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# Probiotics reduce the recurrence of asymptomatic bacterial vaginosis in Chinese women

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**Asymptomatic Bacterial Vaginosis(aBV)increases the risk of acquiring multiple sexually transmitted diseases, HPV, gynecologic complications and adverse reproductive outcomes, and is speculated to affect 10 ~ 35% of women. Without intervention, a significant proportion of aBV would progress. Metronidazole is the most widely used treatment for aBV, yet the main challenge has always been the high rate of recurrence. Probiotics may increase the cure rate and reduce the recurrence rate of symptomatic bacterial vaginosis (sBV), while no study has compared the efficacy of probiotics and metronidazole on treating aBV. This study aims to fill the gap in understanding the difference in efficacy of probiotics and metronidazole in treating aBV by a multicenter, randomized, controlled trial. Participants received either a 10-day intravaginal probiotic capsules or a 7-day oral metronidazole. Follow-up were performed at the end of the 1st, 2nd, and 4th week after completing therapy. Women cured by either method were followed up with three additional visits. The primary outcome was the difference of cure rates between the two groups. The secondary outcome was to evaluate the recurrence rates among patients who were successfully cured using either method. 358 participants received probiotics and another 358 participants received metronidazole. The cumulative cure rates at the end of the 1st, 2nd, and 4th week were higher in probiotics group compared to metronidazole group (OR 1.063, *P* = 0.715; OR 1.324, *P* = 0.083; OR 1.338, *P* = 0.071), while the differences were not statistically significant. Women cured (144 in probiotics and 123 in metronidazole) were followed up. The difference of cumulative recurrence rates between the two groups were statistically significant at the end of the 2nd, 3rd, and 4th month (OR 0.212, *P* = 0.000; OR 0.160, *P* = 0.000; OR 0.119, *P* = 0.000). Adverse events were similar in the two groups (8.3%, 9.6% OR 0.858; *P* = 0.584). No life-threatening or severe adverse events were reported. Probiotics emerge as a superior therapeutic option for aBV due to their comparable cure rates, lower recurrence rates, and minimal side effects. Chinese Clinical Trial Registry (ChiCTR1800019436, 11/11/2018 ).**

**Keywords** Asymptomatic bacterial vaginosis, Probiotics, Vaginal lactobacillus, Vaginal microbiota, Metronidazole

**Abbreviations**

| | |
|---|---|
| aBV | Asymptomatic bacterial vaginosis |
| BV | Bacterial vaginosis |
| sBV | Symptomatic bacterial vaginosis |
| STD | Sexual transmitted disease |
| PARP-i | Poly adenosine-diphosphate-ribose polymerase inhibitor |
| AV | Aerobic vaginitis |
| NG | Neisseria gonorrhoeae |
| CT | Chlamydia trachomatis |

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## Background

Bacterial vaginosis (BV) is a condition characterized by a significant decrease in the number of vaginal *Lactobacillus* and a large increase in the number of anaerobic and facultative anaerobic organisms such as *Gardnerella*, *mycoplasmas*, *Ureaplasma*, *Prevotella*, and *Mobiluncus*, resulting in a dysbiosis of the vaginal microbiota[1,2]. BV is highly prevalent[1–4]and is associated with vaginal discharge, malodor, and localized pruritus, which is termed symptomatic bacterial vaginosis (sBV)[5]. However, approximately 50 ~ 84% of bacterial vaginosis (BV) cases are asymptomatic[6–9], and are termed asymptomatic bacterial vaginosis (aBV)[5]. It is speculated that aBV affects 10 ~ 35% of women[1,5,10].

There is a substantial body of evidence supporting the idea that women with BV have an increased risk of acquiring multiple sexually transmitted diseases (STDs)[11,12]. This heightened risk is accompanied by an increased likelihood of gynecologic complications[13,14]and a greater risk of adverse reproductive outcomes[15–18]. As a specific subtype of BV, aBV not only possesses the risks mentioned above but also has its own inherent harmful effects such as concealment. Due to the lack of reminders from symptoms, most patients with aBV cannot realize the presence of this disease and do not seek medical intervention. Without intervention, a significant proportion of aBV cases would progress to various types of vaginitis, compared to the very few patients whose aBV self-cured spontaneously[19–21]. Therefore, an effective clinical intervention for aBV patients is necessary.

Metronidazole has been used as the primary treatment for aBV[22], yet the main challenge has always been the high rate of recurrence. Probiotics have shown promise in supplying exogenous *Lactobacillus*to the vaginal microbiota, aiding in the restoration of normal conditions and maintaining an acidic pH in the vagina[23,24]. Several studies have indicated that probiotics can increase the cure rate and reduce the recurrence rate of sBV to some extent[25–27]. However, there have been no large-scale randomized controlled trials evaluating the efficacy and benefits of probiotics in the treatment of aBV, especially the efficacy on recurrence. Consequently, the present study aimed to investigate the disparities in efficacy and benefits between Probiotics and Metronidazole for the treatment of aBV in Chinese women at general risk.

## Methods

### Study design

This study was a multicenter, randomized, controlled trial comparing the efficacy of Probiotics with Metronidazole on aBV, conducted at five different hospitals in China(Peking University First Hospital, China-Japan Friendship Hospital, Northwest Women's and Children's Hospital, Maternity and Child Health care of Guangxi Zhuang Autonomous region, Jiangdu people's hospital.

The study was preregistered with the Chinese Clinical Trial Registry (ChiCTR1800019436). Ethical approval (No. 92 in 2017, Peking University First Hospital) was granted by the Ethics Committee of the Peking University First Hospital in Beijing, China.

## Participants

All participants were not enrolled from clinics or outpatient department of hospital, but enrolled from examination center of the five hospitals. They were women with a diagnosis of aBV and did not present any complaints of symptoms. Their diagnosis of aBV was confirmed through pelvic examination and the use of Gram stain criteria, specifically the Nugent scores.

Women were excluded from the present study if they reported any of the followings: increased vaginal discharge, itching, burning, odor, hyperemia, or edema of vaginal mucosa; Nugent score of 0~6; Other kinds of infection such as *Vulvovaginal candidiasis* (VVC), *Trichomonas* vaginitis, Aerobic vaginitis (AV), *Chlamydia*, *Neisseria gonorrhoeae* (NG), human papillomavirus, clinically apparent *herpes simplex virus*, or human immunodeficiency virus; pregnancy or breastfeeding; *Chlamydia trachomatis* (CT); an allergy to metronidazole; received any anti-inflammatory/antibiotic/antifungal drugs within the previous 8 weeks; has sexual intercourse in the past three days; poor follow-up compliance. These exclusion criteria were implemented to ensure that the participants were representative of individuals with aBV and to minimize confounding factors that could affect the study outcomes.

All the participants provided signed informed consent.

## Randomization and masking

Eligible participants were allocated sequentially by research secretary in a 1:1 ratio to the two groups (the Probiotics group or the Metronidazole group) of the study, according to a computer-generated random sequence. Block randomization was used (block sizes of four) to ensure masking of randomization status. The allocation sequence was generated by the independent statistician and not available to any member of the research team until databases had been completed and locked.

## Procedures

Before enrolment, potential participants were required to complete a questionnaire to gather information on potential risk factors probably associated with the incidence, development, or recurrence of aBV. Furthermore, a thorough medical history was documented, and a vaginal examination was conducted using a speculum with the patient in the lithotomy position. To assess the vaginal microecological characteristics, two sterile cotton swabs were used to collect discharge specimens from the lateral vaginal walls. One swab was rolled on a glass slide and evaluated using the Vaginal Microecology Evaluation System (a method for assessing the vaginal microecology)[19,28,29]. The other swab was used to test for the presence of NG and CT[30,31]. To accurately describe the characteristics of aBV and minimize interference from other types of inflammation, participants with any

of the following results were excluded from the study: Nugent score of 0 ~ 6; positive test for *Trichomonas* spp., *Candida* spp., AV, NG, or CT.

After the informed consent was obtained, participants were enrolled in the study, and randomly assigned to receive either a 10-day course of intravaginal probiotic capsules (Living Preparation of *Lactobacillus*, *Lactobacillus delbrueckii* subsp. *lactis DM8909*) with a daily dose of $1.0 \times 10^9$ colony-forming units (CFU), or a 7-day course of oral metronidazole at a dose of 0.4 g twice a day. The probiotic capsules and metronidazole used in the study were purchased from the pharmacy of clinical hospitals.

First-stage follow-up visits were performed at the end of the 1st, 2nd, and 4th week after the completion of therapy, and additionally, women who were cured by either method were followed for 3 more visits each month. The questionnaire and a vaginal examination were performed at each visit. Questionnaires addressing behaviors and symptoms were administered along with a pelvic examination, and vaginal specimens were collected for assessment of vaginal microbiota, and all the women were required to maintain their original lifestyle during the study and were not expected to make any changes because of their participation in the research.

The results of each participant in the study were categorized into one of following three outcomes based on their clinical characteristics and Nugent score:

Cured: Patients were defined as cured when the Nugent score was 0 ~ 6, and no clinical symptoms and any other vaginal infection were found.

Progress: Patients were regarded as progress if any clinical symptoms (including abnormal vaginal discharge, malodor, localized pruritus) appeared or other vaginal infections were diagnosed.

No-change: Patients were defined as no-change when no clinical symptoms appeared, and the classification of Nugent score remained 7 ~ 10.

Second-stage follow-up were performed at the end of the 1st, 2nd, and 3rd month after curation. Those women who were cured by either regimen and subsequently exhibited any of the characteristics of BV again were regarded as recurrence:

Recurrence: Patients who were cured had complaints or signs of vaginal inflammation or had a Nugent score of 7 ~ 10 during the following period of visit.

All the methods were performed in accordance with the relevant guidelines and regulations.

## Outcomes

Socio-demographic and clinical data were collected at screening and baseline, before randomization. The primary outcomes were the cure rates at the end of the 1st, 2nd, and 4th week after the completion of therapy in Probiotics group and Metronidazole group, and the difference between the two groups. The secondary outcomes were the recurrence rates at the end of the 1st, 2nd, and 3rd month in the two groups after the participants were cured, and the difference of recurrence rates between the two groups; time to reach successful treatment; factors which may influence cure rates or recurrence rate in different group.

## Statistical analysis

Previous study showed the cure rate of 36% for metronidazole in the treatment of aBV, and setting a difference of 10% or greater is meaningful, at a significance level of 5%, we calculated that a sample of 620 participants would provide the trial with 90% power to detect an absolute between-group difference of 10% points in the outcome. We intended to recruit 720 participants to accommodate a rate of loss to follow-up of 10% and a dropout rate of 5%.

The Data and Safety Monitoring Committee (DSMC) met twice during the study, specifically after 300 and 600 participants were recruited.

Primary and secondary outcomes were assessed in the modified intention-to-treat population. The analyses were performed using IBM SPSS Statistics software (version 26, IBM).

Baseline variables were summarized using standard descriptive statistics. Inter-group comparisons of the cure rates or recurrence rates were performed by chi square test. Logistic regression was used to estimate odds ratios and 95% confidence intervals for the risk of Probiotics as compared with Metronidazole.

For all randomly assigned participants, adverse events were reported to doctors at study sites during follow-up visits and compared descriptively between the groups. All statistical tests were two-sided (significance threshold $p = 0.05$).

This study is registered with the Chinese Clinical Trial Registry (ChiCTR1800019436, 11/11/2018) and is no longer recruiting because enough participants were enrolled.

# Results

## Participants

From December 1, 2018, through November 30, 2020, a total of 942 subjects were screened and 891 (94.5%) were eligible. Of these, 716 (80.4%) were consented to participate and were randomly allocated to Probiotics group or Metronidazole group, with 143 (19.9%) were declined to participate (Fig. 1).

There were similar numbers of discontinuations in the two groups post randomization. Data for the primary outcome analysis were available for 87.3% (625 of 716) of participants. Of the participants, 312 (87.2%) in the probiotics group and 313 (87.4%) in the metronidazole group completed the study and all follow-up visits, although 91 (12.7%) participants dropped out (Fig. 1).

Baseline demographic and clinical characteristics for women in the two groups appeared balanced across groups, with no evidence of systematic differences (Table 1).

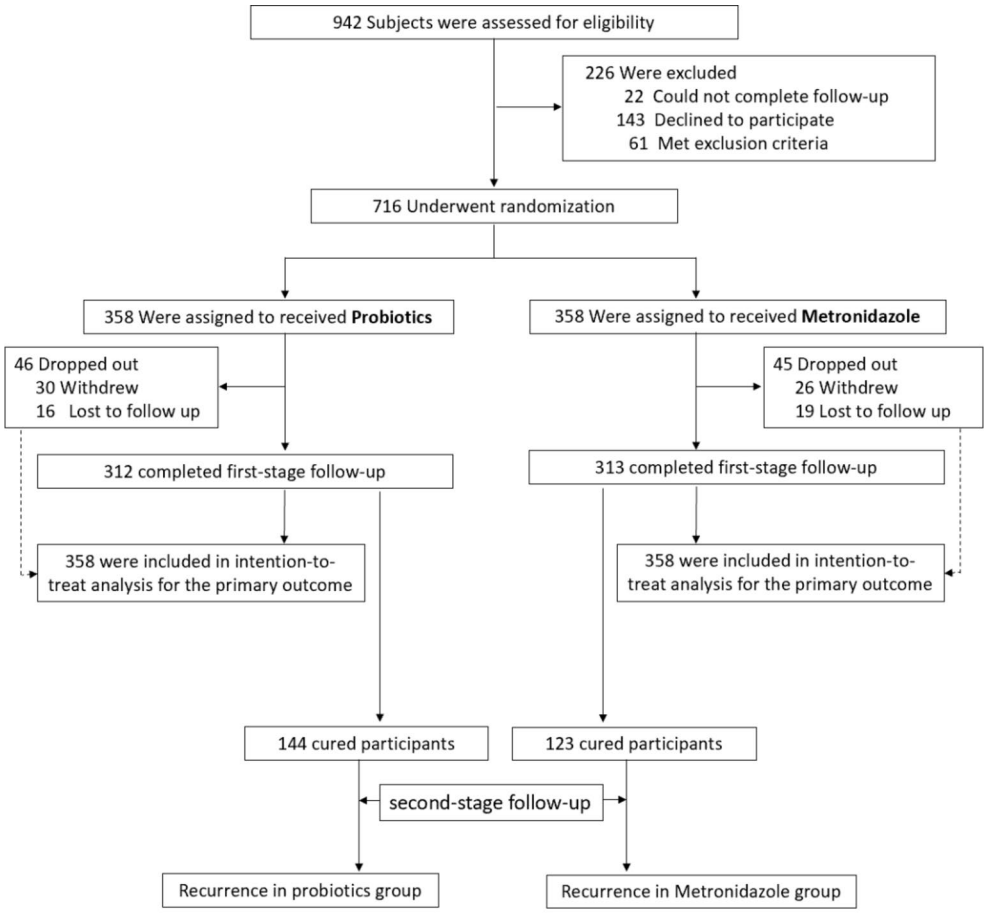


**Fig. 1**. Enrollment, Randomization, and Outcomes.

## Primary outcome

The difference in cumulative cure rates between the two groups was not statistically significant at the end of the 1st, 2nd, and 4th week after the completion of treatments, while it is notable that the cumulative cure rates in the Probiotics group were higher than those in the Metronidazole group (Table 2).

In the 1st week after completing the treatments, approximately 27–30% of patients (30.2% in the Probiotic group, 27.4% in the Metronidazole group) were cured. Furthermore, an additional portion of patients were cured in the 2nd week, and in the next two weeks (Table 2). There was a statistical difference between the cumulative cure rate in the initial two weeks and the first week, and no significant difference was observed between the four weeks and the initial two weeks. As a result, the majority of patients who underwent treatment achieved a cure within the initial two weeks, whereas only a small number experienced a cure in the subsequent two weeks (Table 2).

In subgroup of condom use only, and frequent travel, difference in cure rates between the two groups were statistically significant ($P < 0.05$).

Subgroup analysis was conducted on specific populations such as Postmenopausal, Condom use only, History of BV/STD, Frequent travel, and Vaginal douching, because these factors were indicated to be most possibly related to the outcome of aBV in previous studies.

Significant differences in the cumulative cure rates within the initial two weeks after completing treatment between the Probiotics group and the Metronidazole group were observed in women who had frequent travel (OR 1.889, 95% CI [1.213–2.941], $p = 0.005$), and in women of Condom use only (OR 1.634, 95% CI [1.022–2.613], $p = 0.040$) (Table 2).

Whatever in group of childbearing-age or postmenopausal women, more patients were cured in Probiotics group than those in Metronidazole group ($P > 0.05$). Moreover, whatever in group of Probiotics or Metronidazole, more patients were cured in childbearing-age women than in postmenopausal women ($P > 0.05$). Both the above two differences were not statistically significant (Table 2).

| Characteristic | Probiotic group (*N* = 358) | | Metronidazole group (*N* = 358) | |
|---|---|---|---|---|
| Age (Average, Range) -yrs | 35.9 | (18–70) | 39.4 | (18–71) |
| BMI (Average, Range) -kg/m$^2$ | 21.2 | (14.6–31.9) | 22.1 | (15.3–28.4) |
| Postmenopausal | 99 | 40.2% | 106 | 42.5% |
| Often oral antibiotics | 150 | 50.8% | 150 | 54.7% |
| Regular yogurt | 124 | 42.5% | 111 | 47.2% |
| Tampons regularly | 129 | 48.6% | 116 | 43.9% |
| HPV positive in cervix | 91 | 38.0% | 80 | 35.2% |
| More than 3 partners | 105 | 41.9% | 84 | 36.3% |
| Unemployed | 207 | 69.6% | 226 | 76.0% |
| Smoking | 114 | 44.4% | 97 | 39.9% |
| Alcohol | 89 | 37.4% | 98 | 40.2% |
| Vaginal douching [§] | 136 | 50.6% | 122 | 46.9% |
| Condom use only[a] | 131 | 49.2% | 154 | 55.9% |
| History of BV/STD[b] | 93 | 38.5% | 71 | 32.7% |
| Partner's history of STD | 92 | 38.3% | 108 | 43.0% |
| One regular female sex partner | 167 | 59.2% | 163 | 58.4% |
| New sex partner in past 3 months | 42 | 24.3% | 43 | 24.9% |
| Frequent travel[*] | 167 | 40.2% | 168 | 42.5% |

**Table 1**. Baseline characteristics of participants in two groups. * Frequent travel was defined as no less than 7 days in each month; § Vaginal douching was specified as flushing the vagina twice or more per week;[a] Condom use only was defined as using a condom every time when they had sexual contact with anyone;[b] sexually transmitted diseases.

| Outcome and Subgroup | Probiotic group (*N* = 358) | | Metronidazole group (*N* = 358) | | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|---|---|
| All participants–no./total no. (%) | | | | | | |
| 1 week | 108/358 | 30.1% | 98/358 | 27.3% | 1.146 (0.829–1.585) | 0.409 |
| 2 weeks | 144/358 | 40.2%[*] | 123/358 | 34.4%[#] | 0.893 (0.645–1.219) | 0.475 |
| 4 weeks | 151/358 | 42.2%[**] | 129/358 | 36.0%[##] | 1.295 (0.958–1.750) | 0.092 |
| Subgroup analysis–no./total no. (%) (cure rate in 2 weeks) | | | | | | |
| Postmenopausal | 40/100 | 40.0% | 35/99 | 35.4% | 1.219 (0.686–2.165) | 0.499 |
| Childbearing-age | 104/212 | 49.1% | 88/214 | 41.1% | 1.379 (0.940–2.022) | 0.100 |
| Vaginal douching | 51/136 | 37.5% | 56/122 | 45.9% | 0.707 (0.430–1.163) | 0.172 |
| Condom use only | 73/131 | 55.7% | 67/154 | 43.5% | 1.634 (1.022–2.613) | 0.040 |
| History of BV/STD | 30/93 | 32.2% | 27/71 | 38.0% | 0.776 (0.406–1.482) | 0.442 |
| Frequent travel | 80/167 | 47.9% | 55/168 | 32.7% | 1.889 (1.213–2.941) | 0.005 |

**Table 2**. Cumulative cure rates at different time and subgroup analysis of cure rates in 2 weeks. [*] Statistical significance versus the 1st week (OR 1.366, 95% CI [1.006–1.854], $p = 0.045$). [#] Statistical significance versus the 1st week (OR 1.389, 95% CI 1.010–1.910], $p = 0.043$). [**] No statistical significance versus the 2 weeks (OR 1.236, 95% CI [0.921–1.658], $p = 0.157$). [##] No statistical significance versus the 2 weeks (OR 1.076, 95% CI [0.792–1.463], $p = 0.639$).

## Secondary outcomes

After the completion of treatments, women who were successfully cured by either Probiotics or Metronidazole were followed up to investigate the recurrence rates in the two groups. The baseline characteristic factors were well matched between the groups (Table 3).

The cumulative recurrence rates between the Probiotics and Metronidazole group showed statistically significant differences at the end of the 2nd, 3rd, and 4th month after the completion of treatments, although the difference at the end of the 1st month after completion of treatments was not statistically significant (Table 4).

Based on subgroup analysis conducted on the recurrence rates within the initial 2 months, significant differences were observed between the Probiotics group and the Metronidazole group in specific subgroups. Notably, postmenopausal women, childbearing-age women, and women who solely rely on condoms for

| Characteristic | Probiotic group (*N* = 144) | | Metronidazole group (*N* = 123) | | *P* value |
|---|---|---|---|---|---|
| Age (Average, Range) -yrs | 38.6 | 18–70 | 36.9 | 19–71 | |
| BMI (Average, Range) -kg/m$^2$ | 22.1 | 15.3–31.9 | 22.9 | 16–28 | |
| Postmenopausal | 40 | 27.78% | 35 | 28.46% | 0.902 |
| Often oral antibiotics | 69 | 47.92% | 52 | 42.28% | 0.356 |
| Regular yogurt | 61 | 42.36% | 50 | 40.65% | 0.777 |
| Tampons regularly | 61 | 42.36% | 39 | 31.71% | 0.073 |
| HPV positive in cervix | 40 | 27.78% | 24 | 19.51% | 0.115 |
| More than 3 partners | 44 | 30.56% | 26 | 21.14% | 0.081 |
| Unemployed | 78 | 54.17% | 80 | 65.04% | 0.072 |
| Smoking | 51 | 35.42% | 44 | 35.77% | 0.952 |
| Alcohol | 53 | 36.81% | 41 | 33.33% | 0.554 |
| Vaginal douching | 51 | 35.42% | 56 | 45.53% | 0.093 |
| Condom use only | 73 | 50.69% | 67 | 54.47% | 0.538 |
| History of BV/STD | 30 | 20.83% | 27 | 21.95% | 0.824 |
| Partner's history of STD | 51 | 35.42% | 34 | 27.64% | 0.174 |
| One regular female sex partner | 79 | 54.86% | 65 | 52.85% | 0.742 |
| New sex partner in past 3 months | 24 | 16.67% | 17 | 13.82% | 0.520 |
| Frequent travel | 80 | 55.56% | 55 | 44.72% | 0.077 |

**Table 3**. Baseline characteristics of patients who were cured in two groups.

| Cumulative recurrence rate | Probiotic group (*N* = 144) | | Metronidazole group (*N* = 123) | | Odds ratio (95% CI) | *P* Value |
|---|---|---|---|---|---|---|
| All participants — no./total no. (%) | | | | | | |
| 1 month | 1/144 | 0.7% | 1/123 | 0.8% | 0.853 (0.053–13.784) | 0.911 |
| 2 months | 10/144 | 6.9% | 32/123 | 26.0% | 0.212 (0.099–0.453) | 0.000 |
| 3 months | 17/144 | 11.8% | 56/123 | 45.5% | 0.160 (0.086–0.297) | 0.000 |
| 4 months | 19/144 | 13.2% | 69/123 | 56.1% | 0.119 (0.065–0.217) | 0.000 |
| Subgroup analysis–no./total no. (%) (recurrent rate in 2 months) | | | | | | |
| Postmenopausal | 6/40 | 15.00% | 14/35 | 40.00% | 0.265 (0.088–0.795) | 0.015 |
| Childbearing-age | 4/104 | 3.84% | 18/88 | 20.45% | 0.150 (0.049–0.461) | 0.000 |
| Condom use only | 4/73 | 5.48% | 12/67 | 17.91% | 0.266 (0.081–0.870) | 0.021 |
| History of BV/STD | 5/30 | 16.67% | 9/27 | 33.33% | 0.400 (0.115–1.396) | 0.144 |
| Frequent travel | 8/80 | 10.00% | 10/55 | 18.18% | 0.500 (0.184–1.361) | 0.169 |
| Vaginal douching | 8/51 | 15.69% | 13/56 | 23.21% | 0.676 (0.305–1.496) | 0.327 |

**Table 4**. Cumulative recurrence rates and subgroup analysis of recurrence rates in 2 months.

contraception displayed significant variations in recurrence rates between the Probiotics and Metronidazole groups ($P < 0.05$). Conversely, in subgroups such as women with a history of BV/STD, women who frequently travel, and women who practice vaginal douching, the differences in recurrence rates did not reach statistical significance (Table 4).

## Safety

Adverse events were also documented and the findings revealed that 8.3% (26 out of 312) of participants who received probiotics experienced adverse events, compared to 9.6% (30 out of 313) of participants who received Metronidazole (OR 0.858; 95% CI, [0.495–1.487], $P = 0.584$). It is important to note that no life-threatening or severe adverse events were reported during the study (Table 5).

## Discussion

In the past, people tended to visit a doctor only when they experienced noticeable symptom or discomfort. This approach led to a limited number of women being diagnosed with aBV. As a result, most studies focused on women with sBV[32], and research on aBV was relatively scarce[33]. However, in recent years, as living standards have improved, an increasing number of women who do not exhibit any symptoms have started seeking medical examinations. This shift has allowed for the detection of many women with aBV who would have otherwise gone undiagnosed. Consequently, there is a growing demand for reliable research findings regarding the management

| | Probiotic Group (*N* = 358) | | Metronidazole Group (*N* = 358) | |
|---|---|---|---|---|
| Grade | | | | |
| Mild | 24 | 6.70% | 26 | 7.26% |
| Moderate | 2 | 0.56% | 3 | 0.84% |
| Severe | 0 | 0.00% | 1 | 0.28% |
| Life threatening | 0 | 0.00% | 0 | 0.00% |
| Nausea | 0 | 0.00% | 12 | 3.35% |
| Vomit | 0 | 0.00% | 4 | 1.12% |
| Dizzy | 1 | 0.28% | 7 | 1.96% |
| Diarrhea | 2 | 0.56% | 2 | 0.56% |
| Urinary tract infection | 18 | 5.80% | 0 | 0.00% |
| Vagainl bleeding | 2 | 0.60% | 0 | 0.00% |
| Rash | 0 | 0.00% | 1 | 0.28% |
| Abdominal pain | 3 | 1.00% | 1 | 1.12% |

**Table 5**. Adverse events.

and treatment of aBV. Unlike previous studies[12,25,34–40], we conducted this prospective, large-scale, multicenter, randomized controlled trial involving 716 Chinese women with aBV who were at general risk to compare the efficacy of two therapeutic options, and the result showed that probiotics had a comparable therapeutic cure rate to Metronidazole, but a significantly lower rate of recurrence when compared to Metronidazole (Table 2).

## The primary outcome

The first randomized, double-blind, placebo-controlled trial compared the efficacy of metronidazole gel with placebo for the treatment of aBV was published in 2000 by Schwebke[32], and the result showed an improvement in efficacy in metronidazole group compared to the placebo group. However, the difference between the two group was not statistically significant. Another study published in 2007 aimed to investigate whether treatment of aBV with intravaginal metronidazole gel could reduce the incidence of sexually transmitted diseases STDs, and the results demonstrated that prophylactic treatment of aBV with metronidazole gel led to a significantly lower incidence of chlamydia cases compared to the observation group[38]. A placebo-controlled randomized clinical trial investigated the effects of administering edible vitamin D on the cure rate of aBV in women with vitamin D deficiency. And according to the result, the administration of edible vitamin D was found to significantly increase the cure rate of aBV[41]. Since participants in the above studies were selected from special populations, such as women from STD clinics, or women with vitamin D deficiency, so the results may not be generalizable to general women[42].

In our study, volunteers were selected from a general population, and a large sample size, long-term follow-up, and randomized control group were used to provide valuable insights into the management of aBV that can be applicable to a broader range of individuals. Furthermore, standard treatment regimen, not placebo, was used as control group. While the difference in cure rates between the probiotics group and the Metronidazole group may not be statistically significant in our study, it is noteworthy that the probiotics group had higher cumulative cure rates. This suggests that probiotics may have a potential advantage over Metronidazole in terms of effectiveness for treating aBV.

Although the exact causes and mechanisms of BV are not clearly defined, there is a general consensus regarding its diagnostic criteria. It is currently believed that the essence of BV is a syndrome caused by a disruption in vaginal microbiota. This raises the possibility that BV is a result of multiple etiological factors leading to microbial imbalance, and its pathogenesis may also be diverse. As a specific type of BV, aBV may similarly have a range of potential causes and mechanisms. Investigating a syndrome resulting from different causes and pathophysiological mechanisms as a whole may lead to significant heterogeneity in results. This may be an important reason for the insignificance in cure rates between the two groups. Another reason for the insignificance in cure rates may be insufficiency of the sample size.

Different strains or species of *Lactobacillus*can have distinct biological functions[43], and the therapeutic response to interventions can vary among different populations with a particular disease[44]. As a result, selection of appropriate patient population who may significantly benefit from probiotics therapy may substantially improve the efficacy of cure rate. Indeed, the approach of selecting specific patient populations for targeted therapies has shown promising effects in various areas of medicine, including cancer treatment. The example about the use of Olaparib (a poly adenosine-diphosphate-ribose polymerase inhibitor, PARP-i) in women with familial and somatic BRCA1/2 mutations and high-grade serous ovarian cancer is a notable one. When women with high-grade serous ovarian cancer and BRCA1/2 mutations were selected as the patient population, Olaparib demonstrated significant improvements in progression-free survival (PFS)[45]. This means that these patients experienced a longer period without disease progression compared to those who did not receive Olaparib or who did not have the specific BRCA1/2 mutations. This personalized approach can lead to significant improvements in patient outcomes and represents a promising direction for future research and clinical practice.

Consequently, subgroup analyses were conducted based on various factors such as menstruation status (Postmenopausal or not), history of BV/STD, habits of vaginal douching, condom use only, and frequent travel to identify specific populations that may benefit greatly from probiotics in the context of aBV. The results of these subgroup analyses indicated that probiotics had a significant improvement in cure rates for women who used condoms or women who had frequent travel. This suggests that the efficacy of probiotics would be much higher than that of antibiotics if appropriate aBV patients with certain characteristics were properly selected. Further research and larger-scale studies are needed to validate these findings.

The present study also aimed to determine the optimal time point for evaluating the efficacy of a treatment for aBV. Since there is no global consensus on this issue, we analyzed the cure rates at different time points: the 1st week, the 1st two weeks, and the 1st four weeks after completion of treatments. Based on the findings of our study, in the Probiotic group, the cure rate during the 1st two weeks after treatment was significantly higher than that during the 1st week ($p = 0.009$), however, the difference in the cure rate between the 1st four weeks and the 1st two weeks was not statistically significant ($p = 0.575$). A similar trend was observed in the Metronidazole group, and there was no statistical significance. Consequently, 2 weeks after completing treatments may be the optimal time point for assessment of efficacy of aBV.

In previous studies, the use of Metronidazole to treat sBV resulted in a cure rate of 80–90% one month after treatment[46], However, in the present study, the cure rate for aBV in the Metronidazole group was only 41% one month after completing therapy. The most principal reason for the disparity in cure rates between the two trials might be the difference in study populations. Another possible reason may be that aBV might have a different pathogenesis, response to antibiotic therapy, or complication rates from sBV, and aBV might be a milder form of infection than sBV[19,33,47].

## The second outcome

Although several treatment regimens have been found to be generally effective in curing bacterial vaginosis (BV), the main challenge in managing this condition has always been the high rate of recurrence[48–50]. As a subtype of BV, there is limited research specifically focused on the recurrence of aBV. In a study by Sobel[51], participants with aBV were randomly assigned to receive either metronidazole vaginal gel or a placebo. The results demonstrated that at the end of the third month after completing the therapy, the recurrence rate was 25.5% in the metronidazole group and 59.1% in the placebo group. This indicates a significant difference in recurrence rates between the two groups (relative risk [RR] = 0.43, $p = 0.001$). It's worth noting that the participants with aBV in the above research, were enrolled from another clinical trial, and these women, to some extent, represented high risk women.

In our study, participants with general risk were enrolled, and the results showed the recurrence rates in three months after completing therapy were 45.5% in the Metronidazole group and 11.8% in the Probiotics group, which suggested that probiotics, compared to metronidazole, was more effective in preventing recurrences. These findings indicate that local use of metronidazole gel may be more effective than oral metronidazole in preventing recurrences of aBV. To sum up, the recurrence rate of the probiotic group is indeed lower than that of the metronidazole group, regardless of whether the treatment is local (intravaginal) or oral medication, which suggests that probiotics may be more effective in preventing recurrences of aBV compared to metronidazole.

The mechanism of relapse of BV has been extensively studied, and several factors have been identified as potential contributors to BV recurrence. These factors include: failure to recolonize with Lactobacillus species, persistent colonization with BV-associated bacteria, formation and persistence of biofilms, and potential re-exposure to BV-associated bacteria from sexual partners, and reinfection[48,49,52,53]. Several strategies are currently used to reduce the recurrence of BV. They include use of probiotics (alone or in combination with antibiotics), acidifiers, and biofilm disruptors, and Suppressive therapy and periodic presumptive treatment (PPT)[49], extended course of metronidazole treatment[54].

However, as a special type of BV, it is currently unclear whether the mechanism of the recurrence of aBV differs from that of BV. This uncertainty arises from the lack of published research specifically investigating the recurrence mechanisms of aBV[55]. Therefore, it remains to be determined through clinical trials whether the strategies employed to reduce BV recurrence would also be effective in preventing the recurrence of aBV, and further research is needed to shed light on the recurrence mechanisms and evaluate the efficacy of preventive strategies for aBV[49,55].

The use of probiotics, which supplement vaginal *Lactobacillus*instead of targeting pathogenic bacteria, has emerged as a promising therapeutic approach for infectious diseases. Researches have indicated that probiotics can effectively reduce the recurrence rate of BV[25,47,56–59]. In the present study, probiotics were evaluated as a standalone treatment for aBV for the first time, and the results demonstrated that probiotics significantly decreased the cumulative recurrence rate of aBV. This finding highlights the potential of probiotics as a valuable strategy for preventing the recurrence of aBV.

Previous studies have indicated that a history of BV/STD, vaginal douching habits, and frequent travel are highly correlated with the recurrence of BV[25,55,60]. In light of these findings, subgroup analyses were conducted in the present study to identify potential risk factors that may influence the recurrence rate of aBV and identify populations that may benefit more from specific treatments. Surprisingly, the results showed that there was no significant difference in the recurrence rates of aBV between the Probiotics and Metronidazole treatment groups in the subgroups of individuals with a history of BV/STD, vaginal douching habits, and frequent travel. However, the menstrual status and condom use were found to significantly affect the recurrence rates. This suggests that women who exclusively use condoms may have a lower risk of aBV recurrence. These findings highlight the importance of considering condom use as potential factors in the prevention of aBV recurrence.

Metronidazole, as an antibiotic, functions by eliminating anaerobic bacteria, thereby restoring the balance of vaginal microbiota[48]. On the other hand, probiotics work through a different mechanism, which involves supplementing the vaginal lactobacillus to restore the microbial balance[48]. Although the mechanism of action

of probiotics and metronidazole seems different, there is no significant difference in cure rates between the two groups. This finding indicated that multiple mechanisms may be involved in the pathogenesis of aBV, and investigation of aBV could be performed from various perspectives.

The present study had several limitations. Firstly, the follow-up time was relatively short. Enrolling an adequate number of women with aBV proved challenging. Asymptomatic BV is a type of vaginal infection that often goes unnoticed by individuals, particularly when no symptoms are present. Consequently, only a small proportion of women with aBV were willing to participate in the study. Requesting a longer follow-up period would have likely resulted in decreased compliance among participants.

Secondly, the effects of the sequential use of Metronidazole and probiotics were not investigated in this study. Since Metronidazole and probiotics work through different mechanisms in treating vaginal flora disorders, some scholars have proposed that combining the two therapies may have a superimposed effect[59,61]. It would be valuable for future research to explore the effects of sequential use of Metronidazole and probiotics to determine if there are any synergistic benefits when these treatments are used in combination. This could provide valuable insights into the optimal management of vaginal flora disorders.

Thirdly, in a study by Schwebke, treatment and prophylactic use of intravaginal metronidazole gel can result in significantly fewer cases of chlamydia, while the rate of cure and recurrence were not mentioned[38]. In the present study, cure rate and recurrence rate, rather than the potential harmful outcomes were the endpoint. As the number of cured individuals increases and the number of relapses decreases, is it possible to definitively state that all potentially harmful outcomes from aBV will disappear? This requires designing new research to explore in future studies.

In summary, the study provides evidence supporting the use of a 10-day course of intravaginal probiotic capsules as an effective treatment for aBV in Chinese women, with comparable cure rates to metronidazole and significantly lower recurrence rates. This finding positions probiotics as a promising therapeutic option for the management of aBV.

## Conclusions

The potential harm of aBV to women is significant, and finding a way to cure it and reduce its recurrence has always been a topic of exploration. The results of this study demonstrate that probiotics, when used to treat aBV, not only achieved the same cure rate but also significantly reduced the recurrence rate, making it a promising clinical treatment option.

## Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Author contributions
R Z contributed to the study conception and design. Q L and Z L were responsible for the funding acquisition. R Z, Z L, Y Z, L M, D Z, Y L and Q L developed the study protocol including modification of the questionnaire. Rui Zhang, Zhaohui Liu, Yan Zhang, Lan Mi, Dai Zhang, Yang Li and Qinping Liao were responsible for the recruitment of participants, assessment of eligibility, and collection of clinical data. Rui Zhang, Yan Zhang and Dai Zhang planned, undertook, and interpreted the statistical analysis of the data. Rui Zhang, and Yang Li were responsible for writing of the original draft. All authors had full access to all the data and had final responsibility for the decision to submit the manuscript for publication.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Additional information
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