Factors Associated with Bacterial Vaginosis in Women of Reproductive Age

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Abstract

The BV is the most prevalent alteration of the vaginal microbiota and constitutes an important public health problem, the prevalence varies by age, ethnicity, socioeconomic factors, and geographic region and the risk factors associated with this condition, are included the number of sexual partners, the use of the intrauterine device, use of vaginal douche, hygiene habits and sexual practices/behaviors. To determine the prevalence and factors associated with bacterial vaginosis in women of reproductive age. Cross-sectional study was developed in Botucatu, Brazil, including 315 women. Data were obtained by applying a questionnaire and gynecological examination, with sample collection for analysis of the vaginal microbiota pattern, according to the Nugent scoring system. The association between independent variables and outcome was performed using a regression model. The variables that most influenced the outcome ($p<0.20$) were taken to the multiple logistic regression model and those independently associated with the outcome ($p<0.05$) were identified. The prevalence of bacterial vaginosis was 32% (101/315) and the associated variables were: not living with a partner [1.13(1.02-1.25), $p=0.021$], use of vaginal douche [1.42(1.22-1.64), $p=0.000$] and genital hair removal [1.19(1.01-1.42), $p=0.042$]. Results point to the importance of screening for this vaginal dysbiosis in routine gynecological care and suggest the importance of health professionals conducting guidance on proper intimate hygiene practices for women.

Keywords: Women; Bacterial vaginosis; Prevalence; Risk factors

Introduction

Vaginal dysbiosis and lower genital tract infections in women, with or without an active sex life, are the main complaints in gynecological care, which can be highlighted the bacterial vaginosis (BV), candidiasis, and trichomoniasis [1].

The BV is the most prevalent alteration of the vaginal microbiota [2] and constitutes an important public health problem because due to its gynecological complications such as pelvic inflammatory disease [3,4], endometritis and recurrence of urinary tract infections [5]. In addition, it increases the risk for acquisition and transmission of sexually transmitted infections (STI), such as Human Immunodeficiency Virus (HIV) [6-8].

In the obstetric scenario, numerous studies [4,6,9,10] have confirmed the relationship between diagnosed BV during pregnancy and the occurrence of unfavorable outcomes such as chorioamnionitis, intra-amniotic infection and preterm delivery. Also, BV is established as the most common cause

of abnormal vaginal contents in women in menacme [2,6,11] and is the most prevalent vaginal dysbiosis among women of reproductive age worldwide [12]. The prevalence varies by age, ethnicity, socioeconomic factors, and geographic region, but the same protocol is used for its assessment [2].

Systematic review and meta-analysis pointed out prevalences of BV by regions of the world ranging from 22.8% to 29.0% [13]. Brazilian studies showed prevalences ranging from 20.0% to 43.1% in women of reproductive age [14-16]. Among the risk factors associated with this condition, are included the number of sexual partners [17,18], the use of the intrauterine device, use of vaginal douche [19-21], as well as hygiene habits and sexual practices/behaviors [22]. Also, there are indirect risk factors associated with this dysbiosis, such as the use of illicit drugs and alcohol, since the use of these substances is usually related to the non-use of condoms and poor intimate hygiene [17].

Therefore, considering the high prevalence of BV, its important repercussions on female reproductive health, and the scarcity of brasilian studies on its associated factors, was proposed to determine the prevalence and factors associated with bacterial vaginosis in women of reproductive age.

Methods

A cross-sectional study was developed in Botucatu, southeastern Brazil, carried out including samples from 315 women collected between July 2018 and January 2019. The samples were collected in basic health units through routine cytopathological examination, and the inclusion criteria were: active sexual life, being in reproductive age, over 18 years old, non-pregnant, not having used a vaginal douche previously, not having had sexual intercourse, and not having used vaginal creams in the latest 72 hours and 24 hours respectively or oral antibiotics within 30 days before the exam. The exclusion criteria were: not being physically able to perform the gynecological exam and mentally unable to answer the questionnaire or not signing the Informed Consent Form.

The sociodemographic, gynecological, and behavioral data, as well as clinical findings at the time of the inclusion, were obtained through a questionnaire, applied by trained nursing professionals.

The gynecological examination involved inspection of the external genitalia to detect vulvar and perianal lesions, and after insertion of a non-lubricated speculum, the vaginal walls samples and cervix were examed, followed by the measurement of vaginal pH using Merck 4-7 indicator paper (Merck Millipore, Burlington, MA, USA), smear collection from the mid-third of the vaginal wall with a swab in glass microscope slides to evaluate the vaginal microbiota pattern, and performance of the amine test with drops of 10% of potassium hydroxide.

The vaginal smears were evaluated by microscopic classification in Gram- staining according to Nugent scoring system. The balance of the vaginal microbiota was characterized by the predominance of *Lactobacillus*, in the absence or presence of rare leukocytes, and the absence of mycotic elements and *Trichomonas vaginalis*. BV diagnoses were obtained by scores ranging from 7 - 10, considering the morphology and staining of the microorganisms observed and their respective semi-quantifications. The tests were performed at the Maternal-Fetal Relationship Immunopathology Laboratory, Pathology Department, Botucatu Medical School (FMB), Universidade Estadual Paulista (UNESP). All women diagnosed with abnormal vaginal microbiota received treatment.

All data were entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). Data were analyzed by descriptive statistics and the association between independent variables and diagnosis of BV was performed by a simple logistic regression model. Then, the variables that had the most influence on the outcome ($p<0.20$) were taken to the multiple logistic regression model, in a way to identify those independently associated with the outcome ($p<0.05$). All analyses were performed using SPSS 21 software (IBM, Armonk, NY, USA).

This study was reviewed and approved by the Research Ethics Committee of of Botucatu Medical School, São Paulo State University (FMB-UNESP), under protocol number 2.763.891 and all participants signed a consent form after being informed about the study's aims and procedures.

Results

Among the 315 women included in the study, 48.3% were between 18 and 30 years of age, and most frequent white (59.7%), with 8 or more years of education (66.3%), living with a partner (58.7%) and with a paid employment (67.0%). Most participants had sexual heterosexual intercourse in the last 6 months (92.1%). They mostly did not use condoms (81.9%), 19.7% practiced anal sex, 8.9% used sexual accessories, and 8.9% and 4.1% had changed sexual partners in the last 6 and 3 months, respectively. The use of hormonal contraceptives was reported by 39.0% of women and 14.3% had smoking habits (Table 1).

Variables		n	Prevalence (%)
Age	18-30	152	48.3
	31-40	95	30.2
	≥41	68	21.5
Race/Ethnicity	White	188	59.7
	Non-white	127	40.3
Marital status	Married/living with partner	185	58.7
	Single	130	41.3
Schooling (years)	< 8	106	33.7
	≥ 8	209	66.3
Employed	Yes	211	67
	No	104	33
Sexual intercourse (last 6 months)	Absence	15	4.8
	Only men	290	92.1
	Only women	6	1.9
	Both men and women	4	1.2
Sexual partnership (last 3 months)	≤ 1	287	91.1
	> 1	28	8.9
Vaginal sex	Yes	314	99.7
	No	1	0.3
Anal sex	Yes	62	19.7
	No	253	80.3
Use Condom	Yes	57	18.1
	No	258	81.9
Use of sex toys	Yes	28	8.9
	No	287	91.1
Contraceptive method	Hormonal	123	39
	Other*	192	61
Smoking	Yes	45	14.3
	No	270	85.7

*Intrauterine device, condom, tubal ligation, coitus interruptus and vasectomy.
Table 1: Sociodemographic and behavioral characteristics of women were included in the study (n=315).

Table 2 displays the intimate hygiene habits of the population study, and we can highlight that the majority (91.1%) shaved their genital region, more than one-third of women (34.3%) washed their underwear in the bathroom and 21.6% did not dry them in the sun.

Variables		n	Prevalence (%)
Vaginal douche	Yes	41	13
	No	274	87
Intimate soap	Yes	106	33.6
	No	209	66.4
Tampon	Yes	98	31.1
	No	217	68.9
Panty liner	Yes	54	17.1
	No	261	82.9

Daily washing of the external genitalia	≤ 1	71	22.5
	> 1	244	77.5
Human waste management	Toilet paper	236	74.9
	Wet wipe	20	6.3
	Shower	59	18.8
Post-intercourse management	Toilet paper	24	7.6
	Wet wipe	14	4.4
	Shower	277	88
Genital hair removal	Yes	287	91.1
	No	28	8.9
Underwear washing in the bathroom	Yes	108	34.3
	No	207	65.7
Sun-drying of underwear	Yes	247	78.4
	No	68	21.6

Table 2: Hygiene habits of women included in the study (n=315).

The majority presented a normal microbiota (52,7%) and BV was the most prevalent vaginal dysbiosis (32.1%), followed by Flora II (7.3%) and *Candida* spp. infection (4.1%) (Table 3).

Vaginal microbiota*	n	Prevalence (%)
Normal microbiota	166	52.7%
Intermediate microbiota	23	7.3%
Bacterial vaginosis	101	32.1%
Cytolytic vaginosis	4	1.3%
Vaginal candidiasis	13	4.1%
Other alterations#	8	2.5%

*Microbiota evaluated according to Nugent score [23].
#Cocci, Diplococci, Diphtheroid Bacilli, sparse microbiota.
Table 3: Vaginal microbiota* classification of women included in the study (n=315).

Table 4 displays the simple logistic regression between bacterial vaginosis and the sociodemographic variables, sexual behavior, substance use, and intimate hygiene habits. The variables that showed were more associated with BV were education less than eight years of study [1.09 (0.98-1.21), p=0.123], having no partner [1.13 (1.02-1.25), p=0.021], smoking habit [1,16 (1.00-1.34), p=0.053], more than one partner in the past 6 months [1.22 (1.02-1.46), p=0.032], vaginal douche [1.43 (1.24-1.66), p=0.000] and genital hair removal [1.17 (0.98-1.40), p=0.090].

Variables		Bacterial Vaginosis No (n=214) Yes (n=101)						
		n	%	n	%	Total	OR† (CI#95%)	p‡
Age	≥41	49	72,1	19	27,9	68	1	
	31-40	63	66,3	32	33,7	95	1,06(0,92-1,22)	0,438
	18-30	102	67,1	50	32,9	152	1,05(0,92-1,20)	0,466
Race/Ethnicity	White	131	69,7	57	30,3	188	1	
	Non-white	83	65,4	44	34,6	127	1,04(0,94-1,16)	0,419
Schooling (years)	≥8 anos	148	70,8	61	29,2	209	1	
	<8 anos	66	62,3	40	37,7	106	1,09(0,98-1,21)	0,123

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Marital status	Married/living with partner	135	73,0	50	27,0	185		
	Single	79	60,8	51	39,2	130	1,13(1,02-1,25)	0,021
Employed (yes)		67	64,4	37	35,6	104	1,05(0,94-1,18)	0,348
Sexual intercourse (last 6 months)	Absence	11	73,3	4	26,7	15		
	Only men	196	67,6	94	32,4	290	1,06(0,83-1,35)	0,641
	Only women	5	83,3	1	16,7	6	0,90(0,58-1,41)	0,657
	Both men and women	2	50,0	2	50,0	4	1,26(0,76-2,11)	0,373
Anal sex (yes)		42	67,7	20	32,3	62	1,00(0,88-1,14)	0,971
Vaginal sex (yes)							1,38(0,55-3,45)	0,491
Use Condom (no)		173	67,1	85	32,9	258	1,05(0,92-1,20)	0,475
Use of sex accessories (yes)		17	60,7	11	39,3	28	1,08(0,90-1,30)	0,390
Smoking (yes)		25	55,6	20	44,4	45	1,16(1,00-1,34)	0,053
Sexual partnership (last 6 months)	≤1	200	69,7	87	30,3	287		
	>1	14	50,0	14	50,0	28	1,22(1,02-1,46)	0,032
Sexual partnership (last 3 months)	≤1	206	68,2	96	31,8	302		
	>1	8	61,5	5	38,5	13	1,07(0,83-1,39)	0,614
Contraceptive method	Hormonal	88	71,5	35	28,5	123		
	Other*	126	65,6	66	34,4	192	1,06(0,95-1,18)	0,271
Vaginal douche (yes)		15	36,6	26	63,4	41	1,43(1,24-1,66)	0,000
Intimate soap (yes)		74	69,8	32	30,2	106	0,97(0,87-1,08)	0,611
Tampon (yes)		64	65,3	34	34,7	98	1,04(0,93-1,16)	0,501
Panty liner (yes)		38	70,4	16	29,6	54	0,97(0,85-1,11)	0,674
Washinh of the genitals a day	≤1	52	73,2	19	26,8	71		
	>1	162	66,4	82	33,6	244	1,07(0,95-1,21)	0,276
Genital hair removal (yes)							1,17(0,98-1,40)	0,090
Human waste management	Toilet paper	40	67,8	19	32,2	59		
	Wet wipe	14	70,0	6	30,0	20	0,98(0,77-1,24)	0,855
	Shower	160	67,8	76	32,2	236	1,00(0,88-1,14)	1,000
Post-intercourse management	Shower	186	67,1	91	32,9	277		
	Not shower	28	73,7	10	26,3	38	0,94(0,80-1,10)	0,418
Underwear washing in the bathroom (yes)		70	64,8	38	35,2	108	1,05(0,94-1,17)	0,391
Sun-drying of underwear		43	63,2	25	36,8	68	1,31(0,75-2,30)	0,349

*Intrauterine device, condom, tubal ligation, coitus interruptus, vasectomy; †OR- odds ratio; #CI- confidence interval;
‡*p*-p-value
Table 4: One-variate analysis for sociodemographic, behavioral and hygiene habits with the bacterial vaginosis of women included in the study (n=315).

In the multivariate analysis, the independent variables associated with BV were: being single [1.13 (1.02-1.25), *p*=0.021], use of vaginal douche [1.42 (1.22-1.64), *p*=0.000] and genital hair removal [1.19 (1.01-1.42), *p*=0.042] (Table 5).

Variables		OR† (CI#95%)	p‡
Schooling (years)	<8	1.10 (0.99-1.22)	0.088
	≥8	1	
Marital status	Married/living with partner	1	
	Single	1.13 (1.02-1.25)	0.021
Smoking	Yes	1.06 (0.91-1.22)	0.463
	No	1	
Sexual partnership (last 6 months)	≤1	1	
	>1	1.15 (0.96-1.37)	0.129
Vaginal douche	Yes	1.42 (1.22-1.64)	0
	No	1	
Genital hair removal		1.19 (1.01-1.42)	0.042

†OR- odds ratio; #CI- confidence interval; ‡p-p-value
Table 5: Multivariate analysis of variables for bacterial vaginosis risk.

Discussion

The present investigation identified a high prevalence of this infection with socio- demographic and intimate hygiene variables independently associated with this important and classic vaginal dysbiosis.

The BV prevalence rate (32.1%), diagnosed according to Nugent criteria [23], was similar to other studies. A recent systematic review of the literature demonstrated overall prevalences ranging from 23.0% to 29.0% [13] and another study in Tanzania with women in the age group between 17 and 18 years showed a prevalence of 33.0% [24].

A study with a similar population showed a 30.1% BV prevalence [21]. However, a same located study conducted with women who have sex with women [25] and international studies in Nigeria [26] and South Africa [27], indicated higher prevalences (36.0%, 40.1%, and 48.0%), respectively. This difference may be related to population characteristics, since previous studies have reported higher prevalence in the black population [9,28] and in women who have sex with women [29,30].

Among the factors associated with BV, in agreement with another findings [21,31], it was demonstrated that not having a regular partner increased the chance of vaginal dysbiosis, diverging from other prior investigations that did not show such association [9,32]. Differences in study designs may account for the discrepancies found, as the present study considered two categories (with and without a partner), whereas previous studies considered separated, divorced, and widowed women as a distinct category. The data may suggest that not living with a partner predisposes women to have contact with other microbiotas, suggesting

sexual transmission of BV, since most of them did not use condoms in their sexual relations. However, this study was not designed for this purpose, which limits us to suggest this association, and further studies should properly define this question.

Female intimate hygiene habits are both related to and influenced by cultural, social, and religious issues. The present investigation found an association between the use of vaginal douche with BV, corroborating the findings of several studies with women of reproductive age [9,19,33,34]. However, this finding is still contested by other studies [20,25,35], with no association of this practice with BV being observed. It has been postulated that vaginal douching promotes change in the vaginal microbiota, facilitating the establishment of BV and an increase in the inflammatory environment of the lower genital tract, with the associated release of inflammatory cytokines. This inflammatory environment favors the recruitment and activation of defense cells, increasing mucosa vulnerability to pathogens and HIV during vaginal intercourse [36]. We also observed the association of BV with the genital hair removal. North American studies sought to associate factors, prevalence, and characteristics for the genital hair removal, demonstrating that this practice is associated mainly with young women, relationship status, and sexual practices [37,38]. However, there are no studies relating this hygienic practice to BV, thus providing no comparison of data.

The study's limitations consisted of it being carried out with a non-randomized sample and restricted to users of the public health system who voluntarily sought routine gynecological care. However, this work shows important factors associated with vaginal dysbiosis on women's reproductive health.

Conclusion

The study showed a high prevalence of BV in reproductive-age women in primary health care, pointing to the importance of its screening in routine gynecological care. Its association with marital status, vaginal douching, and genital hair removal suggest that health professionals should be attentive to the intimate hygiene habits of women, guiding appropriate practices, as well as encouraging the use of condoms.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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