**Vaginal dysbiosis, human papillomavirus and cervical cancer: systematic review and meta-analysis.**

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### Condensation: This study supports a causal link between vaginal dysbiosis and cervical cancer along the oncogenic HPV acquisition, persistence, and cervicovaginal dysplasia development pathway.

**Short title:** Bacterial vaginosis, HPV and cervical cancer

**AJOG at a glance:**

1. We conducted this systematic review and meta-analysis to assess if vaginal dysbiosis affects the risk of becoming infected with HPV; clearning HPV and developing cervical dysplasia.
2. Vaginal dysbiosis seems to be a risk factor for acquiring HPV, persistence of HPV and cervical dysplasia
3. This study is the first to pool evidence from longitudinal studies only, while previous systematic reviews were based on cross-sectional data only. We also included all diagnostic techniques to assess the vaginal microbiome (microscopy/molecular).

**ABSTRACT (262 words)**

### Objective: The vaginal microbiota proposedly influence the association between human papillomavirus and cervical cancer. Our aim was to assess if vaginal dysbiosis affects human papilloma virus acquistion, persistence, and progression to related cervical pre-malignancy.

### Data soruces: MEDLINE, Embase, CINAHL, Cochrane Library, and Web of Science (inception until June 2018). The study protocol was registered at PROSPERO (CRD42016035620).

### Study eligibility criteria: This systematic review included all observational studies reporting on incident human papilloma virus, persistent human papilloma virus and/or related cervical disease, in women with or without vaginal dysbiosis prior to outcome assessment.

### Study appraisal and synthesis methods: We used random effects models for meta-analyses and report pooled relative risks with 95% confidence intervals. The risk for incident and/or persistent human papilloma virus, or related cervical disease based on longitudinal results.

**Results**: Out of 1,645 unique articles, 15 mainly prospective cohort studies were included, published between 2003 and 2017, including a total of 101,049 women. Vaginal dysbiosis was associated with an increased risk of incident human papilloma virus (overall relative risk=1.33, 1.18–1.50, I2=0%; among young women relative risk=1.43, 1.10–1.85, I2=0%), human papilloma virus persistence (overall relative risk=1.14, 1.01–1.28, I2=44.2%; for oncogenic types relative risk=1.18, 1.01–1.38, I2=0%), and high-grade lesions and cancer (relative risk=2.01, 1.40–3.01, I2=0%), but women with lesions/cancer were compared to those without regardless of their oncogenic human papilloma virus status. Overall, comparable results were found in the molecular vaginal microbiota studies.

### Conclusions: This study supports a causal link between vaginal dysbiosis and cervical cancer along the oncogenic human papillomavirus acquisition, persistence, and cervicovaginal dysplasia development pathway.

**Key words**: human papillomavirus; hpv; bacterial vaginosis; microbiome;vaginal dysbiosis.

**Introduction**

Cervical cancer is considered a largely preventable disease due to population-based screening and more recent vaccination programs in high-income countries, though it remains the fourth most common cancer in women worldwide.[1](#_ENREF_1),[2](#_ENREF_2) Of the estimated 527,600 new cases and 265,700 related deaths annually, most occur to women in low-and middle-income countries.[1](#_ENREF_1),[2](#_ENREF_2)

It is well established, both epidemiologically and mechanistically, that cervical cancer and its premalignant precursor stages (cervical intraepithelial neoplasia or CIN) are causally related to oncogenic types of the human papillomavirus (HPV).[3](#_ENREF_3),[4](#_ENREF_4) However, from a public health perspective, the association is less-than-straightforward. Most women across the globe are infected at least once with one or more HPV types in their lifetime, but demonstrable persistence of oncogenic HPV types poses a direct risk of progression to premalignancy and invasive cervical cancer in only some individuals.[5](#_ENREF_5) This suggests that other, largely undetermined co-factors are at play,[6](#_ENREF_6) with vaginal dysbiosis emerging as a potential driver of HPV-related disease outcomes.[6](#_ENREF_6),[7](#_ENREF_7) In two meta-analyses of mostly unadjusted cross-sectional data, dysbiosis was associated with prevalent HPV infection and prevalent CIN.[8](#_ENREF_8),[9](#_ENREF_9) The latter studies did not allow for causal inferences, while also prone to confounding, as vaginal dysbiosis and HPV infection share a number of risk factors,including sexual behavior and smoking.[6](#_ENREF_6)

**Objective**

We postulate that vaginal dysbiosis is a putative, potentially modifiable[10](#_ENREF_10) risk factor to HPV acquisition, persistence, and related cervical disease, and have systematically reviewed the available evidence obtained through relevant longitudinal studies.

**Methods**

**Eligibility criteria, information sources, search strategy**

We conducted a systematic literature review and meta-analysis according to the PRISMA statement,[11](#_ENREF_11) and searched MEDLINE (1966 onwards), EMBASE (1946 onwards), CINAHL (1997 onwards), Cochrane Database (1999 onwards), and Web of Science (1955 onwards), without limits or language restriction, up to June 11, 2018. The Boolean search string used in Web of Science, was “(microbiome or microbiota or flora or microflora or vaginitis or vaginosis or dysbiosis or dysbacteriosis) and (vaginal or vagina or cervix or cervical or cervicovaginal or female or women or woman) and (alpha-papillomavirus or HPV or human papillomavirus or uterine cervical neoplasms or cancer or dysplasia or neoplasia or squamous intraepithelial lesions or LSIL or HSIL or CIN)”. Full search strings for all databases are detailed in A.1. Only full original manuscripts were included, but relevant conference abstracts were cross-checked for corresponding full-text papers. For each eligible study, we cross-checked cited references, as well as citing references in Web of Science. Initial eligibility screening of all records retrieved was performed in triplicate (by HV, SS, and NB), and no conflicts arose.

**Study selection**

As described based on the PICOS acronym for Population (P), Intervention/Exposure (I), Comparison (C), Outcome (O) and Study Design (S), we included original studies with longitudinal cohort designs as well as nested case-control studies (S), that compared women (P) with or without vaginal dysbiosis (E & C) and assessed the risk of at least 1 of the following outcomes (O) a) HPV incidence (defined as detection of an HPV type, not previously identified in a given woman), and/or b) HPV persistence (defined as absence of clearance or significantly delayed clearance relative to a reference group), and/or c) related squamous epithelial lesions provided vaginal dysbiosis was considered as a risk factor by study design. Studies were eligible if at least two measurement points were described for at least one outcome of interest (a minimal time-interval was not pre-defined).

Vaginal dysbiosis was broadly defined as deviation from a *Lactobacillus*-dominated microbiota[12](#_ENREF_12) as assessed by microscopy or molecular techniques, specifically *16 rRNA* gene or *cpn60* gene sequencing. Preferably, microscopy-based assessment of dysbiosis (generally denoted bacterial vaginosis) relied on Gram stain-based methods,[13](#_ENREF_13),[14](#_ENREF_14) but the Amsel criteria[15](#_ENREF_15) and Papanicolaou’s smear-based diagnosis[16](#_ENREF_16),[17](#_ENREF_17) were also included. Assessment through vaginal pH measurement alone was not sufficient for inclusion. Studies that applied molecular techniques were expected to generate a variable number of clusters through compositional dissimilarity approaches based on taxonomy-specific relative abundances,[12](#_ENREF_12) with low-*Lactobacillus* abundance states to be categorized as dysbiosis, unless otherwise specified. Cytology grading of cervical lesions was eligible when relying on the Bethesda System (including the categories low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL)), but the comparable Dutch KOPAC grading system was also allowed, as previously specified.[8](#_ENREF_8),[9](#_ENREF_9) Histology assessment on biopsy or surgical specimens was expected to follow the CIN histology system (dysplasia incrementally graded as CIN1, 2, and 3, respectively). The study protocol has been registered with the International Prospective Register of Systematic Reviews (<http://www.crd.york.ac.uk/prospero>) under reference CRD42016035620.

**Data extraction and assessment of risk of bias**

A standardized, pilot-tested form was used to extract data from possibly eligible studies for assessment of risk of bias, and for evidence synthesis. All studies were independently assessed in triplicate (by HV, JVDW, and NB) through a customized component approach addressing a series of methodological hallmarks particular to the associations for which evidence was sought (A.2). Specifically, a numerical score was assigned for selected study characteristics up to a total of eight points to each study, and subsequently categorized high (0-3 points), moderate (4-5 points), and low (6-8 points) risk of bias. No conflicts arose for quality assessment through this approach.

**Data synthesis**

Random-effect meta-analyses were performed with STATA (StataCorp LLC, version 14.2/MP4) to calculate the pooled relative risk for each outcome, and to visualize the results by means of forest plots. Relative risk (RR) was used to denote all extracted or calculated ratio measures of effect, and was pooled and presented with 95% confidence intervals (CI). These ratios included odds ratios, risk ratios, rate ratios (or incidence density ratios, transition rate ratios)), and hazard ratios, and all were considered to approximate the same relative risk. If different crude and adjusted estimates were reported in a study, the model adjusted for the largest number of confounders was selected. Since odds ratios may seemingly overestimate the effect if the outcome is prevalent (>10%), risk ratios were preferred if reported or if crude numbers were available for assessing incident and persistent HPV. Transformation of odds ratios into risk ratios was considered if this was not reported,[18](#_ENREF_18),[19](#_ENREF_19) by using the formula risk ratio=odds ratio/[(1-p)+(odds ratio\*p)], with p being the proportion of the unexposed developing the outcome.[18](#_ENREF_18) If insufficient data were available for this transformation, the odds ratio was used.

Subgroup analyses by effect measure, type of analyses and different definitions of exposure, outcomes and subpopulations were performed if reported in two or more studies. Statistical heterogeneity was assessed by means of Cochran’s Q and I-squared tests, which represent the percentage of variation attributable to heterogeneity, and was categorized as low (25-50%), moderate (51-75%), or high (>75%).[20](#_ENREF_20) The presence of small study effects or publication bias was not evaluated because of the low number of eligible studies.[21](#_ENREF_21)

**Results**

**Study selection**

Fifteen cohort studies published in English between 2003 and 2017 met the inclusion criteria (Fig 1),[22-36](#_ENREF_22) of which all but one (with insufficient data)[30](#_ENREF_30) were included in the meta-analyses.

**Study characteristics**

Study characteristics of all 15 eligible studies are summarized in detail in A.3. Most studies originated from the United States (*n=7*)[22](#_ENREF_22),[28](#_ENREF_28),[30](#_ENREF_30),[31](#_ENREF_31),[33](#_ENREF_33),[34](#_ENREF_34),[36](#_ENREF_36) and Europe (*n=5*)[24](#_ENREF_24),[25](#_ENREF_25),[27](#_ENREF_27),[29](#_ENREF_29),[32](#_ENREF_32), with two studies potentially overlapping[33](#_ENREF_33),[36](#_ENREF_36). Recruitment age ranged from 13 to 73 years, with eight studies including post-menopausal women,[22-26](#_ENREF_22),[28](#_ENREF_28),[35](#_ENREF_35),[36](#_ENREF_36) with mean age ranging between 19-39 years (reported in 9 studies[22](#_ENREF_22),[24](#_ENREF_24),[26](#_ENREF_26),[27](#_ENREF_27),[29-33](#_ENREF_29)) and median age between 16-50 years (reported in 4 studies[23](#_ENREF_23),[25](#_ENREF_25),[34](#_ENREF_34),[36](#_ENREF_36)). Ethnic diversity within cohorts ranged from 100% Caucasian[24](#_ENREF_24) to 100% black African/Caribbean/Afro-American[33](#_ENREF_33),[35](#_ENREF_35). Six studies relied on only two measurement points[23-26](#_ENREF_23),[32](#_ENREF_32),[35](#_ENREF_35), while the other nine studies were based on repeated assessment, with a maximum of 32 measurements per woman.[22](#_ENREF_22) Sampling-intervals ranged from twice weekly to an average of 4 years, but typically 4- to 6-month intervals were handled in eight studies. Four studies excluded women with sexually transmitted infections (STIs) other than HPV from the study or analyses,[25](#_ENREF_25),[32](#_ENREF_32),[33](#_ENREF_33),[35](#_ENREF_35) and another three studies adjusted for the presence of these other STIs.[31](#_ENREF_31),[34](#_ENREF_34),[36](#_ENREF_36)

**Risk of bias of included studies**

Risk of bias was considered high in six studies (A.4).[24-26](#_ENREF_24),[30](#_ENREF_30),[32](#_ENREF_32),[35](#_ENREF_35) Vaginal dysbiosis was defined by microscopy in 11 studies,[23](#_ENREF_23),[25-32](#_ENREF_25),[34](#_ENREF_34),[36](#_ENREF_36) by molecular techniques in three studies, and by both approaches in one study.[35](#_ENREF_35) However, the reported molecular data of the latter study were insufficiently detailed to be included in our meta-analyses. Microscopy-based assessment consisted of Nugent scoring in five studies,[28](#_ENREF_28),[32](#_ENREF_32),[34-36](#_ENREF_34) wet mount microscopy in two studies,[30](#_ENREF_30),[31](#_ENREF_31) and cervical Papanicolaou-stained smears in five studies.[23](#_ENREF_23),[25-27](#_ENREF_25),[29](#_ENREF_29) The baseline prevalence of vaginal dysbiosis ranged from 3% to 54%. All three molecular studies included in the meta-analyses designated *Lactobacillus crispatus* dominance as the reference group.[22](#_ENREF_22),[24](#_ENREF_24),[33](#_ENREF_33) Polymerase-chain reaction (PCR) techniques were used to identify HPV types as summarized in S3. HPV involvement was not assessed in the three studies that had dysplasia or cancer as the outcome.[23](#_ENREF_23),[25](#_ENREF_25),[29](#_ENREF_29)All but one of the 12 other studies specified the HPV types screened for,[26](#_ENREF_26) ranging between 13 to 49 different HPV types. Only three studies reported results for high-risk HPV types only.[26](#_ENREF_26),[32](#_ENREF_32),[34](#_ENREF_34)

**Synthesis of results**

Seven studies reported on the association between vaginal dysbiosis and incident HPV,[22](#_ENREF_22),[28](#_ENREF_28),[30-33](#_ENREF_30),[36](#_ENREF_36), including two molecular studies.[22](#_ENREF_22),[33](#_ENREF_33).One study did not provide sufficiently detailed numerical data on incident HPV to be included in the meta-analysis.[30](#_ENREF_30). In the four microscopy studies (Fig 2) the overall risk of incident HPV was higher among women with vaginal dysbiosis (pooled RR=1.35, 95%CI 1.18 to 1.50, *N=4*), and this tended to be most prominent among young women (pooled RR=1.43, 95%CI 1.10 to 1.85, *N=2*) (Table 1). In the two molecular studies, using *L. crispatus* dominated vaginal microbiota as reference, the risk of incident HPV was higher when not dominated by *L. crispatus* (RR=1.85, 95%CI 0.47 to 7.32) (Table 1). Only one study reported sufficiently detailed information on different groups not dominated by *L. crispatus* incident HPV,[22](#_ENREF_22) so no meta-analysis could be conducted. Statistical heterogeneity was low among microscopy studies (I2=0%), and moderate across the molecular studies (I2=56).

Nine studies examined the association between vaginal dysbiosis and HPV persistence,[22](#_ENREF_22),[24](#_ENREF_24),[26-28](#_ENREF_26),[32](#_ENREF_32),[34-36](#_ENREF_34)including two molecular studies (Fig 3, Table 1).[22](#_ENREF_22),[24](#_ENREF_24) The seven microscopy studies showed a pooled RR of 1.14 (95%CI 1.01 to 1.28, *N=7*) in women with vaginal dysbiosis. The risk was most apparent among asymptomatic women (RR=1.86, 95%CI 1.05 to 3.28, *N=2*) (Table 1). When only high-risk HPV types were accounted for, the pooled RR was 1.18 (95%CI 1.01 to 1.38, *N=3*), compared to an RR of 1.15 (95%CI 0.96 to 1.37, *N=4*) for all HPV types together. Statistical heterogeneity was low to moderate in all analyses.

In both molecular studies (with *L. crispatus* dominance as the reference group), the pooled risk was highest for anaerobic dysbiosis (RR=2.00, 95%CI 1.05 to 3.81), and lowest for *Lactobacillus gasseri* dominance (RR=0.63, 95%CI 0.10 to 3.86).

Three microscopy studies reported on the association between vaginal dysbiosis and HPV-related cervical cytological or histological changes.[23](#_ENREF_23),[25](#_ENREF_25),[29](#_ENREF_29) The large Dutch screening study provided three estimates comparing the risk of atypical squamous cells of undetermined origin (ASCUS), LSIL, and HSIL with the normal cytology category. All three estimates showed a significantly increased risk for women with vaginal dysbiosis, correlating with the degree of dysplasia from 1.44 for ASCUS, 1.85 for LSIL and 2.00 for HSIL (pooled RR=1.63, 95%CI 1.32 to 2.01).[25](#_ENREF_25) The other two studies enrolled HIV-positive women only. One study compared the risk of HSIL or squamous cell carcinoma (SCC) with a combined reference group (including normal, ASCUS, and LSIL categories).[29](#_ENREF_29) The other study provided two different risk estimates for disease progression from normal to LSIL to HSIL/SCC where ASCUS was added either to the normal or to the LSIL category.[23](#_ENREF_23) The results for the subgroups based on the reference and categorization of ASCUS (as described above) are presented separately (Fig 4). All effect estimates showed a trend towards an increased risk of disease progression associated with vaginal dysbiosis (except for the analyses where ASCUS was grouped with LSIL), but none of them reached statistical significance.[23](#_ENREF_23) The two studies that assessed the risk of HSIL/SCC compared to normal (including ASCUS/LSIL in one study), showed a doubled risk among women with vaginal dysbiosis (pooled RR= 2.01, 95%CI 1.40 to 3.01) and low heterogeneity (I2=0%)(Table 1).[25](#_ENREF_25),[29](#_ENREF_29)

**COMMENT**

Our meta-analyses provide evidence that sexually active women with vaginal dysbiosis are a) at increased risk of acquiring HPV infection, b) more prone to HPV persistence, and/or c) at increased risk of progression to associated premalignant and malignant cervical disease.

These findings are consistent with previous systematic reviews and meta-analyses based on cross-sectional data.[7-9](#_ENREF_7),[12](#_ENREF_12) However, our study is the first to address this research topic through a systematic appraisal of longitudinal studies exclusively, retrieved through a comprehensive literature search of five major databases, complemented by backward and forward citation tracking. The longitudinal study designs were such that it was possible to identify incident and persistent HPV infections as opposed to just prevalent infections. However, in all relevant studies that we identified, women with (pre)malignant cervical disease were compared with those without such disease regardless of their oncogenic HPV status. It was therefore not possible to determine whether vaginal dysbiosis increased acquisition and persistence of oncogenic HPV infection only, or also increased risk of carcinogenesis after infection.

The associations that we identified may be confounded by the presence of common risk factors to vaginal dysbiosis and HPV. Most studies enrolled at-risk groups for HPV acquisition, i.e. young, sexually active women and women with high-risk sexual behavior, which inevitably led to marked convergence of interrelated risk factors, and hence to a high risk of confounding. The extent to which the studies adjusted for confounding was highly variable. For example, only half of the studies considered the presence of other STIs or urogenital infections by exclusion at enrolment or through statistical model adjustment, and very few studies controlled for all potentially concomitant urogenital infections. For example, only one study reported to have accounted for (culture-positive) vaginal *Candida.*[28](#_ENREF_28) When urogenital infections were taken into account, this was often done imperfectly by relying on self-report of symptomatic vaginal infections, by using insensitive screening assays (for example, culture for *Trichomonas vaginalis* instead of culture or a PCR-based test*)*, or by not repeating assessments after baseline. Similarly, little information was provided on how treatment, if any, of vaginal dysbiosis, *Candida*, STIs, and HPV-related lesions affected outcome. While confounding requires due attention, we also observed little difference in the sub-analyses restricted to adjusted estimates compared to the overall risk estimates. In addition, several associations tended to be rather consistent across different study populations.

However, caution is warranted in interpreting our pooled risk estimates, as we detected several potential sources of bias throughout the relevant literature base. The eligible studies were implemented in diverse study settings, and included a wide variety of women in terms of age, socio-economic status, ethnicity, past or current sexual risk behaviors, and co-infections, which was also reflected in the wide-ranging prevalence of vaginal dysbiosis. Notably, five of the 15 eligible studies included a high proportion of HIV-positive women.[23](#_ENREF_23),[28](#_ENREF_28),[29](#_ENREF_29),[33](#_ENREF_33),[36](#_ENREF_36) Statistical heterogeneity in our analyses was nonetheless very low to moderate.

Of further concern were differences in exposure and outcome assessments. The number of visits, duration of follow-up, and length of intervals between measurement points varied considerably across studies. Fluctuations in vaginal microbiota status,[37-39](#_ENREF_37) as well as in HPV detectability,[40](#_ENREF_40),[41](#_ENREF_41) may lead to misclassification bias in studies that had few and/or large intervals between measurement points. There was also considerable variability in the HPV types taken into consideration. Metagenomic analysis recently revealed the complexity of the HPV virome, which is only partially visible with common HPV detection methods,[42](#_ENREF_42) with more than half of HPV-positive women showing coinfection with two or three HPV types. Co-occurrence patterns specifically revealed that colonization with a single, even low-risk HPV type may predispose to additional high-risk HPV types. It is also known that coinfection with multiple HPV types is a risk factor for acquiring additional types and for HPV persistence.[43](#_ENREF_43),[44](#_ENREF_44) Only three of the eligible studies reported analyses confined to high-risk HPV types, so it may be that our main associations are diluted by inclusion of HPV-types with a low carcinogenic potential. Misclassification bias relating to exposure assessment may have been an even more pertinent threat to the validity of some studies. Gram stain Nugent scoring, which was applied in five studies, has been the gold standard of microscopic microbiota assessment in research settings.[13](#_ENREF_13),[14](#_ENREF_14) The Amsel criteria (two studies) are a valid diagnostic approach, but have low sensitivity as a screening tool in asymptomatic women.[15](#_ENREF_15),[45](#_ENREF_45) In the HPV and cervical cancer research field, vaginal dysbiosis is often diagnosed when clue cells are seen on Papanicolaou smears (five studies), and the reported accuracy of this approach is inconsistent.[16](#_ENREF_16),[17](#_ENREF_17),[46](#_ENREF_46),[47](#_ENREF_47) However, overall, the associations between vaginal dysbiosis and HPV/cancer were in the same direction and of a similar magnitude for studies that assessed the vaginal microbiota by molecular methods and those that used molecular techniques.

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While we clearly show associations between vaginal dysbiosis and HPV infection, it is remarkable that only few HPV epidemiology studies have accounted for this prevalent risk factor. Different sexually transmitted pathogens employ different infection strategies, but vaginal dysbiosis has been shown to be a risk factor for most STIs,[48](#_ENREF_48) as was documented most extensively for HIV.[49](#_ENREF_49) However, contrary to HIV-1 virions that can penetrate both intact squamous and columnar epithelial barriers,[50](#_ENREF_50) HPV capsids are not able to bind or infect intact epithelia. They possibly take advantage of the mucosal barrier failure that has been observed with vaginal dysbiosis.[51](#_ENREF_51) In addition, dysbiosis of mucosa-associated microbiota is increasingly recognized as a driver of cancer development in humans through a variety of mechanisms including mucosal barrier failure and inflammation.[51](#_ENREF_51),[52](#_ENREF_52) Chronic mucosal inflammation is also considered central to HPV-induced carcinogenesis.[53](#_ENREF_53) At least hypothetically, this may concur with the numerous molecular signatures of mucosal inflammation and barrier failure with vaginal dysbiosis.[54](#_ENREF_54) Of note, recent *in vitro* data do point to a protective, anti-tumoral effect exerted by vaginal lactobacilli on the cervical epithelium.[55-57](#_ENREF_55)

Reverse causation should be considered since HPV may also promote the development of vaginal dysbiosis. HPV displays a number of immune evasion and silencing mechanisms, and subtle changes in the mucosal micro-environment may lead to alterations in the vaginal microbiota.[58](#_ENREF_58) Thus, it cannot be excluded that such dynamics are at play in the presence of HPV and/or associated squamous lesions. Nonetheless, our data, based on longitudinal measurements, support the hypothesis that the vaginal microbiota have a role in the pathway from HPV to cervical cancer at one or more disease stages. As such, our data support the hypothesis that interventions that restore and maintain *Lactobacillus* dominated vaginal microbiota might reduce the HPV-related disease burden,[56](#_ENREF_56),[57](#_ENREF_57),[59-61](#_ENREF_59)thereby possibly reducing obstetric morbidity related to excisional and ablative procedures.[62](#_ENREF_62)

Further prospective studies of vaginal dysbiosis as a cofactor of HPV-related disease would be valuable but should assess vaginal dysbiosis and HPV infection in more detail using molecular methods and more frequently over time, and should compare women who have developed cervicovaginal (pre) malignancy with those without (pre) malignancy but with persistent oncogenic HPV infection. Such studies might clarify the role of vaginal dysbiosis in the different steps in the causal pathway, and may also elucidate several previously unexplained observations. Ethnic disparity in cervical cancer burden has been attributed to increased HPV persistence in young African American women relative to European American women [63](#_ENREF_63). However, vaginal microbiota differences between women of African or European descent might also explain this disparity.[64](#_ENREF_64) Similarly, the disproportionately high HPV prevalence among young African women[65](#_ENREF_65) might be directly related to the high prevalence of vaginal dysbiosis in Sub-Saharan Africa.[66](#_ENREF_66) The vulnerability of sexually active adolescents to HPV also warrants further scrutiny, specifically as it may relate to the putative association between cervical ectopy and vaginal dysbiosis[67](#_ENREF_67) and to the role of the adolescent vaginal microbiota in mucosal immune homeostasis.[68](#_ENREF_68),[69](#_ENREF_69)

In conclusion, vaginal dysbiosis likely is a largely understudied yet important risk factor in HPV and cervical cancer epidemiology. Improved HPV vaccination coverage and vaginal dysbiosis prevention and management will likely reduce cervical cancer disease burden significantly. Expanding the evidence-base may also lead to novel primary and secondary preventive strategies.

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**References**

**1.** Small W, Jr., Bacon MA, Bajaj A, et al. Cervical cancer: A global health crisis. *Cancer.* Jul 1 2017;123(13):2404-2412.

**2.** Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends--An Update. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* Jan 2016;25(1):16-27.

**3.** Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol.* Apr 2002;55(4):244-265.

**4.** Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: a cancer hallmarks analysis. *Cell Host Microbe.* Mar 12 2014;15(3):266-282.

**5.** Sudenga SL, Shrestha S. Key considerations and current perspectives of epidemiological studies on human papillomavirus persistence, the intermediate phenotype to cervical cancer. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases.* Apr 2013;17(4):e216-220.

**6.** Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. *The Lancet. Infectious diseases.* Dec 2010;10(12):862-874.

**7.** Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? *Microbiome.* Nov 01 2016;4(1):58.

**8.** Gillet E, Meys JF, Verstraelen H, et al. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC infectious diseases.* Jan 11 2011;11:10.

**9.** Gillet E, Meys JF, Verstraelen H, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. *PloS one.* 2012;7(10):e45201.

**10.** Senok AC, Verstraelen H, Temmerman M, Botta GA. Probiotics for the treatment of bacterial vaginosis. *The Cochrane database of systematic reviews.* Oct 7 2009(4):CD006289.

**11.** Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine.* june 2009 2009;6(6):doi:10/1371/journal.pmed.1000097.

**12.** van de Wijgert JH, Borgdorff H, Verhelst R, et al. The vaginal microbiota: what have we learned after a decade of molecular characterization? *PloS one.* 2014;9(8):e105998.

**13.** Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of clinical microbiology.* Feb 1991;29(2):297-301.

**14.** Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sexually transmitted infections.* Dec 2002;78(6):413-415.

**15.** Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *The American journal of medicine.* Jan 1983;74(1):14-22.

**16.** Platz-Christensen JJ, Larsson PG, Sundstrom E, Wiqvist N. Detection of bacterial vaginosis in wet mount, Papanicolaou stained vaginal smears and in gram stained smears. *Acta obstetricia et gynecologica Scandinavica.* Jan 1995;74(1):67-70.

**17.** Discacciati MG, Simoes JA, Amaral RG, et al. Presence of 20% or more clue cells: An accurate criterion for the diagnosis of bacterial vaginosis in Papanicolaou cervical smears. *Diagnostic cytopathology.* Apr 2006;34(4):272-276.

**18.** Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *Jama.* Nov 18 1998;280(19):1690-1691.

**19.** Greenland S. Model-based estimation of relative risks and other epidemiologic measures in studies of common outcomes and in case-control studies. *American journal of epidemiology.* Aug 15 2004;160(4):301-305.

**20.** Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed.).* Sep 6 2003;327(7414):557-560.

**21.** Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed.).* Sep 13 1997;315(7109):629-634.

**22.** Brotman RM, Shardell MD, Gajer P, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *The Journal of infectious diseases.* Dec 01 2014;210(11):1723-1733.

**23.** Denslow SA, Westreich DJ, Firnhaber C, Michelow P, Williams S, Smith JS. Bacterial vaginosis as a risk factor for high-grade cervical lesions and cancer in HIV-seropositive women. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics.* Sep 2011;114(3):273-277.

**24.** Di Paola M, Sani C, Clemente AM, et al. Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection. *Scientific reports.* Aug 31 2017;7(1):10200.

**25.** Engberts MK, Verbruggen BS, Boon ME, van Haaften M, Heintz AP. Candida and dysbacteriosis: a cytologic, population-based study of 100,605 asymptomatic women concerning cervical carcinogenesis. *Cancer.* Oct 25 2007;111(5):269-274.

**26.** Guo YL, You K, Qiao J, Zhao YM, Geng L. Bacterial vaginosis is conducive to the persistence of HPV infection. *International journal of STD & AIDS.* Aug 2012;23(8):581-584.

**27.** Kero K, Rautava J, Syrjanen K, Grenman S, Syrjanen S. Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology.* Nov 2017;36(11):2215-2219.

**28.** King CC, Jamieson DJ, Wiener J, et al. Bacterial vaginosis and the natural history of human papillomavirus. *Infectious diseases in obstetrics and gynecology.* 2011;2011:319460.

**29.** Lehtovirta P, Paavonen J, Heikinheimo O. Risk factors, diagnosis and prognosis of cervical intraepithelial neoplasia among HIV-infected women. *International journal of STD & AIDS.* Jan 2008;19(1):37-41.

**30.** Mao C, Hughes JP, Kiviat N, et al. Clinical findings among young women with genital human papillomavirus infection. *American journal of obstetrics and gynecology.* Mar 2003;188(3):677-684.

**31.** Moscicki AB, Ma Y, Jonte J, et al. The role of sexual behavior and human papillomavirus persistence in predicting repeated infections with new human papillomavirus types. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* Aug 2010;19(8):2055-2065.

**32.** Oakeshott P, Aghaizu A, Reid F, et al. Frequency and risk factors for prevalent, incident, and persistent genital carcinogenic human papillomavirus infection in sexually active women: community based cohort study. *BMJ (Clinical research ed.).* Jun 22 2012;344:e4168.

**33.** Reimers LL, Mehta SD, Massad LS, et al. The Cervicovaginal Microbiota and Its Associations With Human Papillomavirus Detection in HIV-Infected and HIV-Uninfected Women. *The Journal of infectious diseases.* Nov 01 2016;214(9):1361-1369.

**34.** Samoff E, Koumans EH, Markowitz LE, et al. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. *American journal of epidemiology.* Oct 01 2005;162(7):668-675.

**35.** Shannon B, Yi TJ, Perusini S, et al. Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal immunology.* Sep 2017;10(5):1310-1319.

**36.** Watts DH, Fazzari M, Minkoff H, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. *The Journal of infectious diseases.* Apr 01 2005;191(7):1129-1139.

**37.** Ness RB, Kip KE, Soper DE, Stamm CA, Rice P, Richter HE. Variability of bacterial vaginosis over 6- to 12-month intervals. *Sexually transmitted diseases.* Jun 2006;33(6):381-385.

**38.** Brotman RM, Ravel J, Cone RA, Zenilman JM. Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. *Sexually transmitted infections.* Aug 2010;86(4):297-302.

**39.** Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Science translational medicine.* May 2 2012;4(132):132ra152.

**40.** Liu SH, Cummings DA, Zenilman JM, Gravitt PE, Brotman RM. Characterizing the temporal dynamics of human papillomavirus DNA detectability using short-interval sampling. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* Jan 2014;23(1):200-208.

**41.** Shew ML, Ermel AC, Tong Y, Tu W, Qadadri B, Brown DR. Episodic detection of human papillomavirus within a longitudinal cohort of young women. *Journal of medical virology.* Dec 2015;87(12):2122-2129.

**42.** Ma Y, Madupu R, Karaoz U, et al. Human papillomavirus community in healthy persons, defined by metagenomics analysis of human microbiome project shotgun sequencing data sets. *Journal of virology.* May 2014;88(9):4786-4797.

**43.** Mendez F, Munoz N, Posso H, et al. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. *The Journal of infectious diseases.* Oct 1 2005;192(7):1158-1165.

**44.** Rousseau MC, Pereira JS, Prado JC, Villa LL, Rohan TE, Franco EL. Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. *The Journal of infectious diseases.* Dec 15 2001;184(12):1508-1517.

**45.** Gallo MF, Jamieson DJ, Cu-Uvin S, Rompalo A, Klein RS, Sobel JD. Accuracy of clinical diagnosis of bacterial vaginosis by human immunodeficiency virus infection status. *Sexually transmitted diseases.* Apr 2011;38(4):270-274.

**46.** Davis JD, Connor EE, Clark P, Wilkinson EJ, Duff P. Correlation between cervical cytologic results and Gram stain as diagnostic tests for bacterial vaginosis. *American journal of obstetrics and gynecology.* Sep 1997;177(3):532-535.

**47.** Greene JF, 3rd, Kuehl TJ, Allen SR. The papanicolaou smear: inadequate screening test for bacterial vaginosis during pregnancy. *American journal of obstetrics and gynecology.* May 2000;182(5):1048-1049.

**48.** Unemo M, Bradshaw CS, Hocking JS, et al. Sexually transmitted infections: challenges ahead. *The Lancet. Infectious diseases.* Aug 2017;17(8):e235-e279.

**49.** Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet (London, England).* Jul 19 2014;384(9939):258-271.

**50.** Carias AM, McCoombe S, McRaven M, et al. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. *Journal of virology.* Nov 2013;87(21):11388-11400.

**51.** Schwabe RF, Jobin C. The microbiome and cancer. *Nature reviews. Cancer.* Nov 2013;13(11):800-812.

**52.** Garrett WS. Cancer and the microbiota. *Science (New York, N.Y.).* Apr 3 2015;348(6230):80-86.

**53.** Fernandes JV, TA DEMF, JC DEA, et al. Link between chronic inflammation and human papillomavirus-induced carcinogenesis (Review). *Oncology letters.* Mar 2015;9(3):1015-1026.

**54.** Borgdorff H, Gautam R, Armstrong SD, et al. Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. *Mucosal immunology.* May 2016;9(3):621-633.

**55.** Vielfort K, Weyler L, Soderholm N, Engelbrecht M, Lofmark S, Aro H. Lactobacillus decelerates cervical epithelial cell cycle progression. *PloS one.* 2013;8(5):e63592.

**56.** Motevaseli E, Shirzad M, Akrami SM, Mousavi AS, Mirsalehian A, Modarressi MH. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. *Journal of medical microbiology.* Jul 2013;62(Pt 7):1065-1072.

**57.** Wang KD, Xu DJ, Wang BY, Yan DH, Lv Z, Su JR. Inhibitory Effect of Vaginal Lactobacillus Supernatants on Cervical Cancer Cells. *Probiotics and antimicrobial proteins.* Oct 25 2017.

**58.** Anahtar MN, Byrne EH, Doherty KE, et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity.* May 19 2015;42(5):965-976.

**59.** Verhoeven V, Renard N, Makar A, et al. Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. *European Journal of Cancer Prevention.* Jan 2013;22(1):46-51.

**60.** Palma E, Recine N, Domenici L, Giorgini M, Pierangeli A, Panici PB. Long-term Lactobacillus rhamnosus BMX 54 application to restore a balanced vaginal ecosystem: a promising solution against HPV-infection. *BMC infectious diseases.* Jan 5 2018;18(1):13.

**61.** Cha MK, Lee DK, An HM, et al. Antiviral activity of Bifidobacterium adolescentis SPM1005-A on human papillomavirus type 16. *BMC Med.* Jul 12 2012;10:72.

**62.** Kyrgiou M, Mitra A, Moscicki AB. Does the vaginal microbiota play a role in the development of cervical cancer? *Translational research : the journal of laboratory and clinical medicine.* Jan 2017;179:168-182.

**63.** Banister CE, Messersmith AR, Cai B, et al. Disparity in the persistence of high-risk human papillomavirus genotypes between African American and European American women of college age. *The Journal of infectious diseases.* Jan 1 2015;211(1):100-108.

**64.** Fettweis JM, Brooks JP, Serrano MG, et al. Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology (Reading, England).* Oct 2014;160(Pt 10):2272-2282.

**65.** Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *The Journal of infectious diseases.* Dec 15 2010;202(12):1789-1799.

**66.** Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. *American journal of obstetrics and gynecology.* Dec 2013;209(6):505-523.

**67.** Junior JE, Giraldo PC, Goncalves AK, do Amaral RL, Linhares IM. Uterine cervical ectopy during reproductive age: cytological and microbiological findings. *Diagnostic cytopathology.* May 2014;42(5):401-404.

**68.** Hwang LY, Scott ME, Ma Y, Moscicki AB. Higher levels of cervicovaginal inflammatory and regulatory cytokines and chemokines in healthy young women with immature cervical epithelium. *Journal of reproductive immunology.* Jan 2011;88(1):66-71.

**69.** Kyongo JK, Crucitti T, Menten J, et al. Cross-Sectional Analysis of Selected Genital Tract Immunological Markers and Molecular Vaginal Microbiota in Sub-Saharan African Women, with Relevance to HIV Risk and Prevention. *Clinical and vaccine immunology : CVI.* May 2015;22(5):526-538.

**Figure headings**

Fig 1. Overview of the systematic literature selection (PRISMA flowchart)

Fig 2. Forest plot showing the association between vaginal dysbiosis and the pooled relative risk of incident human papillomavirus (HPV) using no vaginal dysbiosis as reference.

Fig 3. Forest plot showing the association between vaginal dysbiosis and the pooled relative risk of persistent human papillomavirus (HPV) using no vaginal dysbiosis as reference.

Fig 4. Forest plot showing the association between vaginal dysbiosis and the relative risk of progression of dysplasia into cervical intraepithelial neoplasia using no vaginal dysbiosis as reference.

**Table 1: Vaginal dysbiosis and the risk of human papilloma virus (HPV) incidence, persistence and cervical cancer.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Included studies** | **Number of included individuals** | **Pooled relative risk (95% confidence interval)** | **I2 (%)** | **p** **heterogeneity** |
| ***Microscopy studies - incident HPV*** |  |  |  |  |  |
| Overall | [28](#_ENREF_28),[31](#_ENREF_31),[32](#_ENREF_32),[36](#_ENREF_36) | *5280* | 1.33 (1.18-1.50) | 0.0 | *0.627* |
| Young women | [31](#_ENREF_31),[32](#_ENREF_32) | *1915* | 1.43 (1.10-1.85) | 0.0 | *0.473* |
| HIV+/high risk HIV- | [28](#_ENREF_28),[36](#_ENREF_36) | *4501* | 1.31 (1.14-1.49) | 0.0 | *0.354* |
| Adjusted estimates only | [28](#_ENREF_28),[31](#_ENREF_31),[36](#_ENREF_36) | *4490* | 1.33 (1.17-1.51) | 0.0 | *0.419* |
| Adjusted hazard ratios only | [31](#_ENREF_31),[36](#_ENREF_36) | *3354* | 1.45 (1.20-1.74) | 0.0 | *0.584* |
| Nugent diagnosis | [31](#_ENREF_31),[32](#_ENREF_32),[36](#_ENREF_36) | *4155* | 1.31 (1.16-1.48) | 0.0 | *0.650* |
| Low- and high risk HPV combined | [28](#_ENREF_28),[31](#_ENREF_31),[36](#_ENREF_36) | *4490* | 1.33 (1.17-1.51) | 0.0 | *0.419* |
| ***Molecular studies - incident HPV*** |  |  |  |  |  |
| Not *L. crispatus* dominated vs. *L. crispatus* dominated | [22](#_ENREF_22),[33](#_ENREF_33) | *96* | 1.85 (0.47-7.32) | 55.7 | *0.133* |
| ***Microscopy studies - persistent HPV*** |  |  |  |  |
| Overall | [26-28](#_ENREF_26),[32](#_ENREF_32),[34-36](#_ENREF_34) | *4711* | 1.14 (1.01-1.28) | 44.2 | *0.096* |
| Young women | [27](#_ENREF_27),[32](#_ENREF_32),[34](#_ENREF_34) | *618* | 1.30 (0.77-2.20) | 50.0 | *0.136* |
| HIV+/high risk HIV- | [28](#_ENREF_28),[35](#_ENREF_35),[36](#_ENREF_36) | *3386* | 1.08 (0.97-1.20) | 35.4 | *0.212* |
| HIV- high risk | [28](#_ENREF_28),[35](#_ENREF_35) | *401* | 1.27 (0.57-2.82) | 64.9 | *0.058* |
| Adjusted estimates only | [28](#_ENREF_28),[34](#_ENREF_34) | *1287* | 1.19 (1.03-1.38) | 0.0 | *0.942* |
| Hazard ratios only | [28](#_ENREF_28),[36](#_ENREF_36) | *3365* | 1.09 (0.95-1.25) | 67.7 | *0.078* |
| Risk ratios only | [26](#_ENREF_26),[27](#_ENREF_27),[32](#_ENREF_32),[34](#_ENREF_34),[35](#_ENREF_35) | *1017* | 1.18 (1.01-1.37) | 0.0 | *0.789* |
| Nugent diagnosis | [28](#_ENREF_28),[32](#_ENREF_32),[34-36](#_ENREF_34) | *3675* | 1.05 (1.00-1.11) | 0.0 | *0.418* |
| Pap smear diagnosis | [26](#_ENREF_26),[27](#_ENREF_27) | *1036* | 1.47 (0.85-2.55) | 66.4 | *0.085* |
| Only asymptomatic women | [27](#_ENREF_27),[35](#_ENREF_35) | *350* | 1.86 (1.05-3.28) | 0.0 | *0.328* |
| Including symptomatic women | [26](#_ENREF_26),[28](#_ENREF_28),[32](#_ENREF_32),[34](#_ENREF_34),[36](#_ENREF_36) | *4361* | 1.10 (1.00-1.21) | 35.1 | *0.187* |
| Only high-risk HPV-types | [26](#_ENREF_26),[32](#_ENREF_32),[34](#_ENREF_34) | *996* | 1.18 (1.01-1.38) | 0.0 | *0.599* |
| Low- and high-risk types combined | [27](#_ENREF_27),[28](#_ENREF_28),[35](#_ENREF_35),[36](#_ENREF_36) | *3715* | 1.15 (0.96-1.37) | 61.8 | *0.049* |
| ***Molecular studies – persistent HPV*** |  |  |  |  |  |
| Not *L. crispatus* dominated vs. *L. crispatus* dominated | [22](#_ENREF_22),[24](#_ENREF_24) | *87* | 1.33 (0.63-2.81) | 23.8 | *0.252* |
| *L. gasseri* dominated vs. *L. crispatus* dominated | [22](#_ENREF_22),[24](#_ENREF_24) | *26* | 0.63 (0.10-3.86) | 81.0 | *0.022* |
| *L. iners* dominated vs. *L. crispatus* dominated | [22](#_ENREF_22),[24](#_ENREF_24) | *46* | 1.06 (0.42-2.63) | 0.0 | *0.461* |
| Low lactobacilli mixed aerobe/ anaerobe vs. *L. crispatus* dominated | [22](#_ENREF_22),[24](#_ENREF_24) | *44* | 1.00 (0.23-4.30) | 80.1 | *0.025* |
| Low lactobacilli anaerobe vs. *L. crispatus* dominated | [22](#_ENREF_22),[24](#_ENREF_24) | *69* | 2.00 (1.05-3.81) | 0.0 | *0.391* |
| ***Dysplasia/cancer*** |  |  |  |  |  |
| HSIL vs. normal | [25](#_ENREF_25),[29](#_ENREF_29) | *91,149* | 2.01 (1.40-3.01) | 0.0 | *0.768* |

HIV, human immunodeficiency virus; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus.