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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.

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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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