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Original article

Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis

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Abstract

Bacterial vaginosis (BV) is particularly common in black women, and in Nigeria it is often caused by *Mycoplasma*, as well as *Atopobium*, *Prevotella* and *Gardnerella* sp. Antimicrobial metronidazole oral therapy is poorly effective in eradicating the condition and restoring the *Lactobacillus* microbiota in the vagina. In this study, 40 women diagnosed with BV by discharge, fishy odor, sialidase positive test and Nugent Gram stain scoring, were randomized to receive either two dried capsules containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 each night for 5 days, or 0.75% metronidazole gel, applied vaginally twice a day (in the morning and evening). Follow-up at day 6, 15 and 30 showed cure of BV in significantly more probiotic treated subjects (16, 17 and 18/20, respectively) compared to metronidazole treatment (9, 9 and 11/20: $P = 0.016$ at day 6, $P = 0.002$ at day 15 and $P = 0.056$ at day 30). This is the first report of an effective (90%) cure of BV using probiotic lactobacilli. Given the correlation between BV and HIV, and the high risk of the latter in Nigeria, intravaginal use of lactobacilli could provide women with a self-use therapy, similar to over-the-counter anti-yeast medication, for treatment of urogenital infections.

Keywords: Bacterial vaginosis; Intravaginal probiotics; Metronidazole

1. Introduction

Bacterial vaginosis (BV) is a relatively complex yet extremely common condition characterized by the replacement of lactobacilli by either anaerobic bacteria such as *Atopobium*, *Prevotella*, *Gardnerella* and *Mobiluncus* [1–3], or intracellular *Mycoplasma* [4], or aerobic bacteria such as *E. coli* and enterococci [5]. The net result is an elevated pH, production of sialidase by colonizing bacteria [6], and in many cases discharge and fishy odor caused by amine production by the bacteria [7].

Treatment of BV comprises either oral or vaginal metronidazole or clindamycin, however, the cure rate at 30 days is

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often poor, and recurrences are common [8,9]. This has led to considering the use of long term, low dose suppressive therapy for BV [10]. Failure to eradicate BV is arguably of most concern in sub-Saharan Africa. Black women, for reasons that are not fully understood, have a particularly high incidence of BV [4,11], and in countries like Nigeria, having one quarter of Africa's population, large numbers of females are at risk of HIV acquisition. The presence of BV increases the risk of HIV and other sexually transmitted infections [12,13], and thus it is important to develop new approaches to the retention of a healthy vagina, particularly therapies which can be readily available, not overly expensive and with a higher efficacy in terms of reducing recurrences.

Having shown that certain probiotic lactobacilli organisms can be used effectively to colonize the vagina, and interfere with urogenital pathogenesis [14–16], we hypothesized that vaginal probiotic lactobacilli can be used to treat symptomatic BV. A randomized, comparative clinical study was performed in Benin, Nigeria, to test the efficacy of a capsule containing *Lactobacillus rhamnosus* GR-1 and *L. reuteri* (formerly L. fermentum) RC-14 taken nightly versus 0.75% metronidazole vaginal gel administered twice daily, to treat black women who presented with symptomatic BV.

2. Materials and methods

2.1. Study participants

Three hundred and fifty women who presented to several clinics in Benin City, Nigeria, with symptomatic BV were examined. The study was approved by the Ethics Review Board of the University of Benin. The inclusion criteria for entry into the study were subjects suffering from vaginal discharge, an unpleasant fishy odor, with or without localized irritation and/or discomfort around the vagina. They had a vaginal pH > 4.5, KOH positive test for fishy odor, as well as a Nugent score 7–10 [17] and positive BV Blue sialidase test [18]. Subjects were excluded if they were diagnosed as HIV positive or urogenital infections of another type, such as yeast vaginitis or a sexually transmitted disease. Exclusions also included women who were pregnant, younger than 18, or older than 50 years, or were menstruating at the time of examination or were due to menstruate over the following five treatment days.

2.2. Study groups and randomization

Participants fulfilling the entry criteria were randomized for age, history of previous (past year) urogenital infections (BV, urinary tract infection or yeast vaginitis), and severity of current BV infection, and chosen by a randomization scheme to be treated either with lactobacilli or antibiotic. The sample size of 20 subjects per group was based on the expectation of finding a difference of 50% between the two groups. Study subjects were not blinded to the treatment they received. One group inserted vaginally two gelatin capsules containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (1×10^9 each organism) at bedtime for 5 days. The other group applied 0.75% metronidazole vaginal gel to the vagina twice a day (once in the morning, once in the evening) for 5 days.

2.3. Data collection

Each subject filled out a lifestyle questionnaire, to report each day a self-assessment of presence and severity of their condition (discharge, odor, irritation, other) for the first 5 days, and to note when they believed that their recovery began and was complete. A physician assessed each subject at day 0, 6, 15 and 30, at which time two vaginal swabs were collected for Nugent scoring, KOH testing, and sialidase assessment using the BV Blue test [18]. The clinical assessment and use of KOH and the BV Blue test were carried out at the site of sample collection, whereas all Gram staining for Nugent scoring was carried out blinded by a single scientist, KA, who had experience in this methodology. He reported the results back to the referring physician and to a central database. The results were blinded to all other investigators until the analyses had been complete and 40 subjects had finished the study.

2.3.1. Nugent scoring

Vaginal swab smears were graded by a technician blinded to the subject groups, on a 10-point scale based on the presence or absence of *Lactobacillus* morphotypes under oil immersion (×1000 magnification) as described previously [17,18]. A score of 0–3 was interpreted as consistent with a Normal Gram positive rod dominated microbiota, a score of 4–6 as Intermediate, and a score of 7–10 was considered consistent with BV-like conditions in which the samples were dominated by small Gram negative and Gram-variable straight and curved rods.

2.3.2. BV Blue test

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

3. Results

For this study, 40 subjects enrolled to participate from a pool of over 350 pre-menopausal women who presented with symptoms and signs of BV from five hospitals, including University of Benin Teaching Hospital and Central Hospital Benin City. Table 1 shows the condition of the 40 subjects at the time of commencement of the study. Most (75%) had severe discomfort (75%), vaginal discharge (90%), fishy vaginal odor (75%), while 18 (45%) had vaginal irritation/itching and 12 (30%) had other symptoms such as lower abdominal pain. Most subjects (92.5%) indicated that they have had this problem sometime in the past year, while only three (7.5%) reported having the problem for the first time. The

Table 1
Condition of the subjects at the time of accrual

	N	Percentage (%)
Severity of BV infection		
Very severe	30	75
A little severe	6	15
Bearable	4	10
No discomfort	Nil	Nil
Symptoms presenting today		
Vaginal discharge	36	90
Vaginal odor	30	75
Vaginal irritation	18	45
Others	12	30
Had this problem in the last one year		
Yes	37	92.5
No	3	7.5
Treatment of last episode		
Prescribed vaginal antibiotics	35	87.5
Oral antibiotics	28	70
Injectables	18	45
No treatment	Nil	Nil
Types of contraception		
Oral pill	6	15
Patch		
Condom	8	20
Condom + spermicide		
Diaphragm		
IUD	2	5
None	14	35

majority of subjects (87.5%) had previously been treated for BV with oral antibiotics.

Of the 40 subjects who enrolled, all (100%) returned for a follow-up at day 6 and day 15 (Table 2), and demonstrated compliance by returning empty treatment vials. At 30 day follow-up, despite efforts to contact all subjects, 17/20 from the probiotic group and 18/20 from the metronidazole group returned for consultation.

There was a good correlation between the clinical cure, Nugent score and sialidase test. Chi-squared analysis showed a highly significant difference between the two treatment groups at day 6 ($P = 0.016$), day 15 ($P = 0.002$) and a significant difference at day 30 ($P = 0.056$). This difference was consistent for the BV Blue test.

There was good correlation between the diagnosis of BV using Nugent and sialidase tests which measure microbial parameters microscopically and enzymatically, while the subjects appeared to perceive relief and cure a few days sooner (Table 3). All subjects in both groups noted some relief within 5 days of treatment, but 33% of the metronidazole group stated that the condition had not resolved by day 30, compared to 12% in the probiotic group.

4. Discussion

Recent studies have highlighted the persistent problem of BV, with one resorting to long-term antibiotic therapy to prevent recurrences [10]. This is the first study to show that 5 days intravaginal probiotic lactobacilli treatment can cure BV and restore the vaginal lactobacilli at 30 days follow-up. The probiotic treatment was even more effective than metronidazole vaginal gel, for reasons not investigated in this study. No microbiological analysis was performed on the subjects, but a previous study of this Benin City population showed a high presence of Mycoplasma in women with BV (unpublished data). This organism is treatable with metronidazole gel [9], yet half the subjects who received this drug had BV at the 30 day follow-up. This result is consistent with a recent study also performed in this centre, in which only 40% BV was effectively treated with oral metronidazole [18]. It would seem useful to study the microbiota and immunological parameters of patients from these clinics, before, during and after drug therapy for BV, to try and understand why efficacy is so poor.

Urgent calls for innovative therapies for BV have been made [19], particularly for women at risk of preterm labour or sexually transmitted infections (STI). The presence of BV, the elevated vaginal pH associated with it, and the loss of lactobacilli clearly increase the risk of these complications. The use of probiotics to replenish the vaginal lactobacilli has been the subject of our efforts for the past 25 years, and more recently has led to others attempting to develop strains for human testing. It has been proposed that strains that highly produce hydrogen peroxide are needed to colonize the vagina and treat or prevent BV. However, the two strains used here have been shown to colonize the vagina [14,20,21], displace BV organisms [22] even although only RC-14 produces low levels of hydrogen peroxide. As previously stated by us, hydrogen peroxide production is but one of several important factors involved in urogenital health in women, but it may not be the primary element needed for cure of a diseased state.

Some clinical studies of BV have been criticized for using the Amsel criteria which is based upon vaginal pH and the

Table 2
Results of the BV status (based on Nugent score and BV Blue test) of patients treated with vaginal probiotics GR-1/RC-14 and Metronidazole (0.75%) vaginal gel at day 0, 6, 15 and 30

Probiotic group					Metronidazole (0.75%) group			
BV status	Day 0	Day 6	Day 15	Day 30	Day 0	Day 6	Day 15	Day 30
Signs and symptoms	20	2	2	2	20	8	8	8
Nugent score 7–10	20 (100%)	4 (20%)	3 (15%)	2 (12%)	20 (100%)	11 (55%)	11 (55%)	9 (50%)
Score 4–6	Nil	4	7	4	Nil	3	5	3
Score 0–3	Nil	12	10	11	Nil	6	4	6
Positive BV Blue test	20 (100%)	6 (30%)	3 (15%)	2 (12%)	20 (100%)	15 (75%)	13 (65%)	10 (56%)

Table 3
Responses to lifestyle questionnaires after the treatment for the two groups at day 30

Changes in condition since treatment	Probiotics group ($N = 17$)	Metronidazole group ($N = 18$)
I got relief		
Within 1 day	Nil	Nil
2 days	Nil	Nil
3 days	9	3
4 days	5	5
5 days	3	10
I did not get relief	Nil	Nil
Problem was completely resolved		
Within 1 day	Nil	Nil
2 days	Nil	Nil
3 days	5	2
4 days	2	4
5 days	8	6
Did not resolve	2	6
Any side effect?		
Yes	2	11
No	15	7
Number of sex partners for this one month		
0	9	7
1	8	11
2	Nil	Nil
More than 2	Nil	Nil

presence of fishy odor and discharge, with the latter two not being present in many women with this condition [23]. The Nugent scoring system has been criticized for not being sufficiently sensitive and for the fact that a Gram positive organism (upon which a Normal score is based), *Atopobium vaginalis*, has been found to be associated with causation of BV [24,25]. The BV Blue test is relatively new and not yet widely used. In order to be sure that the subjects enrolled here actually had BV, all three methodologies had to be positive before subjects were recruited. Thus, the 40 subjects from 300 assessed, were suitable for the study. The finding that women felt that the treatment improved their condition days before the assays confirmed it, or in one or two cases when the assays showed no improvement, suggest that more quality of life questionnaires might be useful for future studies.

The reduced ability of other STI agents, such as herpes simplex virus, *Neisseria gonnorhea*, and *Chlamydia trachomatis* to infect the vagina when lactobacilli are the dominant organisms [13,25] could be due to pH [14], immune modulation [26], or other factors as yet unknown. If infected with STI agents, the host is at a higher risk of HIV and other complications, such as preterm delivery. Thus, by restoring the lactobacilli microbiota, benefits other than remediation of BV might be achievable, including ultimate reduction in HIV infections [27].

Metronidazole vaginal gel is expensive (NGN7500 per use), prohibitively so for many women in Nigeria, as is the sialidase test. Thus, for many women diagnosis relies on being able to access a physician who can perform Gram stain and KOH test, and treatment of BV relies upon oral tablets of metronidazole or clindamycin, neither of which provides high cure rates. In a country where as many as 1000 women may be at risk each day of acquiring HIV, efforts to reduce this risk by preventing or curing BV need to be seriously considered. The probiotic capsule used in this study, although not yet available in Nigeria, would be priced at a level affordable to many women [26], and it can retain viability at room temperature for up to two years. This makes it a feasible option for empowering women who wish to self-treat upon the commencement of symptoms and signs of BV. As yet, no studies have been undertaken to assess the potential for probiotics to reduce the risk of HIV or other sexually transmitted diseases, but based upon the latest study, such studies appear warranted.

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