Efficiency of Vaginally Administered Probiotic *Lactobacillus Rhamnosus GR-1 and Lactobacillus Reuteri RC-14* versus Oral Metronidazole in the Treatment of Bacterial Vaginosis: Randomized Double Blind Study

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ABSTRACT

Background*: Bacterial vaginosis (BV) is a relatively complex yet extremely common condition characterized by alteration of the vaginal ecology in which the normal flora, dominated by lactobacilli, is replaced by a mixed bacterial flora. BV is associated with adverse pregnancy outcome, increased risk for pelvic inflammatory disease and abnormal cervical cytology. BV can be treated with antibiotics such as metronidazole or clindamycin, either orally or vaginally. However, there is usually a high rate of recurrence. Probiotics are defined as live micro-organisms which, when administered in an adequate amount, confer a health benefit on the host. The aim of this study was to investigate the efficacy of vaginally administered Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 as probiotics in comparison to the standard oral metronidazole therapy in the treatment of symptomatic bacterial vaginosis.* ***Patients & Methods****: The study enrolled 60 women with BV. They were divided into two groups: Group (1) included 30 patients who received oral metronidazole tablets 500mg twice daily for one week and placebo vaginal capsules twice daily for five days. Group (II): included 30 patients who received oral placebo tablets twice daily for one week and vaginal capsules containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14) twice daily for five days. All participants were evaluated before initiation of treatment then 1 and 4 weeks thereafter. Follow up was based on clinical improvement, pH of vagina, whiff test, absence of clue cells in vaginal smears and Nugent score which is considered the standard for research.* ***Results****: After 4 weeks of follow up, the probiotic group had a significantly higher cure rate of BV (88%) than the metronidazole group (51%) (p = 0.01). In addition, according to the Gram-stain Nugent score more women were assessed with "normal: vaginal micrbiota in the Probiotic group both after 1 week (p=0.05) and after 4 weeks (p=0.01). Relapse was higher in the metronidazole treated group (4 out of 29 cases, who completed the study accounting for 13.8%) while there was no relapse in the probiotic treated group. Failure of cure was higher in metronidazole treated group (10 cases accounting for 34.4%) than probiotic treated group (3 cases accounting for 12%).* ***Conclusion:*** *The results of the present study showed that Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14, taken vaginally, were effective in treatment, relapse prevention and normalization of the vaginal flora in women with bacterial vaginosis for which we recommend the use of Probiotics as an effective treatment option. Furthermore, testing sensitivity of isolates of BV for metronidazole resistance is recommended to find reasons of less efficacious metronidazole treatment.*

INTRODUCTION

Bacterial vaginosis (BV) is a condition characterized by alteration of the vaginal ecology in which the normal flora, dominated by lactobacilli, is replaced by a mixed bacterial flora which includes *Gardnerella vaginalis, Mobiluncus spp., Mycoplasma hominis, Bacteroides spp.* and other anaerobes[(1)]. Epidemiologic studies of women with vaginitis show that BV is present in about 27-31% of general population[(2)]. This condition is common in women of reproductive age and may cause malodorous vaginal discharge, although in many women it is asymptomatic. BV is associated with pelvic inflammatory disease[(3)], infections following gynecological surgery[(4)], and preterm birth[(5)].

Currently, metronidazole is the agent of choice for the treatment of bacterial vaginosis. This therapy is only moderately effective against *G vaginalis and Mobiluncus spp*, and is inactive against *Mycoplasma hominis*, but its metabolites are highly active against anaerobes in general including the *Bacteroides species.* Unfortunately, metronidazole is often poorly tolerated due to its side effects and is usually associated with a high rate of recurrence.[(6,7)]

Probiotics are defined as live micro-organisms which, when administered in an adequate amounts, confer a health benefit on the host .Currently, the best studied probiotics are lactic acid producing bacteria, particularly *Lactobacillus species*[8]**.** The actual mechanism of action of probiotics in the vagina is probably multifactorial. The production of lactic acid, bacteriocins, and hydrogen peroxide, as well as inhibition of the adherence of *G. vaginalis* to the vaginal epithelium seems to be important. The administration of these lactobacilli by mouth, intravaginally or both has been shown to be safe and effective in reducing and/or treating urogenital infections[9]**.**

The presence and dominance of lactobacilli in the vagina is associated with reduced risk of BV and urinary tract infection[10]. On the contrary, the loss of vaginal lactobacilli appears to be the major factor in the cascade of changes leading to BV, and relapses are associated with failure to establish healthy lactobacilli dominated vaginal flora. An efficient treatment of BV should result in a long-term restoring of the ecological balance in vagina by increasing the natural bacterial flora while decreasing the growth of potentially pathogenic bacteria[11].

This raises the question as to whether restoration of lactobacilli by vaginal probiotic therapy can restore the normal flora. The aim of this study was to investigate the efficacy of vaginally administered *Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14* as probiotics in comparison to the standard oral metronidazole therapy in the treatment of symptomatic bacterial vaginosis.

PATIENTS & METHODS

The study enrolled 60 women with BV from those attending the Gynecology outpatient clinics in Ain Shams University Hospital and Kafr El Sheikh General Hospital over the period starting from April 2008 to January 2009. They were divided into two groups: Group (1) included 30 patients who received oral metronidazole tablets 500mg twice daily for one week and placebo vaginal capsules twice daily for five days. Group(II): included 30 patients who received oral placebo tablets twice daily for one week and vaginal capsules containing *Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14* ($1x10^9$ CFU viable cells of each strain /capsule) (Fem-Dophilus™ capsules, by Jarrow Formulas, USA) twice daily for five days. Diagnosis of BV was based on Amsel's criteria**,**[12] defined as the presence of three out of the following four criteria:

1. Thin, gray and homogeneous vaginal discharge.
2. Unpleasant fishy odor before or after addition of 10 % KOH.
3. Vaginal pH > 4.5
4. The presence of clue cells in wet or stained smear.

Exclusion Criteria: Subjects were not included if they were pregnant, breast feeding, diabetics, taking steroids, antibiotics or antifungal therapy. Those diagnosed as vulvovaginal candidiasis, trichomoniasis or other genital infection, and those in menses or giving history of allergy to imidazoles were also excluded from the study.

Each patient was subjected to:

a- **Full history taking** (age, marital status, parity, method of contraception & duration of its use, vaginal discharge, sexual habits, vaginal douching, smoking, past history of any operation, medical treatment of Diabetes mellitus, hypertension or hormonal replacement therapy. An informed consent was obtained at enrollment and a data gathering questionnaire was filled during the interview to increase validity of information obtained.

b- **General and abdominal examination.**

c- **Pelvic examination** for evaluation of:

1. **Vaginal discharge** (amount, color, odor).
2. **pH of vaginal fluid** was measured directly in the posterior fornix by dipping a pH indicator strip with a scale graded from 1-14 including distinct color key for each value.
3. **Amine test (Whiff test**) was performed by addition of 10% KOH to a separate swab which was obtained by rolling a sterile cotton tipped wooden shafted swab across the vaginal wall and posterior vaginal fornix and placed on glass slide, a few drops of KOH (10%) were added by a dropper to the swabbed discharge. The release of fishy odor means +ve amine test
4. **Vaginal smear** was taken from the posterior fornix or the lateral fornix by a cotton tipped swab. It was spread immediately on clear serial slides.

Fixation and staining of the smears:

All the slides were left to dry in air then transferred to the microbiologist.

Slides were fixed by heating and stained by Gram stain.

Examination of smears:

The slides were examined microscopically for the presence of clue cells (≥ 20% of epithelial cells).

Nugent scoring (NS):[13]

Vaginal swab smears were graded by a microbiologist blinded to the subject groups, on a 10-point scale based on the presence or absence of *Lactobacillus* morphotypes under oil immersion (x1000 magnification). A score of **0-3** was interpreted as normal flora, a score of **4-6** as Intermediate flora, and a score of **7-10** as BV (in which the samples were dominated by small Gram negative and Gram-variable straight and curved rods).

All patients received the treatment regimen for one week. Then analysis of vaginal discharge, vaginal pH (pH indicator strip), whiff test, plus a vaginal smear that was Gram stained and visualized under oil immersion lens for clue cells and Nugent score (interpreted on a 10–point scale based on the presence or absence of *Lactobacillus* morphotypes, *G. vaginalis, Mobilluncus spp*. and other microorganism according to **Nugent et al 1991,[13]** to assess treatment efficacy. After four weeks, the same procedures were repeated to assess persistence of the condition or recurrence. The investigators remain blinded to all study procedures, until all analyses had been completed.

Statistical analysis:

Descriptive data were presented as mean ± standard deviation (SD). Results were analyzed using unpaired student's t test and Chi square test. Probability (P) value of less than0.05 was considered significant.

RESULTS

In the present study, vaginal discharge was the most presenting symptom in BV patients and there was a significant improvement in the probiotic treated group in comparison to the metronidazole treated group both at the end of treatment course (after one week) and after 4 weeks of follow up. A raised vaginal pH>4.5 (mean 5.18) in both groups was also observed in all cases of BV .There was no significant improvement at the end of treatment course in the two groups. However, pH showed a significant reduction "acidic pH" four weeks later at the follow up visits in the probiotic group. There was no statistical significant difference as regards Whiff test between the two groups throughout the study (p=0.05). The presence of clue cells in wet mount was found to be a specific criterion for the diagnosis of BV. When we compared clue cells in both groups it showed a significant improvement in the probiotic treated group in comparison to the metronidazole treated one, both after 1 week and after 4 weeks of the follow up. Data is shown in **(tables 1 and 2).**

Table (1): Comparison between Probiotics - versus Metronidazole treated BV patients as regards vaginal discharge, amine test and clue cells

	Probiotic group			**Metronidizole Group**		
Observation of BV patients	**Before treatment**	**After 1 week**	**After 4 weeks**	**Before treatment**	**After 1 week**	**After 4 weeks**
	No. of cases (%):	No. of cases (%):	No. of cases (%):	No. of cases (%):	No. of cases (%):	No. of cases (%):
Vaginal Discharge	30 (100%)	14 (46.7%)	5 (16.7%)	30 (100%)	25 (83.3%)	15 (50%)
t	0.000	- 3.009	- 2.608			
P value	1.000 (NS)	0.004 (S)	0.012 (S)			
Positive Whiff Test	27 (90%)	7 (23.3%)	2 (6.7%)	30 (100%)	7 (23.3%)	2 (6.7%)
t	- 1.79	- 0.071	0.111			
P value	0.078 (NS)	0.943 (NS)	0.912 (NS)			
Clue cells	27 (90%)	6 (20%)	3 (10%)	27 (90%)	13 (43.3%)	14 (46.7%)
t	0.30	- 1.88	- 3.184			
P Value	1.30 (NS)	0.064 (S)	0.003 (S)			

NS: Non-significant (S): Significant

Table (2): Comparison between the vaginal pH of both groups of BV patients before and after treatment

Vaginal pH	Probiotic group		Metronidazole group		t	P
	Mean	std	Mean	std		
Before treatment	5.13	0.73	5.23	0.78	0.511	0.611
After 1 week	4.43	0.529	4.55	0.54	0.855	0.395
After 4 weeks	3.90	0.490	4.29	0.57	2.68	0.010

Our results showed an improvement in Nugent scores both after one & four weeks in the probiotic treated group in comparison to the metronidazole treated group **(table 3) and figures: 1 and 2**

Table (3): Nugent sore in BV patients before and after treatment in both probiotic and metronidazole groups

Nugent score	Probiotic group			Metronidazole group		
	Before Treatment (No. of cases)	After 1 week	After 4 weeks	Before Treatment (No. of cases)	After 1 week	After 4 weeks
Score of 0-3 (Normal)	3 10%	23 79.3%	22 88%	3 10%	16 55%	15 51.7%
Score of 4-6 (Intermediate vaginal flora)	5 16.7%	2 (6.9%)	---	3 10%	1 3.4%	2 2.9%
Score of 7-10 (Bacterial vaginosis)	22 73.3%	4 13.8%	3 12%	24 88%	12 41.6%	12 41.6%
Total	**30**	**29**	**25**	**30**	**29**	**29**

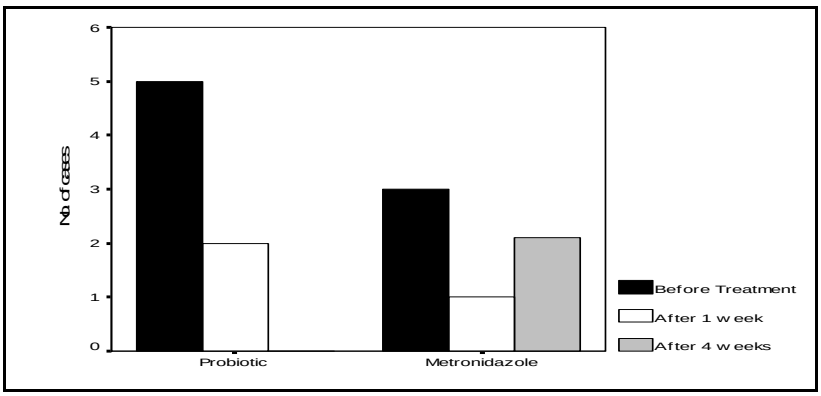

Figure (1): Comparison between Probiotics and Metronidazole in BV patients showing Intermediate vaginal flora (Nugent score 4-6)

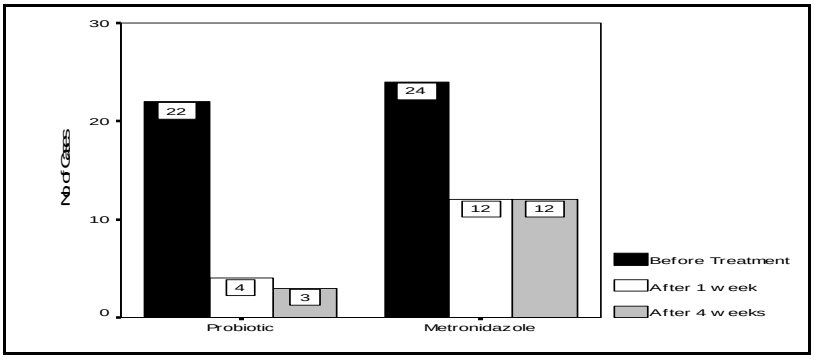

Figure (2): Comparison between Probiotics and Metronidazole in patients showing bacterial vaginosis (Nugent score 7-10)

Follow up of our patients after 4 weeks revealed that cure rate in the probiotic treated group was 88% while in metronidazole treated group, it was 53% which was statistically significant.

Relapse was higher in metronidazole treated group (4 cases out of 29 accounting for 13.8%) while there was no relapse in probiotic treated group.

Failure of cure was higher in metronidazole treated group (10 cases out of 29 accounting for 34.4%) than probiotic treated group (3 cases accounting for 12%) this difference was statistically significant. **(Table 4)**

Table (4): Comparison of cure rates between the two groups of BV patients using Chi2 test

	Probiotic group	**Metronidazole group**
Total no. at start of study	30	30
No. of escaped cases	5	1
Total no. at end of study	25	29
No. of cured patients at the end of 4weeks follow up	22 (88%)	15 (51.7%)
Relapse at the end of 4wks follow up	---	4 (13.8%)
No. of persistent BV patients (not cured)	3 (12%)	10 (34.4%)

Figure (3): Normal vaginal flora smear (Nugent score=0)

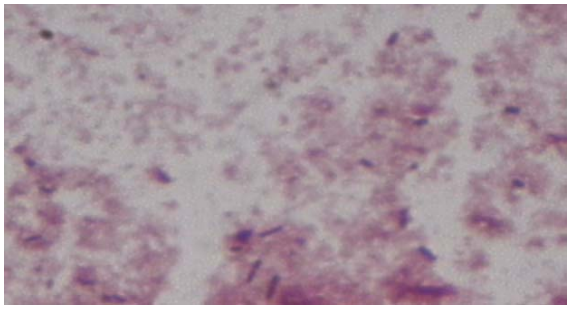

Figure (4): Intermediate vaginal flora smear (Nugent score=5)

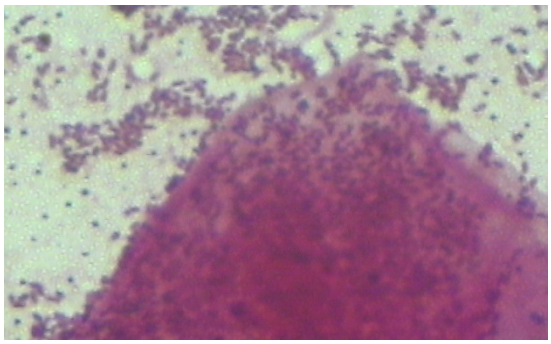

Figure (5): Bacterial vaginosis smear (Nugent score=10)

DISCUSSION

Bacterial vaginosis is the primary cause of abnormal vaginal discharge in women of reproductive age. Pharmaceutical interventions, such as antibiotics, have been suboptimally effective. The absence of lactobacilli in the vagina, a specific feature of BV, raises the question of whether restoration of lactobacilli, by probiotic therapy, can restore the normal flora, improve the cure rate and prevent recurrence of BV[1,7]**.** The rationale for probiotics use in managing BV women is quite strong, where certain lactobacilli strains can safely colonize the vagina after oral and vaginal administration, displace and kill pathogens including *G. vaginalis* and *E. coli*, and modulate the immune response to interfere with the inflammatory cascade that leads to preterm birth[14].

In this study, 60 females complaining of vaginal discharge and diagnosed as having BV were enrolled. They were randomly divided into two groups; group (**I**) received oral metronidazole tablets 500 mg twice a day for one week and placebo vaginal capsules twice a day for five days; and group (**II**) received oral placebo tablets twice a day for one week and vaginal capsules containing *L. rhamnosus GR-1* and *L. reuteri RC-14* (10^9 of each organism/capsule) (Fem-Dophilus™ capsules, by Jarrow Formulas, USA) twice a day for five days. There was no statistical difference between both groups as regards age, contraception methods and obstetric history; however, BV was associated with the use of IUDs as a contraception method (about 30%), this agrees with ***CDC, 2002***[15] finding that IUDs is highly associated with BV**.**

Several studies had been conducted to evaluate the value of probiotics in the treatment of BV. In 1993, **Neri and colleagues** were the first to achieve favorable results using intra-vaginal applications of yogurt in treatment of women with BV in the first trimester of pregnancy; their results indicated that the continuous correction of vaginal pH and lactobacillus flora is crucial for normal vaginal ecology[16].

Later in 1996, **Shalev and colleagues** treated pregnant women suffering from BV with yogurt containing lactobacillus and bifidobacterium (fortified yogurt) every day during pregnancy and conventional pasteurized yogurt. There was a significant reduction in the number of episodes of BV in those who administered fortified yogurt compared to pasteurized yogurt[17]**.**

In another study **Nishijima** and his colleagues' enrolled pregnant women after 34 weeks of pregnancy using oral fermented milk containing $\mathbf{10^9}$ cfu/ml of *Lactobacillus Johnsanii* daily orally for two weeks, as a probiotic, versus placebo, a reduction in BV in 81% of probiotic treated group was the only clinical outcome rather than reduction of preterm labor[18].

In a previous study **Anukam et al (2006a)** compared oral metronidazole versus metronidazole followed by oral probiotics in premenopausal women diagnosed as BV. Follow-up for 30 days showed 88% cure rate in the metronidazole/probiotic treated group compared to 40% in the metronidazole treated group[19]**.** In another study by **Anukam and his colleagues (2006b)**, 40 women diagnosed as BV, were randomized to receive either two dried capsules containing *L. rhamnosus GR-1 and L. reuteri RC-14* (10^9 each organism/capsule) each night for 5 days, or 0.75% metronidazole gel, applied vaginally twice a day (in the morning and evening). Follow-up for 30 days showed an effective (90%) cure of BV using probiotic lactobacilli[20]**.**

In the present study, there was significant improvement in the probiotic treated group in comparison to the metronidazole treated group both at the end of treatment course and after follow up of patients after 4 weeks, which agrees with **Anukam et al., 2006**[19,20] who recommended the use of *L. rhamnosus GR-1 and L. reuteri RC-14* strains, On the other hand **Eriksson et al., 2004**[21]**,** reported no significant difference in prevention of recurrent BV when subjects were treated with clindamycin suppositories for 3 days followed by 5 days of treatment with tampons impregnated with vaginal lactobacilli, or placebo (56% and 62% cure rates, respectively).This discrepancy in results could be due to: difference in lactobacillus strains used; in this study we used *L. rhamnosus GR-1 and L. reuteri RC-14* ,which are known by their ability to adhere to urogenital epithelial cells and production of antimicrobials against Gram negative bacteria, therefore, displacing urogenital pathogens and decreasing amines production associated with the increased vaginal transudation and squamous epithelial exfoliation creating the

typical discharge[22], while Eriksson et al., used a mixture of *L. fermentum, L. casei, L. rhamnosus and L. gasseri,*[21].

In terms of understanding how lactobacilli might intervene against BV **Saunders et al 2007**[23] showed that *L. rhamnosus GR-1* and especially *L. reuteri RC-14* could displace *G. vaginalis* biofilms in vitro. The authors suggeasted that biosurfactants produced by *L. reuteri RC-14* may play a role in displacement ,while production of bacteriocins and signaling molecules may have affected viability and pathogen growth[24]. The current study and the studies of **Anukam et al 2006a & 2006b** emphasize the importance of selecting the right probiotic products.

Another explanation of discrepancy in results could be the different technical handling of vaginal Lactobacilli in the two studies; in this study we used vaginal capsules containing *L. rhamnosus GR-1* and *L. reuteri RC-14* while ***Eriksson and his colleagues*** used tampons impregnated with *L. fermentum*, *L. casei*, *L. rhamnosus* and *L. gasseri* to be used during menstruation**.** Moreover, the concentration of lactobacilli in the treatment given is another factor which was different in both studies. In the current study a concentration of 10^9 of each organism/capsule was used; while in the ***Eriksson*** study, the concentration of Lactobacilli ranged from 10^6 to 10^8/tampon.

Another important factor to be considered is patient compliance, vaginal capsules used in the current study are easy to be inserted into the vagina and left till complete disintegration of its coat; in comparison to tampons. In addition, a lack of effect was observed with usage of tampons, as patients were subjected to a too short exposure time of the lactobacillus-loaded tampons.

This study also coincides with another 2 studies[25,26] In the study done by **Drago and his colleagues 2007**, treatment for 6 days with a douche containing *L.acidophilus* led to improved Nugent scores that remained low during the follow-up period (20 days after the last treatment) for almost all of the patients[25]. **Martinez and coworkers (2009)** showed that the conjoint use of single – dose tinidazole (2 g) with orally administered probiotic L. *rhamnosus GR-1* and *L. reuteri RC-14* augmented cure of Brazilian patients diagnosed with BV. They reported that their findings raise the possibility of combining pharmaceutical agents with probiotics as a means to better manage common infections, perhaps extend the longevity of drugs whose efficacy is warning through bacterial resistance, and reduce the number of women requiring long-term prophylaxis to prevent recurrence[26].

In the present study, a raised vaginal pH >4.5 (mean 5.18 in both groups) was found to be a sensitive indicator of BV. There was no significant improvement in the pH at the end of treatment course (after one week) in the two groups. However, pH showed a significant reduction "acidic pH" four weeks later at the follow up visits in the probiotic group. This could be explained by production of organic acids, and fatty acids by *L. rhamnosus GR-1* and *L. reuteri RC-14*[27], that possibly hadn't reached the optimal levels needed to restore the desired pH after one week. According to **Larrson et al 1995**[28] 1 week follow up is a too short period for meaningful interpretation of individual patient data, and a better time point is one month, as was used in our study. This was proved by the improvement in the pH observed after 4 weeks of treatment.

The last criterion, amine test is a good indicator because it is easily performed, rapid and inexpensive with good sensitivity and less specificity .A negative amine test may be found in early stage of disease in non complainers due to the fact that some may produce amines in late stage of the disease[29]. As regard to amine test, both groups showed marked improvement throughout the study and there was no significant statistical difference between both groups.

The presence of clue cells in wet mount and in Gram stained smears was found to be a specific criterion for the diagnosis of BV. This is in agreement with the finding of **Platz-Chritensen et al., (1995)**[30], who found that this test had 90% sensitivity and 97% specificity. When we compared clue cells in both groups, it showed a significant improvement in the probiotic treated group than the metronidazole treated one, both after 1 week and after 4 weeks of the follow up. This indicates the importance of this microscopic finding in diagnosing BV and its usefulness in follow up of BV patients

Our results showed an improvement in ***Nugent*** score both after 1 week and after 4 weeks in the probiotic treated group compared with the metronidazole treated one , this is in agreement with **Anukam and his colleagues**[19,20], denoting that laboratory criteria (Gram scoring) reflects accurately and

specifically the clinical condition of patients of BV.

Cure was defined as clinical improvement in **Amsel**'s criteria[12] and normal vaginal smear with a NS (0-3). As a follow up, cure rate was evaluated 4 weeks after treatment. In the current study, the probiotic group had a significantly higher cure rate of BV (88%) than the metronidazole treated group (51%). This coincides with the results of **Anukam and his colleagues**[19,20] and denotes the efficacy of probiotics in the treatment of BV. It also supports results of the study done by **Martinez et al 2009** who reported 87.5% cure rate in Probiotic group versus 50% in the placebo group[26].

In the probiotic group, no cure was showed in 3 patients. These patients were not compliant with the use of lactobacillus vaginal capsules on the detailed history. On the other hand, 10 cases in the metronidazole group, where not cured, this could be attributed to lack of patient compliance to take metronidazole due to its metallic taste and GIT upsets associated with it, or perhaps the inability of metronidazole to eradicate persistent infection due to resistance to the drug[31] which needs confirmation by further studies.

Symptomatic BV persists or recurs in 11 to 29% of women at one month[31]. In the present study, none of the probiotic group showed relapse after 4 weeks of follow up, while 4 of metronidazole group showed relapse.This may be explained by failure of vaginal acidification, failure to reduce numbers of *G. vaginalis* and anaerobic micro-flora, failure to recolonize the vagina with protective Lactobacillus spp., re-infection or re-colonization with an unrecognized pathogen or antimicrobial resistance of overgrowing microorganisms[32]. It is well known that with vaginal drug administration, absorption is unaffected by gastrointestinal disturbances. The vaginal route allows administration of lower doses, delivery of a higher load, and less frequent administration than the oral route[33], so it could become the ideal route for probiotic use in gynecology if a Lactobacillus strain with the right properties is available.

In conclusion: The results of the present study showed that *Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14*, taken vaginally, were effective in treatment, relapse prevention and normalization of the vaginal flora in women with bacterial vaginosis. We recommend the use of probiotics as an effective treatment option for bacterial vaginosis. Furthermore, testing sensitivity of isolates of BV for metronidazole resistance is recommended to find reasons of less efficacious metronidazole treatment.

REFERENCES

1. **Spiegel CA. (1991):** Bacterial vaginosis. Clin Microbiol Rev. 4(4):p485- 502.
2. **Jenifer E. Allsworth, Jeffrey F. and Peipert.(2007):** Prevalence of Bacterial Vaginosis National Health and Nutrition Examination Survey Data. ACOG 2007; 109(1):p14-20.
3. **Ness RB, Kip KE, Hillier SL et al. (2005):** A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease. Am J Epidemiol; 162: 585–590.
4. **Larsson PG, Bergstrom M, Forsum U (2005):** Jacobsson B, Strand A , Wolner - Hanssen P. Bacterial vaginosis. Transmission, role in genital tract infection and pregnancy outcome: an enigma. APMIS; 113: 233–245.
5. **Hillier SL, Nugent RP, Eschenbach DA et al. (1995):** Association between bacterial vaginosis and preterm delivery of a lowbirth-weight infant.The Vaginal Infections and Prematurity Study Group. N Engl J Med; 333: 1737–1742.
6. **Lau AH, Lam NP, Piscitelli SC, Wilkes L, and Danziger LH. (1992):** Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. Clin Pharmacokinet.; 23: p328-364.
7. **Bradshaw CS, Morton AN, Hocking J, etal (2006):** High recurrence rates of Bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence.J of Infectious Diseases;193:1478-1486.
8. **Andreu A. (2004):** Lactobacillus as a probiotic for preventing urogenital infections. Reviews in Medical Microbiology; 15:p1-7.
9. **Falagas ME, Betsi GI, and Athanasiou S (2007):** Probiotics for the treatment of women with bacterial vaginosis.Clin Microbiol Infect;13:657-664.
10. **Reid G, and Burton J.(2002):** Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes and Infection; 4:p 319-322.

11. **Reid G, Burton J,and Devillard E.(2004):**The rationale for probiotics in female urogenital healthcare. Medscape General Medicine; 6: p 49-53.
12. **Amsel RP, Totten PA, Spiegel CA, Chen KC, Eschenbach D and Holmes KK.(1983):** Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med; 74(1):p14-22.
13. **Nugent RP, Khron MA and Hillier SL (1991):** Reliability of diagnosing bacterial vaginosis is improved by a standardized method of method of Gram stain interpretation. J. Clin Microbiol; 29: 297-301.
14. **Reid G and Bocking A (2003):** The potential for probiotics to prevent bacterial vaginosis and preterm labor. Am J. Obstet gynecol.; 189(4):p1202-1208.
15. **CDC. Centers for Disease Control and Prevention. (2002):** Sexually transmitted diseases treatment guidelines MMWR Recomm Rep; 51:44-45.
16. **Neri A, Sabah G, and Samra Z. (1993):** Bacterial vaginosis in pregnancy treated with yoghurt. Acta Obstet Gynecol Scand; 72:p17-19.
17. **Shalev E, Battino S, Weiner E, Colodner R, and Keness Y (1996):** Ingestion of yogurt containing Lactobacillus acidophilus compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis Arch Fam Med;5:p593-596.
18. **Nishijima K, Shukunami K, and Kotsuji F. (2005):** Probiotics affects vaginal flora in pregnant women, suggesting the possibility of preventing preterm labor. J Clin Gastroenterol.; 39:p447-448.
19. **Anukam K, Osazuwa E, Ahonkhai I, Ngwu M, Osemene G,Bruce AW and Reid G. (2006a):** Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral Probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. Microbes Infect. 8: 1450–1454.
20. **Anukam K, Osazuwa E, Osemene GI, Ehigiagbe F, Bruce AW, and Reid G (2006b):** Clinical study comparing Probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes Infect. 8: 2772–2776.
21. **Eriksson K, Carlsson B, ForsumU, and Larrson PG. (2005):** A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. Acta Derm. Venereol. 85: 42–46.
22. **Reid G, Peter Cadieux, Pirkka Kirjavainen, Sung O Kim, Kingsley Anukam and Andrew W Bruce (2005):** How do probiotic Lactobacillus GR-1 and RC-14 improve vaginal health?International Yakult Symposium-Understanding the role of probiotics in health. October 13th – 14th 2005. Het Pand, Ghent, Belgium. Abstract book 1:34-35.
23. **Saunders S, Bocking A, Challis J, and Reid G. (2007):** Effect of Lactobacillus on Gardnerella vaginalis biofilms. Colloids Surf.B Biointerfaces, 55: 138–142.
24. **Laughton JM, Devillard E, Heinrichs DE, Reid G, and McCormick JK.(2006):** Inhibition of expression of a staphylococcal superantigen-like protein by a soluble factor from Lactobacillus reuteri. Microbiology, 152: 1155–1167.
25. **Drago L, De Vecchi E, Nicola L, Zucchetti E, Gismondo MR, and Vicariotto F. (2007):** Activity of a Lactobacillus acidophilus-based douche for the treatment of bacterial vaginosis. J.Altern. Complement. Med. 13: 435–438.
26. **Martinez RC, Franceschini SA, Patta MC, Quintana SM, Gomes BC, De Martinis EC, and Reid G (2009):** Improved cure of bacterial vaginosis with single dose of tinidazole (2 g), Lactobacillus rhamnosus GR-1, and Lactobacillus reuteri RC-14: a randomized, double-blind, placebo-controlled trial. Can. J. Microbiol.55: 133-138.
27. **Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D,and O'Halloran S. (2001):** In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. Am J Clin Nutr; 73(2):p386S-392S.
28. **Larsson PG. (1995):** Bacterial vaginosis treatment evaluation before one month is not accurate. Am. J. Obstet. Gynecol. 172: 1638–1639.
29. **Sonnex C. (1995):** The amine test: a simple, rapid, inexpensive method for diagnosing bacterial vaginosis. Br J Obstet Gynaecol. 1995; 102(2):p160-161.

30. **Platz-Christensen JJ, Sundstrom E, and Larsson PG.(1994):** Bacterial vaginosis and cervical intraepithelial neoplasia. Acta Obstet Gynecol Scand; 73:p586-590.
31. **Hanson J M, McGregor J A , Hillier SL , D. Eschenbach DA, Kreutner AK, Galask RP, and Martens M.(2000)**: Metronidazole for bacterial vaginosis. Acomparison of vaginal gel vs. oral therapy. J Reprod Med 2000; 45: p889-896.
32. **Verhelst R. (2006):** Characterization of the vaginal microflora. Faculty of Medicine and Health Sciences, Ghent University, Belgium. P14-170.
33. **Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, Zampagilone E (2004):** Why consider vaginal drug administration? Fertil Steril; 82(1):1-12.

كفاءة العلاج الحيوي البدائي (بروبيوتك) باستخدام البكتيريا اللبنية رامونيسس ج آر-١ والبكتيريا اللبنية ريتيرآرسى-١٤ مهبليا مقابل تناول عقار الميترونيدازول بالفم في علاج الالتهابات البكتيرية المهبلية: تجربة علاجية مزدوجة التعمية

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تتميز الالتهابات البكتيرية المهبلية بحدوث تغير في الوسط البكتيري المهبلي وذلك بإحلال البكتيريا المسببة للعدوى البكتيرية المهبلية محل البكتيريا اللبنية الحمضية الموجودة بالمهبل وتعاني السيدات المصابات بالعدوى البكتيرية المهبلية من إفرازات بيضاء رمادية بالمهبل. وقد أثبتت الدراسات المتعددة وجود علاقة بين الإصابة بالالتهابات البكتيرية المهبلية وحدوث داء الالتهاب الحوضي والتهابات ما بعد استئصال الرحم و حدوث تغيرات غير طبيعية في خلايا عنق الرحم . ويعتبر اختفاء البكتيريا اللبنية من المهبل هو العامل الرئيسي لظهور العدوى البكتيرية المهبلية. يعتبر عقار الميترونيدازول هو الدواء المناسب لعلاج العدوى البكتيرية المهبلية وذلك على الرغم من قدرته المتوسطة على التأثير على البكتيريا المسببة للعدوى البكتيرية المهبلية وحدوث بعض المضاعفات الجانبية للعقار أثناء تناوله كما أن نسبة الشفاء بعد مرور شهر من تناوله لم تتجاوز ٦١% مع احتمال نمو للبكتيريا الممرضة بالمهبل بعد انتهاء فترة العلاج بعقار الميترونيدازول.

البروبيوتكس عبارة عن بكتيريا حية ذات تأثير مفيد على الصحة. وطريقة عمل البروبيوتكس غير واضحة تماما إلا أنها تتميز بإنتاجها لكميات من حمض اللاكتيك و بيروكسيد الهيدروجين والمبيد الجرثومي والتي تؤدي بدورها إلى القضاء على البكتيريا الضارة، ويعتبر تناول هذه المركبات عن طريق الفم أو المهبل من الوسائل المناسبة لعلاج الالتهابات البكتيرية المهبلية

ويكمن الهدف من وراء هذا البحث في المقارنة بين كفاءة تعاطي عقار البروبيوتك البكتيريا اللبنية رامونيسس ج آر-١ والبكتريا اللبنية ريتيرآرسى-١٤ عن طريق المهبل و عقار الميترونيدازول لاستخدامهما في علاج الالتهابات البكتيرية المهبلية.

وقد اشتملت الدراسة على ٦٠ سيدة يعانين من الالتهابات البكتيرية المهبلية تم تقسيمهم إلى مجموعتين:**الأولى**: اشتملت على ٣٠ سيدة تناولن عقار الميترونيدازول عن طريق الفم، وكبسولات مهبلية لم تحتو على المادة الفعّالة. **الثانية**: اشتملت على ٣٠ سيدة تناولن أقراص عن طريق الفم لم تحتو على المادة الفعّالة، وكبسولات مهبلية احتوت على البكتيريا اللبنية رامونيسس ج آر-١والبكتيريا اللبنية ريتيرآرسى- ١٤.

وقد أظهرت الدراسة أن نسبة الشفاء في المجموعة التي تناولت البكتريا اللبنية هي ٨٨% مقارنة بنسبة ٥٣% في المجموعة التي تناولت عقار الميترونيدازول، كما أظهرت الدراسة حدوث انتكاس للمرض في المجموعة التي تناولت عقار الميترونيدازول، بينما لم يحدث ذلك في أي من حالات المجموعة التي تناولت البكتريا اللبنية.كما أن عدد الحالات التي لم تستجيب للعلاج كانت أعلى في مجموعة الميترونيدازول (١٠ حالات، أي بنسبة ٣٤,٤%) مقارنة بالمجموعة التي تناولت البكتريا اللبنية (٣ حالات، أي بنسبة ١٢%)

وقد خلصت الدراسة إلى إمكانية استخدام البروبيوتك البكتريا اللبنية في علاج الالتهابات البكتيرية المهبلية بنسبة أفضل من عقار الميترونيدازول. وأوصت الدراسة باستخدام البروبيوتك البكتريا اللبنية عن طريق المهبل في علاج الالتهابات البكتيريا المهبلية.