV were found in 49.10% of the participants. *T. vaginalis* was detected in 1.78% of women. These results are in contradiction with those of the study by Sobel JD *et al.*, in 2006 [32] who reported that co-infections with BV and *T. vaginalis* were more frequent.

These cases of mixed infections raise the issue of treatment failure against BV. Worldwide, BV is treated with the currently recommended antibiotic therapy of metronidazole and clindamycin according to Javed A *et al.*, in 2018 [33]. Jung HS *et al.*, in 2017, report the recurrence rates of 76% which occur within 06 months of treatment due to antibiotic resistance against the pathogenic bacteria and their biofilms [34].

Co-infection of BV with other pathogenic microorganisms may be the reason for the failure of metronidazole treatments against BV alone as in the study by Carey JC *et al.*, in 2000 [35].

In our study, simple infections with BV represented only 32.14%, against 49.10% of co-infection with *Candida* spp., 13.39% of mixed infections with GBS, 1.78% of infection with *T. vaginalis* and 0.89% of infection associated with *E. coli*.

Some studies have also associated BV with unfavorable pregnancy outcomes. In our study many cases of stillbirth history, such as spontaneous abortion (n = 07), ectopic pregnancy (n = 01), dead egg retention (n = 03), and stillbirth (n = 06) had been recorded.

One of the limitations of this study is that it was conducted only in a public health facility in the city of Dakar. It cannot therefore be considered representative of the general population. In addition, cure rates after treatment were not assessed.

Nevertheless, this study had several strengths, including the fact that it was conducted by investigators experienced in the laboratory diagnosis of bacterial vaginosis. Thus, the results provide useful clinical and epidemiological information that could contribute to improving the quality of management of bacterial vaginosis in pregnant women living in urban areas, attending public health facilities.

Conclusion

This study showed that bacterial vaginosis, which is very common in pregnant women, occurs with adverse pregnancy outcomes.

An association between BV and other pathogens responsible for severe gynecological and neonatal infections is established during pregnancy.

There should be regular laboratory screening for BV during routine prenatal visits. In order to allow adequate screening and early treatment to prevent preterm delivery and related complications. It would be important to conduct more in-depth studies on the pathogenesis of BV infection and the occurrence of pregnancy complications in Senegal.

This study showed that bacterial vaginosis, which is very common in pregnant women, is associated with adverse pregnancy outcomes.

An association between BV and other pathogens responsible for serious gynecological and neonatal infections is established during pregnancy.

A strong national health policy should require regular laboratory screening for BV during pregnancy. In order to allow adequate screening and early treatment to prevent preterm delivery and associated complications. It would be important to conduct more in-depth studies on the pathogenesis of BV infection and the occurrence of pregnancy complications in Senegal.

Abbreviations:

BV: Bacterial vaginosis

VVC: vulvovaginal candidiasis

Ethical Approval and Consent

All ethical considerations and obligations were addressed. The study obtained approval from the National Ethics Committee for Health Research (CNER) of the Cheikh Anta Diop University of Dakar and the management of the health center. Free and informed consent was obtained from the participants. Patient information was coded and kept confidential. All test results were sent to the patients. Those with a positive test for a pathogen were informed by the clinicians and received appropriate treatment.

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