Use of Probiotics as an Adjunct to Metronidazole in the Treatment of Bacterial Vaginosis (BV): a randomized placebo-controlled study.

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Abstract

Context: As bacterial vaginosis (BV) significantly increases the risk of sexually transmitted infections, including HIV, metronidazole is still the agent of choice. The restoration of the depleted lactobacilli in BV patients by the use of probiotics in conjunction with the conventional therapy formed the basis of our study.

Objective: The objective of the present study was to determine the BV status of premenopausal women by detecting elevated vaginal sialidase, before and after oral intake of placebo-controlled probiotics.

Study Design, Setting, Subjects, and Methods: In a randomized placebo-controlled trial, 60 women (18-40 years) diagnosed with BV by clinical signs and detection of elevated vaginal sialidase enzyme, were enrolled. The subjects were given a single oral dose of metronidazole (400mg) twice daily from days 1-7, plus oral Urex cap5 [*Lactobacillus rhamnosus* GR-1 ($2.5x10^9$) and *L. reuteri* RC-14 ($2.5x10^9$)] or placebo twice daily from days 1-15.

Results: A total of 49 subjects returned for 15day follow-up, of which 19 (86%) were cured in the metronidazole/probiotic group (22) compared to 40% in the antibiotic/placebo group (27) ($p<0.001$). Nine (30%) subjects in the placebo group and none in the probiotic group had BV, while 30% in the placebo and 3 (12%) in the probiotic group fell into the Intermediate BV.

Conclusions: The study has demonstrated the effectiveness of treating BV with probiotics as an adjunct to metronidazole. The restoration of the normal vaginal microbiota with probiotics has an important impact in BV, which is a major risk factor of HIV acquisition in sub-Saharan African women.

Key Words: Bacterial Vaginosis, Lactobacilli, Metronidazole, Vaginal Sialidase, [Trop J Obstet Gynaecol, 2006, 23: 95-99]

Introduction

BV is a condition consisting of mucosal inflammation, Gram negative anaerobic bacteria colonizing the vagina of premenopausal women at the expense of lactobacilli [1] elevated pH >4.5, and in some cases odor and discharge. BV significantly increases the risk of sexually transmitted infections, including HIV [2]. BV prevalence in sub-Saharan Africa has been reported in excess of 50% among pregnant women[3] and recent use of molecular method has documented a prevalence of 14.2% in healthy Nigerian women [4]. An estimated 25-30% women have BV at any given time, mostly without signs such as fishy odor or discharge [5]. BV has been associated with various gynecological and obstetric complications including pelvic inflammatory disease (PID), post-caesarian delivery endometritis, chorioamnionitis and premature rupture of membranes, late miscarriage and pre-term labour [6]. A study in pregnant women demonstrated that women who were positive for BV on screening were five times more likely to experience preterm labour or late miscarriage than those without BV [7]. Several bacterial enzymes, including phospholipases , sialidases and proteases, have been suggested as potential virulence determinants in prematurity [8,9].

While the etiology of BV is still not completely clear, it is believed that the loss of lactobacilli is a major component of the condition and its affiliated complications. It is also characterized by an overgrowth of diverse aerobic, anaerobic and micro-aerophilic species such as *Gardnerella vaginalis*, *prevotella spp*., *Peptostreptococcus spp*., *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mobiluncus spp* in very large numbers [10]. Recently *Atopobium vaginae* has been implicated [11] and group B Streptococcus including *Escherichia coli* [12]. The diagnostic methods currently adopted for BV are 'arguably' Amsel signs, characterized by the presence of three of four criteria to define urogenital disease: 1) release of an amine (putrescine, cadaverine, and trimethylamine) or a fishy odour after the addition of 10% potassium hydroxide (whiff test), 2) a vaginal pH >4.5, 3) 20% clue cells in the vaginal fluid, and 4) a milky homogenous, malodorous vaginal discharge [13, 14]. Individually, each of these criteria varies in its sensitivity and specificity

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for the diagnosis of BV. However, the recognition that BV is associated with loss or depletion of indigenous lactobacilli led to the development of a Gram stain method of diagnosis known as Nugent criteria developed by Robert Nugent [15]. The Nugent method is a standardized Gram stain point scoring system (scores from 0 to 10) based on the presence of three bacterial morphotypes: large gram-positive rods (*Lactobacillus* spp.), small Gram-negative or Gram-variable coccobacilli (*Gardnerella* and anaerobic spp.), and curved Gram-variable rods (*Mobiluncus* spp.) Although Amsel and Nugent methods are widely used, both require microscopy and are unable to provide a simple, objective, and rapid means of diagnosis of BV at the bedside and in the rural areas of Nigeria, microscopy would be practically impossible. Recently a qualitative and quantitiative method for the diagnosis of BV have been developed as a result of elevated bacterial sialidase activity associated with BV causing micro-organisms. The test known as BVBlue is a newly developed chromogenic point-of-care test for the diagnosis of BV, which is based on detection of increased vaginal fluid sialidase activity (>7.8 U). This new method is yet to be introduced in Nigeria, besides large published studies in the Northern hemisphere have evaluated its performance to date [16, 17, 18]. Conventional treatment of BV with metronidazole or clindamycin usually leads to recurrence [19].
The objective of the study was to determine the BV status of premenopausal women by detecting elevated vaginal sialidase, before and after oral intake of placebo-controlled probiotic Urex cap5 and metronidazole.

Materials and Methods:

Probiotic Strains: *L. rhamnosus* GR-1 and *L. reuteri* RC-14 were provided by Chr Hansen, Horsholm, Denmark, in gelatin capsules manufactured under Good Manufacturing Practices. Each capsule contained $2.5x10^9$ viable cells of each strain. These probiotic organisms have previously been shown to colonize the vagina following oral intake [20], displace BV pathogens [21] and kill HIV [22].

Subjects and Recruitment: A total of 60 premenopausal women, aged 18 to 45 years, attending urogenital health clinics, were enrolled in Benin City between January and December 2005. The patients presented with clinical symptoms and signs of BV, namely vaginal irritation and discharge with a 'fishy' odor. It was not possible to perform pH assessment at the sites, but a clinician or nurse using a sterile speculum collected a vaginal swab, and elevated vaginal fluid sialidase activity was detected by a positive BV Blue test result [16]. Exclusions included pregnancy, lactation, use of systemic or intravaginal antibacterial agents currently, hypersensitivity to metronidazole, warfarin, lithium or disulfiram and menstruation at time of diagnosis. Each subject consented to the study after thorough explanation by the clinician, or Nurse. The Human Ethics Review Board of the Faculty of Pharmacy, University of Benin gave approval to the study.

BV Blue test: The vaginal swab was placed in the BVBlue vial (Gryphus Diagnostics, L.L.C., Birmingham, Ala.) containing the chromogenic substrate of bacterial sialidase, and a laboratory timer was started. Two drops of BVBlue developer solution were added at 10 min, and a blue-green color was recorded as a positive result and a yellow color were recorded as a negative result. The BVBlue test was performed at room temperature (25 to 28°C).

Randomization: The patients who were positive for BV Blue test were randomized, in a double-blind manner and given one oral dose of metronidazole (400mg) twice daily for 7days, plus either oral lactobacilli strains GR-1 and RC-14 or placebo capsules (cellulose, magnesium stearate) twice daily for fifteen days starting on the first day of metronidazole treatment. The patients were instructed to return the empty antibiotic container and probiotic alotubes at the end of the treatment, at which time a vaginal swab was collected, processed as previously described for BV status.

Primary Outcome: The primary outcome of the study was cure of BV as determined by negative sialidase test and no symptoms or signs of BV at day 15.

Statistical analysis
Statistical analysis was done using Fisher's exact tests.

Results
Of the 60 premenopausal women that enrolled and started the BV treatment, 81.6% (49) returned for the 15 day follow-up visit and all provided evidence that they complied and took the metronidazole and probiotics as required. Only three subjects in the placebo group did not return on day 15, whereas 8/30 that received probiotics did not return. The traditional reason for patients not returning for follow up is that they feel well and have no recurrence of their initial symptoms. This finding was confirmed in several patients who did not return but who were traced by the clinical staff. This is suggestive of BV cure, particularly in the probiotic group, but nevertheless, none of these subjects were included in the final analysis.

No adverse effects were reported, although two subjects in the antibiotic/probiotic group reported mild headaches that resolved after two days of therapy and they also indicated an increased appetite for the first 3 days of treatment.

All the subjects in both groups had positive BV Blue Tests indicative of BV at the beginning of the study, while at the 15 day follow up 19 of the 22 (86%) of the antibiotic/probiotic treated group had negative sialidase results, compared to 7 of 27 (25%) in the antibiotic/placebo group ($p<0.005$)(Table 1). Of the remaining 14% in the antibiotic/probiotics subjects, none had BV but all had mild irritative symptoms, no discharge or odor, a weakly positive sialidase indicative an intermediate BV status. This contrasted with the remaining 20 antibiotic/placebo subjects, of which half had BV and the other half had an Intermediate status. In short, 100% of the probiotic treated patients no longer were diagnosed with BV while 33% of the placebo group were positive ($P<0.003$).

Discussion

This is probably the first randomized placebo-controlled study in Nigeria demonstrating the efficacy of using metronidazole in conjunction with probiotic *L. rhamnosus* GR-1 and *L. reuteri* RC-14 as an adjunct to treat bacterial vaginosis. The treatments recommended by the Centers for Disease Control for BV are either metronidazole or clindamycin administered orally or intravaginally [23]. Metronidazole is a nitroimidazole with activity against anaerobic organisms, while clindamycin, a macrolide, has a broad spectrum of activity against a variety of microbes including aerobic and anaerobic organisms. The present study showed an impressive cure rate of 86% with metronidazole and probiotic lactobacillus GR-1 and RC-14. There was a significant difference when this is compared with the 25% cure rate of the metronidazole and placebo group.

The concept of replenishing the vaginal microbiota with proven probiotic lactobacilli has been considered for the past 60 years. Under normal circumstances, the patient's own lactobacilli would return post-antibiotic therapy, and colonize the vagina, thereby conferring some protection from infection, as seen in the 25% cure rate of the placebo group in this study. The additional use of probiotics is designed to enhance this process, and as noted in the 86% cure rate of the metronidazole/probiotic group, this leads to improvements in disease management and quick recovery. The lactobacilli used as an adjunct may have ascended from the rectal skin to the vagina, as previous study has shown that daily oral intake of probiotic lactobacillus GR-1 and RC-14 resulted in bacterial vaginosis patients reverting to normal lactobacilli dominated vaginal microbiota [20]. In addition, studies have shown that most cases of BV, UTI and yeast vaginitis arise from the host's gastrointestinal tract, as microbes ascend 4-5 cm from the anus to the vagina daily [24]. Also the lactobacilli may have reduced the ascension of BV pathogens from the rectal skin into the vagina, and possibly enhancement of the intestinal mucosal immunity, which affects vaginal immunity, thereby rendering the vaginal environment less receptive to BV organisms. BV Blue test was used in this study for simple and objective diagnosis of BV. Detection of elevated vaginal sialidase enzyme activity has previously been reported [25] to be both sensitive (96%) and specific (96%) for the diagnosis of BV compared to the results of the Nugent criteria (Sensitivity 88%, and specificity, 97%).

Table 1.
Results from clinical study of patients diagnosed using clinical and BV Blue diagnostic systems, then treated with metronidazole plus $2.5x10^9$ Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 or placebo.

	Metronidazole (400mg/twice for day 1-7) plus Lactobacillus (day 1-15)		**Metronidazole (400mg/twice for day 1-7) plus Placebo (day 1-15)**	
	Day 0	Day 15	Day 0	Day 15
Number of Subjects	30	22	30	27
Positive clinically (vaginal irritation, discharge and 'fishy' odor)	30	2* P=.005 compared to placebo	30	9
Positive BV Blue Test (sialidase)	30	3** P=.035 compared to placebo	30	7

* mild irritative symptoms but no discharge or odor ** weakly positive
Statistical analysis using Fisher's exact tests.

The 14% intermediate BV category in the metronidazole/probiotics group raises the question as to whether or not these women will go on to be at risk of infection or revert to a lactobacilli dominated flora. Although not done in the present study, the monitoring of the patients in the metronidazole/probiotic lactobacilli group would be worth assessing to determine if a change to more women having a 'normal' flora is feasible. On the other hand, it is possible that the intermediate flora is actually somehow protective for the subjects, and it represents a 'normal' status in that population. Furthermore, there has been no evidence from epidemiological studies, to suggest that an intermediate microbiota is protective against HIV, unlike normal healthy vagina dominated by lactobacilli that have been found to be protective against BV and HIV [26].

A recent study that tested the augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 in a randomized, double-blind, placebo controlled trial for 30 days, recorded a success rate of 100% [27]. In the present study, we recorded a success rate of 86% for treatment period of 15 days.
Another study tested the efficacy of probiotics combined with antibiotics for BV treatment. Although not published in peer-reviewed literature, the company that performed the study reported that treatment with *Lactobacillus crispatus* CTV-05 resulted in vaginal colonization in 62% of patients at 30 days and satisfactory clinical endpoint was found in 50% patients in probiotic and placebo groups (http://www.themedicinescompany.com). By comparison, the ability of *Lactobacillus* GR-1 and RC-14 to colonize the vagina after 15 days oral intake and the negative vaginal fluid sialidase demonstrates the merits of this strain combination along with seven days metronidazole therapy.

A number of studies have assessed various antibiotic protocols for the treatment of BV in Caucasian, African American, European, Asian and Mexican women, with failure rates as high as 39% [28, 29, 30, 31], but no data exist on African albeit Nigerian women with regard to either metronidazole failure or success rate.

Despite treatment with either metronidazole or clindamycin, the proportion of women who relapse also increases over time. Previous reports have documented that the recurrence rate of BV is approximately 30% at 3 months and approximately 50 to 80% at 1 year following therapy with either drug [19]. However, recurrent rate of BV in this combination of metronidazole and probiotics has not been determined. Current therapy for managing recurrent BV is repeated treatment with antibiotics. An obvious problem and important health issue associated with repeated exposure to the same antibiotic is resistance of those microbes targeted by the drug, which can result in an alteration of normal microbiota and possible persistence of BV-associated pathogens.

Understanding the mechanism of the therapy was not the main focus of the study, but it should be noted with astuteness that metronidazole functions by killing the pathogens, while *Lactobacillus* GR-1 and RC-14 are known to inhibit urogenital pathogen growth and adhesion [32], displace BV organisms [21], and colonize the vagina [20] This may explain the significant difference between the two treatment groups. If the presence of lactobacilli is deemed to confer health benefits, then application of exogenous probiotic Lactobacillus strains to substitute or boost autochthonous strains could be used for restoration and maintenance of the vagina, making the individual less susceptible to infection.

To complicate the clinical picture, bacterial vaginosis is frequently associated with other vaginal infections, such as candidiasis, trichomoniasis, and chlamydia. Based on our present findings we submit that bacterial vaginosis is a disease that is frequently asymptomatic, difficult to diagnose clinically, and associated with other pathology; it clearly requires new and more effective diagnosis such as BV Blue test and treatment option using probiotic augmentation. In a review article published in the West African Journal of Pharmacology and Drug Research, it has been suggested that use of probiotics have the potential of replacing antibiotics as prophylaxis in some infections [33].

Of importance is the fact that this study was undertaken on women with BV and BV has been found to be significantly associated with HIV. Conventional treatment of BV leads to recurrence and continuous administration of antibiotics leads to overgrowth of BV organisms. Our present study has demonstrated the use of probiotics as an adjunct to metronidazole in the management of BV. HIV infection rates are skyrocketing amongst women in sub-Saharan Africa, with over 7,000 new cases reported each day. It is not known if the correlation between BV and HIV is due to elevated inflammation, loss of lactobacilli or some other host affinity change, but it seems logical to test whether eradication of BV with the use of probiotics can reduce the risk of HIV. The current findings provide further support for such a study.

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