doi.org/10.36721/PJPS.2026.39.7.REG.189.1

Effects of nifuratel-based therapy combined with vaginal lactobacillus probiotics on microecological restoration in pregnant women with abnormal vaginal flora: A retrospective cohort study

Ya Luo*, Hong Xu and Xiaohong Ye
Department of Obstetrics and Gynaecology, Maternal and Child Health Hospital Shuangliu District, Chengdu 610200, Sichuan Province, China

Abstract: **Background**: Imbalances in the vaginal flora during pregnancy elevate risks for adverse outcomes. While nifuratel targets pathogens, probiotics restore acidity, and robust cohort evidence supporting their combined safety and efficacy in restoring the microbiome remains limited. **Objectives**: This retrospective cohort study assessed nifuratel-nystatin therapy plus *Lactobacillus* probiotics for restoring vaginal flora in pregnancy. **Methods**: Employing a retrospective cohort design, this study enrolled pregnant women diagnosed with vaginal microecological abnormalities between March 2023 and 2024. Participants were allocated via 1:1 propensity score matching (PSM) into two groups (n=58 each): a combination group receiving nifuratel-nystatin therapy combined with vaginal *Lactobacillus* probiotics and a monotherapy group receiving nifuratel-nystatin alone. All received a 7-day treatment. The primary endpoint was clinical effectiveness assessed one week post-treatment. Secondary outcomes included vaginal pH, *Lactobacillus* and bacterial diversity normalization rates, recurrence, adverse pregnancy outcomes and drug-related adverse events, monitored until delivery. Multivariate logistic regression identified independent predictors of treatment efficacy. **Results**: After PSM, baseline characteristics were balanced ($P>0.05$). The comparative analysis indicated a superior overall efficacy for the combination therapy (93.1%) over monotherapy (77.6%), with statistical significance ($P<0.01$) Both groups exhibited reduced vaginal pH and increased rates of normalized *Lactobacillus* and bacterial diversity, with greater improvements observed in the combination group ($P<0.01$). The recurrence rate was significantly lower in the combination group (8.6% vs. 22.4%; $P<0.05$), as was the total incidence of adverse pregnancy outcomes (6.9% vs. 20.7%; $P<0.01$). No significant difference in adverse drug reactions was found. Multivariate analysis identified combination therapy as an independent protective factor for clinical efficacy (aOR=4.25, 95% CI: 1.42-12.71, $P<0.05$). **Conclusions**: In pregnant women with vaginal dysbiosis, combining nifuratel-nystatin with *Lactobacillus* probiotics safely enhances clinical efficacy, normalizes pH and flora and reduces adverse pregnancy outcomes.

Keywords: Abnormal vaginal microecology; Nifuratel; Probiotics; Pregnancy; Propensity score matching; Vaginal *Lactobacillus*

Submitted on 26-12-2025 – Revised on 24-02-2026 – Accepted on 04-03-2026

INTRODUCTION

Due to the changes in hormone levels during pregnancy, the vaginal microecological balance is easily disrupted, leading to a significant increase in the incidence of vaginal microecological abnormalities, mainly manifested as a reduction in the number of *Lactobacillus*, an increase in vaginal pH value and a disorder of bacterial diversity, which can be accompanied by clinical symptoms such as vaginal pruritus and abnormal leucorrhea. Some patients may also be complicated by specific vaginitis, such as bacterial vaginosis and aerobic vaginitis (Dube-Zinatelli *et al.*, 2025; Chopra *et al.*, 2022). Abnormal vaginal microecology not only reduces the quality of life of pregnant women, but is also linked to detrimental pregnancy outcomes including preterm birth, membrane rupture and intrauterine infection, which poses a significant threat to mother and child. Therefore, it is of great clinical significance to promptly implement effective treatment measures to restore the balance of vaginal microecology (Gupte *et al.*, 2024; Swarna *et al.*, 2023; Ahrodia *et al.*, 2022).

For pregnant patients with abnormal vaginal microbiota, antibacterial monotherapy remains the predominant first-line treatment in clinical practice. Nifuratel and nystatin are commonly used drugs, that have inhibitory effects on a variety of pathogenic microorganisms and can relieve clinical symptoms. However, it is difficult to effectively reconstruct the vaginal microecological barrier by using antibiotics alone and some patients have problems such as poor effect and recurrence after treatment (Yefet *et al.*, 2025; Wang *et al.*, 2024). *Lactobacillus* probiotics contribute to restoring vaginal microecological balance by supplementing beneficial bacterial counts (Petricevic *et al.*, 2023; Zhang *et al.*, 2022). However, more clinical data are needed to confirm the clinical effect of its combination with nifuratel and nystatin in the treatment of vaginal microecological abnormalities during pregnancy.

Based on the above clinical background, this retrospective

**Corresponding author:* e-mail: ly641557686@hotmail.com

cohort study used propensity score matching (PSM) to minimize confounding while evaluating the efficacy of nifuratel-nystatin therapy, with or without vaginal Lactobacillus probiotics, for managing vaginal microecological imbalances during pregnancy. At the same time, to inform the optimization of clinical management for antenatal vaginal dysbiosis, this study also monitored vaginal microecological parameters and recorded adverse pregnancy outcomes in both cohorts. The study hypothesized that, compared to nifuratel-nystatin monotherapy, the combination with vaginal Lactobacillus probiotics would lead to: (1) a higher total clinical effectiveness rate; (2) greater improvement in vaginal microecological parameters (pH reduction, Lactobacillus colonization and diversity restoration); (3) lower recurrence rates; and (4) a reduced incidence of adverse pregnancy outcomes, without increasing drug-related adverse events. This study, which was expected to reduce confounding bias through propensity score matching, showed that nifuratel-nystatin plus lactic acid bacteria therapy not only promoted clinical and microbiologic recovery but was also associated with reduced recurrence and adverse pregnancy outcomes, thus providing a basis for improving maternal and infant health in this vulnerable population.

MATERIALS AND METHODS

Study design

This retrospective cohort study utilized medical records of pregnant women diagnosed with vaginal microecological abnormalities during obstetric examinations at our hospital's gynecology clinic from March 2023 to March 2024. Patients were grouped based on the actual treatment they received during their clinical care. According to the treatment regimen, the subjects were divided into a combination group (nifuratel-nystatin therapy combined with vaginal Lactobacillus probiotics) and a single-drug group (nifuratel-nystatin alone). A 1:1 PSM analysis using nearest-neighbor matching was conducted to balance baseline characteristics, with a caliper of 0.02. This resulted in 58 cases per group and the detailed workflow is presented in Fig.1.

Ethics statement

This retrospective study, which analyzed anonymized clinical archives, was approved by Maternal and Child Health Hospital Shuangliu District, Chengdu ethics committee in accordance with the Declaration of Helsinki (Approval No: 2022- (ky)-6). The need for informed consent was waived due to the use of pre-existing anonymized data.

Inclusion and exclusion criteria

Inclusion criteria (Weldegebreal *et al.*, 2025): (1)singleton pregnant women aged ≥18 years old; (2)Gestational age ranged from 14 to 28 weeks; (3)meet the diagnostic criteria for vaginal microecological abnormalities in the "Expert Consensus on the Clinical application of Vaginal Microecological Evaluation" and have relevant clinical symptoms (such as abnormal secretion, peculiar smell, etc.) (Collaborative Group on Infectious Diseases, 2016); (4)The patient had no use of antibiotics within 7 days, no vaginal irrigation or medication within 15 days and the uterine and cervical functions were normal; (5)Complete clinical records.

Exclusion criteria (Kim *et al.*, 2022; Li *et al.*, 2025): (1) History of habitual procedures or adverse pregnancy outcomes; (2) Congenital diseases of the reproductive system; (3) complicated with gestational hypertension, gestational diabetes mellitus and other middle and late pregnancy complications; (4) History of allergy to nifuratel-nystatin, nystatin or *Lactobacillus* preparations; (5) with severe cardiopulmonary dysfunction; (6)Recurrent vaginitis.

Treatment methods

The choice of treatment regimen (combination or monotherapy) reflected the standard care options at our institution during the study period. Patients in the combination group received nifuratel and nystatin vaginal soft capsules (containing nifuratel 500 mg and nystatin 200 000 units), one capsule per night, combined with live *Lactobacillus* vaginal capsules (containing viable bacteria ≥0.25×106 CFU), one capsule per night for 7 days. The monotherapy group only received nifuratel and nystatin vaginal soft capsules (containing nifuratel 500mg and nystatin 200,000 units), 1 capsule per night for 7 days. All treatments were completed according to standard procedures or guidelines (Polatti *et al.*, 2003; Husain *et al.*, 2020).

Observation indicators

Data for the following observation indicators were retrospectively collected from the hospital's electronic medical records, communication systems and nursing documentation.

Baseline data

Demographic data (age, BMI), obstetrical characteristics (gestational age, gravidity, parity, previous abortion history), medical history characteristics (types of vaginal microecological abnormalities, previous history of vaginitis) and microecological laboratory indicators (vaginal pH value, nugent score, *Lactobacillus* classification and flora diversity) were extracted. All baseline data were obtained from admission medical records, evaluation records and archived preoperative imaging data.

Main outcome measures

The results of one week after drug withdrawal were extracted from the case system and the criteria for determining the clinical total effective rate (Fan *et al.*, 2024).

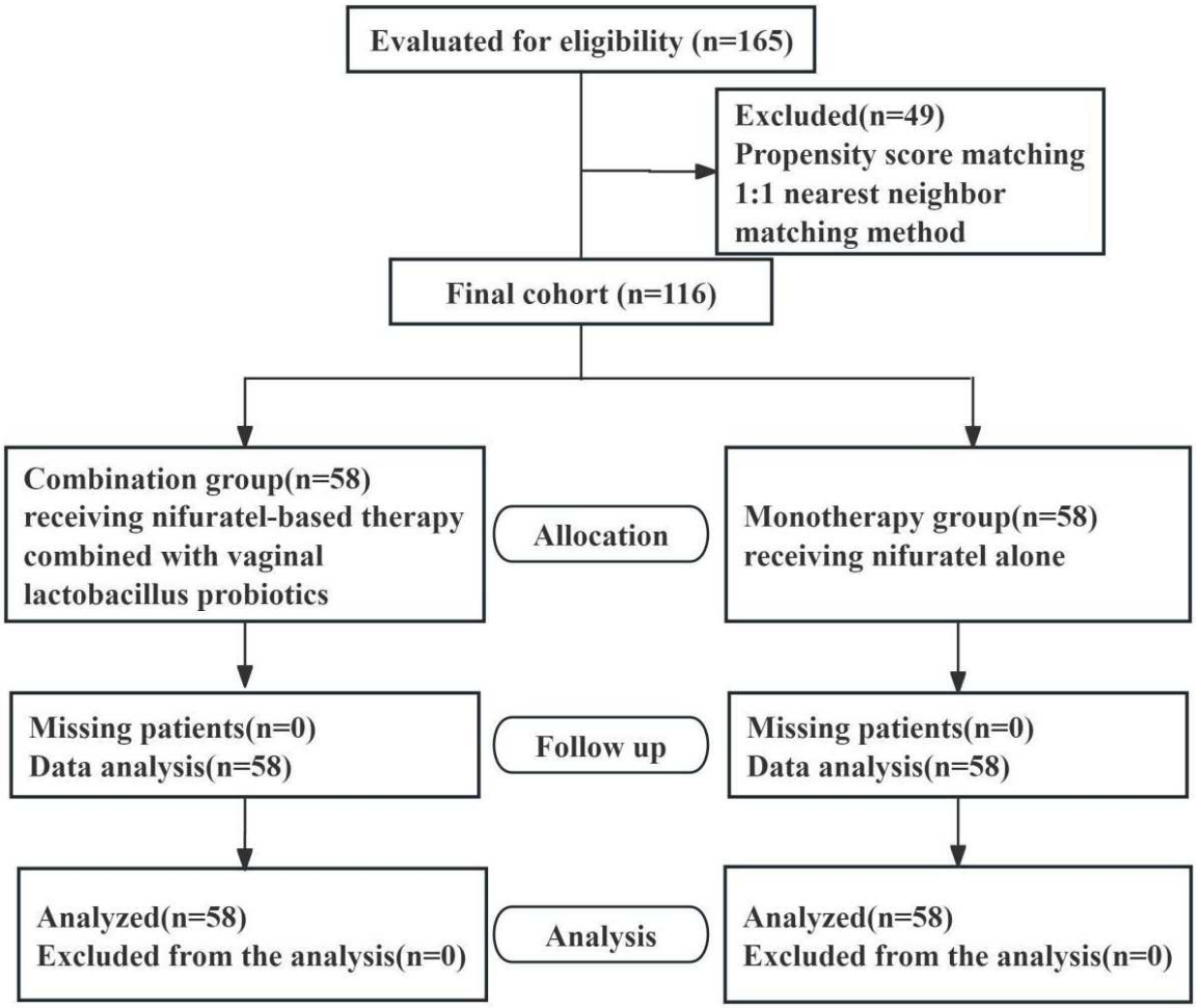

Fig. 1: Flow chart of the study

Cured (symptoms and signs disappeared, vaginal microecology test results were negative), markedly effective (patients' clinical symptoms and signs were significantly reduced compared with those before treatment, vaginal microecology test results were negative), effective (patients' clinical discomfort symptoms and signs were reduced, vaginal microecology test results were negative), ineffective (patients' clinical symptoms were not alleviated or aggravated, vaginal microecology test results were positive). Total effective rate = (cured + markedly effective + effective) cases/total cases × 100%.

Secondary outcome measures

(1) Vaginal microecology-related indicators: including vaginal pH before treatment and 1 week after drug withdrawal, the normal rate of *Lactobacillus* (defined as the grade I-II of *Lactobacillus* in the smear) and the normal rate of microflora diversity (defined as the density and diversity of microflora were normal)(Collaborative Group on Infectious Diseases, 2016).

(2) Symptom relief time: Through retrospective review of nursing records and subsequent medical records, the time (days) from the beginning of treatment to the first recorded "disappearance" or "significant relief" of the following core symptoms/signs was recorded: abnormal leucorrhea, vaginal mucosal congestion, vaginal itching and vaginal pain(Konkov *et al.*, 2024).

(3) Recurrence rate: the proportion of patients with recurrent clinical symptoms related to vaginal microecological abnormalities confirmed by laboratory tests from the end of treatment to delivery.

(4) Adverse pregnancy outcomes: based on follow-up data through delivery, this outcome was defined as the occurrence of preterm delivery, premature rupture of membranes, intrauterine infection, or similar related events.(Gerede *et al.*, 2024).

(5) Incidence of adverse drug reactions: adverse events such as dizziness and nausea, vulvar burning and vaginal bleeding recorded during treatment were extracted.

Vaginal microecological assessment

Vaginal flora was assessed microscopically using the

Nugent scoring system (0-10) for bacterial vaginosis, Lactobacillus grading (I–IV) and semi-quantitative evaluation of flora diversity on Gram-stained smears, following standard clinical laboratory protocols. It should be noted that these conventional microscopic methods, while clinically established and providing immediate diagnostic information, are semi-quantitative and their interpretation can vary between observers. More precise molecular techniques (e.g., 16S rRNA gene sequencing) were not employed in this retrospective clinical data analysis.

Sample size calculation

The sample size calculation was based on the primary outcome, the total clinical response rate, with reference to prior relevant studies (Chen, 2020). The overall efficacy rate was approximately 78.9% in the monotherapy group and 94.4% in the combination group for treating vaginal microecological abnormalities. Set α=0.05 (two-sided test), β=0.2 (power 80%) and the sample size ratio of the two groups was 1:1. The required sample size for each group was 52 calculated by PASS 15.0 software. Given the potential missing data and loss to follow-up in the retrospective study, the sample size was increased by 10%. Finally, 58 cases were planned for each group, for a total sample size of 116.

Statistical analysis

Statistical analyses were conducted using SPSS 26.0. Normally distributed continuous data are presented as mean ± SD and compared using independent samples *t*-tests; non-normal data are expressed as median (IQR) and analyzed with the Mann-Whitney U test. Categorical data are shown as n (%) and assessed via chi-square or Fisher's exact tests. To balance baseline characteristics including age, gestational age, parity, abortion history, prior vaginitis, vaginal pH and Nugent score, 1:1 propensity score matching with a caliper of 0.02 was performed. Vaginal pH changes and symptom relief times were compared between groups with independent t-tests and within groups with paired t-tests. Rates of clinical efficacy, *Lactobacillus* normalization, diversity normalization, recurrence, adverse pregnancy outcomes and adverse drug reactions were compared between groups with chi-square/Fisher's tests and within groups using McNemar's test. Multivariate logistic regression (forward stepwise likelihood ratio method) was performed to identify independent predictors of clinical effectiveness, including variables with $P<0.1$ in univariate analysis and clinically relevant factors. Results are reported as adjusted odds ratios (aOR) with 95% confidence intervals (CI). Statistical significance was defined as a two-tailed P-value < 0.05 for all tests.

RESULTS

Comparison of baseline data

The study initially included 165 pregnant women with vaginal microecological abnormalities, with 86 assigned to combination therapy and 79 to monotherapy.To address potential confounders, PSM was applied via a 1:1 nearest-neighbor approach, using a caliper of 0.02. The matching variables included age, gestational age, parity, miscarriage history, previous history of vaginitis, type of vaginitis, pre-treatment vaginal pH and Nugent score. After matching, 116 patients (58 per group) were included in the final analysis. Table 1 demonstrates that after matching, all observed baseline characteristics showed no statistically significant differences between the two groups (all $P>0.05$, SMD<0.1). This indicates that PSM effectively balances the known confounding factors between the treatment and control groups and significantly improves comparability between the groups. Therefore, this allows subsequent intergroup differences in treatment response, microecology and pregnancy outcomes to be attributed to the treatment difference, not to baseline imbalance.

Clinical efficacy

The clinical efficacy results of the two groups are shown in table 2. The combined treatment group achieved a significantly higher total clinical efficacy rate compared to the monotherapy group (P=0.016). There were no significant differences in the cure rate (55.2% vs. 41.4%), significant efficiency (25.9% vs. 20.7%) and effective rate (12.1% vs. 15.5%) between the two groups at 1 week after drug withdrawal (all $P>0.05$). However, a significantly lower failure rate was observed with combination therapy (6.9%) versus monotherapy (22.4%) (P=0.022). This suggests that the efficacy advantage of nifuratel-nystatin combined with vaginal *Lactobacillus* probiotic is mainly due to the significant reduction in the risk of treatment failure (no effect), thereby improving the overall clinical response rate.

Improvement of microecological indicators

Comparison of changes in vaginal flora before and after treatment

The two groups showed comparable baseline levels of key microecological indices, including Lactobacillus and bacterial diversity normality (all $P>0.05$). As shown in table 3, after treatment, the composition of vaginal microbiota was significantly improved in both groups. The normal rates of *Lactobacillus* and bacterial diversity were significantly increased from pretreatment levels in both treatment groups (all $P<0.001$). However, the quality of microbiota reconstitution was significantly better with combination therapy than with monotherapy. The post-treatment normal rates for *Lactobacillus* (91.4% vs. 75.9%; P=0.023) and bacterial diversity (87.9% vs. 70.7%; P=0.022) were both significantly higher in the combination group compared to the monotherapy group. Nifuratel-nystatin alone can effectively improve vaginal flora, but when combined with vaginal Lactobacillus probiotics, it can achieve better microecological reconstruction.

Table 1: Comparison of baseline characteristics between groups after PSM

Indicators	Combination group (n=58)	Monotherapy group (n=58)	Statistic	*P* Value	SMD
Demographics					
Age (years, $\bar{x}\pm s$)	28.8 ± 3.4	29.1 ± 3.6	t=-0.50	0.620	0.086
BMI (kg/m², $\bar{x}\pm s$)	23.3 ± 2.7	23.4 ± 2.9	t=-0.21	0.836	0.036
Higher education, n (%)	38 (65.5)	37 (63.8)	χ^2=0.04	0.847	0.036
Obstetric Profile					
Gestational age (weeks, $\bar{x}\pm s$)	20.5 ± 3.6	20.8 ± 3.6	t=-0.49	0.624	0.083
Gravidity (times, $\bar{x}\pm s$)	1.8 ± 0.8	1.8 ± 0.8	t=0.12	0.903	0.000
Parity (times, $\bar{x}\pm s$)	1.4 ± 0.7	1.4 ± 0.7	t=0.13	0.897	0.000
History of abortion, n (%)	14(24.1)	13 (22.4)	χ^2=0.04	0.841	0.041
Medical History					
Prior vaginitis, n (%)	28 (48.3)	30 (51.7)	χ^2=0.14	0.707	0.068
Current vaginitis type, n (%)			χ^2=0.40	0.823	0.069
- Bacterial vaginosis	36 (62.1)	38 (65.5)			
- Aerobic vaginitis	22 (37.9)	20 (34.5)			
Laboratory Indicators					
Vaginal pH ($\bar{x}\pm s$)	5.1 ± 0.4	5.1 ± 0.3	t=0.62	0.534	0.000
Nugent score ($\bar{x}\pm s$)	7.3 ± 1.4	7.2 ± 1.3	t=0.27	0.790	0.074
Lactobacillus grade, n (%)			-	1.000	0.000
- Grade I-II	3(5.2)	3(5.2)			
- Grade III-IV	55 (94.8)	55 (94.8)			
Abnormal diversity, n (%)	54 (93.1)	53 (91.4)	χ^2=0.12	0.729	0.061
Abnormal Vaginal Flora, n (%)	58 (100.0)	58 (100.0)	-	-	-

Note: BMI: Body Mass Index.

Table 2: Clinical efficacy at one week after treatment cessation [n (%)]

Group	n	Cured	Markedly effective	Effective	Ineffective	Total effective rate
Combination group	58	32 (55.2)	15 (25.9)	7 (12.1)	4 (6.9)	54 (93.1)
Monotherapy group	58	24 (41.4)	12 (20.7)	9 (15.5)	13 (22.4)	45 (77.6)
χ^2 Value	-	2.239	0.423	0.318	5.242	5.828
P Value	-	0.135	0.516	0.573	0.022	0.016

Note: Total effective rate = (Cured + Markedly Effective + Effective) cases / total cases × 100%; χ^2 test was used for between-group comparisons.

Table 3: Comparison of vaginal flora changes before and after treatment[n (%)]

Group	n	Normal *Lactobacillus* rate				Normal bacterial diversity rate			
		Pre-treatment	Post-treatment	χ^2 Value	*P* Value	Pre-treatment	Post-treatment	χ^2 Value	*P* Value
Combination group	58	3 (5.2)	53 (91.4)	48.03	<0.001	4 (6.9)	51 (87.9)	43.05	<0.001
Monotherapy group	58	3 (5.2)	44(75.9)	38.03	<0.001	5 (8.6)	41 (70.7)	30.03	<0.001
χ^2 Value	-	0.00	5.18	-	-	0.14	5.24	-	-
P Value	-	1.000	0.023	-	-	0.710	0.022	-	-

Note: Within-group comparisons (pre- vs. post-treatment) were performed using McNemar's test (χ^2 values reported). The chi-square test was used to analyze differences between the groups.

Table 4: Comparison of vaginal pH values before and after treatment($\bar{x}\pm s$)

Group	n	Pre-treatment	Post-treatment	t Value	*P* Value
Combination group	58	5.12 ± 0.36	4.15 ± 0.23	49.788	<0.001
Monotherapy group	58	5.08 ± 0.33	4.49 ± 0.25	39.256	<0.001
t Value	-	0.653	-7.669	-	-
P Value	-	0.515	<0.001	-	-

Note: Within-group comparisons (pre-vs. post-treatment) were analyzed using paired-sample t-tests. Group comparisons were conducted using independent-samples t-tests.

Comparison of vaginal pH value before and after treatment

Both groups had comparable baseline vaginal pH levels, with no statistically significant difference (P=0.515). As shown in table 4, after treatment, vaginal pH decreased significantly compared with that before treatment in both groups (all P<0.001 for intra-group comparisons). The combination therapy group exhibited a significantly lower post-treatment vaginal pH (4.15±0.23) than the monotherapy group (4.49±0.25) (P < 0.001). These results indicate that nifuratel-nystatin monotherapy is effective in reducing vaginal pH, but the combination of nifuratel-nystatin with vaginal *Lactobacillus* probiotic can reduce it to a more ideal physiological range.

Comparison of symptom relief time

Regarding symptom relief speed, the combination group showed a significant advantage over the single-drug group. As shown in table 5, for the four core symptoms and signs of abnormal leucorrhea, vaginal mucosal congestion, vaginal pruritus and vaginal pain, the average remission time in the combination therapy group was significantly shorter than that in the monotherapy group and all the differences were highly significant (P<0.001). The results showed that nifuratel-nystatin combined with vaginal *Lactobacillus* probiotic treatment could significantly accelerate the remission process of clinical symptoms and signs related to vaginal microecological abnormalities during pregnancy. Combination therapy has shown clear advantages in rapid relief of subjective discomfort (especially itching and pain) and objective inflammatory signs.

Recurrence rate

Across all follow-up time points, the combination therapy group had a lower cumulative relapse incidence than the monotherapy group (Table 6). At the 4-week follow-up, the recurrence rate was 5.2% for the combination group and 12.1% for the monotherapy group. The number of new recurrences decreased over time in both groups, but the combination group consistently had fewer recurrences at each follow-up time point. By the end of follow-up (delivery), the total recurrence rate was significantly lower in the combination treatment group (8.6%, 5/58) than in the monotherapy group (22.4%, 13/58) (P=0.040). These results suggest that, compared with nystatin-nifuratel alone, combined treatment with vaginal Lactobacillus and probiotics is more effective at reducing the risk of recurrence of vaginal microecological abnormalities during pregnancy. This advantage occurs early after treatment ends and can be maintained throughout the pregnancy.

Comparison of adverse pregnancy outcomes

The combination group exhibited a reduced incidence of each adverse pregnancy outcome relative to the monotherapy group, with trends toward lower risks for preterm birth (3.4% vs. 8.6%), premature rupture of membranes (1.7% vs. 6.9%) and intrauterine infection (1.7% vs. 5.2%). As shown in table 7, although the between-group differences in each outcome did not achieve statistical significance (all P>0.05), the combination group experienced a significantly lower total incidence of adverse pregnancy outcomes (6.9%) compared to the monotherapy group (20.7%) (P=0.033). The combined strategy demonstrated dual benefits: A positive trend in reducing multiple specific adverse pregnancy risks and a significant reduction in the total incidence, relative to nifuratel-nystatin monotherapy.

Drug safety analysis

Drug safety profiles for both regimens are summarized in table 8. During treatment, no severe adverse events occurred in any participant. The overall incidence of adverse events was low and comparable between the combination and monotherapy groups. The adverse reactions reported by patients in both groups were dizziness and nausea, vulvar burning sensation, vaginal bleeding, etc. The incidence of adverse events showed no statistically significant difference between the groups (P>0.05), indicating that no new or more serious safety issues were observed with nifuratel-nystatin combined with vaginal *Lactobacillus* probiotic on the basis of anti-infective treatment, suggesting that this combination therapy has a good safety profile.

Multivariate logistic regression analysis of clinical total effective rate

Univariate Logistic regression analysis showed that the P value for the relationship between treatment plan, parity, and a previous history of vaginitis and the total clinical effective rate was less than 0.1. Among them, the combination treatment plan and parity (≥1) are potential protective factors and the previous history of vaginitis may also have a positive effect on the efficacy. To control the interaction between variables, the above three variables were included in the multivariate Logistic regression model (using the stepwise forward method). As shown in table 9, treatment regimen was an independent protective factor for clinical total response rate (adjusted OR = 4.05, 95% CI: 1.25-13.15, P=0.020). Following adjustment for treatment regimen, neither parity nor history of vaginitis demonstrated independent statistical significance (P>0.05). Multivariate analysis confirmed that after controlling for potential confounders such as parity and medical history, patients treated with nifuratel-nystatin combined with vaginal *Lactobacillus* probiotic were 4.05 times more likely to have clinical response than those treated with nifuratel-nystatin alone. This suggests that combination therapy is an independent favorable factor for improved clinical efficacy.

Table 5: Comparison of symptom resolution time between groups ($\bar{x}\pm s$)

Symptom / Sign	Combination group (n=58)	Monotherapy group (n=58)	t Value	*P* Value
Abnormal discharge	5.1 ± 1.2	7.8 ± 1.5	-10.393	<0.001
Vaginal mucosal redness	6.5 ± 1.5	9.3± 1.8	-8.895	<0.001
Vaginal itching	4.3 ± 1.0	6.9 ± 1.3	-12.001	<0.001
Vaginal pain	3.8 ± 0.9	5.7 ± 1.1	-9.810	<0.001

Note: Intergroup differences were analyzed with independent-sample t-tests.

Table 6: Comparison of recurrence rates between groups [n (%)]

Group	n	Recurrence at 4 weeks	Recurrence at 8 weeks	Recurrence at 12 weeks	Total recurrence
Combination group	58	3 (5.2)	1 (1.7)	1 (1.7)	5 (8.6)
Monotherapy group	58	7 (12.1)	4 (6.9)	2 (3.4)	13 (22.4)
P Value	-	0.332	0.369	1.000	0.040

Note: Between-group comparisons were analyzed using Fisher's exact test.

Table 7: Comparison of adverse pregnancy outcomes between groups [n (%)]

Group	n	Preterm birth	Premature rupture of membranes	Intra-amniotic infection	Total incidence
Combination group	58	2 (3.4)	1 (1.7)	1 (1.7)	4 (6.9)
Monotherapy group	58	5 (8.6)	4 (6.9)	3 (5.2)	12 (20.7)
P Value	-	0.438	0.361	0.618	0.033

Note: Between-group comparisons were performed using Fisher's exact test.

Table 8: Incidence of adverse drug reactions in both groups[n (%)]

Adverse event	Combination group (n=58)	Monotherapy group (n=58)	*P* Value
Dizziness/Nausea	2 (3.4%)	3 (5.2%)	0.650
Vulvar burning	3 (5.2%)	1 (1.7%)	0.650
Vaginal bleeding	1 (1.7%)	1 (1.7%)	1.000
Severe adverse events	0 (0%)	0 (0%)	-

Note: P values were calculated using Fisher's exact test.

Table 9: Multivariate logistic regression analysis of factors affecting total clinical effectiveness

Factor	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* Value	aOR (95% CI)	*P* Value
Treatment (Combination vs. Mono)	4.12 (1.28 ~ 13.27)	0.018	4.05 (1.25 ~ 13.15)	0.020
Parity (≥1 vs. 0)	2.10 (1.05 ~ 4.20)	0.036	1.95 (0.96 ~ 3.98)	0.066
Prior vaginitis (Yes vs. No)	1.80 (0.95 ~ 3.41)	0.072	1.65 (0.86 ~ 3.18)	0.134
Age (per year increase)	0.96 (0.83 ~ 1.10)	0.533	-	-
Gestational age (per week)	1.02 (0.92 ~ 1.13)	0.677	-	-
Pre-treatment pH	0.81 (0.43 ~ 1.53)	0.514	-	-

Note: OR = odds ratio; aOR = adjusted odds ratio; CI = confidence interval. Multivariate analysis was conducted with forward stepwise selection based on the likelihood ratio test, incorporating variables identified as significant (P<0.1) in the univariate analysis. "–" indicates that the variable was not retained in the final multivariate model.

DISCUSSION

This study evaluated the efficacy and safety of nifuratel-nystatin combined with vaginal *Lactobacillus* probiotic versus nifuratel-nystatin alone in the treatment of vaginal microecological abnormalities during pregnancy in a PSM retrospective cohort analysis. The combination therapy showed significant advantages in improving the total clinical effectiveness rate, accelerating symptom relief, promoting recovery of microecological indicators, reducing the long-term recurrence rate, and improving pregnancy outcomes, without increasing drug-related risks. This series of findings provides important clinical evidence for optimizing the management strategy of vaginal infection during pregnancy.

The study revealed a significantly higher total clinical effective rate in the combination therapy group compared to the monotherapy group, which is consistent with the synergistic effect reported for the "antimicrobial therapy followed by probiotics" approach in non-pregnant populations (Liu *et al.*, 2022; Jeng *et al.*, 2020; Sartaj, 2025). Our results extend these findings to the pregnant population. The advantage of combination therapy in our study was primarily driven by a significantly lower rate of treatment failure (6.9% vs. 22.4%), underscoring its potential to enhance overall treatment success. Furthermore, the combination group demonstrated significantly faster resolution of core symptoms such as vaginal itching, pain, abnormal discharge and mucosal redness. This finding aligns with with a previous study (Li *et al.*, 2019), which reported that a topical agent combined with probiotics significantly shortened symptom relief time in pregnant women with bacterial vaginitis. Rapid symptom alleviation is of direct clinical value in reducing the physical and mental burden on pregnant women and may improve treatment adherence.

At the microbiological level, the combination group achieved significantly lower post-treatment vaginal pH and higher rates of normalized Lactobacillus colonization and bacterial diversity than monotherapy. This indicates that the combined regimen not only suppresses pathogens but also appears to support the reconstruction of a Lactobacillus-dominant microecological barrier more effectively. A healthy vaginal microbiota is typically characterized by a high proportion of *Lactobacillus*, which maintains an acidic environment (pH ~3.8-4.5) by secreting lactic acid and producing substances such as bacteriocins and H_2O_2 to inhibit pathogen colonization (Baud *et al.*, 2023). While in vitro studies suggest that nifuratel-nystatin may have minimal inhibitory effect on lactobacilli (Togni *et al.*, 2011), our clinical observation of superior *Lactobacillus* recovery with the combination regimen suggests that the exogenous probiotics may actively compensate for any potential disruption and facilitate faster ecological restoration. This "anti-pathogen and pro-microbiome" approach may help avoid a post-antibiotic "ecological vacuum," potentially reducing opportunities for pathogen recolonization and providing a biological basis for the lower recurrence rate observed (Ma *et al.*, 2023).

Moreover, the combination therapy was associated with a significantly lower overall incidence of adverse pregnancy outcomes (6.9% vs. 20.7%). Extensive evidence links vaginal dysbiosis, particularly bacterial vaginosis, to an increased risk of preterm birth, premature rupture of membranes and intra-amniotic infection (Kenfack-Zanguim *et al.*, 2023; Ng *et al.*, 2023). The mechanisms may involve ascending infection, inflammation-mediated uterine contractions and enzymatic weakening of fetal membranes. Our findings suggest that achieving a more robust and stable restoration of vaginal microecology through combination therapy may contribute to mitigating these risks. A healthier vaginal microenvironment could better resist the ascent of ascending pathogens and modulate local inflammatory responses, thereby creating a more favorable environment for maintaining pregnancy. This perspective is supported by other research advocating for integrated management of vaginal infections in pregnancy (Jahic, 2022). Furthermore, the concept of prenatal probiotic intervention for maternal and infant health is gaining support. A large-scale randomized controlled trial demonstrated that specific probiotic supplementation in late pregnancy reduced maternal infections and benefited infants through vertical transmission, underscoring the potential broader health value of microecological interventions in pregnancy (Mu *et al.*, 2023).

Safety is the primary consideration for any pregnancy intervention. During this study, no severe adverse events were observed in any participant and the frequency of common adverse reactions was comparable between the two groups. This suggests that under standard use, the vaginal application of nifuratel-nystatin and *Lactobacillus* preparations is safe and well tolerated during pregnancy. Existing data support the safety profile of nifuratel-nystatin during pregnancy (Neut *et al.*, 2015). Vaginal *Lactobacillus* probiotics, as physiological modulators, have also demonstrated a favorable safety record in pregnancy and lactation (Sheyholislami *et al.*, 2021). This study further confirms the short-term safety of this combination. Therefore, this combination regimen achieves a good balance between efficacy and local safety, conforms to the basic principles of medication during pregnancy and provides clinicians with a reliable option.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, its retrospective design, despite the use of PSM to control for known confounders, cannot eliminate the potential influence of unmeasured or residual confounding factors. Second, as a single-center study, the patient population and clinical protocols were relatively homogeneous, which may affect the generalizability of the results to other settings. Third, the assessment of vaginal microecology relied on conventional microscopy-based methods (Nugent score, *Lactobacillus* grading). While clinically practical, these are semi-quantitative and operator-dependent. The absence of molecular profiling (e.g., 16S rRNA gene sequencing) limits deeper insights into compositional changes in the microbiome. Fourth, although the matched sample size was adequate for the primary analysis, it may be underpowered to detect significant differences in individual, less frequent adverse pregnancy outcomes. Future prospective, multi-center studies with larger sample sizes and molecular microbiome analysis are warranted to confirm these findings and better elucidate the causal pathways involved.

CONCLUSION

In conclusion, this retrospective cohort study suggests that for pregnant women with vaginal microecological abnormalities, combining nifuratel-nystatin with vaginal Lactobacillus probiotics is associated with enhanced clinical and microbiological effectiveness, faster symptom relief, lower recurrence and a potentially reduced risk of adverse pregnancy outcomes, without increasing adverse reactions. These findings support considering the integration of probiotic supplementation into the management of vaginal dysbiosis during pregnancy. Future research should focus on validating these results in prospective, randomized settings and exploring the long-term impacts on maternal and infant health.

Acknowledgments
None

Authors' contributions
Ya Luo: Developed and planned the study, performed experiments and interpreted results. Edited and refined the manuscript with a focus on critical intellectual contributions.
Hong Xu: Participated in collecting, assessing and interpreting the data. Made significant contributions to data interpretation and manuscript preparation.
Xiaohong Ye: Provided substantial intellectual input during the drafting and revision of the manuscript.

Funding
None

Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
This study was approved by Maternal and Child Health Hospital Shuangliu District, Chengdu Ethics Committee (Approval No: 2022- (ky) -6). This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest
The authors declare that they have no financial conflicts of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1777634872.pdf

REFERENCES

Ahrodia T, Yodhaanjali JR and Das B (2022). Vaginal microbiome dysbiosis in preterm birth. *Prog. Mol. Biol. Transl. Sci.,* **192**(1): 309-29.

Baud A, Hillion KH, Plainvert C, Tessier V, Tazi A, Mandelbrot L, Poyart C and Kennedy SP (2023). Microbial diversity in the vaginal microbiota and its link to pregnancy outcomes. *Sci Rep.*, **13**(1): 9061.

Chopra C, Bhushan I, Mehta M, Koushal T, Gupta A, Sharma S, Kumar M, Khodor SA and Sharma S (2022). vaginal microbiome: Considerations for reproductive health. *Future Microbiol.*, **17**(18): 1501-13.

Collaborative Group on Infectious Diseases Society of Obstetrics and Gynecology, Chinese Medical Association. (2016). Expert consensus on the clinical application of vaginal microecology assessment. *Chin J Obstet Gynecol*, **51**(10): 721-23.

Dubé-Zinatelli E, Cappelletti L and Ismail N (2025). The vaginal microbiome in bacterial vaginosis: Pathogenesis, reproductive impacts and emerging therapies. *J Reprod Immunol.*, **172**:104804.

Fan Y, Gu Y, Xian Y, Li Q, He Y, Chen K, Yu H, Deng H, Xiong L, Cui Z, Yang Y and Xiang Y (2024). Efficacy and safety of different drugs for the treatment of bacterial vaginosis: A systematic review and network meta-analysis. *Front Cell Infect Microbiol*, **14**: 1402346.

Gerede A, Nikolettos K, Vavoulidis E, Margioula-Siarkou C, Petousis S, Giourga M, Fotinopoulos P, Salagianni M, Stavros S, Dinas K, Nikolettos N and Domali E (2024). Vaginal microbiome and pregnancy complications: A review. *J Clin Med*, **13**(13): 3875.

Gupte S , Purandare CN, Nazareth AK, Chatla D, Deulkar MG, Kirdat K, Arora P and Shah S (2024). The role of microbiome in pregnancy: Exploring links between vaginal health, preterm birth and preeclampsia. *IJISRT.,* pp.1966-68.

Husain S, Allotey J, Drymoussi Z, Wilks M, Fernandez-Felix BM, Whiley A, Dodds J, Thangaratinam S, McCourt C, Prosdocimi EM, Wade WG, de Tejada BM, Zamora J, Khan K and Millar M (2020). Effects of oral probiotic supplements on vaginal microbiota during pregnancy: A randomised, double-blind, placebo-controlled trial with microbiome analysis. *Bjog*, **127**(2): 275-84.

Jahic M (2022). Aerobic vaginitis caused by *Enterococcus faecalis* - clinical features and treatment. *Mater Sociomed*, **34**(4): 291-95.

Jeng HS, Yan TR and Chen JY (2020). Treating vaginitis with probiotics in non-pregnant females: A systematic review and meta-analysis. *Exp Ther Med*, **20**(4): 3749-65.

Kenfack-Zanguim J, Kenmoe S, Bowo-Ngandji A, Kenfack-Momo R, Thierry Ebogo-Belobo J, Kengne-Ndé C, Serge Mbaga D, Zeuko'o Menkem E, Lontuo Fogang R, Tchatchouang S, Laure Ndzie Ondigui J, Irma Kame-Ngasse G, Nina Magoudjou-Pekam J, Roussel Takuissu G, Nkie Esemu S, Nantcho Clavela N, Barriere Fodjo A, Ndip L, Bigoga J, Megnekou R and Esemu LF (2023). Systematic review and meta-analysis of maternal and fetal outcomes among pregnant women

with bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol.*, **289**: 9-18.

Kim MJ, Lee S, Kwon MY and Kim M (2022). Clinical significance of composition and functional diversity of the vaginal microbiome in recurrent vaginitis. *Front Microbiol*, **13**: 851670.

Konkov D , Starovier A, Bulavenko O, Dan N and Buran O (2024). The evaluation of the clinical efficacy of prodexyn vaginal suppositories in the treatment of recurrent bacterial vaginosis. *Reproductive endocrinology,* **75**: 37–43.

Li C, Xie Z and Xu W (2019). Clinical curative effects and safety of self-made cortex phellodendri lotion combined with beneficial microorganisms in treating bacterial vaginitis in pregnant women after 37-week pregnancy *Hebei Med J.*, **41**(5): 721-23+27.

Li Z, Zhang Y, Li X, Zhou S, Gao Q, Wu C and Meng L (2025). Stage-specific vaginal microbial alterations in pregnancy-associated vulvovaginal candidiasis. *Microbiol Spectr*, **13**(12): e0214425.

Liu HF and Yi N (2022). A systematic review and meta-analysis on the efficacy of probiotics for bacterial vaginosis. *Eur Rev Med Pharmacol Sci*, **26**(1): 90-98.

Ma S, Wang W, Su Y, Sun W and Ma L (2023). Antibiotics therapy combined with probiotics administered intravaginally for the treatment of bacterial vaginosis: A systematic review and meta-analysis. *Open Med (Wars)*, **18**(1): 20230644.

Mu J, Guo X, Zhou Y and Cao G (2023). The effects of probiotics/synbiotics on glucose and lipid metabolism in women with gestational diabetes mellitus: A meta-analysis of randomized controlled trials. *Nutrients*, **15**(6): 1375.

Neut C, Verrière F, Nelis H and Coenye T (2015). Topical treatment of infectious vaginitis: Effects of antibiotic, antifungal and antiseptic drugs on the growth of normal vaginal lactobacillus strains. *Open J Obstet Gynecol.*, **5**: 173-80.

Ng BK, Chuah JN, Cheah FC, Mohamed Ismail NA, Tan GC, Wong KK and Lim PS (2023). Maternal and fetal outcomes of pregnant women with bacterial vaginosis. *Front Surg*, **10**:1084867.

Petricevic L, Rosicky I, Kiss H, Janjic N, Kaufmann U, Holzer I and Farr A (2023). Effect of vaginal probiotics containing *Lactobacillus casei* rhamnosus (Lcr Regenerans) on vaginal dysbiotic microbiota and pregnancy outcome, prospective, randomized study. *Sci Rep.*, **13**(1): 7129.

Polatti F, Nappi RE, Brundu B, Fantuzzi M and Frisenda L (2003). Clinical study on the dose-effect relationship of a nifuratel-nystatin combination in the treatment of vulvo-vaginal infections. *Arzneimittelforschung*, **53**(10): 730-7.

Sartaj Ali (2025). Probiotics in the management of autoimmune disorders (Multiple Sclerosis): A new approach. *JPBT*, **1**(1): 35-46.

Sheyholislami H and Connor KL (2021). Are probiotics and prebiotics safe for use during pregnancy and lactation? A systematic review and meta-analysis. *Nutrients*, **13**(7): 2382.

Swarna SR, Vidyameena R, Bharathi T and Jeyakumari D (2023). Association between vaginal microbiota dysbiosis with asymptomatic uti in pregnant woman and adverse pregnancy outcome- a case report. *Indian J. Microbiol. Res.*, **9**(4): 299-303.

Togni G, Battini V, Bulgheroni A, Mailland F, Caserini M and Mendling W (2011). *In-vitro* activity of nifuratel on vaginal bacteria: Could it be a good candidate for the treatment of bacterial vaginosis? *Antimicrob Agents Chemother*, **55**(5): 2490-2.

Wang Y, Zhang Z, Chen Q and Chen T (2024). Simultaneous application of oral and intravaginal probiotics for helicobacter pylori and its antibiotic-therapy-induced vaginal dysbacteriosis. *NPJ Biofilms Microbiomes*, **10**(1): 49.

Weldegebreal F, Ayana DA, Wilfong T, Dheresa M, Yadeta TA, Negesa AS, Demmu YM, Tesfa T, Alemu TN, Eticha TG, Geremew A, Roba KT, Abdissa A, Assefa N, Negash AA, Cools P and Tura AK (2025). Relationship between vaginal and gut microbiome and pregnancy outcomes in eastern Ethiopia: A protocol for a longitudinal maternal-infant cohort study (the Ethiomics Study). *BMJ Open*, **15**(1): e092461.

Yefet E, Mirin D, Massalha M, Alter A and Nachum Z (2025). Screening for and treatment of bacterial vaginosis reduced preterm delivery in high-risk pregnant women: A systematic review and meta-analysis. *Gynecol Obstet Invest*, **90**(4): 353-62.

Zhang H, Jin S, Ji A, Zhang C and Shi S (2022). Correlation between vaginal microecological status and prognosis of cin patients with high-risk hpv infection. *Biomed Res Int*, **2022**: 3620232.