Comparative efficacy of oral lactobacillus rhamnosus (protexin) against metronidazole (flagyl) in the treatment of bacterial vaginosis: A ramdomized clinical trial*

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ABSTRACT

Background: Bacterial vaginosis (BV) is a very common gynecologic infection associated with a vast number of complications both in gynecologic and obstetric patients. One of the major concerns in its treatment is a high recurrence rate which was multifactorial and the choice of the suitable antimicrobial is important to decrease the treatment failure.

Methods: All gynecologic patients aged 18 years old and above in a tertiary hospital diagnosed with bacterial vaginosis according to Amsel's criteria. A total of 80 patients were randomly assigned into two groups; one group to receive oral Probiotics (Protexin) while the other group to receive Metronidazole. The patients will be followed up accordingly on Days 1, 3, 7 and 30 and will be graded according to Amsel's criteria. The primary endpoint of the study is the treatment of bacterial vaginosis based on the mentioned criteria. (Anukam, 2006)

Results: The results showed that there was a significant improvement in the character of the vaginal discharge based on the Amsels criteria on Day 1 of treatment for the Metronidazole group (0/40; 100%, p value <0.001) and Day 3 for Oral Lactobacillus arms. (7/40; 20%, p value 0.01). The Metronidazole arm showed a significant improvement in the fishy odor on vaginal examination with addition of 10% KOH on day 1 (0/40; 100%, p value <0.001) and Day 3 for oral Lactobacilus (0/40; 100%, p value 1.00). Then vaginal pH was noted to be more acidic in the Metronidazole compared to the Protexin arm on Day 1 of treatment (0/40; 0% and 40/40; 100% p value <0.001 respectively). However, both groups had no significant difference of vaginal pH in Days 3-30 (0/40; 100% p value 1.0). There was a note of less number of recurrence rate under the Protexin arm after 30 days of treatment (5/40; 12.5% p value <0.001) as reflected in the decreased number of clue cells.

Conclusion: The Metronidazole remains to be the standard treatment for Bacterial vaginosis. There was also faster recovery and clinical improvement in the character of the vaginal discharge, amount and smell based on the Amsel's criteria as early as Day 1 of follow-up; however, there was a small number of population with poor compliance resulting to higher recurrence rate which was evident on the 30th day of follow-up. The oral lactobacillus rhamnosus showed advantage over Metronidazole due to lower recurrence rate of BV as noted on Day 30 of follow up.

Keywords: Bacterial vaginosis, oral lactobacillus, metronidazole

INTRODUCTION

Bacterial vaginosis (BV) results from the breakdown of the physiological equilibrium that exists between the local *Lactobacillus* microenvironment and a pool of other resident anaerobic or facultative anaerobic bacteria in the vagina. Bacterial vaginosis has been shown to be a risk factor in the development of pelvic inflammatory disease, progression of squamous intraepithelial lesion (SIL), chorioamnionitis and high incidence of preterm delivery in pregnancy. The recurrence rate of BV is up to 30% after traditional antimicrobial therapy as early as 2 weeks.[1] There seems to be an association between the absence of, or low concentrations of vaginal lactobacilli and the development of BV. Many studies have suggested that the presence of H_2O_2-producing vaginal lactobacilli may protect against BV. The importance of the microflora of the vagina is important in that there exists a balance, which could either lead to a healthy and diseased state. Factors, such as hormone levels, douching, sexual practices, innate immunity, as well bacterial interactions and host defenses all play a role in this delicate balance.[2] The role of innate immunity cannot be stressed enough. Bacterial vaginosis was also said to be associated with apparent reduced expression of host antimicrobial factors especially apparent in post-menopausal women.

Lactobacilli are the most common organisms used as probiotics. In medicine, they have been used orally to

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alleviate gastroenteritis and in this study, as a modality to prevent BV. Since the vaginal microbiota of women with BV has been found to contain diminished levels of lactobacilli in comparison with healthy women, it has been theorized that lactobacilli administered orally or intra-vaginally can be an effective means in the restoration of the normal flora of the vagina and in effect, curing women with BV, or at least preventing its recurrence.[3] When probiotic *L. rhamnosus* GR-1 was administered to the vagina of premenopausal women, it resulted in 3536 gene expression changes and increased expression levels of some antimicrobial defenses against pathological flora. The organisms associated with BV are theorized to form dense biofilms on the vaginal epithelium which lead to increased resistance to lactobacilli-produced lactic acid and hydrogen peroxide (H_2O_2) which are normally antagonistic to them.[4] The biofilms are also able to induce host expression of certain inflammatory factors, such as IL-1 and IL-8 which lead to a decreased immune response against them.

Among 10 strains of lactobacilli being evaluated for use in a probiotics tablet, (Mastromarino et al) found, in vitro, that *Lactobacillus gasseri* 335 and Lactobacillus salivarius FV2 were able to coaggregate with *G. vaginalis*. When these strains of lactobacilli were combined with Lactobacillus brevis in a vaginal tablet, adhesion of *G. vaginalis* was reduced by 57.7%, and 60.8% of adherent cells were displaced. Boris et al. found that the adherent properties G. vaginalis were similarly affected by *Lactobacillus acidophilus*.[5]

The purpose of this study therefore is to compare in a randomized clinical trial, the effectiveness in terms of cure and prevention of recurrence of BV using Oral Probiotics (Protexin) VS Metronidazole (Flagyl) in the treatment of Bacterial Vaginosis.

METHODOLOGY

The randomized clinical trial will be carried out on 80 female patients with sexual contact aged 18 years old and above, diagnosed with Bacterial vaginosis based on the Amsel's criteria with a scoring system of 4: (1. Fluid with pH >4.5, 2. Thin, homogeneous, grayish-white adherent discharge, 3. Fishy odor on addition of potassium hydroxide 10% w / v to the discharge (positive amine test or sniff / whiff test), 4. Clue cells on saline wet mount). Number of patients will be divided equally according to the group A (Oral probiotics-Protexin) and group B (Metronidazole) which would serve as the control. They will be monitored based on follow up re-evaluation of the BV. Women who underwent antimicrobial therapy for conditions other than BV, pregnancy, presence of urogenital infections, sexually transmitted diseases, co-morbid conditions, immunocompromised patients and use of contraceptive methods were excluded from the study. Using OpenEpi, Version 2 software program to compute the sample size, it was computed that a minimum number of 80 subjects are required for 80% power (alpha of 0.05). In a study done by Anukam in 2006, those who received oral Lactobacillus showed 64.7% clinical and microbiological cure as measured by a normal Nugent score, more pronounced after the 30 day follow up, while those who received Metronidazole had 33.3% cure. These data are reflective of the possible beneficial outcomes of Oral Lactobacillus particularly in preventing recurrent Bacterial Vaginosis. According to their per-protocol analysis, 11 of the 17 (64.7%) women in the probiotic arm had normal Nugent scores on day 30 compared to 6 out of 18 (33.3%) in the metronidazole arm: OR 0.27 (95% CI 0.07 to 1.10).

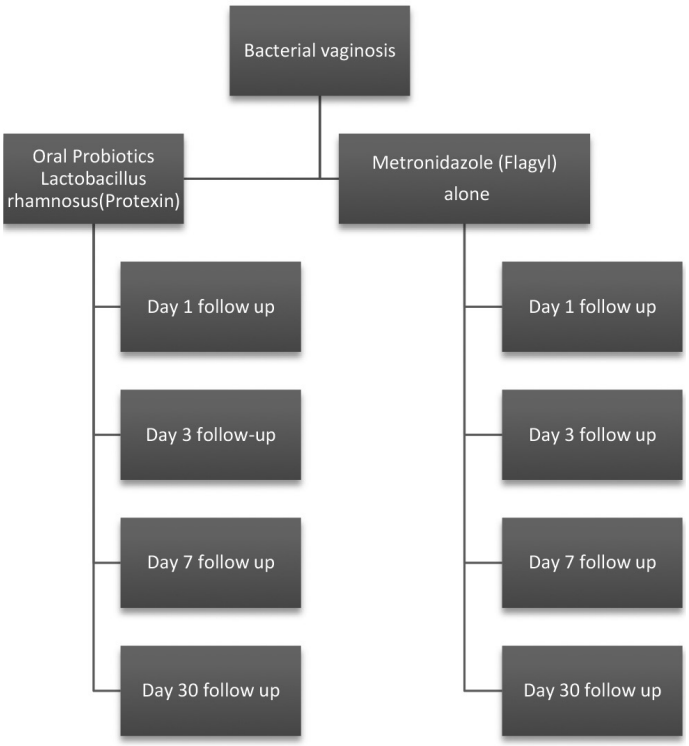

Figure 1. Flowchart of Methodology

The participants will receive either Oral Lactobacillus rhamnosus (Protexin) or Metronidazole (Flagyl) as per the instructions in the sealed white envelopes that will be opened upon recruitment of the patient to the study. Group A will be the treatment group and Group B as the comparator group. Group A will be given Oral Probiotics (Protexin) tablet once a day for one week. Group B will be given Metronidazole 500 mg/tab (Flagyl), 1 tab twice daily for one week. Follow up will be assigned as Day 1, which is the onset of treatment, Day 3, Day 7 and Day

30 and will be re-evaluated based on the resolution of BV based on the Amsel's criteria (0-2/4). During their scheduled visits, an unmoistened sterile speculum was inserted into the vagina so that vaginal walls, fornices, and the cervix could be evaluated for erythema, color, and viscosity of discharge. The pH value of the vaginal walls and of the lateral fornices was measured by colorimetric paper with 8 comparison colors for pH values between 3.6 and 6.1. Vaginal samples were collected from lateral fornices by a wooden Ayre's spatula; the samples were mixed with saline and 10% potassium hydroxide on two different slides and then immediately observed under a microscope. The identification of clue cells will be from the obtained vaginal smear and will be studied under the microsope under the supervision of a medical staff from the Pathology Department of a tertiary hospital. Overall, the respone to treatment for Bacterial vaginosis as the endpoint and goal of both study groups will be based on Amsel's criteria point system from which a minimum of 3 out of 4 of the criteria is needed for the diagnosis of Bacterial vaginosis and a score of 1 to 2 out of 4 would mean recovery from the infection. The treatment endpoint is to attain a score of 0-2 out of 4 which means disease improvement/treatment. A persistent score of 3-4 out of 4 signifies disease persistence or treatment failure. Differences between groups determining association will be analyzed using the T student test, chi^2 and fisher exact tests. The difference between the two groups will be considered significant if P<0.05 after intention to-treat analysis.

RESULTS

Table 1 shows the comparison of the demographic characteristics between the two groups. The results showed that there was no significant difference noted as proven by all p values >0.05.

Table 1. Comparison of the Demographic Characteristics Between the Two Groups

	Metronidazole (n=40)	Protexin (n=40)	p-value*
Age Mean ± SD	34.05 ± 10.03	34.05 ± 13.19	0.70 (NS)
Gravida Primigravida Multigravida	 24 (60.0%) 16 (40.0%)	 23 (57.5%) 17 (42.5%)	 0.82 (NS)
Amsel's Scoring 4	40 (100%)	40 (100%)	1.00 (NS)

* p>0.05- Not significant; p ≤0.05-Significant

With initiation of treatment, as shown in Table 2, there was a significant difference in the proportion of subjects with discharge on days 1 and 3 as shown by the p values <0.001 and 0.01 respectively. Significantly more proportion of subjects given oral Lactobacillus rhamnosus (Protexin) had discharge with 100% and 20% on days 1 and 3 respectively. However, there was no significant difference noted in the proportion of subjects with discharge at days 7 and 30 as reflected by all p values >0.05. It can be seen from the table that vaginal discharge significantly improved on day 7 in both groups.

Table 2. Comparison of the Amount of Discharge Between the Two

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Day 1	0 (0%)	40 (100%)	<0.001 (S)
Day 3	0 (0%)	7 (20.0%)	0.01 (S)
Day 7	0 (0%)	0 (0%)	1.00 (NS)
Day 30	0 (0%)	0 (0%)	1.00 (NS)

* p>0.05- Not significant; p ≤0.05-Significant

Grossly, the appearance of the discharge in majority of the subjects are comparable during examination particularly on day 7 and 30 of the follow up. Below are representative pictures of the vaginal smear added with 10% KOH solution.

Figure 1. Day 1 post treatment. Demonstration of vaginal discharge of a patient with BV on Day 1 of treatment. It has a characteristic yellowish mucoid fishy like smelling disharge upon addition of KOH

Figure 2. Day 3 post treatment. Demonstration of vaginal discharge of a patient with BV on Day 3 of treatment showing a significant decrease in the amount of vaginal discharge and with improvement in the fishy-like odor of the discharge on addition of KOH

Figure 3. Day 7 of treatment. Shows further decrease in the amount of whitish mucoid with complete resolution of the foul smelling vaginal discharge when mixed with 10% KOH solution

Figure 4. Day 30 after treatment. Shows further decrease in the amount of whitish mucoid with complete resolution of the foul smelling vaginal discharge when mixed with 10% KOH solution

Table 3 shows the comparison of the proportion of subjects according to pH of vaginal flora between the two groups. The results showed that there was a significant difference in the proportion of subjects at day 1 ($p<0.001$). Significantly more proportion of subjects given protexin had alkalinepH of vaginal discharge with 100%. However, there was no significant difference noted in the proportion of subjects with acidic pH of vaginal flora at days 3, 7 and 30 as proven by all p values >0.05. It can be seen from the table that pH of vaginal flora was significantly improved in acidity on day 3 in both groups.

Table 3. Comparison of the Proportion of Subjects According to pH of Vaginal Flora Between the Two Groups

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Day 1	0 (0%)	40 (100%)	<0.001 (S)
Day 3	0 (0%)	0 (0%)	1.00 (NS)
Day 7	0 (0%)	0 (0%)	1.00 (NS)
Day 30	0 (0%)	0 (0%)	1.00 (NS)

* p>0.05- Not significant; p ≤0.05-Significant

Table 4 shows the comparison of the proportion of subjects with presence of clue cells between the two groups. The results showed that there was a significant difference in the proportion of subjects with clue cells at day 1 ($p<0.001$). Significantly more proportion of subjects given protexin had clue cells with 87.5%. However, there was no significant difference noted in the proportion of subjects with pH of vaginal flora at days 3, and 7 as proven by all p values >0.05. It can be seen from the table the number of clue cells significantly decreased on day 3 in both groups. On day 30, there was a re-appearance of cells among those given Metronidazole with 12.5% which was statistically significant (p=0.05).

Table 4. Comparison of the Proportion of Subjects with Presence of Clue Cells Between the Two Groups

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Day 1	0 (0%)	35 (87.5%)	<0.001 (S)
Day 3	0 (0%)	0 (0%)	1.00 (NS)
Day 7	0 (0%)	0 (0%)	1.00 (NS)
Day 30	5 (12.5%)	0 (0%)	0.05 (S)

* p>0.05- Not significant; p ≤0.05-Significant

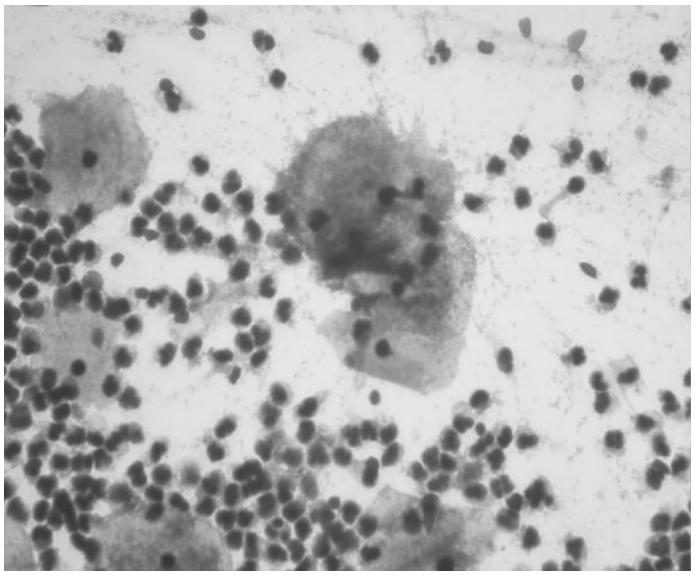

Figure 5. Demonstration of Vaginal smear with Bacterial vaginosis (Day 1 of treatment) Squamous cells have been identified surrounded by gram negative cocobacilli

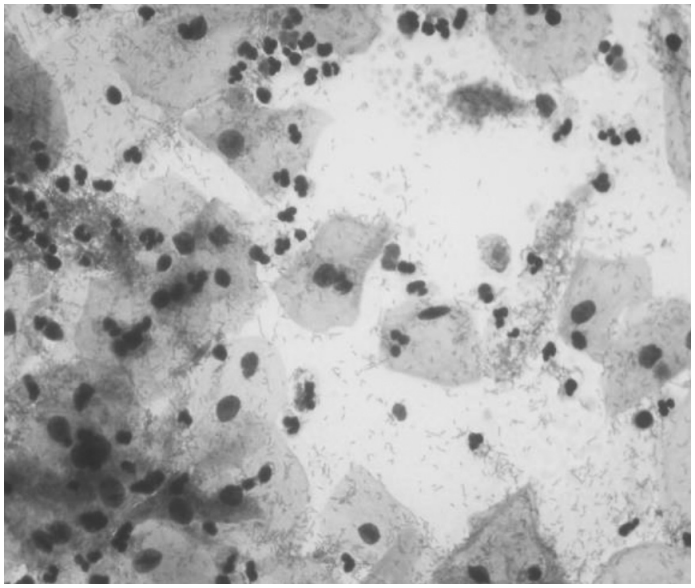

Figure 6. Demonstration of Vaginal smear with Bacterial vaginosis (Day 3 of treatment). The gram negative coccobacilli was noted to be decreased in number and intermediate cells becomes more apparent

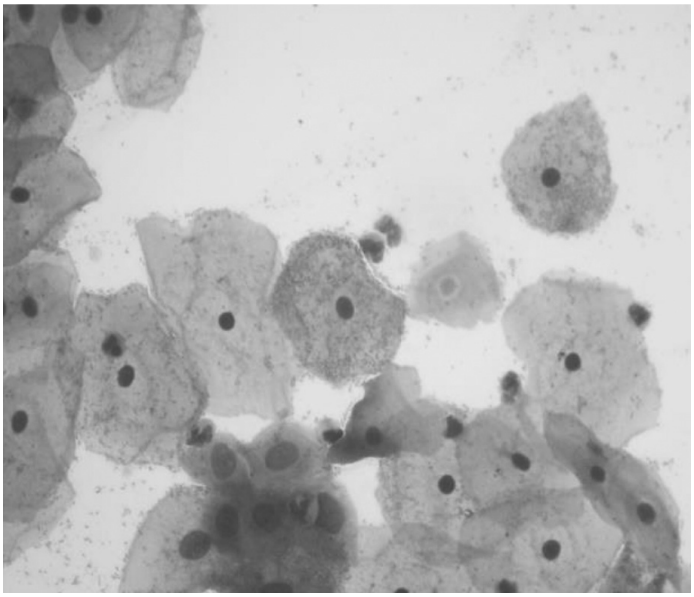

Figure 7. Demonstration of Vaginal smear with Bacterial vaginosis (Day 7 of treatment). The gram negative coccobacilli were very scant in number and intermediate cells becomes more visible

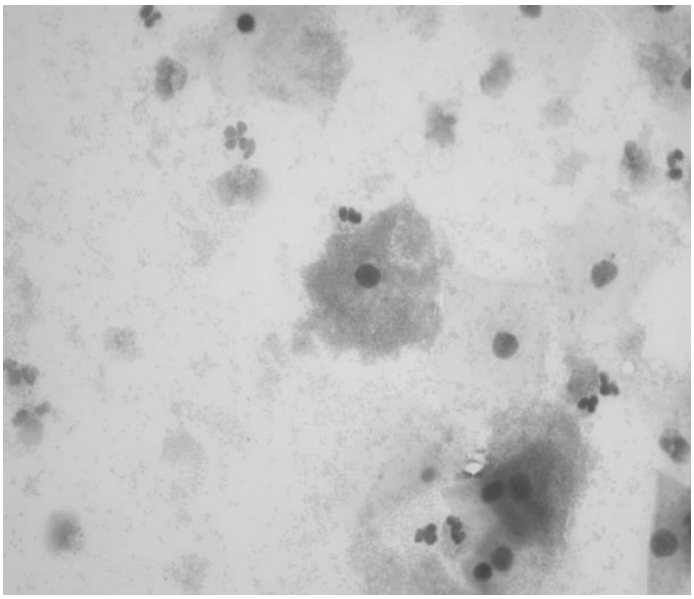

Figure 8. Demonstration of Vaginal smear with Bacterial vaginosis (Day 30 of treatment). The gram negative coccobacilli discretely exists surrounding the intermediate cells.

Table 5 shows the comparison of the proportion of subjects with fishy odor on KOH between the two groups. The results showed that there was a significant difference in the proportion of subjects at day 1 (p<0.001). Significantly more proportion of subjects given protexin had persistence of the fishy odor on KOH with 100% with no significant difference on days 3, 7 and 30 as proven all p values >0.05 with disappearance of the foul smelling vaginal discharge. It can be seen from the table that fishy odor on KOH already gone on day 3 in both groups. For

the metronidazole group, there was complete resolution of the fishy odor starting on day 1 of treatment until days 3, 7 and 30.

Table 5. Comparison of the Proportion of Subjects with Fishy Odor on KOH Between the Two Groups

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Day 1	0 (0%)	40 (100%)	<0.001 (S)
Day 3	0 (0%)	0 (0%)	1.00 (NS)
Day 7	0 (0%)	0 (0%)	1.00 (NS)
Day 30	0 (0%)	0 (0%)	1.00 (NS)

* p>0.05- Not significant; p ≤0.05-Significant

Table 6 shows the comparison of the days before complete resolution of the different criteria between the two groups. The results showed that there was a significant difference noted (p<0.001). The day of resolution was significantly shorter in the Metronidazole group than the protexin group. However, the recurrence was significantly noted only among those given Metronidazole.

Table 7 shows the comparison of scores at different follow-up between the two groups. The results showed that there was a significant difference in the proportion of subjects according to Amsel's scoring at days 1, 3 and 30 as shown by all p values <0.05. Significantly higher scores were noted among subjects given protexin. However, there was no significant difference noted in the score at days 7 (p=1.00). On day 30, there was a recurrence noted in the presence of clue cells among those given Metronidazole with 12.5% which was statistically significant (p=0.05).

Table 8 shows the comparison of recurrence between the two groups. The results showed that there was a significant difference in the proportion of subjects with recurrence among those given Metronidazole with 12.5% which was statistically significant (p=0.05).

Table 9 shows the comparison of the days before complete resolution of signs and symptoms between the two groups. The results showed that there was a significant difference in the proportion of subjects according to days before complete resolution (p<0.001). The day of resolution was significantly shorter in the Metronidazole group than the protexin group. However, the recurrence was significantly noted only among those given Metronidazole.

Table 6. Comparison of the Days Before Complete Resolution Between the Two Groups

	Metronidazole (n=40)	Protexin (n=40)	p-value*
Amount of Discharge			
<1 day	40 (100%)	0 (0%)	
1 day	0 (0%)	33 (82.5%)	<0.001 (S)
3 days	0 (0%)	7 (17.5%)	
pH of Vaginal Restoration			
<1 day	40 (100%)	0 (0%)	<0.001 (S)
1 day	0 (0%)	40 (100%)	
Presence of Clue Cells			
<1 day	35 (87.5%)	5 (12.5%)	
1 day	0	35 (87.5%)	<0.001 (S)
Recurrence	5 (12.5%)	0	
Fishy Odor with KOH			
<1 day	1 (2.5%)	0	
1 day	39 (97.5%)	0	<0.001 (S)
3 days	0	40 (100%)	

* p>0.05- Not significant; p ≤0.05-Significant

Table 7. Comparison of the Score Between the Two Groups

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Day 1 Amsels score			
0	40 (100%)	0	
3	0	5 (12.5%)	<0.001 (S)
4	0	35 (87.5%)	
Day 3 Amsels score			
0	40 (100%)	33 (82.5%)	0.01 (S)
1	0	7 (17.5%)	
Day 7 Amsels score			
0	40 (100%)	40 (100%)	1.00 (NS)
Day 30 Amsels score			
0	35 (87.5%)	40 (100%)	0.05 (S)
1	5 (12.5%)	0	

* p>0.05- Not significant; p ≤0.05-Significant

Table 8. Comparison of Recurrence Between the Two Groups

	Metronidazole (n=40)	Protexin (n=40)	p-value*
Recurrence			
(+)	5 (12.5%)	0	0.05 (S)
(-)	35 (87.5%)	40 (100%)	

Table 9. Comparison of the Days Before Complete of Resolution of Signs and Symptoms Between the Two Groups

Follow-up	Metronidazole (n=40)	Protexin (n=40)	p-value*
Over-all Resolution			
<1 day	35 (87.5%)	0	
1 day	0	33 (82.5%)	<0.001 (S)
3 days	0	7 (17.5%)	
Recurrence	5 (12.5%)	0	

* p>0.05- Not significant; p ≤0.05-Significant

DISCUSSION

The mechanisms whereby lactobacilli function as anti-infective defenses are still not fully understood. This may involve production of antimicrobial factors and maintenance of a vaginal pH of ≤4.5. It could also be due to bio-surfactants that alter the surrounding surface tension of the vagina which leads to reduced adhesion of pathologic bacteria. This might explain the relative lack of exposed epithelial cells noted in healthy women. (8) In addition, lactobacilli have been shown to bind and aggregate with some pathogens, interfering with their adhesion, and directly killing them through production of antimicrobials thus limiting their effect and spread to other organs.

Studies have also shown that production of H_2O_2 by lactobacilli has a clinically protective role against BV such that it is believed to have a protective role against colonization of other flora. Studies have shown that an oral dose of over one billion organisms per day was found to maintain a lactobacilli-dominated vaginal presence leading to a decreased incidence of BV. The onset of effect is obviously longer than direct vaginal instillation, and will depend on viability of the strains as they pass through the stomach and gut. However, an advantage of the oral approach may be the ability of the lactobacilli to reduce the transfer of yeast and pathogenic bacteria from the rectum

to the vagina, which could potentially lower the risk of infection. It is highly recommended that in comparing the treatment outcome for Bacterial vaginosis, a 3rd arm can be utilized which will include Metronidazole and oral Lactobacillus rhamnosus combined as the standard treatment for Bacterial vaginosis. The Oral lactobacillus rhamnosus will serve as an adjunct in the treatment of BV to help prevent recurrence which is proven to be its significant effect in the treatment of BV together with Metronidazole, the standard drug.

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CONCLUSION

The Metronidazole remains to be the standard treatment for Bacterial vaginosis. There was also faster recovery and clinical improvement in thecharacter of the vaginal discharge, amount and smell based on the Amsel's criteria. The advantage of the Oral Lactobacillus rhamnosus is evident in the lowering of the recurrence rate of BV for Protexin arm (0 out of 40 on the 30 fo the follow-up). ■

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