Review Article

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20240866

Bacterial vaginosis and the role of prebiotics and probiotics: current practices

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Received: 09 February 2024 **Accepted:** 07 March 2024

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ABSTRACT

Bacterial vaginosis (BV) is characterized by overgrowth of opportunistic bacteria and a decrease in the levels of *Lactobacilli*. BV is commonly encountered by gynaecologists practicing in India. The opinions of 21 gynaecologists across India regarding diagnosis and management of BV were sought via focussed group discussions. In Indian women, BV is more common in the reproductive age group, perimenopausal women, women with polycystic ovarian disease (PCOD), pregnant women, and in teenagers/pubertal age group. BV is often underestimated as it is not diagnosed by family physicians. The panel of experts opined that they preferred to conduct screening for BV in all high-risk pregnant cases, women with bad obstetric history such as premature abortions and women with a history of tuberculosis. One challenge faced by clinicians in the real-world setting is recurrent infections of BV in their patients. For the treatment of BV, antibiotics can be prescribed along with pre-probiotics to avoid dysbiosis and to prevent recurrence of BV. Pre-probiotics should be administered in sufficient amounts for adequate management of BV. More than 80% of *Lactobacilli* species should be present in an ideal pre-probiotic to ensure adequate production of acid and bacteriocins for the destruction of unhealthy bacteria and prevention of microfilm formation. The results with the pre-probiotics used will depend on the quality of the formulation and the maintenance of the cold chain. Prebiotic -probiotic supplementation may be considered a new adjuvant treatment for BV.

Keywords: Bacterial vaginosis, Prebiotic-probiotic supplementation, Dysbiosis, Bacteriocins

INTRODUCTION

Bacterial vaginosis (BV) is one of the most common causes of abnormal vaginal discharge in women in the reproductive age group and among pre-menopausal women. BV is characterized by overgrowth of opportunistic bacteria and a decrease in the levels of *Lactobacilli*. A healthy vaginal flora is dominated by *Lactobacilli*, which contribute to 90-95% of total bacteria. However, research also indicates that some healthy women do not possess a *Lactobacillus*-dominated vaginal microbiota. In cases of BV, mainly anaerobic microorganisms such as gram-positive cocci and gramnegative bacilli dominate the vaginal flora. Common

opportunistic bacteria include *Prevotella* species, *Gardnerella vaginalis* and *Mobiluncus* species.²

The prevalence of BV varies from 15% to 30% in non-pregnant women and up to 50% in pregnant women. The prevalence of BV can vary from country to country.³ A high prevalence has been reported in South Africa, a moderate prevalence in Asia and South Asia and a low prevalence in Finland, Australia, New Zealand and Western Europe.⁴⁻⁷ Cultural factors may play a role in these observed differences. Additionally, there may be differences in surveillance techniques used and BV may not be a reportable disease in every country. Diagnostic techniques vary depending on the availability of resources. Currently, the prevalence of BV has been observed to be rising. The risk factors associated with the high prevalence

of BV include unprotected sexual exposure, frequent use of condoms, douching, and diabetes. ¹

Bacterial vaginosis is very commonly encountered by Indian gynaecologists practicing in the real-world setting. BV has been reported in 38% of Indian women (*Bhalla*). The approach to diagnosis and management of BV can vary. Although there is a high prevalence of BV in India, many asymptomatic women with BV are less likely to seek treatment for the morbidity and thus are more likely to acquire other sexually transmitted infections (STIs). Women attending various healthcare facilities should be screened and treated for bacterial vaginosis to reduce the risk of acquisition of other STIs. Hence the opinions of gynaecologists across India were sought through a discussion about the approach to early diagnosis and appropriate treatment of BV in Indian women

A meeting of 21 gynaecologists from India was conducted to discuss the issues pertaining to BV diagnosis and management followed in the real-world setting.

EPIDEMIOLOGY

The vaginal microbiome is an intricate and dynamic microecosystem that constantly undergoes fluctuations during the female menstrual cycle and the woman's entire life. It is affected by menstrual cycle, progesterone and estradiol levels, glycogen content in the vaginal epithelium, vaginal pH, and immune responses. A healthy female genital tract harbours a microbiome dominated by lactic acid and hydrogen peroxide-producing Lactobacilli, which provide protection against infections by maintaining a low pH. Changes in the bacterial compositions of the vaginal microbiome can lead to BV, which is often associated with vaginal inflammation.⁸ A healthy vaginal microbiome is dominated by Lactobacillus species such as L. crispatus, L. gasseri, L. jensenii, and L. iners and to a lesser extent species such as L. acidophilus, L. brevis, L. delbrueckii, L. fermentum, L. mucosae, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus and L. vaginalis, which produce various antimicrobial compounds. BV is associated with dysbiosis of vaginal microbiome where Lactobacilli are displaced by predominantly polybacterial species such as Gardnerellavaginalis, anaerobic Atopobiumvaginae, Prevotella and Porphyromonas, Mobiluncus (Leptotrichia), spp., Sneathia Mycoplasma hominis.9

New insights about the asymptomatic nature of BV in some women has been a matter of research. The reason why women can still maintain "healthy" vaginal environments is yet unknown, but it has been postulated that anaerobic bacteria such as *Atopobium* spp., *Leptotrichia* spp. and *Megasphaera* spp. may produce lactic acid similar to *Lactobacillus* spp. This substitution of an ecological role by anaerobic bacteria may partially explain why there is a high percentage of asymptomatic BV and may also contribute to the misdiagnosis of BV. Therefore, the diagnosis of BV should not be focused on

only the abundance of *Lactobacilli* and other anaerobic bacteria.

Vaginal douching has been reported to be associated with BV in observational studies. Douching causes an increased risk of BV due to disruption of vaginal flora. ¹⁰

CLINICAL PRESENTATION

BV is a highly prevalent disorder among women of reproductive age.¹¹ Almost 50% of patients with BV may be asymptomatic. Women of reproductive age group may present with symptoms such as vaginal discharge, itching, and malodour. Women may also present with history of infertility and sexually transmitted diseases such as human immunodeficiency virus (HIV), *Neisseria gonorrhea* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and herpes simplex virus-2 (HSV-2).^{2,9}

Expert opinion

The prevalence of BV is increasing due to change in lifestyles and excessive use of douches and washes. Women from the high socioeconomic strata too present with symptoms of BV. This may be attributed to excessive washing and douching, which displaces the normal protective microbacterial flora. Use of tampons and menstrual cups can increase chances of BV. Hence maintaining appropriate personal hygiene is important. Proper menstrual hygiene is important. The rise in prevalence of BV observed in Indian women is in concurrence with the rising prevalence of asymptomatic disease reported in literature

Patients in India are unaware of the disorder of bacterial vaginosis. They are aware that the symptom of discharge associated with vaginal itching and malodour is not normal. However, in India, the taboos associated with vaginal discharge prevent women from seeking treatment.

In Indian women, BV is more common in the reproductive age group, perimenopausal women, pregnant women and teenager/pubertal age group. Globally, a high prevalence of BV has been reported in women of the reproductive age group. ¹²

BV patients are obese, and they have polycystic ovarian disease and insulin resistance. Although current evidence about association of BV and PCOD is scarce, and the experts did find any association between PCOD and BV in the real world setting in India.¹³

CLINICAL IMPLICATIONS OF BV

Although BV is considered to be a mild disease, it may be associated with uterine infections and adverse pregnancy outcomes such as premature labour and spontaneous abortions. BV-associated complications include pelvic inflammatory disease (PID), several STIs, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, HSV-2,

and an increased risk of human immunodeficiency virus-1 (HIV-1) acquisition. BV has also been linked with human papillomavirus (HPV). Women with BV may experience a decreased quality of life.

DIAGNOSIS

BV is currently diagnosed using Amsel's clinical criteria and microbiological criteria (Nugent's scoring). The Nugent's scoring is considered to be the gold standard. However, many clinics in India do not have microscopy facilities and skilled microbiologists. Hence a greater reliance on clinical evaluation is observed in the real world setting as it is easy and does not require special facilities or equipment.¹⁴ Although the Amsel criteria method is a convenient and inexpensive means of diagnosing bacterial vaginosis, it is not always reliable. Amsel's criteria involve saline microscopy and has been improved over time to include the presence of a thin watery homogenous discharge, elevated vaginal pH (>4.5), the presence of more than 20% of clue cells (vaginal epithelial cells) and a fishy odour after the addition of 10% potassium hydroxide to vaginal secretions ("whiff test") for a positive BV diagnosis. A combination of Amsel's criteria and Nugent scoring may be beneficial for an accurate diagnosis of BV due to an assessment on both clinical symptoms and microbial morphology.

Another diagnostic method based on Gram-stained vaginal smears, the Ison-Hav classification criteria was described 2002, which allows simplified grading and characterization of the vaginal microflora based on the number of Lactobacilli morphotypes compared with Gardnerella morphotypes. A vaginal microflora with a Nugent score of 4 to 6, known as an intermediate score or intermediate microflora is classified in grade II by the Ison-Hay criteria. An intermediate microflora was initially thought as a transitional step between a normal vaginal microflora and BV, or vice versa, but remains an uncharacterized category and is a challenge in the diagnosis of BV due to unknown clinical implications. Alternative reliable and inexpensive diagnostic methods that unify clinical and microbiological parameters, thus increasing sensitivity while retaining specificity, are needed.8,15

Expert opinion

BV is often underestimated as it is not diagnosed by family physicians. General practitioners often have a syndromic approach for all urogenital infections and prescribe empirical antifungals and antibiotics to all patients. Current evidence indicates that syndromic management is based on the identification of a combination of symptoms presented during a clinical examination. Syndromic management of STIs and BV has its benefits, especially in resource-limited settings where invasive procedures and laboratory tests are not available. The success of syndromic management relies on accurate information including the sexual history of the patient and a thorough

clinical examination. BV is diagnosed based on vaginal discharge syndrome (VDS) and is treated with a stat dose of metronidazole. The disadvantages of the syndromic approach in resource-limited settings are antibiotic overusage; there are limited opportunities for routine surveillance complicating the treatment and resolution of infections.⁸

Normal Gram staining is gold standard to distinguish between various forms of urogenital infections. Gram positive thick bacilli are *Lactobacilli* and their presence signifies normal vaginal microflora. Lowering of *Lactobacilli* and presence of clue cells are diagnostic of BV. Motile flagella are diagnostic of trichomoniasis. Normal vaginal swab and saline wet mount specimens must be collected after sterile isolation. Motility of trichomoniasis can be only assessed by saline wet mount.

The higher pH (>4.5) indicates growth of unhealthy anaerobes.

The smell of discharge is a good diagnostic sign for BV. If there is a diagnostic dilemma between BV and trichomoniasis, the speculum and wet mount examination can differentiate between the two disorders. This clinical insight is similar to the recommendations from clinical studies

The panel of experts opined that they preferred screening for BV in all women with high-risk pregnancy, women with bad obstetric history such as premature abortions, and women with a history of tuberculosis. Hormonal changes in pregnancy increase the chance of infections as the *Lactobacilli* content is reduced, and there is an increase in unhealthy pathogenic strains like *Streptococci*. Hence, it is important to rule out these infections during pregnancy. Universal screening is not required. Vaginal pH screening can be done followed by wet mount or else Gram staining can be done. The panel opinion was in concurrence with the evidence-based approach from inferences of well-designed clinical trials. ¹⁶

Identifying appropriate patients for screening, such as pregnant women, women planning pregnancy, and women with multiple and/or new sexual partners, is imperative for treatment.¹⁷

TREATMENT OF BV

Antibiotics are the preferred first line of treatment for a patient with BV. The antibiotics commonly prescribed include metronidazole and clindamycin. They can cause 80% improvement in symptoms of patients after 4 weeks of treatment. Although these antibiotics are safe, several side effects have been reported with their use such as nausea, vomiting, and abdominal pain. Secondly, recurrence of BV may occur in 40% to 50% of cases after 12 months after antibiotic treatment. Another challenge associated with BV treatment strategies is that the impact of vaginal microbiota disruption on the disease occurrence,

has not been considered. The dysbiosis of vaginal microbiome is further exacerbated by the antibiotics used. Hence the principles of management of BV must be three pronged: relieving the patients' clinical symptoms, reducing the recurrence rate by addressing the risk factors for recurrence, and regenerating the vaginal microbiome.

Use of prebiotics/probiotics to regenerate the vaginal microbiome

The effect of metronidazole/clindamycin combined with pre/probiotics or probiotics alone on treating BV has been studied recently. Several clinical trials (Table 1) and meta-analysis (Table 2) have been conducted to evaluate the effects of prebiotics/probiotics in BV.

Expert opinion

Most cases of BV are asymptomatic and when symptomatic, diagnosis can be made by foul smelling discharge. Clinical criteria of discharge are very important. The pH test is also important for BV diagnosis. The Nugent's criteria can offer definitive diagnosis of BV. However, clinicians often rely on speculum examination and the foul-smelling discharge as diagnostic pointers. Clue cells can also be an incidental finding in cytology. Trichomoniasis needs differentiation from BV when pH is more than 4.5. Therefore, a wet mount is required.

Antibiotic stewardship is very important in India because it is the biggest hub of infectious diseases and also multidrug resistant (MDR) bacteria (e.g. extended spectrum beta lactamase [ESBL]). Because metronidazole is an inducer of ESBL, it should be used only if it is absolutely necessary, and topical route should be preferred. Clindamycin is notorious for antibiotic associated diarrhoea. If *Clostridium difficile* is implicated, then toxic megacolon may ensue, which can be fatal. All antibiotics are associated with side effects and hence prudent use is advisable. Therefore, pre-probiotics should be preferred to restore normal vaginal microbiota and reduce or avoid the overuse of antibiotics.

Antibiotics can be co-prescribed with pre-probiotics to avoid dysbiosis. In pregnant women, antibiotics plus pre-probiotics are prescribed for 2 weeks. Prolonged probiotics can be given for 30-32 weeks till the salvage of the foetus is possible in high-risk cases such as women with more than 2 miscarriages. The experts preferred a pre-probiotic formulation after first line antibiotic therapy. They opined that combination of antibiotic plus pre-probiotic was most likely to give high cure rates. Several studies have ratified the use of pre-probiotics along with antibiotics.

Deviation from standard management guidelines by healthcare workers and the habit of relying on their own clinical judgement also contributes to insufficient treatment of vaginal infections. Syndromic management may lead to the misdiagnosis of BV, and treatment failure

and high rates of recurrence may contribute to antibiotic resistance.8

In women with tubal infertility, there is high incidence of BV. BV could be important cause in 20-25% of patients with infertility. Sometimes prophylactic use of prebiotics-probiotics for 10-20 days may be advised in these women.

Another advantage of the use of pre-probiotics is improvement in the oocyte quality. In recurrent *in vitro* fertilization (IVF) failures, pre-probiotics may be prescribed prior to egg pickup.

The experts recommended using pre-probiotics at the initiation of endometrial preparation in all embryo transfer cases, and they advised the regimen could be continued till the pregnancy test is positive i.e. up to 1 month after IVF.

The recurrence rate of BV is 30% at the end of 3 months, and almost 50 % at the end of a year. Recurrent BV may require prolonged treatment to return the vaginal flora to a normal predominately lactobacilli-dominated environment. 19

One challenge faced by clinicians in the real-world setting is recurrent infections of BV in their patients. The treatment of the male partner has been recommended in women with recurrent BV. However, husbands are often reluctant to take treatment.

Antibiotics used along with pre-probiotic offer faster relief of symptoms and higher clinical cure and can also help prevent recurrences. Pre-probiotics should be in sufficient amounts for adequate management. More than 80% of *Lactobacilli* should be present in an ideal pre-probiotic to ensure adequate production of acid, and bacteriocins for the destruction of unhealthy bacteria and prevention of microfilm formation.

It is better to use vaginal specific pre-probiotics for BV management. *Lactobacillus rhamnosus* plus some other species need to be part of the pre-probiotic for the treatment of BV. Gut specific pre-probiotics will only take care of gut related disorders. The expert panel opined that they prescribe pre-probiotics for 10-15 days when a patient is diagnosed with BV.

The vaginal route for pre-probiotics is preferred because the probiotics are delivered at the target site. However, the final decision is based on the patient preference as compliance to treatment is important. Patients often prefer the oral route of treatment due to convenience. The experts concurred and stated that their patients preferred oral preprobiotics.

The results with the pre-probiotics used will depend on the quality of the formulation and the maintenance of the cold chain.

Table 1: Clinical trials of probiotics in BV.

Study	Study design	Patient population	Treatment	Outcomes
Hemalatha et al ^{20,21}	Double-blind study	67 patients with BV, 50 with intermediate flora, 42 with normal vaginal flora	Group 1: probiotic Lactobacilli vaginal tablets (L. brevis CD2, L. salivarius subsp. salicinius, L. plantarum) or group 2: vaginal pH tablet (active comparator)	Cure rate after 8 days of treatment. Group 1 versus group 2: 80% versus 74% Effect on IL-1β and IL-6 vaginal cytokines. Group 1: significant reduction in IL-1β and IL-6 (p<0.001), group 2: no effect
Tomusiak et al ²²	Multicentre, randomized, double-blind, and placebo-controlled trial	160 women were randomly allocated to receive either the probiotic preparation inVag(®), or a placebo (one capsule for seven consecutive days vaginally)	Women were randomly allocated to receive either the probiotic preparation or a placebo (one capsule for seven consecutive days vaginally)	Probiotics caused a significant decrease (between visits) in both vaginal pH (p<0.05) and Nugent score (p<0.05), and a significant increase in the abundance of <i>Lactobacillus</i> between visit (p<0.05)
Russo ²³	Double blind, randomised clinical trial	48 adult women with BV	Women received metronidazole (500 mg twice daily) for 7 days and randomly assigned to take simultaneously either probiotics plus lactoferrin or placebo (2 capsules/day for 5 days followed by 1 capsule/day for 10 consecutive days; induction phase). The treatment was repeated each month (maintenance phase) during the six months of follow-up starting the first day of menstrual cycle.	Significant improvement in symptoms (vaginal discharge and itching), Nugent score and recurrence rate in women receiving probiotics + lactoferrin versus placebo. It was a safe and effective remedy for the restoration of healthy vaginal microbiota in preventing recurrent BV
Heczko et al ²²	Multicentre, randomised, double-blind, placebo-controlled trial	241 women with BV	Probiotic containing three <i>Lactobacillus</i> strains, n=118; placebo, n=123	Probiotics lengthened the time to clinical relapse of bacterial vaginosis (BV) and aerobic vaginitis (AV). symptoms up to 51% (p<0.05) versus placebo. BV relapse was delayed by up to 76% (p<0.05). Probiotic use also reduced and maintained low vaginal pH and Nugent score, and increased vaginal <i>Lactobacillus</i> counts following standard treatment

BV: bacterial vaginosis; IL: interleukin

Table 2: Meta analysis of role of probiotics in BV.

Author	Number of studies	Inferences
Jeng et al ²⁴	30 studies	Probiotics have a significant short-term effect in the treatment of common vaginal infections in non-pregnant females. Probiotics interventions were significantly associated with a lower recurrence rate of vaginitis [pooled OR=0.27, 95% CI: 0.18-0.41, p<0.001] and higher cure/remission rate (pooled OR=2.28, 95% CI: 1.20-4.32, p=0.011).
Li et al ²⁵	13 studies, 1258 patients	Probiotics may have a positive effect on the treatment of BV. More data is required.
Liu et al ²⁶	18 studies, 1651 patients	In comparison with short-term probiotics treatment (<1 month), long-term probiotics treatment (1-3 months) yields superior beneficial outcomes and efficacy in the treatment of BV. Compared with antibiotics, probiotics significantly decreased the recurrence rate of BV (at <1 month, 1-3 months, and overall analysis) as well as the incidence of AEs (at <1 month) and increased the cure/remission rate of BV (at 1-3 months)
Chen et al ²⁷	14 studies	Probiotics may play a positive role in the treatment of BV, but more strong evidence is needed.
López- Moreno ²⁸	35 clinical studies	Vaginal probiotic doses administered were similar to oral probiotics protocols, ranging from $\geq 10^7$ CFU/day to 2.5×10^{10} CFU/day, but were highly variable regarding the treatment duration timing

AES: adverse events; BV: bacterial vaginosis; CFU: colony forming unit; CI: confidence interval; OR: odds ratio

CONCLUSION

Bacterial vaginosis is common in women in the reproductive age group. Prebiotic-probiotic supplementation is associated with several advantages such as restoration of the normal vaginal microbiome and prevention of recurrent BV. Prebiotic-probiotic supplementation may be considered a new adjuvant treatment for bacterial vaginosis.

ACKNOWLEDGEMENTS

Authors would like to acknowledge Clinical Medicine Infomatics India for their help in medical writing of this manuscript. They would also like to acknowledge the support provided by Abbott India Ltd.

Funding: No funding sources Conflict of interest: None declared Ethical approval: Not required

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Cite this article as: Gupta M, Tiwari B. Bacterial vaginosis and the role of prebiotics and probiotics: current practices. Int J Res Med Sci 2024;12:1327-33.