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CLINICAL ARTICLE

Cervicovaginal bacterial count and failure of metronidazole therapy for bacterial vaginosis

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ABSTRACT

Objective: To evaluate whether total bacterial count in cervicovaginal fluid is associated with failure of metronidazole therapy for bacterial vaginosis. *Methods:* In a cross-sectional study, women attending a primary health center in Botucatu, São Paulo, Brazil, for routine cervical screening between September 2012 and October 2013 were enrolled. Women who tested positive for bacterial vaginosis (Nugent classification) were offered oral metronidazole. Women who completed metronidazole treatment and an equal number of control women with normal vaginal flora at initial screening were included in analyses of total bacterial count, assessed by flow cytometry of cervicovaginal fluid samples. *Results:* Of 287 women who enrolled, 49 were excluded because they tested positive for trichomoniasis, chlamydial endocervicitis, gonorrhea, or candidiasis. Among the remaining 238, 85 (35.7%) had bacterial vaginosis. Among 36 women evaluated at follow-up, 23 (63.9%) had successfully restored lactobacilli-dominant flora, 12 (33.3%) had persistent bacterial vaginosis, and 1 (2.8%) had vaginal candidiasis (excluded from flow cytometry). Total bacterial count did not differ between 35 women with bacterial vaginosis and 35 with normal vaginal flora ($P = 0.62$). Total bacterial count did not differ at enrollment between women who went on to have persistent bacterial vaginosis and those who had successful treatment ($P = 0.78$). *Conclusion:* Failure of oral metronidazole therapy for bacterial vaginosis was not associated with total bacterial count in cervicovaginal fluid.

1. Introduction

Vaginal flora is considered normal when *Lactobacillus* spp. outnumber other colonizing bacteria [1]. Bacterial vaginosis, the most common type of abnormal vaginal flora, is a condition characterized by partial or total replacement of vaginal lactobacilli by other bacterial species, mostly anaerobes [2]. This condition is particularly important among reproductive-aged women because it increases the risk of both poor pregnancy outcomes and the acquisition of several sexually transmitted infections, including HIV [3–5].

Although bacterial vaginosis has serious implications for women's reproductive health, treatment of this condition remains a challenge in clinical practice. The short-term persistence of bacterial vaginosis after therapy with metronidazole is reported to be up to 36.0% [6,7]. Moreover, the rate of therapy failure can be higher than 50.0% at 6 months after treatment [8]. Despite the high persistence rates of bacterial vaginosis after metronidazole therapy, the microbial features associated with difficulties in re-establishing the lactobacillar flora remain unknown.

Studies have shown that the bacterial diversity of cervicovaginal fluid among women with bacterial vaginosis is significantly higher than the diversity of normal lactobacilli-dominated flora [9,10]. However, the possible association between the microbiological features of bacterial vaginosis and the response to treatment for this condition remains to be established [11,12]. In addition to the increased bacterial diversity, a higher bacterial load, as determined by the number of bacterial colony-forming units (CFUs) or by taxon-directed quantitative PCR, is also present in the cervicovaginal fluid of women with bacterial vaginosis [13–15]. Thus, it is possible that the vaginal bacterial load, rather than the bacterial composition, might contribute to failure of the recommended metronidazole therapy for this condition [6].

Because most bacterial species colonizing the vaginal milieu are fastidious or non-cultivable, methods other than traditional microbiological culture are needed to determine the actual load of cervicovaginal bacteria [10]. High-throughput sequencing facilitates the identification of several vaginal bacterial species that have not been previously recovered by culture methods; however, it is not useful for determining the total bacterial count. Despite advances in the microbial characterization of bacterial vaginosis, therefore, information regarding the bacterial load of cervicovaginal fluid remains scarce.

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Recently, flow cytometry has been successfully used to determine the total bacterial count in cervicovaginal samples [16], as well as other body fluids [17], because it does not depend on bacterial growth in vitro. Given that the microbiological aspects involved in the treatment failure of bacterial vaginosis remain unknown, the aim of the present study was to evaluate whether the total bacterial count in cervicovaginal fluid is associated with the subsequent failure of metronidazole therapy for bacterial vaginosis.

2. Materials and methods

From September 1, 2012, to October 31, 2013, women of reproductive age (18–49 years) who were attending a primary medical care unit in Botucatu, São Paulo, Brazil, for a routine screening test for cervical cancer were enrolled into a cross-sectional study. Women who reported vaginal bleeding, urinary incontinence, recent sexual intercourse (<3 days ago), breastfeeding, use of intrauterine device, antibiotics (<30 days ago), and confirmed/possible pregnancy were not considered for enrollment. After an explanation of the aims and procedures of the study, all participating women provided written informed consent. The study was reviewed and approved by the Ethics Committee of Botucatu Medical School (protocol 306.547).

Participants underwent a physical examination and answered a standardized questionnaire that included information regarding demographics, behavioral characteristics, and clinical history. During the physical examination, two trained nurses assessed the vaginal pH (4.0–7.0) with pH indicator strips (Merck, Darmstadt, Germany) and performed a 10% potassium hydroxide test using a nonlubricated sterile speculum. Microscopic evaluation of the vaginal flora was performed via midlateral vaginal wall smears, which were Gram-stained and classified by Nugent's score as normal (0–3 score), intermediate (4–6), or bacterial vaginosis (7–10) [18]. An additional vaginal smear was wet-mounted to detect the presence of *Trichomonas vaginalis* and *Candida* spp. morphotypes. Cervical brush samples were taken for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing by PCR as previously described [19]. Cervicovaginal fluid samples were also obtained from all women by placing 3 mL sterile NaCl 0.9% (w/v) in the vagina in contact with the lateral vaginal wall and ectocervix. The samples were recovered by using sterile plastic pipettes and kept at −80 °C until analysis.

After these tests, women who tested positive for *C. trachomatis*, *N. gonorrhoeae*, concurrent *C. trachomatis* and *N. gonorrhoeae*, *T. vaginalis*, and vaginal candidiasis were excluded from the study. Women with intermediate flora at baseline were also excluded.

Participants with bacterial vaginosis were offered treatment with metronidazole, which was doses of 500 mg twice daily for 7 days, as recommended by the Center of Disease Control and Prevention [6]. All participants were asked to return for follow-up 45–60 days after the end of therapy.

At follow-up, women who confirmed that they had performed the treatment according to the recommendations, did not forget the medication at any time, and had not had sexual intercourse during the progress of treatment were included in the analysis. To ensure that their answers were correct, the women were asked about these topics in different ways with varying orders of questions.

Flow cytometry analyses were performed on cervicovaginal fluid samples obtained at enrollment from women with bacterial vaginosis who completed the treatment and returned for follow-up. For each woman who completed treatment and returned for follow-up, the next woman who had been enrolled and found to have normal flora was used as a control individual in flow cytometry analyses.

In accordance with the protocol of Schellenberg et al. [16], frozen samples were thawed on ice and 400-μL aliquots were fixed overnight

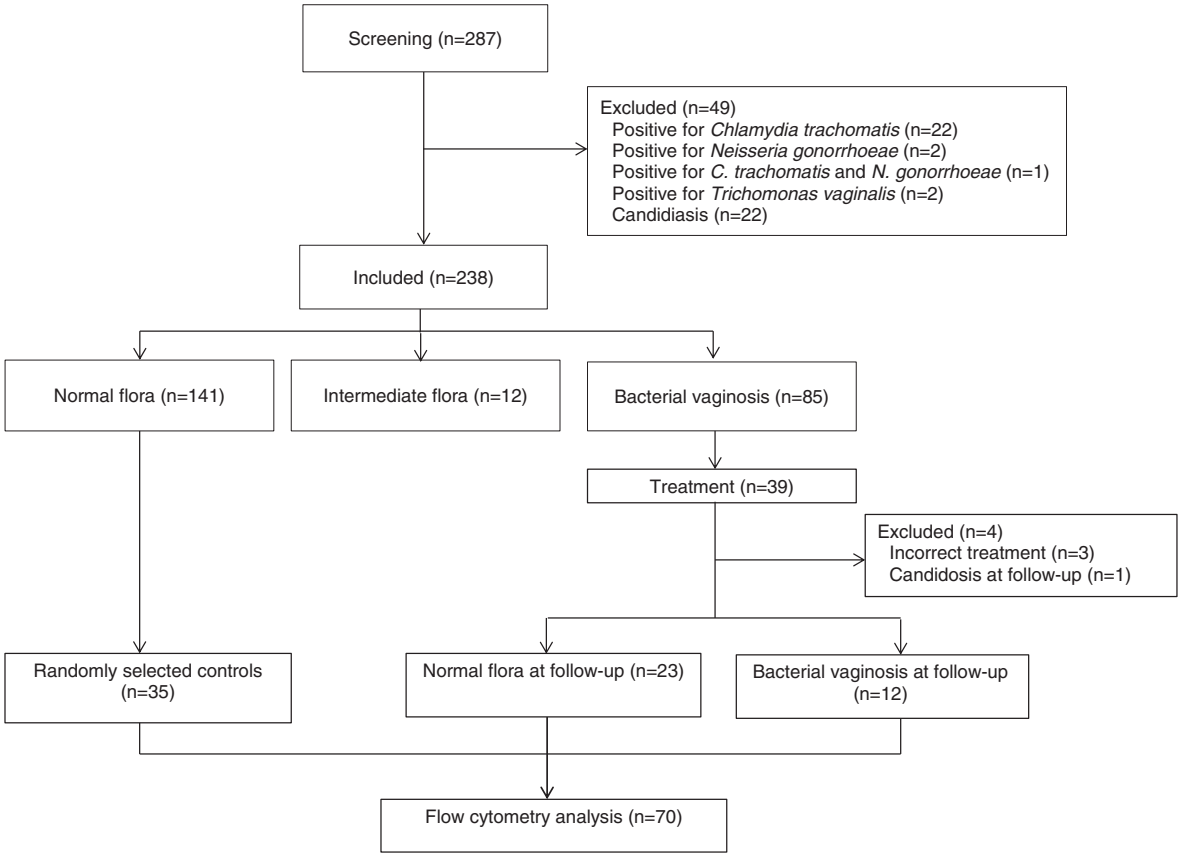

Fig. 1. Flow of patients through the study.

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