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CLINICAL ARTICLE

Prevalence of and risk factors for bacterial vaginosis among women of reproductive age attending cervical screening in southeastern Brazil

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ABSTRACT

Objective: To determine the prevalence of and risk factors for bacterial vaginosis. *Methods:* A cross-sectional study of women aged 14–54 years attending 18 primary healthcare units in Botucatu, Brazil, for cervical screening was undertaken between September 1, 2012, and January 31, 2013. Data on sociodemographics, sexual behavior, and medical history were obtained by interview. Vaginal swabs were taken to classify the vaginal flora according to the Nugent scoring system. *Candida* sp. hyphae and infection by *Trichomonas vaginalis* were also evaluated by microscopy and culture, respectively. Stepwise logistic regression analysis was performed to identify risk factors independently associated with bacterial vaginosis. *Results:* Among 1519 women included in analyses, 457 (30.1%) had bacterial vaginosis. Variables independently associated with bacterial vaginosis were a single marital status (OR 1.4; 95%CI 1.1-1.8), partner infidelity (OR 1.5; 95%CI 1.2-1.9), abnormal discharge in the previous year (OR 1.5; 95%CI 1.2-2.0), and concurrent trichomoniasis (OR 4.1; 95%CI 1.5-11.5). Current use of hormonal contraception (OR 0.7; 95%CI 0.5-0.9), luteal phase of menstrual cycle (OR 0.8; 95%CI 0.6-0.9), higher income (OR 0.8; 95%CI 0.6-0.9), and vaginal candidiasis (OR 0.5; 95%CI 0.3-0.9) all had protective effects. *Conclusion:* The prevalence of bacterial vaginosis in the study population is high. The epidemiological data provide evidence of the sexual transmissibility of bacterial vaginosis.

1. Introduction

Bacterial vaginosis—characterized by the loss of local lactobacillus-predominant vaginal flora—is the most common type of vaginal flora abnormality among women of reproductive age [1]. Although its symptoms are considered a leading cause for women to seek medical care, up to 50% of affected women are asymptomatic [1], which is particularly troublesome given the disease's association with numerous gynecologic complications, such as postoperative infection, pelvic inflammatory disease, and increased risks of sexually transmitted infections (STIs) [2,3]. Indeed, several studies have linked bacterial vaginosis with the most frequently occurring STIs—i.e. infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and HIV [4–6].

Nevertheless, the etiology of bacterial vaginosis remains to be elucidated, although several sociodemographic and behavioral factors have been shown to be associated with the development of this condition. It is significantly more frequent among black women and among those with a lower income and educational level [1,7–9]. Moreover, a recent meta-analysis [10] showed that an increase in the number of sex partners, a history of female sex partners, and irregular condom use are also associated with bacterial vaginosis, all of which provide increasing evidence of the sexual transmissibility of this disorder.

The prevalence of bacterial vaginosis and the factors associated with this condition remain underestimated, particularly in Brazil. The few reported studies have had small population sizes [11], were limited to high-risk populations [12], or did not evaluate the independent risk factors for the disease [11,12]. Considering the scarce data available on the prevalence of bacterial vaginosis in Brazilian women, a population with a high prevalence of STIs, the aim of the present study was to assess the prevalence of and risk factors for bacterial vaginosis among women of reproductive age attending primary health care units in Botucatu, São Paulo, Brazil.

2. Materials and methods

A cross-sectional study was undertaken at 18 primary healthcare units in Botucatu, southeastern Brazil. These primary health care units are part of the Brazilian public health system and provide free-of-charge medical care to the population of Botucatu. The units are located in both urban (15 units) and rural (3 units) areas of the town.

Women of reproductive age (14–54 years) attending the general gynecology clinics for routine cervical smear tests between September 1, 2012, and January 31, 2013, were invited to participate, unless they

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reported HIV infection, were pregnant, or were postmenopausal. The study aims were explained and women were asked to provide written informed consent. The study was approved by the Research Ethics Committee of Botucatu Medical School, São Paulo State University (FMB-UNESP), under protocol number 4121–2012.

Women contacting the gynecology services to arrange an appointment for a cervical smear test were asked to attend a minimum of 5 days following the end of menses. Women were asked to refrain from sexual activity for at least 72 hours in advance of their appointment, and to confirm they had not received any oral or topical antibiotics in the previous 30 days. If women did not meet these criteria on presentation for the smear test, an alternative appointment was scheduled for an appropriate date.

Self-reported information regarding sociodemographics, sexual behavior, and medical and reproductive history was obtained during individual face-to-face interviews using an extensive standard questionnaire with closed questions. All interviews were performed informally in an easily understandable way by trained nursing staff from FMB-UNESP. In cases of uncertainty or discomfort when answering a question, women were advised to leave it unanswered, which would have no repercussions on their inclusion in the study.

During the routine physical examination for cervical smear collection, additional vaginal samples were taken to assess the presence of bacterial vaginosis, vaginal candidiasis, and infection by *Trichomonas vaginalis*. Following insertion of a non-lubricated speculum, mid-vaginal wall samples were collected with cotton swabs and spread onto glass microscope slides to detect *Candida* sp. morphotypes and to classify vaginal flora after Gram-staining according to the Nugent scoring system (normal: 0–3; intermediate: 4–6; bacterial vaginosis: 7–10) [13]. Women with Nugent scores of 7 or higher were deemed to have bacterial vaginosis, irrespective of the presence of symptoms or clinical findings. Microscopic classification of the vaginal flora was performed onsite, immediately after sample collection. At least one of the four experienced microscopists from the central laboratory at FMB-UNESP attended the units on the dates on which cervical smear tests were scheduled. From the total number of vaginal smears evaluated, 10% were re-checked by a different microscopist. The overall κ pairwise agreement for diagnosis of bacterial vaginosis using the Nugent score was above 0.9.

For *T. vaginalis* detection, vault vaginal samples were collected and transported to the central laboratory at FMB-UNESP within a maximum period of 4 hours after sampling, and kept at 25 °C–30 °C. Samples were incubated in Diamond's medium using CPLM medium base at 37 °C, prepared according to the manufacturer's instructions (Himedia, Mumbai, India). The presence of motile protozoa was assessed daily, for up to 3 days, using fresh wet-mount microscope slides prepared with aliquots from the culture. A positive culture of *T. vaginalis* was maintained in the laboratory and cultured concomitantly as a positive control.

For statistical analyses, the median and range of continuous variables (i.e. age, income, income per family member, and ages at menarche and at first sexual intercourse) were determined together with the frequency of bacterial vaginosis for each categorical variable. The strategy of analysis was the adjustment of a stepwise logistic regression model to describe the likelihood of positivity for bacterial vaginosis in relation to all continuous and categorical variables, controlling for possible confounders. SAS version 9.2 (SAS Institute, Cary, NC, USA) was used. $P < 0.05$ was considered statistically significant.

3. Results

Overall, 1521 non-pregnant, non-menopausal women who did not report HIV seroconversion were considered eligible. Laboratory results were available for 1519 of the 1521 women. Despite having the choice of not responding to questions which they felt uncomfortable answering, the vast majority of participants completed the entire questionnaire; exceptions were questions regarding a current paid activity and last menstrual period, which were answered by 1459 (96.1%) individuals. The median age was 33 years (range 14–54), and 941 (61.9%) defined themselves as white and 1420 (93.5%) reported living in an urban area. Most participants were married or living with a partner (1247 [82.1%] women), and had a formal or informal job (969 [66.4%]) at the time of enrollment. Regarding education, 798 (52.5%) had completed high school, but only 202 (13.3%) had attended college or completed a degree.

Laboratory results from the 1519 women enrolled showed that 457 (30.1%) had bacterial vaginosis. Additionally, *Candida* sp. hyphae were detected on vaginal smears from 75 (4.9%) women during microscopic evaluation of the Gram-stained smears. Finally, *T. vaginalis* was identified in 21 (1.4%) women.

Analysis of the characteristics associated with bacterial vaginosis showed that single women were at increased risk of this condition, whereas a higher income per family member exerted a protective effect (Table 1). Participants who reported previous or current partner infidelity were more likely to present with bacterial vaginosis (Table 1). Use of hormonal contraceptives for at least the preceding 4 months and being in the luteal phase of the menstrual cycle were protective factors (Table 1). When inquiring about observed episodes of abnormal vaginal discharge in the preceding year, women who answered "yes" were at increased risk of current bacterial vaginosis (Table 1). Detection of *Candida* sp. hyphae on vaginal smears was inversely associated with concurrent bacterial vaginosis, while women with positive culture results for *T. vaginalis* were at increased risk of presenting with abnormal vaginal flora (Table 1).

4. Discussion

The present study aimed to investigate the prevalence of and risk factors for bacterial vaginosis in a large Brazilian population cohort. The results indicate that approximately 30% of women in this population have bacterial vaginosis. Additionally, several risk factors for this condition have been identified, including a single marital status, a low income, abnormal discharge in the past year, and partner infidelity, thus adding evidence that bacterial vaginosis is sexually transmissible.

Previous studies with similar aims have been conducted in Brazil [11,12], although these were limited to specific populations and did not provide any information regarding the factors associated with bacterial vaginosis. Thus, the prevalence of this vaginal disorder in Brazil could not be accurately compared with the largest studies performed worldwide.

The prevalence of bacterial vaginosis of 30.1% in the present study is similar to rates reported in similar populations in the USA (29.2%) [1], Uganda (34.3%) [14], and Peru (40.8%) [15], but much higher than prevalences reported in Finland (8.6%) [16], Canada (7.1%) [17], and China (5.9%) [18]. Because bacterial vaginosis increases the risk of several STIs [4–6], the present data show that more than one-third of women of reproductive age in Brazil are highly vulnerable to such infections.

Numerous previously published studies failed to demonstrate an association between bacterial vaginosis and marital status [1,7,19]. However, the present findings show that single women are at increased risk of bacterial vaginosis compared with those living with a husband or a partner. This discrepancy could be attributed to differences in study design: in the present study, only two marital status categories were considered, whereas previous studies considered divorced, widowed, and separated women as a distinct category [1,19].

Another divergence between the present findings and the literature is the absence of an association between bacterial vaginosis and self-defined ethnic origin. Although previous studies have consistently shown that black women present with an increased risk of bacterial vaginosis [1,7,9], even when controlling for several demographic and behavioral variables [7], this association was not confirmed in the present investigation. In fact, miscegenation is extremely frequent among Brazilians, and categorizing the population based on self-defined ethnic

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Table 1
Characteristics and their associations with bacterial vaginosis determined by stepwise logistic regression analysis.

Variable	BV negative (n = 1062)[a]	BV positive (n = 457)[a]	Adjusted odds ratio (95% confidence interval)[b]
Sociodemographics			
Age, y	32 (14–54)	33 (15–53)	–
Ethnic origin			–
White	676 (63.7)	265 (58.0)	
Afro-Brazilian	366 (34.5)	188 (41.1)	
Asian/Indigenous	20 (1.9)	4 (0.9)	
Marital status			
Single	347 (32.7)	191 (41.8)	1.4 (1.1–1.8)
Married/living with partner	981 (67.3)	266 (58.2)	Ref.
Current paid activity [c]			–
Yes	688 (67.5)	281 (63.9)	
No	331 (32.5)	159 (36.1)	
Educational level			–
Elementary school (any)	386 (36.3)	142 (31.1)	
High school, but incomplete	190 (17.9)	90 (19.7)	
Completed high school	410 (38.6)	186 (40.7)	
Completed college	163 (15.3)	39 (8.5)	
Income (number of minimum wages)	2.2 (0.0–29.5)	1.9 (0.0–15.0)	–
Number of minimum wages per family member	0.6 (0.0–11.1)	0.5 (0.0–5.2)	0.8 (0.6–0.9)
Residence			–
Rural area	67 (6.3)	32 (7.0)	
Urban area	995 (93.7)	425 (93.0)	
Behavioral characteristics			
Active sex life			–
Yes	967 (91.1)	412 (90.2)	
No	95 (8.9)	45 (9.8)	
Number of partners in preceding year	1 (1–15)	1 (1–5)	–
Current or previous partner infidelity			
Yes	378 (32.8)	246 (53.8)	1.5 (1.2–1.9)
No	714 (67.2)	211 (46.2)	Ref.
History of a female sexual partner			–
Yes	11 (1.0)	13 (2.8)	
No	1051 (99.0)	444 (97.2)	
Frequency of sexual intercourse per wk			–
<1	241 (22.7)	115 (25.2)	
1–3	634 (59.7)	262 (57.3)	
≥4	187 (17.6)	80 (17.5)	
Regular anal sex			–
Yes	261 (24.6)	121 (26.5)	
No	801 (75.4)	336 (73.5)	
Postcoital genital washing			–
Yes	968 (91.1)	411 (89.9)	
No	94 (8.9)	46 (10.1)	
Vaginal douching			–
Yes	75 (7.1)	35 (7.7)	
No	987 (92.9)	422 (92.3)	
Tobacco smoking			–
Yes	184 (17.3)	103 (22.5)	
No	878 (82.7)	354 (77.5)	
Alcohol consumption			–
Yes	378 (35.6)	194 (42.5)	
No	684 (64.4)	263 (57.5)	
Frequency of alcohol consumption			–
Never	681 (64.1)	261 (57.1)	
Socially	377 (35.5)	191 (41.9)	
Daily	4 (0.4)	5 (1.0)	
Current use of illicit drugs (any)			–
Yes	25 (2.4)	19 (4.1)	
No	1037 (97.6)	438 (95.8)	
Current use of marijuana			–
Yes	19 (1.8)	14 (3.1)	
No	1043 (98.2)	443 (96.9)	
Current use of cocaine			–
Yes	7 (0.7)	4 (0.9)	
No	1055 (99.3)	453 (99.1)	
Current use of crack cocaine			–
Yes	3 (0.3)	4 (0.9)	
No	1059 (99.7)	453 (99.1)	
Reproductive and sexual history			
Age at menarche, y	13 (8–18)	13 (9–17)	–
Age at first sexual intercourse, y	16 (0–45)	16 (9–36)	–
Ever pregnant			–
Yes	849 (80.0)	361 (79.0)	
No	213 (20.0)	96 (21.0)	

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Table 1 (*continued*)

Variable	BV negative (n = 1062)[a]	BV positive (n = 457)[a]	Adjusted odds ratio (95% confidence interval)[b]
History of sexually transmitted infections			–
Yes	92 (8.7)	43 (9.4)	
No	970 (91.3)	414 (90.6)	
Oral hormonal contraception for at least the previous 4 months			
Yes	429 (40.8)	146 (31.9)	0.7 (0.5–0.9)
No	623 (59.2)	311 (68.1)	Ref.
Injectable hormonal contraception for at least the previous 4 months			–
Yes	96 (9.1)	45 (9.8)	
No	956 (90.9)	412 (90.2)	
Intrauterine device			–
Yes	15 (1.4)	7 (1.5)	
No	1047 (98.6)	450 (98.5)	
Consistent condom use			–
Yes	209 (19.7)	84 (18.4)	
No	853 (80.3)	373 (81.6)	
Clinical findings at enrollment			
Phase of menstrual cycle [d]			
Luteal	501 (48.9)	182 (41.9)	0.8 (0.6–0.9)
Follicular	524 (51.1)	252 (58.1)	Ref.
Itching complaint			–
Yes	518 (48.8)	242 (53.0)	
No	544 (51.2)	215 (47.0)	
Abnormal discharge (preceding year)			
Yes	638 (60.1)	323 (70.7)	1.5 (1.2–2.0)
No	424 (39.9)	134 (29.3)	Ref.
Abnormal discharge (preceding week)			–
Yes	437 (41.1)	226 (49.5)	
No	625 (58.9)	231 (50.5)	
Candida sp. hyphae			
Yes	59 (5.6)	16 (3.5)	0.5 (0.3–0.9)
No	1003 (94.4)	441 (96.5)	Ref.
Trichomoniasis			
Yes	7 (0.7)	14 (3.0)	4.1 (1.5–11.5)
No	1055 (99.3)	443 (97.0)	Ref.

Abbreviation: BV, bacterial vaginosis.
[a] Data are median (range) or number (percentage).
[b] Shown only for variables that reached significance in the stepwise selection for the final logistic model.
[c] BV negative: n = 1019; BV positive: n = 440.
[d] BV negative: n = 1025; BV positive: n = 434.

origin might not result in accurate data. Supporting this hypothesis, Mascarenhas et al. [12] also failed to demonstrate an association between bacterial vaginosis and ethnic origin in a Brazilian population.

Several studies have shown that a lower educational level increases the risk of bacterial vaginosis [1,8,9]. Although this association was initially observed in the present investigation, it did not persist after eliminating confounders by logistic regression. Regarding socioeconomic status, a higher income (estimated as the number of minimum wages per family member) was a protective factor against the presence of bacterial vaginosis in the present study, which is in agreement with literature findings demonstrating that a lower household income is a risk factor for bacterial vaginosis [9].

An important finding of the present study was the significantly higher prevalence of bacterial vaginosis among women who reported infidelity of the current or past sexual partner; this association remained significant even after controlling for other sexual behavior variables. These data corroborate the recent report by Kenyon et al. [20], who demonstrated that the presence of bacterial vaginosis is associated with male concurrency, and with another study that found an association of this condition with partner infidelity in the preceding year [15]. Additionally, first evidences of the sexual transmission of bacterial vaginosis were provided by Gardner and Dukes [21], who demonstrated that bacterial vaginosis can be induced in healthy women following inoculation with vaginal samples from women with bacterial vaginosis. Indeed, a recent report showed the shared carriage of cohesive *Gardnerella vaginalis* strains between symptomatic women with bacterial vaginosis and their respective partners using the highly specific and sensitive fluorescent in situ hybridization technique [22].

Finally, in line with the present findings, several studies have observed that women who have sex with women are disproportionally affected by bacterial vaginosis [8,9]. Nevertheless, the correlation no longer remained significant after controlling for possible confounders in multivariate analyses in the present study. It is worth mentioning that, although the independent association of bacterial vaginosis with a previous female sex partner was not confirmed in the present study, only 1.6% of the population recruited declared ever having had sex with a woman.

The protective role of hormonal contraceptive intake against bacterial vaginosis has previously been demonstrated by several studies [8,19] and was confirmed by the present results. One of the most accepted hypotheses for this association is the increased accumulation of glycogen in vaginal epithelial cells, which serve as local lactobacilli substrates for conversion to lactic acid, previously shown to suppress the bacterial species associated with bacterial vaginosis [23]. Other plausible evidence of the contribution of estrogen toward a lactobacilli-dominated flora is the inverse association of bacterial vaginosis with the luteal phase of the menstrual cycle, which occurs right after the peak of estrogen during ovulation. In addition, the vaginal flora is disrupted during menses in most women and, because the vaginal flora is highly dynamic, lactobacilli restoration is more likely to be achieved during the second phase of the menstrual cycle [24].

The prevalence of bacterial vaginosis was also significantly higher among women who reported to have experienced episodes of abnormal vaginal discharge in the preceding year, irrespective of whether they had sought medical care (which was not established in the present study). Nevertheless, other investigators have failed to demonstrate

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this association [1,10]. No association was determined between itching complaints and bacterial vaginosis; however, women with *Candida* sp. hyphae detected by microscopy were less likely to present with concurrent bacterial vaginosis. This negative association between vaginal *Candida* sp. morphotypes and bacterial vaginosis corroborates previous findings showing that vaginal candidiasis was protective against recurrent cases of bacterial vaginosis [25]. This is expected, because *Candida* sp. presents optimum growth under acid pH and bacterial vaginosis is associated with significantly increased vaginal pH. Several STIs have been positively associated with bacterial vaginosis, including vaginal trichomoniasis [6]. Indeed, a strong association was observed between trichomoniasis and bacterial vaginosis in the present study, independently of other factors.

A limitation of the present study is that it was restricted to women who rely on the public health system and who voluntarily contacted the study units for cervical smear testing. Thus, these data might not be generalizable to the entire Brazilian population. However, the public health system has good coverage in Botucatu, São Paulo, Brazil, so the present study probably reached a substantial proportion of the population of women of reproductive age. Another limitation is that these data do not provide information regarding women who do not routinely visit gynecology services for cervical smear tests. Further studies need to reach this part of the population to assess the frequency and risk factor for bacterial vaginosis.

In conclusion, the present study shows that the prevalence of bacterial vaginosis in Brazilian women attending cervical screening in primary health care units is high and that routine assessment of the vaginal flora could benefit this population by preventing the gynecologic complications associated with abnormal types of vaginal flora, such as increased STI acquisition. The present epidemiological study provides further information concerning high-risk behaviors in this population and corroborates previous findings regarding the role of estrogen in maintaining a lactobacilli-dominated flora as well as the sexual transmissibility of bacterial vaginosis.

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Conflict of interest

The authors have no conflicts of interest.

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