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Original Article

A cross-sectional study on the current status and influencing factors of high-risk HPV infection, vaginal microbiota imbalance, and cervical lesions in eligible women in the community

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Abstract: Cervical cancer remains a leading cause of cancer mortality among women globally, with persistent high-risk human papillomavirus (HPV) infection being the primary etiologic factor. However, the role of vaginal microbiota in modulating cervical lesion risk remains incompletely understood. This cross-sectional study enrolled 450 women aged 25-65 years from a community in Harbin, China, to investigate the prevalence of high-risk HPV infection, vaginal microbiota imbalance, and cervical lesions, and to examine their combined associations with cervical lesion risk. Participants underwent high-risk HPV genotyping, cervical liquid-based cytology (TCT), and vaginal microecological assessment including Lactobacillus grading, pH, bacterial vaginosis (BV), and microbial diversity. Participants with abnormal screening results (TCT ≥ atypical squamous cells of undetermined significance [ASC-US] or HPV16/18 positive) underwent colposcopy-directed biopsy. Cervical lesions were defined as histologically confirmed cervical intraepithelial neoplasia grade 1 or above (CIN1+). The prevalence of high-risk HPV was 16.2% (73/450), vaginal microbiota imbalance 40.2% (181/450), and CIN1+ 9.6% (43/450). Across the four groups defined by HPV and microbiota status, the CIN1+ detection rates were 2.1% in the double-negative, 8.6% in the microbiota-imbalance-only group, 19.4% in the HPV-only group, and 47.6% in the co-positive group (P<0.001). Adjusted odds ratios (aOR) for CIN1+ were 4.47 (95% CI: 1.52-13.14) for microbiota imbalance only, 12.09 (95% CI: 3.35-43.60) for HPV only, and 39.14 (95% CI: 13.13-111.73) for co-positive versus double-negative. The additive interaction indices were not statistically significant: relative excess risk due to interaction (RERI) = 23.58 (95% CI: -2.28 to 139.30), attributable proportion (AP) = 0.602 (95% CI: -0.078 to 0.882), and synergy index (SI) = 2.62 (95% CI: 0.925-9.873). Early sexual debut (<20 years, aOR=2.27) and ≥3 sexual partners (aOR=2.41) were independent risk factors. Although the co-occurrence of high-risk HPV and vaginal microbiota imbalance was strongly associated with cervical lesions, we found no statistical evidence of a synergistic interaction. These exploratory findings underscore the need for prospective studies to validate the observed associations and to investigate potential microbiota-targeted interventions.

Keywords: High-risk human papillomavirus (HPV), vaginal microbiota, cervical lesions, cross-sectional study, interaction

Introduction

Cervical cancer is the fourth most common malignant tumor among women worldwide, with an estimated 604,000 new cases and 342,000 deaths in 2020. China accounts for approximately one-fifth of the global burden. Persistent infection with high-risk human papillomavirus (HR-HPV) is widely recognized as the primary etiologic factor for cervical cancer [1, 2]. However, only a small minority of HR-HPV infected women eventually develop cervical intraepithelial neoplasia or invasive cancer, suggesting that host factors and the reproductive tract microenvironment may modulate viral pathogenesis [3, 4]. In healthy women, the vaginal microbiota is a complex ecosystem dominated by hydrogen-peroxide-producing Lactobacillus species, which maintain a low pH and inhibit pathogen colonization [5, 6]. Vaginal

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microbiota imbalance is characterized by a reduction or loss of lactobacilli, elevated pH, and overgrowth of anaerobic and facultative anaerobic bacteria, with bacterial vaginosis (BV) being the most common presentation [7]. Although previous studies have suggested a positive association between BV and HR-HPV infection, population-level evidence regarding their combined effect on cervical lesion risk remains limited and inconclusive [3, 8]. In addition, most previous cross-sectional surveys have focused on single factors (HPV or microbiota) and have rarely examined the interactive effects of both exposures, particularly in community-based general populations [9].

In most regions of China, cervical cancer screening relies primarily on HPV testing or cytology, and vaginal microbiota assessment has not yet been incorporated into routine screening system [10, 11]. Clarifying the combined effect of HR-HPV infection and vaginal microbiota imbalance may help optimize risk stratification, identify high-risk populations, and provide personalized interventions. Therefore, this community-based cross-sectional study aimed to describe the prevalence of HR-HPV infection, vaginal microbiota imbalance, and cervical lesions in women aged 25-65 years, with a particular focus on evaluating the potential synergistic effects of combined exposure on cervical lesions.

Data and methods

General information

This cross-sectional study employed a cluster sampling to recruit permanent female residents aged 25-65 years from a community affiliated with Harbin Medical University Cancer Hospital between June 2024 and May 2025.

Inclusion criteria were: (1) age 25-65 years; (2) residing in the community for at least 6 months; (3) having a sexual history; (4) voluntary participation with written informed consent. Exclusion criteria were: (1) pregnancy or lactation; (2) history of hysterectomy or cervical surgery; (3) sexual intercourse, vaginal douching, or vaginal medication within 48 hours; (4) known immunodeficiency or long-term immunosuppressant use. A total of 450 eligible women were enrolled. This study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital (Approval No. YD2024-15). All participants provided written informed consent prior to enrollment.

Data collection

Trained interviewers collected general information, including age, age at first sexual intercourse, lifetime number of sexual partners, and oral contraceptive use (defined as continuous use for ≥6 months) via face-to-face questionnaires. Sample size was estimated based on logistic regression requirements. Given an anticipated CIN1+ prevalence of approximately 10% and up to 8 predictors in the final model, we applied the Events Per Variable (EPV) criterion [12]. An EPV of at least 10 is generally recommended for reliable logistic regression. Thus, a minimum of 80 events (CIN1+ cases) was required. Accounting for the expected prevalence, we targeted a sample of approximately 400-450 participants to ensure adequate statistical power for multivariable modeling. The final sample of 450 participants, with 43 CIN1+ events, yielded an EPV of 5.4 (43 events/8 predictors), which is suboptimal and we acknowledge this as a limitation.

Before the investigation, all investigators received unified training and assessment. Laboratory testing was performed blinded to participants' clinical information. Pathological diagnosis was conducted using a double-blind method. Data entry was performed using EpiData with dual and consistency verification.

All participants underwent the following examinations: (1) High-risk HPV typing: PCR-reverse dot hybridization (Tiangen Biochemical Technology, Beijing, China) was used to detect 15 high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 82); (2) Cervical liquid-based thin-layer cytology (TCT): Results were interpreted using the Bethesda system; (3) Vaginal microecological assessment [12]: This included Lactobacillus grading (wet mount), vaginal pH (precision pH paper), BV diagnosis using Amsel's criteria (presence of ≥3 of the following: homogeneous discharge, clue cells, pH>4.5, and positive amine test), and microbial diversity (Gram stain). Vaginal microbiota imbalance was defined as the presence of at least one of the following: Lactobacillus grade IIb-III, pH>4.5, BV positivity, or microbiota diversity grade II-III.

Participants with TCT results of ASC-US or higher, or those positive for HPV16/18, were referred for colposcopy-directed biopsy. Histological diagnosis of CIN1+ was made by two pathologists independently, with discrepancies resolved by a third senior pathologist. Importantly, participants without these screening abnormalities did not undergo biopsy and were assumed to be free of cervical lesions. This differential verification (verification bias) is a major limitation, as biopsy referral depended on exposure (HPV) and screening test status, potentially leading to outcome misclassification, particularly among HPV-negative women.

To assess effect modification, an interaction term between age (continuous) and the combined exposure groups was planned a priori.

Participants were divided into four groups based on HPV and vaginal microbiota status: (1) Double negative (HPV-negative and microbiota-balanced); (2) Microecological imbalance only (HPV-negative and microecological-imbalanced); (3) HPV positive only (HPV-positive and microecological-balanced); (4) Co positive (HPV-positive and microbiota-imbalanced). Covariates included age (continuous, years), age of first sexual intercourse (<20 years), number of sexual partners (≥3), and long-term oral contraceptives use (yes/no).

For a more detailed understanding, separate analyses for each component of the microbiota imbalance (e.g., BV alone, pH>4.5 alone) were also conducted as secondary analyses (Supplementary Table 1).

All *p*-values for multiple comparisons were corrected using the Bonferroni method to control the family-wise error rate.

There were no missing data for any of the variables included in the primary analysis.

Statistical analysis

Statistical analyses were performed using Python 3.14.3. The original Python script is provided in Attachment 1. Continuous variables were first assessed for normality using Shapiro-Wilk test and homogeneity of variance (Levene's test). Data conforming to normal distribution were expressed as mean ± standard deviation, and intergroup comparisons were performed using independent samples t-test. Categorical variables were expressed as numbers and percentages, and compared using chi-square test, or Fisher's exact test when expected frequencies were <5. Univariate and multivariate logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI). For multivariable logistic regression, variables with P<0.10 in univariate analysis were entered into the initial model, and a backward stepwise elimination procedure (likelihood ratio criterion) was applied to select the final model. The multivariate model included age, age at first intercourse, number of sexual partners, and oral contraceptive use as potential confounders.

For the four-group comparisons (double negative, micro-ecological imbalance only, HPV only, co-positive), global chi-square tests were performed. Post-hoc pairwise comparisons were adjusted using the Bonferroni correction.

Additive interaction was evaluated using the following measures: relative excess risk due to interaction (RERI) = OR_{11} - OR_{10} - OR_{01}+1; attributable proportion (AP) = RERI/OR_{11}; and synergy index (SI) = (OR_{11} -1)/[(OR_{10} -1)+(OR_{01} -1)]. Here, OR_{10}, OR_{01}, and OR_{11} are the adjusted odds ratios of the HPV-only, microbiota-imbalance-only, and co-positive groups, respectively, relative to the double-negative group. Use Bootstrap method (1000 resamples) were used to estimate the 95% CIs for RERI, AP, and SI. A statistically significant interaction was indicated if the 95% CIs for RERI and AP excluded 0, and the 95% CI for SI excluded 1. All tests were two-sided, with statistical significance set at α=0.05. Model performance was assessed using the area under the receiver operating characteristic curve (AUC) and the Hosmer-Lemeshow goodness-of-fit test.

Results

Overall prevalence

A total of 450 community-dwelling women aged 25-65 years were included, with a mean age of 45.5±11.9 years. As shown in **Table 1**, the HR-HPV positivity rate was 16.2% (73/450), and the vaginal microbiota imbalance rate was 40.2% (181/450). The detection rate of CIN1+ was 9.6% (43/450), of which CIN2+ accounted for 4.9% (22/450). Detailed individual micro-

Table 1. Overall prevalence rates

Indicator	N	n (%)	95% CI (Wilson)
HR-HPV Positive	450	73 (16.22%)	(13.10%, 19.91%)
Vaginal Micro-ecological Imbalance	450	181 (40.22%)	(35.79%, 44.82%)
Lactobacillus Reduction (IIb-III)	450	127 (28.22%)	(24.24%, 32.60%)
Vaginal pH>4.5	450	112 (24.89%)	(21.07%, 29.14%)
BV Positive	450	60 (13.33%)	(10.52%, 16.76%)
Microbial Diversity Increased (II-III)	450	113 (25.11%)	(21.13%, 29.07%)
Cervical Lesion CIN1+	450	43 (9.56%)	(7.17%, 12.62%)
Cervical Lesion CIN2+	450	22 (4.89%)	(3.25%, 7.29%)

Note: Among the 450 participants, the HR-HPV positive rate was 16.2% (95% CI: 13.1%-19.9%), and the vaginal micro-ecological imbalance rate was 40.2% (95% CI: 35.8%-44.8%). Regarding individual micro-ecological indicators, lactobacillus reduction (28.2%), elevated pH (24.9%), BV positivity (13.3%), and increased microbial diversity (25.1%) were observed. The CIN1+ detection rate was 9.6% (95% CI: 7.2%-12.6%), and CIN2+ was 4.9% (95% CI: 3.3%-7.3%).

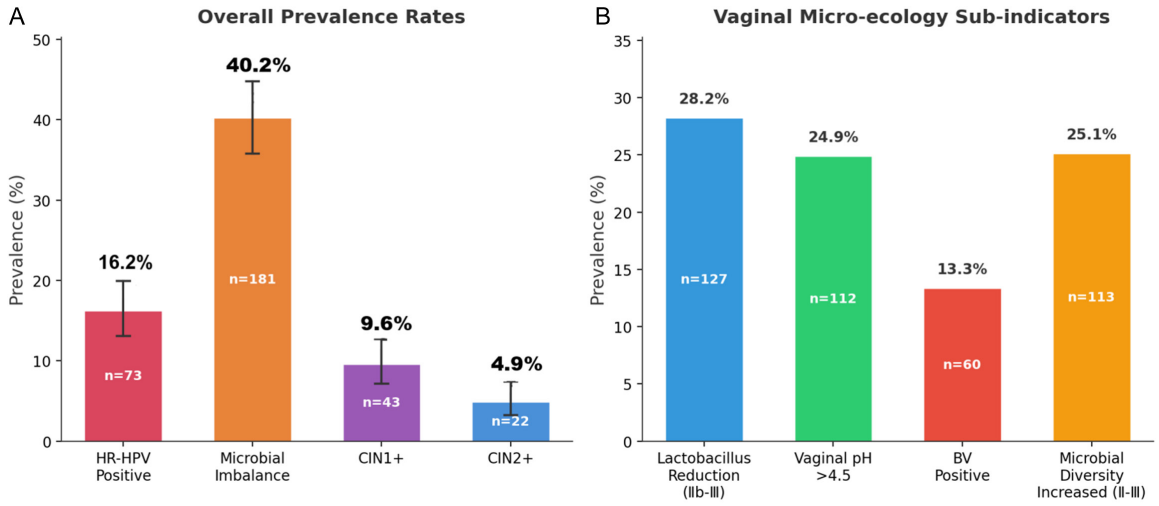


Figure 1. Overall prevalence visualization. A. Overall prevalence of HR-HPV, microbiota imbalance, and cervical lesions; B. Prevalence of individual vaginal microecological indicators.

ecological indicators with their 95% Wilson confidence intervals are presented in **Table 1** and visualized in **Figure 1**.

The cervical lesion detection rates by joint exposure group were as follows: double-negative (n=238, 52.9%) - CIN1+ 2.1% (5/238); microbiota imbalance only (n=139, 30.9%) - CIN1+ 8.6% (12/139); HPV positive only (n=31, 6.9%) - CIN1+ 19.4% (6/31); and co-positive (n=42, 9.3%) - CIN1+ 47.6% (20/42) (global χ^2=139.0, P<0.001). The corresponding CIN2+ rates were 0.4%, 3.6%, 16.1%, and 26.2%, respectively (**Table 2**).

Baseline characteristics

The study participants were divided into two groups according to the presence or absence of CIN1+. As shown in **Table 3**, compared with women without CIN1+ (n=407), those with CIN1+ (n=43) had higher proportions of women with early sexual debut (<20 years: 53.5% vs. 31.2%, P=0.005), multiple sexual partners (≥3: 37.2% vs. 20.6%, P=0.022), HR-HPV positivity (60.5% vs. 11.5%, P<0.001), and vaginal microbiota imbalance (74.4% vs. 36.6%, P<0.001). No significant differences were observed in age (P=0.585) or long-term oral contraceptive use (P=1.000). After Bonferroni correction for multiple comparisons (adjusted α=0.05/6≈0.008), only HR-HPV positivity (P<0.001) and vaginal microbiota imbalance (P<0.001) remained statistically significant. The associations for early sexual debut and multiple partners were not significant after correction.

Table 2. CIN1+ and CIN2+ by joint exposure groups

Group	N	CIN1+ n (%)	CIN2+ n (%)	*p*-value
Double negative	238	5 (2.10%)	1 (0.42%)	--
Micro-ecological imbalance only	139	12 (8.63%)	5 (3.60%)	--
HR-HPV positive only	31	6 (19.35%)	5 (16.13%)	--
Co-positive	42	20 (47.62%)	11 (26.19%)	<0.001
				χ^2=139.0, <0.001**

Note: The CIN1+ detection rate increased progressively from the double negative group (2.1%) through micro-ecological imbalance only (8.6%) and HPV positive only (19.4%) to the co-positive group (47.6%). The chi-square test showed highly significant differences across groups (P<0.001), indicating that the joint presence of HR-HPV infection and vaginal micro-ecological imbalance is associated with a substantially higher risk of cervical lesions. A similar trend was observed for CIN2+. **P<0.01.

Table 3. Baseline characteristics by CIN1+ status

Variable	Total (N=450)	CIN1- (n=407)	CIN1+ (n=43)	*P*-value
Age (years), mean ± SD	45.5±11.9	45.6±11.8	44.6±12.5	0.585[1]
Early sexual debut (<20 y), n (%)	150 (33.3%)	127 (31.2%)	23 (53.5%)	0.005**
Multiple partners (≥3), n (%)	100 (22.2%)	84 (20.6%)	16 (37.2%)	0.022*
Long-term OCP use, n (%)	73 (16.2%)	66 (16.2%)	7 (16.3%)	1.000
HR-HPV positive, n (%)	73 (16.2%)	47 (11.5%)	26 (60.5%)	<0.001***
Vaginal micro-ecological imbalance, n (%)	181 (40.2%)	149 (36.6%)	32 (74.4%)	<0.001***

Note: [1]Independent samples t-test; all others: χ^2 test. After Bonferroni correction (adjusted α=0.008), only HR-HPV positivity and vaginal micro-ecological imbalance remained statistically significant. *, **, and *** indicate statistical significance at the 10%, 5%, and 1% levels, respectively (two-tailed tests).

Table 4. Univariate logistic regression results (CIN1+)

Variable	OR	95% CI	*P*-value
Age (per year)	0.99	(0.97, 1.02)	0.5850
Early sexual debut (<20 y)	2.54	(1.34, 4.78)	0.0040
Multiple partners (≥3)	2.28	(1.17, 4.42)	0.0150
Long-term OCP use	1.00	(0.43, 2.35)	0.9920
HR-HPV Positive	11.71	(5.92, 23.19)	<0.001
Microbial Imbalance	5.04	(2.47, 10.29)	<0.001
Group: Micro. Only (ref=Double Neg.)	4.40	(1.52, 12.78)	0.0060
Group: HPV Only (ref=Double Neg.)	11.18	(3.18, 39.29)	<0.001
Group: Co-positive (ref=Double Neg.)	42.36	(14.49, 123.88)	<0.001

Note: In univariate analysis, HR-HPV infection (OR=11.71, 95% CI: 5.92-23.19, P<0.001) and vaginal micro-ecological imbalance (OR=5.04, 95% CI: 2.47-10.29, P<0.001) were the strongest individual risk factors for CIN1+. Early sexual debut (OR=2.54, P=0.004) and multiple partners (OR=2.28, P=0.015) were also significant. When analyzed as joint groups, the co-positive group showed the highest odds (OR=42.36, 95% CI: 14.49-123.88), suggesting a synergistic effect.

Cervical lesion rates by joint exposure groups

The four-group comparison is detailed in **Table 2**. The CIN1+ detection rates increased progressively across groups (χ^2=139.0, P<0.001), and a similar trend was observed for CIN2+.

Univariate logistic regression analysis

As shown in **Table 4**, HR-HPV positivity (OR= 11.71, 95% CI: 5.92-23.19), vaginal microbiota imbalance (OR=5.04, 95% CI: 2.47-10.29), early sexual debut <20 years (OR=2.54, 95% CI: 1.34-4.78), and ≥3 sexual partners (OR=2.28, 95% CI: 1.17-4.42) were all associated with CIN1+. Using the double-negative group as reference, the ORs for the microbiota-imbalance-only, HPV-only, and co-positive groups were 4.40 (95% CI: 1.52-12.78), 11.18 (95% CI: 3.18-39.29), and 42.36 (95% CI: 14.49-123.88), respectively.

Table 5. Multivariate logistic regression results (CIN1+)

Variable	aOR	95% CI	*P*-value	Significance
Microbial Imbalance Only	4.47	(1.52, 13.14)	0.0060	**
HR-HPV Positive Only	12.09	(3.35, 43.60)	<0.001	***
Co-positive (Both)	39.14	(13.13, 116.73)	<0.001	***
Age (per year)	0.99	(0.96, 1.02)	0.6010	ns
Early sexual debut (<20 y)	2.27	(1.10, 4.68)	0.0270	*
Multiple partners (≥3)	2.41	(1.11, 5.23)	0.0270	*
Long-term OCP use	1.15	(0.43, 3.07)	0.7790	ns

Note: Model adjusted for all listed variables. Reference group for joint exposure: double negative. Model Fit: McFadden pseudo R^2=0.2624, AUC=0.85, Hosmer-Lemeshow P=0.32. *, **, and *** indicate statistical significance at the 10%, 5%, and 1% levels, respectively (two-tailed tests).

Table 6. Additive interaction measures

Measure	Estimate	Bootstrap 95% CI	Interpretation
RERI	23.58	(-2.28, 139.30)	Not statistically significant
AP	0.602	(-0.078, 0.882)	Not statistically significant
SI	2.62	(0.925, 9.873)	Not statistically significant

Multivariate logistic regression analysis

After adjusting for age, age at first intercourse, number of sexual partners, and oral contraceptive use, the adjusted odds ratios (aOR) for CIN1+ remained significantly elevated: microbiota imbalance only - aOR=4.47 (95% CI: 1.52-13.14, P=0.006); HPV only - aOR=12.09 (95% CI: 3.35-43.60, P<0.001); co-positive - aOR=39.14 (95% CI: 13.13-111.73, P<0.001). Early sexual debut <20 years (aOR=2.27, 95% CI: 1.10-4.68, P=0.027) and ≥3 sexual partners (aOR=2.41, 95% CI: 1.11-5.23, P=0.027) remained independent risk factors, whereas age and oral contraceptive use did not (**Table 5**). The model showed good discrimination (AUC=0.85) and calibration (Hosmer-Lemeshow P=0.32).

Additive interaction

Using the adjusted ORs from the multivariable model (OR_{01}=4.47, OR_{10}=12.09, OR_{11}=39.14), the additive interaction indices were: RERI= 23.58 (Bootstrap 95% CI: -2.28 to 139.30), AP=0.602 (95% CI: -0.078 to 0.882), and SI=2.62 (95% CI: 0.925-9.873). As the 95% CIs for RERI and AP included 0, and the 95% CI for SI include 1, there was no statistically significant additive interaction. However, the point estimates suggest a possible synergistic effect that warrants further investigation. Due to the verification bias inherent in the study design, the observed effect sizes, especially the high aOR for the co-positive group, may be overestimated (**Table 6**).

Sensitivity analysis

Due to the limited number of CIN2+ events (n=22), multivariable adjustment would lead to severe overfitting (EPV<5). Therefore, we present only univariate logistic regression results for CIN2+ in **Table 7**. The unadjusted ORs were: microecological imbalance only - 9.75 (95% CI: 1.13-84.26, P=0.038); HPV only - 44.82 (95% CI: 5.06-396.79, P=0.001); co-positive - 81.46 (95% CI: 10.26-647.20, P< 0.001). These results should be interpreted with caution owing to the small case number and wide confidence intervals. Interaction indices were not estimated for CIN2+ because of insufficient power.

Subgroup analysis

In a pre-specified analysis, we tested for interaction between age (continuous) and the combined exposure groups by including a product term in the logistic model. The interaction term was not statistically significant (P for interaction = 0.23), suggesting that the combined exposure effect does not vary significantly with age.

Table 7. Univariate Logistic Regression for CIN2+ (descriptive, unadjusted)

Variable	OR	95% CI	*P*-value
Microbial Imbalance Only	9.75	(1.13, 84.26)	0.038
HR-HPV Positive Only	44.82	(5.06, 396.79)	0.001
Co-positive (Both)	81.46	(10.26, 647.20)	<0.001
Age (per year)	1.00	(0.97, 1.04)	0.827
Early sexual debut (<20 y)	2.91	(1.20, 7.05)	0.018
Multiple partners (≥3)	1.10	(0.40, 3.04)	0.857
Long-term OCP use	1.24	(0.41, 3.76)	0.706

Note: Only 22 CIN2+ events; multivariable adjustment is not attempted due to severe overfitting (EPV ~3.1). These estimates should be interpreted with extreme caution and are considered purely exploratory.

Discussion

This cross-sectional study of community-dwelling women describes the prevalence of HR-HPV infection, vaginal microbiota imbalance, and cervical lesions, and explores their potential combined association. Our findings indicate that the co-occurrence of HR-HPV and vaginal microbiota imbalance is strongly associated with an increased risk of CIN1+. However, the additive interaction indices were not statistically significant, and these findings should be interpreted as exploratory and hypothesis-generating.

Compared with previous studies, the HR-HPV positivity rate in this study (16.2%) is consistent with the range reported in multiple community-based surveys in China (10%-20%) [13-15]. The vaginal microbiota imbalance rate (40.2%) is slightly higher than the reported 30% to 35% in some healthy populations, which may be due to the inclusion of more asymptomatic individuals with intermediate microbiota status (e.g., Lactobacillus grade IIb and microbiota diversity grade II) in this study [16]. The CIN1+ detection rate was 9.6%, which is higher than that reported in some general population-based studies [17]. One possible explanation is that our screening strategy - using HPV testing combined with TCT with more sensitive biopsy indications (ASC-US or higher, or HPV16/18 positive) - may have detected more low-grade lesions. However, this may also be a consequence of significant verification bias, as outcome status was not independently confirmed for participants with negative screening results.

The most critical limitation is the severe verification bias. By only performing biopsies on women with abnormal screening results, we have likely created a scenario where the detection of CIN1+ is dependent on exposure status. This differential misclassification of the outcome could substantially inflate the observed associations and is a major threat to the validity of our conclusions. Although the point estimates for interaction are suggestive, they are derived from data with this inherent bias and thus cannot be considered conclusive.

Several important confounders, including smoking, parity, HPV vaccination status, and socioeconomic factors, were not collected. These are known to influence both vaginal microbiota and HPV persistence, and their absence may have led to residual confounding. In particular, HPV vaccination status is a critical missing variable that could have significantly impacted HPV positivity rates and lesion risk.

Regarding the combined effect of vaginal microbiota and HPV, previous meta-analyses have suggested a positive association between BV and HPV infection [3, 8, 18]; however, direct evaluation of their interaction on cervical lesions is relatively limited. Mitra et al. reported in a case-control study that vaginal microbiota diversity was significantly higher in women with cervical lesions than in healthy controls, and that Lactobacillus abundance was negatively correlated with lesion severity [19]. However, that study did not systematically calculate additive interaction indices. Another population-based study from China reported that the risk of CIN2+ was significantly increased when BV coexisted with HR-HPV, but the interaction effect was not further quantified [20]. Research on Korean women also reported a significant synergistic effect between cervical microbiota dysbiosis and HPV (RERI=15.9, SI=1.93) [21]. Although that study explored cervical micro-

biota while ours focuses on vaginal microbiota, both support the notion that microbiota imbalance is a key synergistic factor in the progression of HPV-related lesions. In addition, this study provides estimates of RERI and SI in a community-based population, and the results are comparable to those of the aforementioned studies. In contrast, our study did not find statistically significant additive interaction, which may be due to insufficient statistical power, verification bias, and the heterogeneous composite exposure definition.

Although mechanistic hypotheses suggest that microbiota imbalance may impair local immunity and facilitate HPV persistence [22, 23], our cross-sectional design and non-significant interaction indices preclude causal inference. Reverse causality (HPV-induced microbiota alteration) is equally plausible, and the temporal sequence remains unresolved.

Despite the methodological limitations, our results suggest that simultaneous assessment of vaginal microbiota status in HPV-positive women might help refine risk stratification. For co-positive individuals, even with normal cytology, closer follow-up or additional evaluation could be considered, but this requires prospective validation. Currently, vaginal microbiota assessment is not part of routine screening guidelines [10, 11]. Our exploratory findings do not warrant a change in clinical practice but highlight a promising research avenue. If causal relationships are confirmed in future well-designed prospective cohorts, microbiota-targeted interventions (e.g., probiotics) might become adjunctive strategies [24].

There are several shortcomings in this study. First, the cross-sectional design precludes causal inference; Second, single-timepoint microbiota measurement may not reflect long-term exposure; Third, important unmeasured confounders (smoking, parity, HPV vaccination, socioeconomic status) may have resulted in residual confounding; Fourth, the CIN2+ analysis was underpowered (only 22 events) and should be considered purely descriptive; Fifth, the generalizability is limited to a single community in Harbin; Sixth, verification bias is a major threat to validity, likely inflating effect estimates; Seventh, the EPV for the primary CIN1+ model was only 5.4, below the recommended minimum of 10, reducing the reliability of the adjusted odds ratios. We have provided the full analysis code as supplementary material to ensure transparency.

In summary, this cross-sectional study suggests that HR-HPV infection and vaginal microbiota imbalance are common among community-dwelling women. Their co-occurrence is strongly associated with an increased risk of cervical lesions, although the additive interaction was not statistically significant. Simultaneous assessment of vaginal microbiota status in HPV screening may improve risk stratification accuracy. However, further prospective studies with rigorous design are needed to verify the causal relationship and explore whether microbial ecological interventions can effectively reduce the risk of HPV-related cervical lesions.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Univariate and HPV-adjusted associations of individual vaginal microecological components with CIN1+

Component	n (%)	Unadjusted OR (95% CI)	Adjusted for HPV* OR (95% CI)
Lactobacillus reduction (IIb-III)	127 (28.2%)	1.85 (0.95-3.62)	1.42 (0.70-2.88)
Vaginal pH>4.5	112 (24.9%)	3.24 (1.70-6.18)	2.56 (1.29-5.08)
BV positive	60 (13.3%)	4.68 (2.42-9.05)	3.40 (1.70-6.80)
Microbial diversity increased (II-III)	113 (25.1%)	2.51 (1.28-4.92)	1.98 (0.97-4.02)

Note: *Adjusted for HPV status (positive/negative). All ORs were derived from univariate logistic regression models; adjustment for HPV was performed by including HPV status as a covariate.