# Vaginal microecological characteristics in women across different age, maternal and disease states and their correlation with HPV infection

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**Abstract.** The aim of the present study was to investigate variations in vaginal microecology across female populations, elucidate alterations in vaginal microecology across age groups and physiological states, and provide evidence to support clinical prevention, diagnosis and treatment of vaginal diseases to safeguard women's reproductive health. A total of 200 women who underwent routine health check-ups at the Third Affiliated Hospital of Jinzhou Medical University between March and September 2024 were enrolled. According to age, subjects were divided into five groups: Group A (21-29 years, n=40), Group B (30-39 years, n=40), Group C (40-49 years, n=40), Group D (50-59 years, n=40), and Group E (≥60 years, n=40). Based on maternal status, the participants were categorized into a pregnant/postpartum group (defined as women during pregnancy or within 6 weeks postpartum, n=40) and a non-pregnant/non-postpartum group (women not in pregnancy or postpartum, n=40). Within the same age group, subjects were further divided into the vaginal disease group (women with vaginal diseases, n=40) and the healthy group (women with no vaginal diseases, n=40). All participants underwent tests including vaginal pH measurement, bacterial community analysis, vaginal microecological functional assays, and HPV detection. Indicators of pH, flora structure, and functional test results were compared among different age groups, maternal statuses, and disease states. The correlation between vaginal microecology and HPV infection was analyzed.

Significant differences were observed in the rates of individuals with a vaginal pH >4.6, the distributions of dominant bacteria, and the positive rates of hydrogen peroxide, leukocyte esterase, sialidase (SA), and coagulase among different age groups, maternal statuses, and disease states (all P<0.05). No significant difference was observed in the proportion of individuals with a vaginal pH >4.6 between the HPV-positive and HPV-negative groups (P>0.05). HPV infection was significantly associated with abnormal microbial diversity (Grade I or Grade IV, that is, reduced or excessive species richness) and abnormal microbial density (Grade I or Grade III, that is, insufficient or excessive bacterial load); the proportions of both abnormal diversity and abnormal density were significantly higher in HPV-positive than in HPV-negative women (P<0.05). HPV infection was also associated with SA positivity, but with lower rates of vulvovaginal candidiasis and hydrogen peroxide positivity (all P<0.05). Age, pregnancy/postpartum status, vaginal diseases, and HPV infection were closely correlated with vaginal microecology. Clinical emphasis should be placed on vaginal microecological monitoring in middle-aged and elderly women, pregnant and postpartum women, and those with a history of vaginal diseases, to timely detect and diagnose reproductive tract infections and protect women's health.

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## Introduction

The female vagina is a dynamic, relatively enclosed cavity harboring a complex and diverse microbial community that constitutes the vaginal microecosystem (1). This ecosystem is maintained by intricate interactions among vaginal flora, host epithelial cells, immune responses and endocrine status, and its delicate balance is critical for female reproductive health (2). *Lactobacillus*, the dominant genus in healthy vaginal microecology, exerts protective effects by producing lactic acid (maintaining a between pH 3.8-4.5), hydrogen peroxide ($H_2O_2$), bacteriocins, and biosurfactants to inhibit pathogenic colonization (3). Disruption of this balance (dysbiosis) increases the risk of vaginal infections [such as bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), trichomoniasis] and ascending infections such as pelvic inflammatory disease.

Vaginal infections affect 20-40% of reproductive-aged women globally, with higher rates in perimenopausal and elderly populations (4). Persistent dysbiosis is also associated with infertility, preterm birth, and increased HPV susceptibility, highlighting the need for accurate and timely diagnosis of microecological disorders.

Traditional diagnosis of vaginal infections relies on microscopic examination of vaginal secretions, which assesses vaginal cleanliness (Grades I-IV) based on the presence of lactobacilli, leukocytes and pathogens (5). However, this method is subjective, observer-dependent, and cannot comprehensively evaluate microecological functional indicators (such as $H_2O_2$ production and pathogen enzyme activity) (6), leading to high rates of missed or misdiagnosed cases, especially for subclinical dysbiosis, mixed infections, or early-stage imbalance (7,8). Comprehensive vaginal microecological assessment relies on combined morphological detection of flora and cellular features, alongside functional biomarkers including pH, $H_2O_2$, leukocyte esterase (LE), sialidase (SA) and coagulase (COA) to fully profile vaginal microenvironmental status. Combined detection strategies deliver higher sensitivity and specificity for vaginal dysbiosis screening than routine single microscopic examination, yet widespread clinical application is hampered by inadequate epidemiological data covering heterogeneous female populations. Notably, vaginal microecology exhibits significant variability across physiological stages and demographic groups (9). Age-related changes (such as declining estrogen levels in perimenopause/menopause, leading to vaginal atrophy and *Lactobacillus* reduction), hormonal fluctuations during pregnancy (which alter glycogen metabolism and flora composition), and the presence of preexisting vaginal diseases can all potentially modulate the structure and function of the vaginal microbiome (10). However, most existing studies have adopted a single-factor analytical framework: They either focused exclusively on reproductive-age women or on specific disease cohorts or investigated only the impact of a single factor (such as age alone or pregnancy alone) on vaginal microecology. No study to date has systematically and simultaneously evaluated the independent and combined effects of age, pregnancy/postpartum status, vaginal diseases, and HPV infection on vaginal microecological profiles in a single cohort. This critical gap prevents a comprehensive understanding of microecological variations across diverse female populations and hinders the development of population-specific diagnostic and intervention strategies. To address these gaps, a total of 200 women undergoing routine health check-ups at our hospital were enrolled and stratified by age (21-29, 30-39, 40-49, 50-59, and ≥60 years), maternal status (pregnancy/postpartum vs. non-pregnancy/postpartum), and disease status (vaginal disease vs. healthy). Key vaginal microecological parameters (pH, flora structure, and functional enzyme activity) and their correlation with HPV infection were analyzed. The aim of the present study was to: (i) characterize vaginal microecological profiles across female subgroups; (ii) identify factors associated with microecological variations; (iii) explore the association between dysbiosis and HPV infection; and (iv) provide population-specific evidence to optimize microecological evaluation and improve vaginal disease management. The present study is the first to simultaneously evaluate the four aforementioned factors in a single cohort, and its findings fill an important gap in the literature and support the optimization of clinical diagnostic protocols for vaginal diseases (Fig. 1).

## Materials and methods

*General information*. A total of 200 independent female participants who underwent routine health check-ups at the Third Affiliated Hospital of Jinzhou Medical University between March and September 2024 were enrolled. No sample overlap existed across any grouping dimensions. Participants were stratified into five groups by age: Group A (21-29 years, n=40), Group B (30-39 years, n=40), Group C (40-49 years, n=40), Group D (50-59 years, n=40), and Group E (≥60 years, n=40). Additionally, individuals were categorized by maternal status into the pregnant and postpartum group (defined as women during pregnancy or within 6 weeks postpartum, n=40) and the non-pregnant, non-postpartum group (women not in pregnancy or postpartum, n=40). From the total cohort, 80 age-matched participants were selected (independent of the maternal status subgroup) to form two subgroups (n=40 per group): The vaginal disease group (women diagnosed with vaginal diseases) and the healthy control group. This stratification was conducted within the same age stratum to ensure balanced age distribution between the two groups.

The inclusion criteria were as follows: (i) Clinically healthy without a history of chronic systemic diseases (such as diabetes mellitus and hypertension); (ii) with a history of sexual activity; (iii) voluntarily agreed to undergo vaginal pH testing, functional assays, and microbiota structure analysis; (iv) no sexual activity, vulvar/vaginal medication use, or vaginal douching within the 3 days prior to enrollment; (v) intact uterus with at least one functional ovary; (vi) regular menstrual cycles for participants <40 years old; and (vii) fully informed of the study protocol and signed the written informed consent form. Separate inclusion criteria for healthy controls and vaginal disease participants were used as follows: Healthy participants, no clinical symptoms of reproductive tract infection, vaginal cleanliness Grade I-II. For the vaginal disease group the inclusion criteria were as follows: Enrolled after a confirmed clinical diagnosis of BV, VVC, trichomonas vaginitis (TV), or aerobic vaginitis (AV) according to standard clinical diagnostic criteria. The exclusion criteria were: (i) Malignant tumors or severe dysfunction of major organs (such as liver and kidney); (ii) history of vaginal medication, douching, or local treatment within 2 weeks prior to enrollment; (iii) previous uterine or cervical surgery; (iv) menstruation at the time of enrollment; and (v) mental or cognitive disorders that prevented cooperation with the study procedures.

The sample size was calculated based on the primary outcome of the difference in SA positivity rates between

$$n = \frac{\left(Z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{p_1(1-p_1)+p_2(1-p_2)}\right)^2}{(p_1-p_2)^2}$$

HPV-positive and HPV-negative groups, which was the most robust association identified in preliminary investigations and previous relevant studies (11). For two independent sample proportion comparisons using the chi-square test, the sample

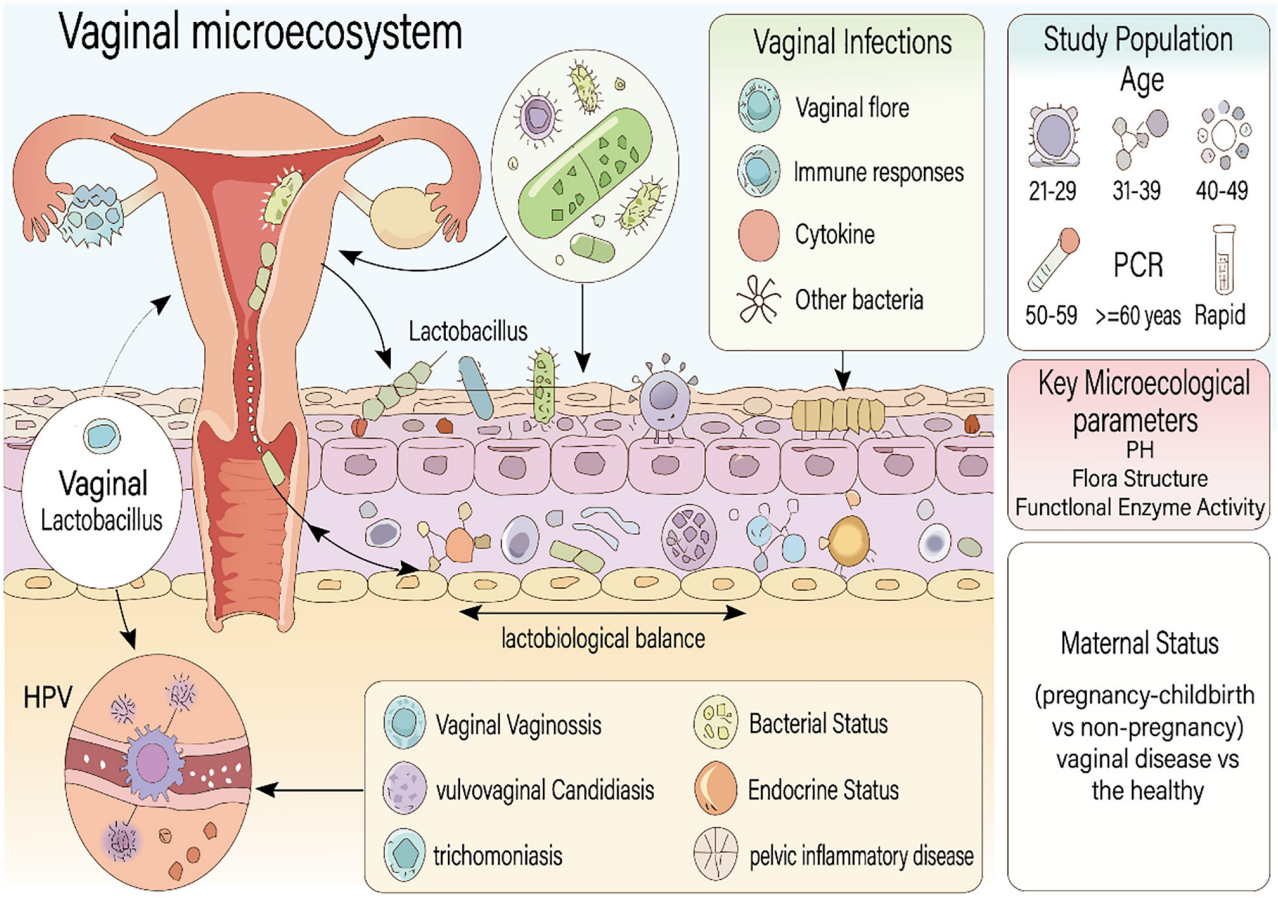



Figure 1. Schematic overview of the vaginal microecosystem, associated disorders, and key research dimensions. A schematic of the vaginal microecosystem's structure and dynamics, where vaginal *Lactobacillus* acts as the pivotal microbe sustaining lactobiological homeostasis in the vaginal mucosa. Dysregulation of this balance links to vaginal pathologies: infections (driven by flora shifts, immune/cytokine changes, opportunistic bacteria), disorders (for example, bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis), HPV infection, and comorbidities such as pelvic inflammatory disease (tied to bacterial/endocrine/inflammatory status). Integrated research frameworks were also outlined: (i) age-stratified populations (21-29 to ≥60 years) analyzed via PCR/rapid assays; (ii) key microecological parameters (pH, flora structure, enzyme activity) for microenvironment assessment; (iii) maternal status comparisons (pregnant/postpartum vs. non-pregnant/non-postpartum; diseased vs. healthy) to define microecosystem variations.

size per group was calculated using the following standard formula:

where: Significance level $\alpha$=0.05 (two-sided), corresponding to Z1-$\alpha$/2=1.96; statistical power 1-$\beta$=0.8, corresponding to Z1-$\beta$=0.84; p1=38.0% (estimated SA positivity rate in the HPV-positive group); p2=10.0% (estimated SA positivity rate in the HPV-negative group); and $\bar{p}$=(p1+ p2)/2=24.0% (pooled positive rate).

Substituting the above parameters, the calculated minimum sample size was 36 participants per group. Considering a 10% potential loss to follow-up and a missing data rate, which was rounded up to 40 participants per subgroup. For the age-stratified analysis spanning the full adult female lifespan, 40 participants were enrolled in each of the 5 age groups (21-29, 30-39, 40-49, 50-59, ≥60 years), yielding a total sample size of 200. This sample size provides ≥80% statistical power to detect significant differences in all key vaginal microecological parameters (pH, flora structure, functional enzyme activity) between groups at the $\alpha$=0.05 significance level.

The present study used convenience sampling, but all participants were strictly screened against the pre-specified inclusion and exclusion criteria to minimize selection bias.

*Baseline characteristics of participants*. The baseline characteristics of all 200 independent participants are summarized in Table I. The overall mean age of the cohort was 44.2±13.7 years (range, 21-78 years). The mean ages of the five age-stratified groups were: Group A, 25.1±2.3 years (range, 21-29 years); group B, 34.5±2. years (range, 30-39 years); group C, 44.3±2.7 years (range, 40-49 years); group D, 54.2±2.6 years (range, 50-59 years): and group E, 67.8±5.4 years (range, ≥60 years), respectively, with significant inter-group age differences (P<0.001) as designed.

Among all participants, 40 (20.0%) were pregnant or within 6 weeks postpartum, with the highest proportion in Group A (35.0%) and Group B (30.0%), consistent with the age distribution of reproductive events. The vaginal disease group (n=40) comprised 8 cases of BV (20.0%), 9 cases of VVC (22.5%), 10 cases of AV (25.0%), 7 cases of TV (17.5%) and 7 cases with two or more of these conditions (17.5%); because a participant could carry more than one pathogen, these categories are not mutually exclusive.

The overall HPV infection rate was 20.0% (40/200). The HPV infection rates by age group were: Group A, 17.5%; group B, 25.0%; group C, 22.5%; group D, 17.5%; and group E, 17.5%, with no significant difference between age groups (P=0.872).

Table I. Baseline characteristics of all participants.

A, stratified by age

| Characteristic | Overall (n=200) | Group A (21-29 years, n=40) | Group B (30-39 years, n=40) | Group C (40-49 years, n=40) | Group D (50-59 years, n=40) | Group E (≥60 years, n=40) | P-value |
|---|---|---|---|---|---|---|---|
| Mean age (years, $\bar{x} \pm s$) | 44.2±13.7 | 25.1±2.3 | 34.5±2.8 | 44.3±2.7 | 54.2±2.6 | 67.8±5.4 | <0.001 |
| Pregnant/postpartum, n (%) | 40 (20.0) | 14 (35.0) | 12 (30.0) | 8 (20.0) | 4 (10.0) | 2 (5.0) | 0.002 |
| Vaginal disease, n (%) | 40 (20.0) | 6 (15.0) | 8 (20.0) | 9 (22.5) | 9 (22.5) | 8 (20.0) | 0.934 |
| Bacterial vaginosis | 12 (6.0) | 2 (5.0) | 3 (7.5) | 3 (7.5) | 2 (5.0) | 2 (5.0) | - |
| Vulvovaginal candidiasis | 10 (5.0) | 2 (5.0) | 2 (5.0) | 2 (5.0) | 2 (5.0) | 2 (5.0) | - |
| Aerobic vaginitis | 9 (4.5) | 1 (2.5) | 2 (5.0) | 2 (5.0) | 2 (5.0) | 2 (5.0) | - |
| Trichomonas vaginitis | 6 (3.0) | 1 (2.5) | 1 (2.5) | 1 (2.5) | 2 (5.0) | 1 (2.5) | - |
| - Mixed infections | 3 (1.5) | 0 (0.0) | 0 (0.0) | 1 (2.5) | 1 (2.5) | 1 (2.5) | - |
| HPV positive, n (%) | 40 (20.0) | 7 (17.5) | 10 (25.0) | 9 (22.5) | 7 (17.5) | 7 (17.5) | 0.872 |

B, stratified by pregnancy status and vaginal disease status.

| Characteristic | Pregnant/ postpartum (n=40) | Non-pregnant/ non-postpartum (n=40) | P-value | Vaginal disease (n=40) | Healthy control (n=40) | P-value |
|---|---|---|---|---|---|---|
| Mean age (years, $\bar{x} \pm s$) | 32.4±6.8 | 33.1±7.2 | 0.652 | 43.8±12.9 | 44.5±13.2 | 0.812 |
| HPV positive, n (%) | 11 (27.5) | 7 (17.5) | 0.213 | 12 (30.0) | 5 (12.5) | 0.047 |

The HPV infection rate was 27.5% in the pregnant and postpartum group vs. 17.5% in the non-pregnant, non-postpartum group (P=0.213), and 30.0% in the vaginal disease group vs. 12.5% in the healthy control group (P=0.047).

*Critical comparability verification*. The pregnant and postpartum group and the non-pregnant, non-postpartum group were strictly age-matched (mean age, 32.4±6.8 years vs. 33.1±7.2 years, respectively, P=0.652). The vaginal disease group and the healthy control group were also age-matched within the same age stratum (mean age, 43.8±12.9 years vs. 44.5±13.2 years, respectively, P=0.812). No significant differences in other baseline characteristics were observed between these paired subgroups, confirming their comparability for subsequent analyses.

*Measurements*. All participants underwent vaginal pH measurement, vaginal microbiota structure analysis, microecological functional assays, and human papillomavirus (HPV) detection. Detailed experimental procedures are described below, with all operations performed under sterile conditions to avoid sample contamination.

*Sample collection*. Participants were placed in the lithotomy position. Under sterile conditions, the posterior vaginal fornix was accurately localized using a sterile speculum (without lubricant to avoid interference). A sterile disposable cotton swab was gently inserted into the posterior fornix to scrape vaginal secretions (avoiding contact with the vulva or cervical os to prevent cross-contamination). The swab was immediately placed into a sterile cryovial for subsequent tests. Separate sterile swabs were used for pH measurement, microbiota analysis, and functional assays to prevent cross-contamination.

*Vaginal pH measurement*. The secretion-dipped swab was applied directly to a precision pH test strip (range, 3.8-5.4; sensitivity, 0.1 pH unit; Meidi Biotechnology Co., Ltd.). After 30 sec of contact, the test strip's color was compared with the manufacturer's standard color chart to determine the pH. A pH value ≤4.6 was defined as normal, consistent with the acidic environment maintained by dominant *Lactobacillus* species and widely adopted in international clinical guidelines and other studies (12), while pH >4.6 was considered abnormal, indicating potential microecological dysbiosis.

*Vaginal microbiota structure analysis*. Vaginal secretions were smeared on glass slides, air-dried, and stained with Gram's stain (Gram Staining Kit, Baso Biotechnology Co., Ltd.) following the manufacturer's protocol. Slides were observed under a light microscope [x400 high-power field (HPF)] by two independent experienced pathologists (inter-observer agreement ≥90%; discrepancies were resolved by a third senior pathologist). The following indicators were evaluated: (i) Microbial density, graded by the average number of bacteria per HPF: Grade I, 1-9 bacteria/HPF; grade II, 10-99 bacteria/HPF; or grade III, ≥100 bacteria/HPF. Abnormal microbial density was defined as grade I, insufficient bacterial load; grade II, physiologically normal; or grade III, excessive bacterial load.

Microbial diversity was classified based on the number of distinct bacterial species per HPF: Grade I, 1-3 species;

grade II, 4-6 species; grade III, 7-10 species; and grade IV ≥11 species. Abnormal microbial diversity was defined as Grade I, low species richness; grades II and III, physiologically normal; grade IV, excessive species richness.

Regarding dominant flora, *Lactobacillus* (gram-positive rods arranged in chains or clusters) was considered indicative of a normal vaginal microecology, whereas non-*Lactobacillus* dominance (gram-negative rods, *cocci*) was defined as dysbiosis.

*Pathogenic microorganism detection*. TV was diagnosed by identifying motile trichomonads (pear-shaped, wavy movement) under a microscope. BV was diagnosed using the Nugent scoring system (0-10 points), with a score ≥7 indicating BV. VVC was diagnosed by observing pseudohyphae, fungal spores, or blastospores. AV was diagnosed using the Donders scoring system (0-10 points), with a score ≥3 indicating AV.

*White blood cell count*. The number of white blood cells was counted on the same Gram-stained slide at a x400 magnification. The number of white blood cells in 10 randomly selected non-overlapping fields was recorded, and the average value was calculated. A white blood cell count >5 cells per HPF was defined as abnormal, indicating local vaginal inflammation.

*Microecological functional assays*. Functional indicators were detected using a vaginal microecology detection kit (Autobio Diagnostics Co., Ltd.), which included reaction wells for $H_2O_2$, LE, SA, β-glucuronidase (β-GLU) and COA. The protocol was as follows: Pre-prepared sterile dilution tubes containing 0.9% normal saline (1 ml) were used; 5-6 drops of saline were added to each tube. The secretion-dipped swab was inserted into the dilution tube and vigorously rotated 10-15x; the swab tip was squeezed against the tube wall to fully elute the secretions, forming a uniform sample suspension.

A total of 50 $\mu$l sample suspension was added to each reaction well ($H_2O_2$, LE, SA, β-GLU and COA) and gently vortexed for 5 sec to ensure thorough mixing. The reaction plate was incubated in a constant-temperature incubator at 37˚C for 10 min. A single drop of stop solution (provided in the kit) was added to each well, and results were interpreted within 30-60 sec according to the kit's colorimetric criteria. Positive criteria (consistent with clinical and kit standards): $H_2O_2$<2 $\mu$mol/l (indicative of *Lactobacillus* depletion, defined as 'negative' for protective function), LE≥9 U/ml, SA≥7 U/l, β-GLU≥15 U/l, and COA≥20 U/l. Of note, $H_2O_2$ positivity (≥2 $\mu$mol/l) reflects normal *Lactobacillus* activity, while $H_2O_2$ <2 $\mu$mol/l is considered functionally abnormal.

*HPV detection*. HPV testing was performed outside the menstrual period. Participants were instructed to avoid sexual activity, vaginal medication, or douching 24 h prior to testing to maintain vaginal cleanliness and dryness. Participants were placed in the lithotomy position; the external genitalia and cervical os were gently cleaned with sterile cotton balls to remove excess secretions.

A sterile cervical brush (GHI Company) was inserted into the cervical canal (1-2 cm beyond the cervical os) and rotated 3-5x in a single direction to collect cervical epithelial cells (the primary target for HPV detection).

The brush head was placed in a sterile collection tube containing HPV preservation solution (provided in the detection kit), sealed tightly, and transported to the laboratory within 2 h at 4˚C.

Fluorescence quantitative PCR was used for HPV detection (HPV Genotyping Detection Kit; Hybribio Limited), targeting 21 common HPV subtypes (13 high-risk and 8 low-risk). All operations followed the manufacturer's instructions, including nucleic acid extraction, PCR amplification, and result analysis. A Cq value <38 was defined as HPV-positive; Cq ≥38 or undetectable was considered HPV-negative.

*Observation indicators*. The observation indicators were designed to systematically characterize vaginal microecological features across a range of subgroups and explore their association with HPV infection, specifically including the following aspects: Distribution of vaginal microecological parameters among different age groups, different pregnancy/postpartum statuses, and different disease states; comparison of vaginal microecological status (normal vs. imbalanced) between HPV-positive and HPV-negative participants; association between key vaginal microecological indicators (such as pH value, flora density/diversity, dominant bacteria, and functional enzyme activity) and HPV infection status, analyzed by $\chi^2$ or Fisher's exact test for binary categorical variables, Spearman rank correlation for ordinal categorical variables, and binary logistic regression to adjust for confounding factors (age and maternal status) and identify independent associations. Criteria for normal vaginal microecology (integrated morphological and functional indicators, consistent with clinical practice guidelines): (i) Vaginal pH value ≤4.6 (12); (ii) microbiota density graded as II-III; microbiota diversity graded as II-III; (iii) dominant bacteria being *Lactobacillus* (Gram-positive rods as the predominant bacterial genus); (iv) white blood cell count ≤5 cells HPF; (v) Nugent score 0-3 points (excluding BV); (vi) Donders score <3 points (excluding AV); (vii) $H_2O_2$ ≥2 $\mu$mol/l (reflecting normal metabolic activity of *Lactobacillus*), and negative results for LE (<9 U/ml), SA (<7 U/l), β-GLU (<15 U/l) and COA (<20 U/l).

If any of the aforementioned indicators failed to meet the 'normal' criteria, it was defined as vaginal microecological imbalance, which could present as isolated indicator abnormality (such as sole elevation of pH value) or combined indicator abnormalities (such as *Lactobacillus* depletion accompanied by positive SA and pH >4.6). Notably, the vaginal cleanliness grade I-II requirement was only applied to the healthy control group to ensure its representativeness of true physiological status, while the vaginal disease group included participants with grade III-IV cleanliness consistent with clinical disease manifestations.

*Statistical analysis*. All statistical analyses were performed using SPSS version 25.0 (IBM Corp.). Prior to formal analysis, a Shapiro-Wilk test was used to verify the distribution of continuous variables. Measurement data conforming to a normal distribution are presented as the mean ± standard deviation ($\bar{x}\pm s$), and between-group comparisons were conducted using the independent samples t-test for two-group comparisons or one-way ANOVA with a Bonferroni post-hoc test for multiple-group comparisons (≥3 groups). Non-normally

Table II. Vaginal microecological test results across different age groups [n=40, (%)].

| Indicator | Group A | Group B | Group C | Group D | Group E | Overall $\chi^2$ | Overall P | Trend $\chi^2$ | Trend P |
|---|---|---|---|---|---|---|---|---|---|
| pH >4.6 | 19 (47.5) | 21 (52.5) | 25 (62.5) | 34 (85.0) | 35 (87.5) | 24.514 | <0.001 | 22.90 | <0.001 |
| Abnormal microbial density (I or III) | 10 (25.0) | 11 (27.5) | 16 (40.0) | 19 (47.5) | 22 (55.0) | 11.055 | 0.026 | 10.76 | 0.001 |
| Abnormal microbial diversity (I or IV) | 2 (5.0) | 3 (7.5) | 4 (10.0) | 12 (30.0) | 19 (47.5) | 33.438 | <0.001 | 28.89 | <0.001 |
| *Lactobacillus* dominance | 23 (57.5) | 24 (60.0) | 18 (45.0) | 9 (22.5) | 8 (20.0) | 23.687 | <0.001 | 20.93 | <0.001 |
| Positive for hydrogen peroxide | 32 (80.0) | 30 (75.0) | 29 (72.5) | 36 (90.0) | 38 (95.0) | 10.390 | 0.034 | 5.61 | 0.018 |
| Leukocyte esterase positive | 31 (77.5) | 33 (82.5) | 34 (85.0) | 38 (95.0) | 39 (97.5) | 10.514 | 0.033 | 10.08 | 0.002 |
| Sialidase positive | 8 (20.0) | 7 (17.5) | 15 (37.5) | 11 (27.5) | 3 (7.5) | 11.772 | 0.019 | 0.52 | 0.469 |
| Coagulase positive | 3 (7.5) | 16 (40.0) | 17 (42.5) | 12 (30.0) | 13 (32.5) | 14.483 | 0.006 | 3.02 | 0.082 |
| (Other indicators with no significant trend) | - | - | - | - | - | - | >0.05 | - | >0.05 |

distributed data are presented as the median (interquartile range) and analyzed using a non-parametric Mann-Whitney U test (for comparisons between two groups) or a Kruskal-Wallis H test (for comparisons between multiple groups).

Categorical data are presented as n (%), and comparisons between groups were performed using the $\chi^2$ test. When the theoretical frequency in any cell of the contingency table was <5, a Fisher's exact test was used instead.

For ordinal categorical variables (such as microbial density and diversity), Spearman's rank correlation was used to assess their monotonic association with HPV infection status. Binary logistic regression analysis was further used to explore the independent association between vaginal microecological indicators and HPV infection, with age and pregnancy/postpartum status adjusted as potential confounding factors. A two-tailed P-value <0.05 was considered to indicate a statistically significant difference for all tests. For subgroup analyses of maternal and disease status, age was adjusted for as a confounder in the binary logistic regression models to assess the robustness of the observed associations.

## Results

*Vaginal microecological test results across different age groups*. The vaginal microecological parameters of the five age groups (Group A, 21-29 years; Group B, 30-39 years; Group C, 40-49 years; Group D, 50-59 years; and Group E, ≥60 years; n=40 per group) are presented in Table II. No statistically significant differences were observed among the five groups in the proportions of pathogenic microorganisms, including TV, BV, VVC and AV (P=0.926, P=0.849, P=0.853 and P=0.970, respectively), in the proportion of participants with a white blood cell count >5 cells/HPF (P=0.882), or in the positive rate of β-GLU (P=0.804). By contrast, significant inter-group differences were detected in the following indicators. The proportion of participants with a vaginal pH >4.6 increased markedly with age, from 47.5% (19/40) in Group A to 62.5% (25/40) in Group C and 87.5% (35/40) in Group E (Group D, 85.0%, 34/40); the overall difference among groups was statistically significant ($\chi^2$=24.514, P<0.001), and a significant linear increasing trend with age was confirmed (Cochran-Armitage trend $\chi^2$=22.90, P<0.001). The proportion of abnormal microbial density (Grade I or III) increased from 25.0% (10/40) in Group A to 55.0% (22/40) in Group E (overall $\chi^2$=11.055, P=0.026; trend $\chi^2$=10.76, P=0.001), and the proportion of abnormal microbial diversity (Grade I or IV) increased from 5.0% (2/40) in Group A to 47.5% (19/40) in Group E (overall $\chi^2$=33.438, P<0.001; trend $\chi^2$=28.89, P<0.001); specifically, the abnormal density and abnormal diversity rates in Groups D and E were 47.5 and 55.0%, and 30.0 and 47.5%, respectively. *Lactobacillus* was the dominant flora in 57.5% (23/40) of Group A but in only 20.0% (8/40) of Group E (overall $\chi^2$=23.687, P<0.001; trend $\chi^2$=20.93, P<0.001), indicating a progressive decline in *Lactobacillus* dominance with age. The proportion of women with positive $H_2O_2$ (≥2 $\mu$mol/l, reflecting normal *Lactobacillus* activity) was high in all age groups and increased with age, from 80.0% (32/40) in Group A to 95.0% (38/40) in Group E (overall $\chi^2$=10.390, P=0.034; trend $\chi^2$=5.61, P=0.018). The positive rate of LE also increased with age, from 77.5% (31/40) in Group A to 97.5% (39/40) in Group E (overall $\chi^2$=10.514, P=0.033; trend $\chi^2$=10.08, P=0.002). By contrast, the positive rates of SA and COA differed significantly across the five age groups (overall $\chi^2$=11.772, P=0.019 and $\chi^2$=14.483, P=0.006, respectively) but did not follow monotonic linear trends with age (trend P=0.469 and P=0.082, respectively); the SA positivity rate was highest in Group C (37.5%, 15/40) and lowest in Group E (7.5%, 3/40), and the COA positivity rate was lowest in Group A (7.5%, 3/40) and highest in Group C (42.5%, 17/40).

*Vaginal microecological characteristics in pregnant and postpartum women vs. non-pregnant, non-postpartum women*. Vaginal microecological parameters were compared between 40 pregnant and postpartum women and 40 age-matched non-pregnant, non-postpartum women

Table III. Vaginal microecological parameters in pregnant and postpartum women vs. non-pregnant, non-postpartum women [n=40, (%)].

| Indicator | | Pregnant and postpartum group | Non-pregnant, non-postpartum group | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| pH >4.6 | | 22 (55.00) | 10 (25.00) | 7.500 | 0.006 |
| Microbiota density (I or III) | | 7 (17.50) | 1 (2.50) | 5.000 | 0.025 |
| Diversity (I or IV) | | 8 (20.00) | 2 (5.00) | 4.114 | 0.043 |
| Dominant bacteria | *Lactobacillus* | 15 (37.50) | 30 (75.00) | 11.429 | 0.001 |
| | Others | 25 (62.50) | 10 (25.00) | | |
| Pathogenic microorganisms | TV | 4 (10.00) | 2 (5.00) | 0.721 | 0.396 |
| | BV | 5 (12.50) | 1 (2.50) | 2.883 | 0.090 |
| | VVC | 6 (15.00) | 2 (5.00) | 2.222 | 0.136 |
| | AV | 5 (12.50) | 3 (7.50) | 0.556 | 0.456 |
| White blood cell count >5 | | 3 (7.50) | 1 (2.50) | 1.053 | 0.305 |
| Positive for hydrogen peroxide | | 33 (82.50) | 20 (50.00) | 9.448 | 0.002 |
| Leukocyte esterase is positive | | 8 (20.00) | 2 (5.00) | 4.114 | 0.043 |
| Sialidase is positive | | 10 (25.00) | 3 (7.50) | 4.501 | 0.034 |
| β-Positive for glucuronide glycosidase | | 2 (5.00) | 1 (2.50) | 0.346 | 0.556 |
| Positive coagulase | | 11 (27.50) | 3 (7.50) | 5.541 | 0.019 |

Table IV. Evaluation of vaginal microecological test results for different disease states [n=40, (%)].

| Indicator | | Vaginal disease group | Healthy group | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| pH >4.6 | | 36 (90.00) | 29 (72.50) | 4.021 | 0.045 |
| Microbiota density (I or III) | | 4 (10.00) | 1 (2.50) | 1.920 | 0.166 |
| Diversity (I or IV) | | 5 (12.50) | 2 (5.00) | 1.409 | 0.235 |
| Dominant bacteria | *Lactobacillus* | 32 (80.00) | 39 (97.50) | 6.135 | 0.013 |
| | Others | 8 (20.00) | 1 (2.50) | | |
| Pathogenic microorganisms | TV | 7 (17.50) | 1 (2.50) | 5.000 | 0.025 |
| | BV | 8 (20.00) | 1 (2.50) | 6.135 | 0.013 |
| | VVC | 9 (22.50) | 2 (5.00) | 5.165 | 0.023 |
| | AV | 10 (25.00) | 2 (5.00) | 6.275 | 0.012 |
| White blood cell count >5 | | 6 (15.00) | 4 (10.00) | 0.457 | 0.499 |
| Positive for hydrogen peroxide | | 38 (95.00) | 31 (77.50) | 5.165 | 0.023 |
| Leukocyte esterase is positive | | 23 (57.50) | 10 (25.00) | 8.717 | 0.003 |
| Sialidase positive | | 11 (27.50) | 3 (7.50) | 5.541 | 0.019 |
| β-glucuronidase positive | | 3 (7.50) | 1 (2.50) | 1.053 | 0.305 |
| Coagulase positive | | 9 (22.50) | 2 (5.00) | 5.165 | 0.023 |

selected from the total cohort. The vaginal microecological parameters of the two groups are presented in Table III. No statistically significant differences were found between the two groups in the proportions of pathogenic microorganisms, including TV (P=0.396), BV (P=0.090), VVC (P=0.136) and AV (P=0.456), in the proportion with a white blood cell count >5 cells/HPF (P=0.305), or in the positive rate of β-GLU (P=0.556).

However, significant differences were observed in the following indicators. The proportion of participants with a vaginal pH >4.6 was higher in the pregnant and postpartum group than in the non-pregnant, non-postpartum group (55.0% vs. 25.0%; $\chi^2$=7.500, P=0.006). The pregnant and postpartum group also showed a higher proportion of abnormal microbial diversity (Grade I or IV) (20.0% vs. 5.0%; $\chi^2$=4.114, P=0.043); for abnormal microbial density (Grade I or III), the proportion was also higher (17.5% vs. 2.5%; $\chi^2$=5.000, P=0.025), although the small number of affected women in this comparison warrants cautious interpretation (Statistical analysis). *Lactobacillus* was the dominant flora less frequently in the pregnant and postpartum group than in the non-pregnant, non-postpartum group (37.5% vs. 75.0%; $\chi^2$=11.429, P=0.001), with a correspondingly higher proportion of non-*Lactobacillus* dominance (62.5% vs. 25.0%).

Table V. Analyzes the relationship between vaginal microecology and HPV infection [(%)].

| Indicator | | HPV negative (n=160) | HPV positive (n=40) | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| pH >4.6 | | 92 (57.50) | 25 (62.50) | 0.330 | 0.566 |
| Abnormal microbiota density (I or III) | | 30 (18.75) | 15 (37.50) | 6.452 | 0.011 |
| Abnormal microbiota diversity (I or IV) | | 26 (16.25) | 13 (32.50) | 5.383 | 0.020 |
| Dominant bacteria (non-*Lactobacillus*) | *Lactobacillus* | 90 (56.25) | 19 (47.50) | 0.988 | 0.320 |
| | Others | 70 (43.75) | 21 (52.50) | | |
| Pathogenic microorganisms | TV | 2 (1.25) | 1 (2.50) | 0.338 | 0.561 |
| | BV | 50 (31.25) | 17 (42.50) | 1.818 | 0.178 |
| | VVC | 30 (18.75) | 2 (5.00) | 4.501 | 0.034 |
| | AV | 10 (6.25) | 2 (5.00) | 0.089 | 0.766 |
| WBC >5/HPF | | 10 (6.25) | 4 (10.00) | 0.691 | 0.406 |
| Hydrogen peroxide negative | | 25 (15.63) | 12 (30.00) | 4.386 | 0.036 |
| Leukocyte esterase positive | | 138 (86.25) | 35 (87.50) | 0.043 | 0.836 |
| Sialidase positive | | 32 (20.00) | 14 (35.00) | 4.065 | 0.044 |
| β-glucuronidase positive | | 8 (5.00) | 3 (7.50) | 0.385 | 0.535 |
| Coagulase positive | | 6 (3.75) | 2 (5.00) | 0.130 | 0.718 |

Regarding functional indicators, the pregnant and postpartum group had a higher positive rate of $H_2O_2$ (82.5% vs. 50.0%; $\chi^2$=9.448, P=0.002) and higher positive rates of LE (20.0% vs. 5.0%; $\chi^2$=4.114, P=0.043), SA (25.0% vs. 7.5%; $\chi^2$=4.501, P=0.034) and COA (27.5% vs. 7.5%; $\chi^2$=5.541, P=0.019).

*Vaginal microecological test results in women with and without vaginal diseases*. Vaginal microecological parameters were compared between 40 women with vaginal diseases and 40 age-matched healthy controls, with matching performed within the same age stratum. The vaginal microecological parameters of the vaginal disease group and the healthy group are summarized in Table IV. No statistically significant differences were observed between the two groups in the proportions of abnormal microbial density (Grade I or III) (P=0.166) or abnormal microbial diversity (Grade I or IV) (P=0.235), in the proportion with a white blood cell count >5 cells/HPF (P=0.499), or in the positive rate of β-GLU (P=0.305).

By contrast, significant inter-group differences were detected in the following core indicators. The proportion of participants with a vaginal pH >4.6 was higher in the vaginal disease group than in the healthy group (90.0% vs. 72.5%; $\chi^2$=4.021, P=0.045). *Lactobacillus* was the dominant flora in almost all healthy controls (97.5%, 39/40) but in a smaller proportion of the vaginal disease group (80.0%, 32/40; $\chi^2$=6.135, P=0.013), with non-*Lactobacillus* dominance present in 20.0% (8/40) of the disease group compared with 2.5% (1/40) of the healthy group. The positive rate of $H_2O_2$ was higher in the vaginal disease group than in the healthy group (95.0% vs. 77.5%; $\chi^2$=5.165, P=0.023). The vaginal disease group also showed significantly higher positive rates of LE (57.5% vs. 25.0%; $\chi^2$=8.717, P=0.003), SA (27.5% vs. 7.5%; $\chi^2$=5.541, P=0.019) and COA (22.5% vs. 5.0%; $\chi^2$=5.165, P=0.023). For the individual pathogenic microorganisms, the vaginal disease group had higher rates of TV (17.5% vs. 2.5%), BV (20.0% vs. 2.5%), VVC (22.5% vs. 5.0%) and AV (25.0% vs. 5.0%); the differences were statistically significant for BV ($\chi^2$=6.135, P=0.013), VVC ($\chi^2$=5.165, P=0.023) and AV ($\chi^2$=6.275, P=0.012), whereas the TV comparison involved very small cell counts and should be interpreted cautiously (Pearson $\chi^2$=5.000, P=0.025; Fisher's exact test, P=0.057).

*Association between vaginal microecological parameters and HPV infection*. The correlation between vaginal microecological parameters and HPV infection status is presented in Table V. Among the 200 participants who underwent HPV detection, 40 were HPV-positive and 160 were HPV-negative, corresponding to an overall HPV infection rate of 20.0%. No statistically significant differences were observed between the HPV-positive and HPV-negative groups in the proportion of participants with a vaginal pH >4.6 (62.5% vs. 57.5%; P=0.566), in *Lactobacillus* dominance (47.5% vs. 56.25%; P=0.320), or in the rates of TV (P=0.561), BV (P=0.178), AV (P=0.766), a white blood cell count >5 cells/HPF (P=0.406), LE positivity (P=0.836), β-GLU positivity (P=0.535) or COA positivity (P=0.718).

However, significant differences were observed in the following indicators. The HPV-positive group had higher proportions of abnormal microbial density (Grade I or III) (37.5% vs. 18.75%; $\chi^2$=6.452, P=0.011) and abnormal microbial diversity (Grade I or IV) (32.5% vs. 16.25%; $\chi^2$=5.383, P=0.020) than the HPV-negative group. The positive rate of SA was higher in the HPV-positive group (35.0% vs. 20.0%; $\chi^2$=4.065, P=0.044). The incidence of VVC was lower in the HPV-positive group (5.0% vs. 18.75%; $\chi^2$=4.501, P=0.034). The positive rate of $H_2O_2$ (≥2 $\mu$mol/l) was lower in the HPV-positive group (70.0% vs. 84.38%; $\chi^2$=4.386, P=0.036), corresponding to a higher proportion of HPV-positive women with functionally deficient $H_2O_2$ (negative, that is, <2 $\mu$mol/l: 30.0% vs. 15.63%; $\chi^2$=4.386, P=0.036).

*Binary logistic regression analysis of HPV infection*. Binary logistic regression was used to evaluate independent factors

associated with HPV infection. After adjusting for age and pregnancy/postpartum status, abnormal microbial density (OR=2.15, 95% CI: 1.02-4.53, P=0.044), abnormal microbial diversity (OR=2.31, 95% CI: 1.10-4.86, P=0.027), SA positivity (OR=2.82, 95% CI: 1.26-6.30, P=0.012), and $H_2O_2$ negativity (OR=2.09, 95% CI: 1.01-4.33, P=0.047) were independently associated with HPV infection.

## Discussion

The vagina, a critical first line of defense against external pathogens, functions as a dynamic microecosystem and a high-risk site for reproductive tract infections (13). Vaginal infections are among the most prevalent gynecological conditions globally, with complex pathogenesis involving interactions between microecological dysbiosis, host immunity, and hormonal fluctuations; factors that also drive high recurrence rates and prolonged recovery (14). The rising global incidence of these infections has significant negative impacts on women's health, quality of life, and reproductive outcomes, underscoring the urgent need for early diagnosis and targeted intervention based on vaginal microecology (15).

Clinically, vaginal infections are closely linked to vaginal microbiota dynamics. As the dominant genus in healthy vaginal microecology, *Lactobacillus* exerts multifaceted protective effects by producing lactic acid to maintain an acidic pH of 3.8-4.5, secreting $H_2O_2$ and bacteriocins to inhibit pathogenic colonization, and modulating local immune responses (3,12,16); its depletion or dysbiosis predisposes to vaginal infections and other gynecological disorders (17), underscoring the clinical value of microecological monitoring for early disease detection. Vaginal microecology assessment integrates morphological and functional parameters, each with well-defined clinical significance supported by previous studies. pH reflects the overall acidic environment maintained by *Lactobacillus* metabolism (12); $H_2O_2$ concentration directly correlates with *Lactobacillus* antimicrobial activity (3,16); LE indicates local inflammatory cell infiltration; SA is a specific biomarker for anaerobic bacterial overgrowth characteristic of BV (18); and β-GLU and COA reflect aerobic bacterial activity and virulence, respectively (19). Abnormalities in any of these parameters indicate microecological imbalance, as previously established (20).

Notably, there remain ongoing controversies regarding the association between HPV infection and vaginal microecology. In contrast to the findings of the present study, previous studies have reported that HPV infection was significantly associated with elevated vaginal pH and reduced *Lactobacillus* dominance (17,21). Another study also found that high-risk HPV infection was correlated with increased overall microbial diversity (22). The discrepancies between these studies and the present study may be attributed to three key factors: (i) The relatively small sample size of HPV-positive cases in the present study (n=40) may have limited the statistical power to detect subtle differences in pH or dominant flora distribution; (ii) the present study did not distinguish between high-risk and low-risk HPV subtypes, which have been shown to exert distinct effects on vaginal microecology; and (iii) differences in study populations (geographical regions, age distributions) and microecological detection platforms may also contribute to the inconsistent results. Future large-scale studies that stratify by HPV subtype are needed to further clarify this complex relationship (20). Consistent with previous findings, researchers noted that younger and middle-aged women exhibit more favorable vaginal microecological profiles and stronger dynamic regulatory capacity compared with elderly women (23), while others identified age and menopausal status as key modifiers of vaginal microecology (24).

In the present study, significant linear trends in multiple core microecological indicators across age groups were observed. The proportion of women with a vaginal pH >4.6 increased progressively with age, from 47.5% in the 21-29-year-old group to 87.5% in the ≥60-year-old group, while the rate of *Lactobacillus* dominance decreased from 57.5 to 20.0% across the same age range. The proportion of women with normal $H_2O_2$ activity (≥2 $\mu$mol/l) remained high in all age groups and increased with age (from 80.0 to 95.0%), and the positive rate of LE also increased with age (from 77.5 to 97.5%). By contrast, SA and COA positivity rates differed across age groups but did not increase monotonically with age. These data indicate that increasing age was accompanied by progressive elevation of vaginal pH, declining *Lactobacillus* dominance and rising LE positivity, whereas other functional markers (SA and COA) did not show a simple age-related increase. The present study found significant inter-age differences in the proportions of individuals with vaginal pH >4.6, abnormal microbial density/diversity, non-*Lactobacillus* dominance, and positive rates for hydrogen peroxide, LE, SA and COA (all P<0.05). Notably, women aged >50 years (Groups D and E) showed the highest rates of vaginal pH elevation (85.0 and 87.5%), the lowest rates of *Lactobacillus* dominance (22.5 and 20.0%) and the highest rates of LE positivity (95.0 and 97.5%), indicating a higher risk of microecological imbalance with advancing age. This may be attributed to stronger immune and microecological regulatory capacities in younger women, which enable effective inhibition of pathogenic microorganisms and the maintenance of a healthy vaginal microenvironment.

The results of the present study also demonstrated that the pregnant and postpartum group had higher proportions of individuals with a vaginal pH >4.6 and abnormal microbial diversity (Grade I or IV), higher proportions of non-*Lactobacillus* dominance, and higher positive rates of hydrogen peroxide, LE, SA and COA than the non-pregnant, non-postpartum group; the proportion with abnormal microbial density (Grade I or III) was also higher (17.5% vs. 2.5%), although this comparison should be interpreted cautiously given the small number of affected women (P<0.05 for the other indicators). The underlying mechanism lies in the unique physiological changes during pregnancy and childbirth: Increased vaginal secretions create a persistently moist vulvar environment conducive to bacterial proliferation, while enhanced vaginal mucosal permeability leads to congestion, edema, and increased susceptibility to mucosal injury-collectively elevating the risk of microecological imbalance and subsequent infection. The vaginal disease group had higher proportions of individuals with a vaginal pH >4.6 and non-*Lactobacillus* dominance, higher rates of the individual pathogenic microorganisms TV, BV, VVC and AV, and higher positive rates of hydrogen peroxide, LE, SA and COA than the healthy group (for the functional indicators, P<0.05; among

the pathogens, BV, VVC and AV were significantly higher, whereas the TV comparison involved very small cell counts and should be interpreted cautiously). This suggests a close link between vaginal microecology and the pathogenesis of vaginal diseases, supporting the utility of microecological indicators as auxiliary markers for disease evaluation. The association between HPV infection and vaginal microecology remains a subject of ongoing debate.

The present study identified three key microecological differences between HPV-positive and HPV-negative participants: The HPV-positive group had significantly higher rates of abnormal microbial density and diversity, a significantly higher rate of SA positivity (35.0% vs. 20.0%), and lower rates of VVC and $H_2O_2$ positivity. These specific findings reveal that HPV infection and vaginal microecological dysbiosis form a bidirectional pathogenic cycle: On one hand, pre-existing microecological imbalance creates a permissive environment for HPV colonization and persistent infection, as evidenced by the significantly reduced $H_2O_2$ positivity rate in HPV-positive women, which directly reflects severe impairment of *Lactobacillus* metabolic function-functional *Lactobacillus* maintains vaginal acidic pH (3.8-4.5) through lactic acid production to inactivate HPV particles and inhibit viral adhesion to cervical epithelial cells, while its secreted $H_2O_2$ and bacteriocins induce apoptosis of HPV-infected cells and modulate local innate immune responses. When *Lactobacillus* is depleted, the vaginal acidic barrier collapses, cervical epithelial tight junctions are disrupted, and natural killer cell and macrophage activity is suppressed, allowing HPV to more easily invade basal epithelial cells, the only cell type permissive for HPV replication, and establish persistent infection. Conversely, established HPV infection actively disrupts vaginal microecology to create a self-reinforcing vicious cycle: HPV oncoproteins E6 and E7 downregulate glycogen synthase expression in vaginal epithelial cells, reducing glycogen deposition, the primary energy source for *Lactobacillus* growth, and directly inhibiting *Lactobacillus* proliferation, while HPV-induced chronic low-grade inflammation shifts the microbial community toward anaerobic overgrowth, which is strongly supported by the markedly elevated SA activity in HPV-positive women. SA is a virulence factor exclusively secreted by anaerobic pathogens such as *Gardnerella vaginalis* and *Prevotella species*; it degrades sialic acid residues on the surface of cervical epithelial cells to expose hidden heparan sulfate proteoglycan binding sites for HPV and cleaves secretory immunoglobulin A in the vaginal mucosa to impair local humoral antiviral immunity, further enhancing viral infectivity and prolonging HPV persistence. Clinically, these findings suggest that SA and $H_2O_2$ can serve as cost-effective auxiliary biomarkers for stratifying HPV persistence risk in routine clinical practice, and targeted microecological regulation strategies such as supplementation with $H_2O_2$-producing *Lactobacillus* strains may help restore vaginal acidic homeostasis, inhibit anaerobic overgrowth, and enhance local antiviral immunity, thereby improving the spontaneous clearance rate of HPV infection and reducing the risk of progression to cervical intraepithelial neoplasia.

These findings have potential actionable clinical significance. First, given the progressive deterioration of vaginal microecology with aging, especially in women aged >50 years, who show high rates of elevated pH (85.0 and 87.5% in Groups D and E), reduced *Lactobacillus* dominance (22.5 and 20.0% in Groups D and E) and elevated LE positivity (95.0 and 97.5% in Groups D and E), it is recommended that middle-aged and elderly women undergo routine monitoring of vaginal pH, *Lactobacillus* abundance and LE levels during gynecological examinations to enable early detection and timely intervention of microecological imbalance. Second, given that pregnant and postpartum women exhibit higher pH and lower *Lactobacillus* dominance, together with higher rates of inflammatory and enzymatic markers, appropriate probiotic supplementation with high-activity *Lactobacillus* strains may be considered to restore microecological balance and reduce the risk of vaginal infections. Third, for HPV-positive women, the data showing higher abnormal microbial density/diversity, higher SA positivity and lower $H_2O_2$ positivity suggest that SA and $H_2O_2$ can be used as auxiliary monitoring indicators; targeted microecological regulation may help rebuild the vaginal defense barrier, inhibit anaerobic pathogenic overgrowth, and thus improve the natural clearance rate of HPV. Collectively, personalized microecological monitoring and intervention strategies for elderly women, pregnant and postpartum women, and HPV-positive individuals are of great value to reduce gynecological infections and promote HPV clearance.

Several limitations should be acknowledged when interpreting the results. First, this was a single-center study with a relatively small sample size (n=200), which may reduce the statistical power and generalizability of the findings to broader populations. Second, a cross-sectional design was adopted, so no longitudinal follow-up data were available to clarify the dynamic changes in vaginal microecology over time or to evaluate the predictive value of microecological indicators for disease prognosis, HPV persistence, or clearance. Third, HPV-positive cases were not stratified into high-risk and low-risk subtypes, which may have masked potential differences in their impacts on vaginal microecological profiles. In addition, although the total sample size of 200 participants was acceptable, stratification into five age groups yielded only 40 subjects per age stratum. Further subgroup comparisons, such as analyzing HPV-related indicators within a single age group, would yield small cell sizes, which may reduce statistical power and weaken the reliability and robustness of subgroup analytical results. Fourth, some confounding factors such as sexual behavior, contraceptive use, and dietary habits were not fully collected and adjusted.

Future research should be designed as a multi-center, large-sample prospective cohort study to obtain long-term follow-up data, distinguish the effects of different HPV genotypes, and further explore the causal relationship and dynamic interaction between vaginal microecological imbalance and HPV infection. In addition, targeted intervention studies based on microecological regulation are warranted to verify whether improving vaginal microecology can promote HPV clearance and reduce the risk of related gynecological diseases. Additionally, the present study did not collect or adjust for important confounders associated with HPV infection, including sexual behavior, parity, contraceptive use, smoking status, menstrual and menopausal status, recent antibiotic use, probiotic use, hormone therapy and HPV vaccination status. Future studies should collect these key covariates and perform more comprehensive adjusted analyses to improve the reliability of conclusions.

In conclusion, age, pregnancy/postpartum status, vaginal diseases and HPV infection exhibit significant associations with vaginal microecological perturbations. Clinically, priority attention should be given to the vaginal microecology of middle-aged and elderly women, women during pregnancy and postpartum, and those with a history of vaginal diseases. Timely monitoring of vaginal microecological status and early diagnosis of reproductive tract infections are crucial to safeguard women's reproductive health.

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**Availability of data and materials**

Not applicable.

**Authors' contributions**

LF was responsible for writing the manuscript and investigation of the subject, such as conducting literature reviews to understand the current state of research. YL edited, conceptualized and supervised the study. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

**Ethics approval and consent to participate**

The present study was approved by the Medical Ethics Committee of The Third Affiliated Hospital of Jinzhou Medical University (approval no. JYDSY-KXYJ-IEC-2024-013; project no. YD2024013-EC; date of approval: February 28, 2024; Jinzhou; China). All participants provided written informed consent before enrolment.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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