Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis

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OBJECTIVE: The vaginal microbiota proposedly influence the association between human papillomavirus and cervical cancer. Our aim was to assess whether vaginal dysbiosis affects human papilloma virus acquisition, persistence, and progression to related cervical premalignancy.

DATA SORUCES: MEDLINE, Embase, CINAHL, Cochrane Library, and Web of Science (inception until June 2018) were used for this study. 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 was determined.

RESULTS: Of 1645 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, $I^2 = 0\%$; among young women relative risk, 1.43, 1.10–1.85, $I^2 = 0\%$), human papilloma virus persistence (overall relative risk, 1.14, 1.01–1.28, $I^2 = 44.2\%$; for oncogenic types relative risk, 1.18, 1.01–1.38, $I^2 = 0\%$), and high-grade lesions and cancer (relative risk, 2.01, 1.40–3.01, $I^2 = 0\%$), but women with lesions/cancer were compared with those without, regardless of their oncogenic human papilloma virus status. Overall, comparable results were found in the molecular vaginal microbiota studies.

CONCLUSION: 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: bacterial vaginosis, HPV, human papillomavirus, microbiome, vaginal dysbiosis

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

It is well established, both epidemiologically and mechanistically, that cervical cancer and its premalignant precursor stages (cervical intraepithelial neoplasia [CIN]) are causally related to oncogenic types of the human papillomavirus (HPV).[3,4] However, from a public health perspective, the association is less than straightforward.

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Most women across the globe are infected at least once with 1 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] This suggests that other, largely undetermined cofactors are at play,[6] with vaginal dysbiosis emerging as a potential driver of HPV-related disease outcomes.[6,7]

In two meta-analyses of mostly unadjusted cross-sectional data, dysbiosis was associated with prevalent HPV infection and prevalent CIN.[8,9] The latter studies did not allow for causal inferences while also prone to confounding because vaginal dysbiosis and HPV infection share a number of risk factors, including sexual behavior and smoking.[6]

We postulate that vaginal dysbiosis is a putative, potentially modifiable[10] risk factor to HPV acquisition, persistence, and related cervical disease and have systematically reviewed the available

AJOG at a Glance

Why was this study conducted?

We conducted this systematic review and meta-analysis to assess whether vaginal dysbiosis affects the risk of becoming infected with HPV, clearning HPV, and developing cervical dysplasia.

Key findings

Vaginal dysbiosis seems to be a risk factor for acquiring HPV and persistence of HPV and cervical dysplasia.

What does this add to what is known?

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).

evidence obtained through relevant longitudinal studies.

Materials and Methods

Eligibility criteria, information sources, and search strategy

We conducted a systematic literature review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[11] and searched MEDLINE (1966 onward), EMBASE (1946 onward), CINAHL (1997 onward), Cochrane Database (1999 onward), and Web of Science (1955 onward), 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 alphapapillomavirus or HPV or human papillomavirus or uterine cervical neoplasms or cancer or dysplasia or neoplasia or squamous intraepithelial lesions or low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL) or CIN.

Full search strings for all databases are detailed in Appendix 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 H.V., S.S., and N.B.), and no conflicts arose.

Study selection

As described based on the Population, Intervention/Exposure, Comparison, Outcome, and Study Design design, we included original studies with longitudinal cohort designs as well as nested case-control studies (Study Design) that compared women (Population) with or without vaginal dysbiosis (Exposure and Comparison) and assessed the risk of at least 1 of the following outcomes (Outcome): (1) HPV incidence (defined as detection of an HPV type, not previously identified in a given woman) and/or (2) HPV persistence (defined as an absence of clearance or significantly delayed clearance relative to a reference group), and/or (3) related squamous epithelial lesions, provided vaginal dysbiosis was considered as a risk factor by study design. Studies were eligible if at least 2 measurement points were described for at least 1 outcome of interest (a minimal time interval was not predefined).

Vaginal dysbiosis was broadly defined as deviation from a *Lactobacillus*-dominated microbiota[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,[13,14] but the Amsel criteria[15] and Papanicolaou's smear-based diagnosis[16,17] 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] 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 LSIL and HSIL), but the comparable Dutch KOPAC grading system was also allowed, as previously specified.[8,9] 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 H.V., J.v.d.W., and N.B.) through a customized component approach addressing a series of methodological hallmarks particular to the associations for which evidence was sought (Appendix 2). Specifically, a numerical score was assigned for selected study characteristics up to a total of 8 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

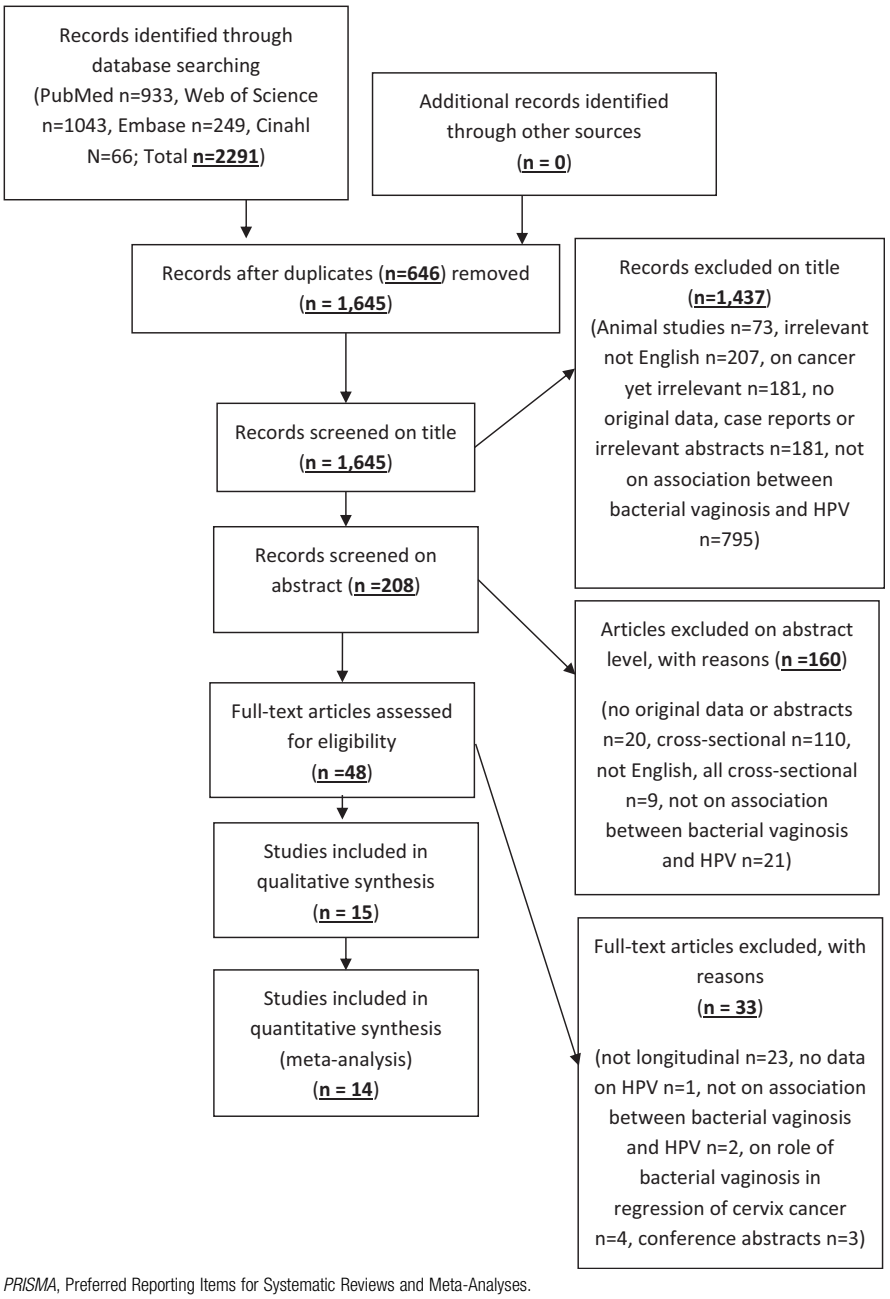
Random-effect meta-analyses were performed with STATA (StataCorp LLC, version 14.2/MP4, College Station, TX)

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.

Because 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[18,19] by using the following formula: risk ratio = odds ratio/[(1 − p) + (odds ratio*p)], with p being the proportion of the unexposed developing the outcome.[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 2 or more studies. Statistical heterogeneity was assessed by means of Cochran's Q and I^2 tests, which represent the percentage of variation attributable to heterogeneity, and was categorized as low (25−50%), moderate (51−75%), or high (>75%).[20] The presence of small study effects or publication bias was not evaluated because of the low number of eligible studies.[21]

FIGURE 1
Overview of the systematic literature selection (PRISMA flowchart)



PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

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Results

Study selection

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

Study characteristics

Study characteristics of all 15 eligible studies are summarized in detail in Appendix 3. Most studies originated from the United States (n = 7)[22,28,30,31,33,34,36] and Europe (n = 5),[24,25,27,29,32] with 2 studies potentially overlapping.[33,36]

Recruitment age ranged from 13 to 73 years, with 8 studies including postmenopausal women,[22−26,28,35,36] with mean age ranging between 19 and 39 years (reported in 9 studies[22,24,26,27,29−33]) and median age between 16 and 50 years (reported in 4 studies[23,25,34,36]). Ethnic diversity within cohorts ranged from 100% white[24] to 100% black African/Caribbean/Afro-American.[33,35]

Six studies relied on only 2 measurement points,[23−26,32,35] while the other 9

studies were based on repeated assessment, with a maximum of 32 measurements per woman.[22] Sampling intervals ranged from twice weekly to an average of 4 years, but typically 4−6 month intervals were handled in 8 studies. Four studies excluded women with sexually transmitted infections (STIs) other than HPV from the study or analyses,[25,32,33,35] and another 3 studies adjusted for the presence of these other STIs.[31,34,36]

Risk of bias of included studies

Risk of bias was considered high in 6 studies (Appendix 4).[24−26,30,32,35] Vaginal dysbiosis was defined by microscopy in 11 studies,[23,25−32,34,36] by molecular techniques in 3 studies, and by both approaches in 1 study.[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 5 studies,[28,32,34−36] wet mount microscopy in 2 studies,[30,31] and cervical Papanicolaou-stained smears in 5 studies.[23,25−27,29] The baseline prevalence of vaginal dysbiosis ranged from 3% to 54%. All 3 molecular studies included in the meta-analyses designated *Lactobacillus crispatus* dominance as the reference group.[22,24,33]

Polymerase chain reaction techniques were used to identify HPV types as summarized in the Table. HPV involvement was not assessed in the 3 studies that had dysplasia or cancer as the outcome.[23,25,29] All but 1 of the 12 other studies specified the HPV types screened for,[26] ranging between 13 and 49 different HPV types. Only 3 studies reported results for high-risk HPV types only.[26,32,34]

Synthesis of results

Seven studies reported on the association between vaginal dysbiosis and incident HPV,[22,28,30−33,36], including 2 molecular studies.[22,33] One study did not provide sufficiently detailed numerical data on incident HPV to be included in the meta-analysis.[30] In the 4 microscopy studies (Figure 2), the overall risk of incident HPV was higher among women with vaginal dysbiosis (pooled RR, 1.35; 95% CI, 1.18−1.50; n = 4), and this tended to be most prominent among young women (pooled RR, 1.43; 95% CI, 1.10−1.85; n = 2) (Table).

In the 2 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, 0.47−7.32) (Table). Only 1 study reported sufficiently detailed information on different groups not dominated by *L crispatus* incident HPV,[22] so no meta-analysis could be conducted. Statistical heterogeneity was low among microscopy studies ($I^2 = 0\%$) and moderate across the molecular studies ($I^2 = 56$).

Nine studies examined the association between vaginal dysbiosis and HPV persistence,[22,24,26−28,32,34−36] including 2 molecular studies (Figure 3 and Table).[22,24] The 7 microscopy studies showed a pooled RR of 1.14 (95% CI, 1.01−1.28; n = 7) in women with vaginal dysbiosis. The risk was most apparent among asymptomatic women (RR, 1.86; 95% CI 1.05−3.28; n = 2) (Table). When only high-risk HPV types were accounted for, the pooled RR was 1.18 (95% CI, 1.01−1.38; n = 3), compared with an RR of 1.15 (95% CI, 0.96−1.37; n = 4) for all HPV types together. The 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, 1.05−3.81) and lowest for *Lactobacillus gasseri* dominance (RR, 0.63; 95% CI, 0.10−3.86).

Three microscopy studies reported on the association between vaginal dysbiosis and HPV-related cervical cytological or histological changes.[23,25,29] The large Dutch screening study provided 3 estimates comparing the risk of atypical squamous cells of undetermined origin (ASCUS), LSIL, and HSIL with the normal cytology category. All 3 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, 1.32−2.01).[25]

The other 2 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] The other study provided 2 different risk estimates for disease progression from normal to LSIL to HSIL/SCC in which ASCUS was added either to the normal or to the LSIL category.[23] The results for the subgroups based on the reference and categorization of ASCUS (as described in the previous text) are presented separately (Figure 4).

All effect estimates showed a trend toward an increased risk of disease progression associated with vaginal dysbiosis (except for the analyses in which ASCUS was grouped with LSIL), but none of them reached statistical significance.[23] The 2 studies that assessed the risk of HSIL/SCC compared with normal (including ASCUS/LSIL in 1 study) showed a doubled risk among women with vaginal dysbiosis (pooled RR, 2.01; 95% CI, 1.40−3.01) and low heterogeneity ($I^2 = 0\%$) (Table).[25,29]

Comment

Our meta-analyses provide evidence that sexually active women with vaginal dysbiosis are as follows: (1) at increased risk of acquiring HPV infection, (2) more prone to HPV persistence, and/or (3) 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.[7−9,12] 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 5 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

TABLE
Vaginal dysbiosis and the risk of HPV incidence, persistence, and cervical cancer

Variables	Included studies	Number of included individuals	Pooled relative risk (95% confidence interval)	I^2, %	*P* heterogeneity
Microscopy studies, incident HPV					
Overall	28, 31, 32, 36	5280	1.33 (1.18–1.50)	0.0	.627
Young women	31, 32	1915	1.43 (1.10–1.85)	0.0	.473
HIV positive/high-risk HIV negative	28, 36	4501	1.31 (1.14–1.49)	0.0	.354
Adjusted estimates only	28, 31, 36	4490	1.33 (1.17–1.51)	0.0	.419
Adjusted hazard ratios only	31, 36	3354	1.45 (1.20–1.74)	0.0	.584
Nugent diagnosis	31, 32, 36	4155	1.31 (1.16–-1.48)	0.0	.650
Low- and high-risk HPV combined	28, 31, 36	4490	1.33 (1.17–1.51)	0.0	.419
Molecular studies, incident HPV					
Not *L crispatus* dominated vs *L crispatus* dominated	22, 33	96	1.85 (0.47–7.32)	55.7	.133
Microscopy studies, persistent HPV					
Overall	26-28, 32, 34-36	4711	1.14 (1.01–1.28)	44.2	.096
Young women	27, 32, 34	618	1.30 (0.77–2.20)	50.0	.136
HIV positive/high-risk HIV negative	28, 35, 36	3386	1.08 (0.97–1.20)	35.4	.212
HIV, high risk	28, 35	401	1.27 (0.57–2.82)	64.9	.058
Adjusted estimates only	28, 34	1287	1.19 (1.03–1.38)	0.0	.942
Hazard ratios only	28, 36	3365	1.09 (0.95–1.25)	67.7	.078
Risk ratios only	26, 27, 32, 34, 35	1017	1.18 (1.01–1.37)	0.0	.789
Nugent diagnosis	28, 32, 34-36	3675	1.05 (1.00–1.11)	0.0	.418
Papanicolaou smear diagnosis	26, 27	1036	1.47 (0.85–2.55)	66.4	.085
Only asymptomatic women	27, 35	350	1.86 (1.05–3.28)	0.0	.328
Including symptomatic women	26, 28, 32, 34, 36	4361	1.10 (1.00–1.21)	35.1	.187
Only high-risk HPV types	26, 32, 34	996	1.18 (1.01–1.38)	0.0	.599
Low- and high-risk types combined	27, 28, 35, 36	3715	1.15 (0.96–1.37)	61.8	.049
Molecular studies, persistent HPV					
Not *L crispatus* dominated vs *L crispatus* dominated	22, 24	87	1.33 (0.63–2.81)	23.8	.252
L gasseri dominated vs *L crispatus* dominated	22, 24	26	0.63 (0.10–3.86)	81.0	.022
L iners dominated vs *L crispatus* dominated	22, 24	46	1.06 (0.42–2.63)	0.0	.461
Low lactobacilli mixed aerobe/anaerobe vs *L crispatus* dominated	22, 24	44	1.00 (0.23–4.30)	80.1	.025
Low lactobacilli anaerobe vs *L crispatus* dominated	22, 24	69	2.00 (1.05–3.81)	0.0	.391
Dysplasia/cancer					
HSIL vs normal	25, 29	91,149	2.01 (1.40–3.01)	0.0	.768

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

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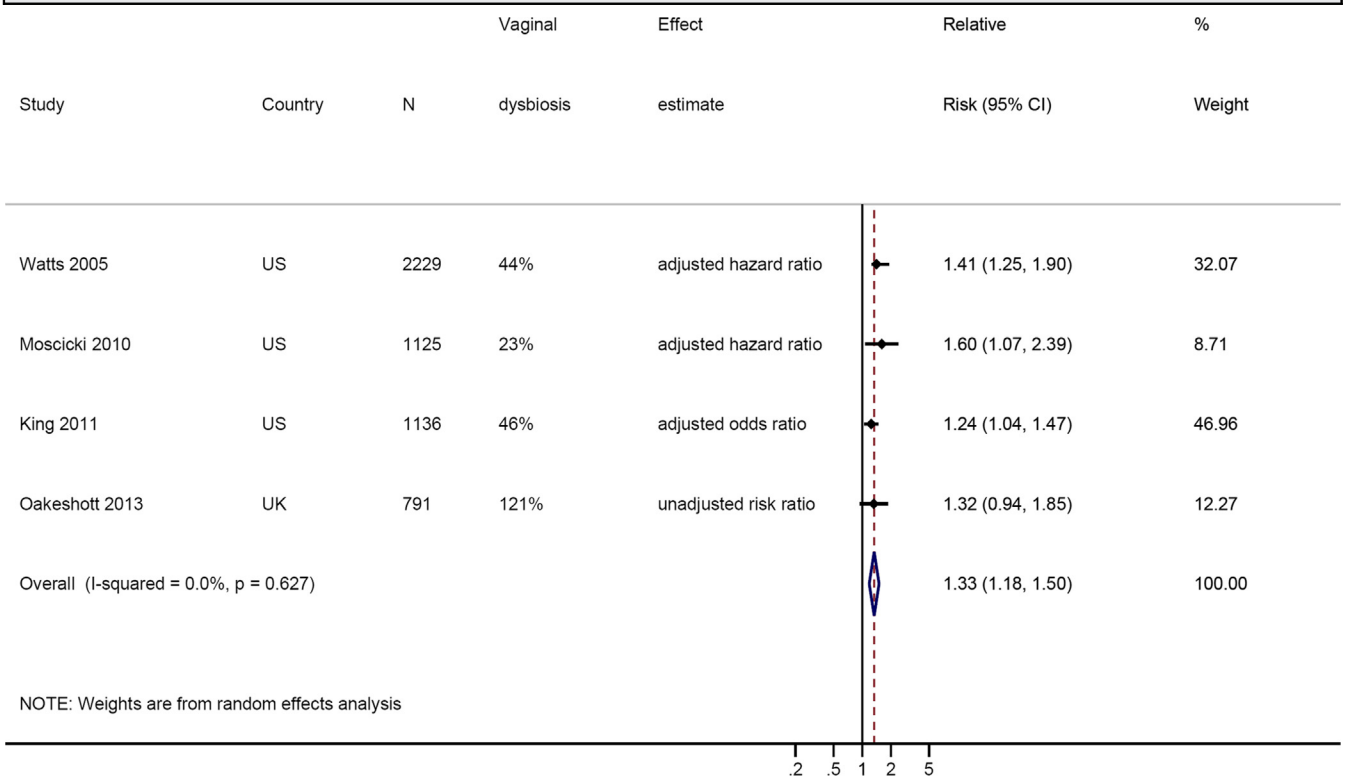
FIGURE 2
Vaginal dysbiosis and incident HPV



Forest plot showing the association between vaginal dysbiosis and the pooled relative risk of incident HPV using no vaginal dysbiosis as reference.

HPV, human papillomavirus.

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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 (ie, 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.

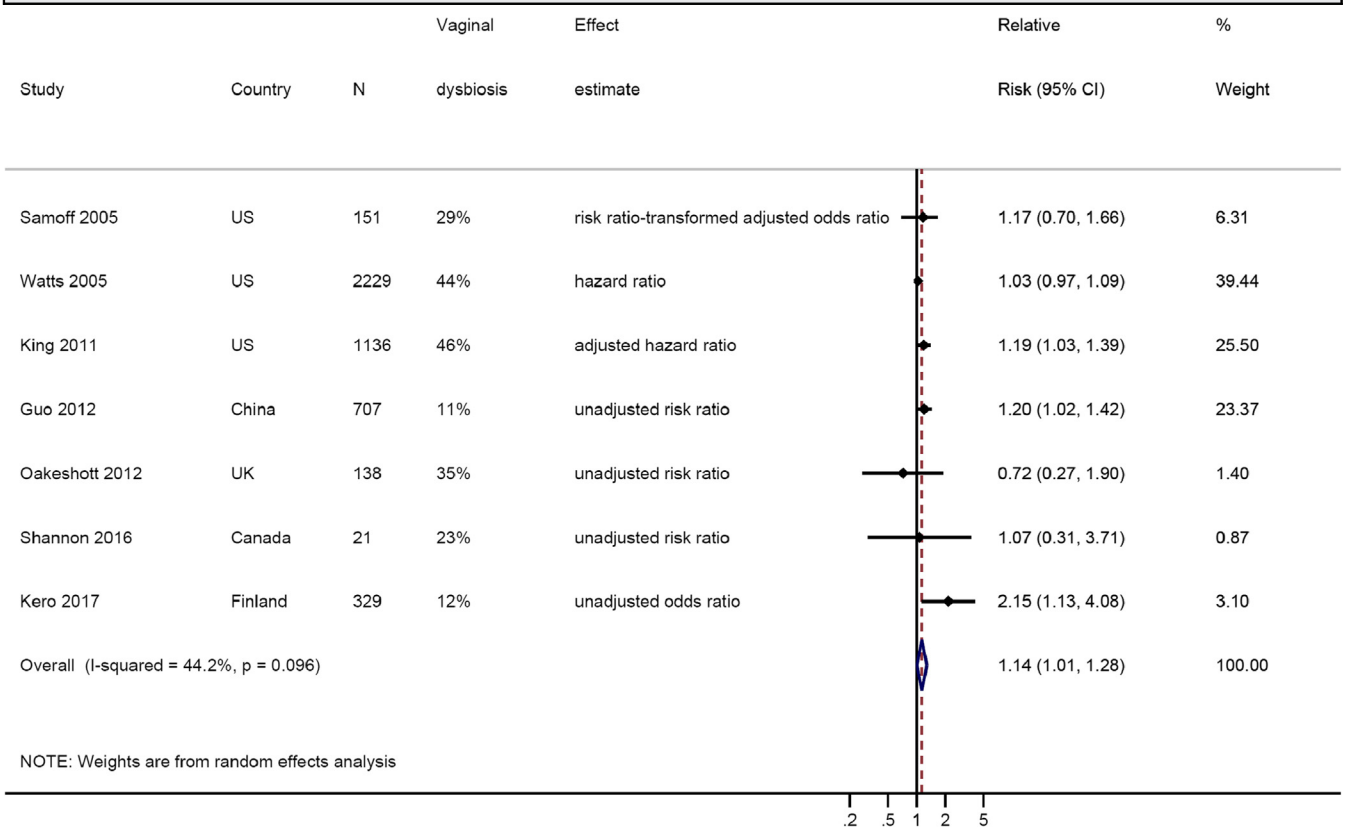
The extent to which the studies adjusted for confounding was highly variable. For example, only half of the studies considered the presence of other STIs or urogenital infections by exclusion at enrollment or through statistical model adjustment, and very few studies controlled for all potentially concomitant urogenital infections. For example, only 1 study reported to have accounted for (culture-positive) vaginal *Candida*.[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 polymerase chain reaction–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 subanalyses restricted to adjusted estimates compared with 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 because 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, socioeconomic status, ethnicity, past or current sexual risk behaviors, and coinfections, which was also reflected in the wide-ranging prevalence of vaginal dysbiosis. Notably, 5 of the 15 eligible studies included a high proportion of HIV-positive women.[23,28,29,33,36] 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] as well as in HPV detectability,[40,41] may lead to misclassification bias in studies that had few and/or large intervals between measurement points. There was also considerable variability in

FIGURE 3
Vaginal dysbiosis and persistent HPV



Forest plot showing the association between vaginal dysbiosis and the pooled relative risk of persistent HPV using no vaginal dysbiosis as reference.

HPV, human papillomavirus.

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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] with more than half of HPV-positive women showing coinfection with 2 or 3 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.[43,44] Only 3 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 5 studies, has been the gold standard of microscopic microbiota assessment in research settings.[13,14] The Amsel criteria (2 studies) are a valid diagnostic approach but have low sensitivity as a screening tool in asymptomatic women.[15,45]

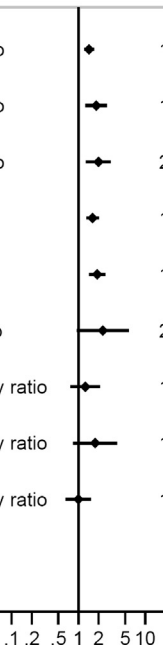
In the HPV and cervical cancer research field, vaginal dysbiosis is often diagnosed when clue cells are seen on Papanicolaou smears (5 studies), and the reported accuracy of this approach is inconsistent.[16,17,46,47] However, overall, the associations between vaginal dysbiosis and HPV/cancer were in the same direction and of a similar magnitude for studies that assessed the vaginal microbiota by molecular methods and those that used molecular techniques.

While we clearly show associations between vaginal dysbiosis and HPV infection, it is remarkable that only a few HPV epidemiology studies have accounted for this prevalent risk factor. Different sexually transmitted pathogens use different infection strategies, but vaginal dysbiosis has been shown to be a risk factor for most STIs,[48] as was documented most extensively for HIV.[49] However, contrary to HIV-1 virions that can penetrate both intact squamous and columnar epithelial barriers,[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]

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.[51,52] Chronic mucosal inflammation is also considered

FIGURE 4
Vaginal dysbiosis and progression to dysplasia and CIN

Study	Country	Population	n	vaginal dysbiosis	Progression	Effect estimate	Relative Risk (95% CI)
Engberts 2007	Netherlands	Screening	92656	6%	Normal > ASCUS	unadjusted odds ratio	1.44 (1.22, 1.71)
Engberts 2007	Netherlands	Screening	91093	6%	Normal > LSIL	unadjusted odds ratio	1.85 (1.28, 2.67)
Engberts 2007	Netherlands	Screening	90996	6%	Normal > HSIL	unadjusted odds ratio	2.00 (1.31, 3.05)
Engberts 2007	Netherlands	Screening	93205	6%	Normal > ASCUS > LSIL > HSIL	pooled odds ratio	1.63 (1.32, 2.01)
Engberts 2007	Netherlands	Screening	91319	6%	Normal > LSIL > HSIL (excl. ASCUS)	pooled odds ratio	1.91 (1.45, 2.53)
Lethovirta 2008	Finland	HIV+	153	12%	Normal/ASCUS/LSIL > HSIL/SCC	adjusted hazard ratio	2.32 (0.95, 5.65)
Denslow 2011	South-Africa	HIV+	503	54%	Normal/ASCUS > LSIL > ASC-H > HSIL	adjusted incidence density ratio	1.27 (0.76, 2.11)
Denslow 2011	South-Africa	HIV+	318	60%	Normal > LSIL > ASC-H > HSIL (excl. ASCUS)	adjusted incidence density ratio	1.78 (0.84, 3.79)
Denslow 2011	South-Africa	HIV+	503	60%	Normal > ASCUS/LSIL ASC-H > HSIL	adjusted incidence density ratio	1.00 (0.65, 1.53)



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.

CIN, cervical intraepithelial neoplasia.

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central to HPV-induced carcinogenesis.[53] At least hypothetically, this may concur with the numerous molecular signatures of mucosal inflammation and barrier failure with vaginal dysbiosis.[54] Of note, recent in vitro data do point to a protective, antitumoral effect exerted by vaginal lactobacilli on the cervical epithelium.[55–57]

Reverse causation should be considered because 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 microenvironment may lead to alterations in the vaginal microbiota.[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 1 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,[56,57,59–61] thereby possibly reducing obstetric morbidity related to excisional and ablative procedures.[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] However, vaginal microbiota differences between women of African or European descent might also explain this disparity.[64]

Similarly, the disproportionately high HPV prevalence among young African women[65] might be directly related to the high prevalence of vaginal dysbiosis in sub-Saharan Africa.[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] and to the role of the

adolescent vaginal microbiota in mucosal immune homeostasis.[68,69]

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 2017;123:2404–12.
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends—an update. Cancer Epidemiol Biomark Prev 2016;25:16–27.
3. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002;55:244–65.
4. Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: a cancer hallmarks analysis. Cell Host Microbe 2014;15:266–82.
5. Sudenga SL, Shrestha S. Key considerations and current perspectives of epidemiological studies on human papillomavirus persistence, the intermediate phenotype to cervical cancer. Int J Infect Dis 2013;17:e216–20.
6. Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect Dis 2010;10:862–74.
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 2016;4:58.
8. Gillet E, Meys JF, Verstraelen H, et al. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. BMC Infect Dis 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:e45201.
10. Senok AC, Verstraelen H, Temmerman M, Botta GA. Probiotics for the treatment of bacterial vaginosis. Cochrane Database Syst Rev 2009:CD006289.
11. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
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:e105998.
13. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297–301.
14. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. Sex Transm Infect 2002;78:413–5.
15. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983;74:14–22.
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 Obstet Gynecol Scand 1995;74:67–70.
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. Diagn Cytopathol 2006;34:272–6.
18. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 1998;280:1690–1.
19. Greenland S. Model-based estimation of relative risks and other epidemiologic measures in studies of common outcomes and in case-control studies. Am J Epidemiol 2004;160:301–5.
20. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ Clin Res Ed 2003;327:557–60.
21. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ Clin Res Ed 1997;315:629–34.
22. Brotman RM, Shardell MD, Gajer P, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis 2014;210:1723–33.
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. Int J Gynaecol Obstet 2011;114:273–7.
24. Di Paola M, Sani C, Clemente AM, et al. Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection. Sci Rep 2017;7:10200.
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 2007;111:269–74.
26. Guo YL, You K, Qiao J, Zhao YM, Geng L. Bacterial vaginosis is conducive to the persistence of HPV infection. Int J STD AIDS 2012;23:581–4.
27. Kero K, Rautava J, Syrjanen K, Grenman S, Syrjanen S. Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. Eur J Clin Microbiol Infect Dis 2017;36:2215–9.
28. King CC, Jamieson DJ, Wiener J, et al. Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol 2011;2011:319460.
29. Lehtovirta P, Paavonen J, Heikinheimo O. Risk factors, diagnosis and prognosis of cervical intraepithelial neoplasia among HIV-infected women. Int J STD AIDS 2008;19:37–41.
30. Mao C, Hughes JP, Kiviat N, et al. Clinical findings among young women with genital human papillomavirus infection. Am J Obstet Gynecol 2003;188:677–84.
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 Epidemiol Biomark Prev 2010;19:2055–65.
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 Clin Res Ed 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. J Infect Dis 2016;214:1361–9.
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. Am J Epidemiol 2005;162:668–75.
35. Shannon B, Yi TJ, Perusini S, et al. Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. Mucos Immunol 2017;10:1310–9.
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. J Infect Dis 2005;191:1129–39.
37. Ness RB, Kip KE, Soper DE, Stamm CA, Rice P, Richter HE. Variability of bacterial vaginosis over 6- to 12-month intervals. Sex Transm Dis 2006;33:381–5.

38. Brotman RM, Ravel J, Cone RA, Zenilman JM. Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. Sex Transm Infect 2010;86:297–302.
39. Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. Sci Transl Med 2012;4:132ra152.
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 Epidemiol Biomark Prev 2014;23:200–8.
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. J Med Virol 2015;87:2122–9.
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. J Virol 2014;88:4786–97.
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. J Infect Dis 2005;192:1158–65.
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. J Infect Dis 2001;184:1508–17.
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. Sex Transm Dis 2011;38:270–4.
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. Am J Obstet Gynecol 1997;177:532–5.
47. Greene JF 3rd, Kuehl TJ, Allen SR. The papanicolaou smear: inadequate screening test for bacterial vaginosis during pregnancy. Am J Obstet Gynecol 2000;182:1048–9.
48. Unemo M, Bradshaw CS, Hocking JS, et al. Sexually transmitted infections: challenges ahead. Lancet Infect Dis 2017;17:e235–79.
49. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet (London, England) 2014;384:258–71.
50. Carias AM, McCoombe S, McRaven M, et al. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. J Virol 2013;87:11388–400.
51. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013;13:800–12.
52. Garrett WS. Cancer and the microbiota. Science (New York, NY) 2015;348:80–6.
53. Fernandes JV, De Medeiros Fernandes TA, De Azevedo JC, et al. Link between chronic inflammation and human papillomavirus-induced carcinogenesis (review). Oncol Lett 2015;9:1015–26.
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. Muco Immunol 2016;9:621–33.
55. Vielfort K, Weyler L, Soderholm N, Engelbrecht M, Lofmark S, Aro H. Lactobacillus decelerates cervical epithelial cell cycle progression. PloS One 2013;8: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. J Med Microbiol 2013;62(Pt 7):1065–72.
57. Wang KD, Xu DJ, Wang BY, Yan DH, Lv Z, Su JR. Inhibitory effect of vaginal Lactobacillus supernatants on cervical cancer cells. Probiot Antimicrob Proteins 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 2015;42:965–76.
59. Verhoeven V, Renard N, Makar A, et al. Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. Eur J Cancer Prev 2013;22:46–51.
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 Infect Dis 2018;18:13.
61. Cha MK, Lee DK, An HM, et al. Antiviral activity of Bifidobacterium adolescentis SPM1005-A on human papillomavirus type 16. BMC Med 2012;10:72.
62. Kyrgiou M, Mitra A, Moscicki AB. Does the vaginal microbiota play a role in the development of cervical cancer? Transl Res 2017;179:168–82.
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. J Infect Dis 2015;211:100–8.
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) 2014;160(Pt 10):2272–82.
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. J Infect Dis 2010;202:1789–99.
66. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol 2013;209:505–23.
67. Junior JE, Giraldo PC, Goncalves AK, do Amaral RL, Linhares IM. Uterine cervical ectopy during reproductive age: cytological and microbiological findings. Diagn Cytopathol 2014;42:401–4.
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. J Reprod Immunol 2011;88:66–71.
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. Clin Vaccine Immunol 2015;22:526–38.

Supplemental Material

APPENDIX 1
Detailed search strategy

Database	Search string	Details	Date of most recent search	Number of hits
PubMed	("Microbiota"[Mesh] OR microbiome[tiab] OR microbiota[tiab] OR flora[tiab] OR microflora[tiab] OR "vaginitis"[Mesh] OR vaginitis[tiab] OR vaginosis[tiab] OR dysbiosis[tiab] OR dysbacteriosis[tiab]) AND (vaginal[tiab] OR vagina[tiab] OR cervix[tiab] OR cervical[tiab] OR cervicovaginal[tiab] OR female[tiab] OR women[tiab] OR woman[tiab]) AND ("Alphapapillomavirus"[Mesh] OR HPV[tiab] or human papillomavirus[tiab] OR human papilloma virus[tiab] OR "Uterine Cervical Neoplasms"[Mesh] OR cancer [tiab] OR dysplasia[tiab] OR neoplasia[tiab] OR squamous intraepithelial lesions[tiab] OR LSIL[tiab] OR HSIL[tiab] OR CIN[tiab])		June 11, 2018	933
Web of Science	(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 (alphapapillomavirus 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)	Search on topic	June 11, 2018	1043
Embase	('microbiome'/exp/mj OR 'microbiota'/exp/mj OR 'flora'/exp/mj OR 'microflora'/exp/mj OR 'vaginitis'/exp/mj OR 'vaginosis'/exp/mj OR 'dysbiosi'/exp/mj OR 'dysbacteriosis'/exp/mj) AND (vaginal OR 'vagina'/exp/mj OR cervix OR cervical OR cervicovaginal OR 'female'/exp/mj OR 'women'/exp/mj OR 'woman'/exp/mj) AND ('alphapapillomavirus'/exp/mj OR 'HPV'/exp/mj OR ('human'/exp/mj AND 'papillomavirus'/exp/mj) OR ('uterine'/exp/mj AND cervical AND 'neoplasms'/exp/mj) OR 'cancer'/exp/mj OR 'dysplasia'/exp/mj OR 'neoplasia'/exp/mj OR (squamous AND intraepithelial AND lesions) OR 'LSIL' OR 'HSIL' OR 'CIN')	Advanced search: map to preferred term in Emtree and explode using narrower Emtree terms, limit to major focus	June 11, 2018	249
Cinahl	(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 (alphapapillomavirus 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)	Search on title or abstract	June 11, 2018	66
Cochrane	(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 (alphapapillomavirus 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)	Advanced search on title, abstract, key words, no additional studies identified	June 11, 2018	174

CIN, cervical intraepithelial neoplasia; *HPV*, human papillomavirus; *HSIL*, high-grade squamous intraepithelial lesion; *LSIL*, low-grade squamous intraepithelial lesion.

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APPENDIX 2

Quality assessment composite grading tool

Quality criteria	Total points
The inception study had a prospective cohort design (including nested case-control study)	1
The study sample was representative for the population specified, not necessarily the community or background population	1
Assessment of vaginal dysbiosis was pursued through Gram-stained–based microscopy or a microbiome-wide sequencing approach	1
The study was adequately powered relative to the incidence of dysbiosis, HPV infection and related laesions	1
Potential confounders (including the major confounders smoking, parity, hormonal contraception, HIV, and concomitant STIs) were properly assessed and accounted for in the analysis	0.5 if several, although not all major confounders were accounted for and 1 point for comprehensive control
The analysis was based on repeated, serial assessment of vaginal microbiota status and incident HPV and/or related laesions (in contrast to a single follow-up visit)	1
Intermittent treatment of vaginal dysbiosis and of CIN was reported and/or accounted for in the analysis	0.5 for each and hence 1 point for both
Loss to follow-up was not likely to impinge on results (1 point)	1

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A total of 8 points can be assigned to each study, categorized as:

1–3: high risk of bias (low-quality evidence)
4–5: moderate risk of bias (moderate-quality evidence)
6–8: low risk of bias (high-quality evidence)

CIN, cervical intraepithelial neoplasia; *HPV*, human papillomavirus; *STI*, sexually transmitted infection.

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APPENDIX 3
Study characteristics of all 15 studies included in the systematic review

	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1	Exclusion criteria	Age range at baseline	Age at baseline	Number of sex partners	Ethnicity	Vaginal dysbiosis detection	Vaginal dysbiosis diagnosis	Vaginal dysbiosis symptoms?	Treatment of vaginal dysbiosis	% vaginal dysbiosis at baseline	% vaginal dysbiosis at follow-	HPV status at baseline	% HPV positive at baseline
2	Sexual inexperience during study period,	18-24 years	19 (mean)	median 1 (lifetime)	79% white, 12% Asian	Microscopy	Amsel (wet mount)	possible	Antibiotics if symptomatic	3%	n.r.	HPV positive or negative	n.r.
3	Pregnant, HIV+, no antibiotics during month before baseline visit	13-19 years	16 (median)	median 3 (lifetime)	95% Afro-American	Microscopy	Nugent (gram stained)	possible	"one dose therapy"	29%	n.r.	HPV positive	100%
4	<1 follow-up visit with HPV data available	16-73 years	35 (median)	median 1 (current)	53 Afro-American, 25% Hispanic, 19% white	Microscopy	Nugent (gram stained)	possible	n.r.	44%	n.r.	HPV positive or negative	52%
5	No subsequent smear, symptomatic	30-60 years	40-50 (median)	n.r.	n.r.	Microscopy	Pap smear/KOPAC	asymptomatic	n.r.	6%	n.r.	n.a.	n.a.
6	Hysterectomy, only 1 follow-up visit	n.r.	30 years (mean)	n.r.	64% European, 19% African, and 13% Asian	Microscopy	Pap smear - clue cells	possible	n.r.	24%	n.r.	n.a.	n.a.
7	Current pregnancy, >5 years sexually active, immunosuppression	13-22 years	19 (mean)	mean 11.6 (at last visit)	42% white, 24% hispanic, 15% Asian, 14% African-American	Microscopy	Amsel (wet mount)	possible	n.r.	23%	n.r.	HPV positive	100%
8	No follow-up visit >=6 months, severely ill. If pregnant: eligible for inclusion 6w after delivery. If symptomatic STI: eligible after treatment	18-65 years	34 (median)	<=4 in 61% (lifetime)	98% black-African.	Microscopy	Pap smear - clue cells (on 2 consecutive tests)	possible	antibiotic treatment is offered if detected	54%	54%	n.a.	n.a.
9	HIV seroconversion during study period, no history of injection drugs or high-risk sexual behaviour, not fluent in English/Spanish, history of AIDS defining conditions	16-55 years	n.r.	median 1 (last six months)	61% Afro-American, 21% Caucasian, 18% hispanic	Microscopy	Nugent (gram stained) (Modified Amsel in <1% if Nugent was	possible	n.r.	46%	n.r.	HPV positive or negative	52%
10	n.r.	19-71 years	39 (mean)	n.r.	n.r.	Microscopy	Pap smear - clue cells	possible	n.r.	11%	9%	High risk HPV positive	100%
11	Age >27, not sexually experienced, pregnant, tested for Chlamydia previous 3 months.	16-27 years	21 (mean)	43% ≥2 parteners in previous year	27% black	Microscopy	Nugent (gram stained)	possible	n.r.	21%	n.r.	HPV positive or negative	18%
12	Not reproductive age	22-53 years	37 (mean)	84% in monogamous relationship	50% Afro-American, 40% white	Molecular	16s RNA on self-collected mid-vaginal swabs	not specified	n.r.	36% low lactobacilli CST	n.r.	HPV positive or negative	77%
13	<8 year follow up at the centre, <6 annual cervicolavage specimens, gonorrhea/syphilis/trichomonas/chlamydia at baseline, exchange of sex for drugs/money at >5 visits during the observation period	100% premenopausal	32 (mean)	median 10-49 (lifetime)	100% Afro-American	Molecular	16s RNA on cervicovaginal lavage	possible	n.r.	unclear	n.r.	HPV positive or negative	n.r.
14	Pregnant, HIV+, immunodeficiency related diseases, previous HPV vaccination	26-64 years	41-45 (mean)	n.r.	100% Caucasian	Molecular	16s RNA on cervicovaginal samples (midwife)	not specified	n.r.	25%	n.r.	HPV negative excluded from analyses (except for 17 control samples)	100%
15	n.r.	n.r.	25 (mean)	mean 2.4 (lifetime)	n.r.	Microscopy	Pap smear/Hay-Ison criteria	asymptomatic	n.r.	12%	n.r.	HPV negative excluded from analyses	100%
16	Incomplete data, current infection with HIV, *N. gonorrhoeae, C.trachomatis*, <16 years, current pregnancy, symtomactic genital infection in previous 3 months	10% menopausal	n.r.	n.r.	100% African/Carribean or other black women	Microscopy/ molecular	Nugent (gram stained) + 16s RNA on self-collected undiluted cervicovaginal	asymptomatic	n.r.	23%	n.r.	HPV negative excluded from analyses	100%

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(continued)

APPENDIX 3
(*Continued*)

	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL
1	**HPV testing**	**HPV types**	**HPV types included**	**Cytology at baseline**	**Cytology categorisation**	**Histology**	**Total population (in longitudinal analyses)**	**Total population (in longitudinal analyses)**	**Total number of visits**	**Interval**
2	PCR (L1 consensus primer MY09/MY11/HMB01)	Overall (18): 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 45, 51-56, 58	Low and high risk combined in analyses	Not reported at baseline nor assessed in study	n.a.	n.a.	516	516	4009 (mean 8)	4 months
3	PCR (Roche Line blot assay)	Combined (27): high risk (20): 16, 18, 26, 31, 33, 35, 39, 45, 51-53, 55, 56, 58, 59, 66, 68, 73, 82, 83; low risk (7): 6, 11, 40, 42, 54, 57, 84.	Low and high risk analysed separately (only high risk in our analyses)	Not reported at baseline	Bethesda	n.a.	151	151	181 follow-up pairs (first visit HPV+, follow-up vist >=6 months)	≥6 months
4	PCR (L1 consensus primer MY09/MY11/HMB01)	Combined (23): high risk (4): 16, 18, 31, 41; intermediate risk (10): 33, 35, 39, 51, 52, 56, 58, 59, 68, 73; low risk (9): 6, 11, 40, 42, 53, 54, 61, 72, 81	Low and high risk combined in analyses	Prevalence of SIL at each visit 6-14%	Bethesda	n.a.	2229	2229	>=2	6 months
5	n.a.	n.a.	n.a.	Excluded: abnormal cytology at baseline: excluded	KOPAC	not available	93205	93,205	2 per person	4 years (mean)
6	n.a.	n.a.	n.a.	Only reported in detail for those with CIN	Bethesda	Biopsie or LEEP resection	153	153	n.r.	6-12 months (mean 9.5 months)
7	PCR (PGMY09/11 primer system)	Overall (29): 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 61, 62, 64, 66-68, 70-73, 81-84, 89	Low and high risk combined in analyses	Excluded: history of ablative/surgical therapy cervix	n.a.	n.a.	1125	1125	13,775 (mean 12)	4 months
8	n.a.	n.a.	n.a.	Excluded: HSIL positive at baseline, hysterectomy or cone biopsy treatment for cervical lesions.	Bethesda	not available	503	503	2 per person	>6 months
9	PCR	Overall (26): 6, 11, 16, 18, 26, 31, 33, 34, 39, 40, 42, 45, 51-56, 58, 59, 66, 68, 73, 82-84	Low and high risk combined in analyses	Excluded: No cervix at baseline or cerival treatment/hysterectomy around enrollment	n.a.	n.a.	1136	1136	max 15 per person	6 months
10	not specified	Only high risk HPV (not specified)	Only high risk in analyses	Included: ASCUS or LSIL 6-12 months before	Bethesda	n.a.	707	707	2 per person	6-12 months (mean 9.5 months)
11	Hybrid Capture 2 assay - PCR (Roche Linear Array)	Combined (37): high risk (13): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; low risk (24): 6, 11, 26, 40, 42, 53-55, 61, 62, 64, 66, 67, 69-73, 81-84, IS39, CP6108)	Only high risk in analyses	Not reported at baseline nor assessed in study	n.a.	n.a.	821	821	2 per person	11-32 months, 16 months median
12	PCR (Roche Linear Array)	Combined (37): high risk (13): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68.	Only high risk in analyses	Not reported at baseline nor assessed in study	n.a.	n.a.	32	32	32 self-collected swabs (twice weekly)	twice weekly
13	PCR (L1 consensus primer MY09/MY11/HMB01)	Combined (49): 6, 11, 13, 16, 18-26, 31, 32, 33, 34, 35, 39, 40, 42, 45, 51-59, 61, 62, 64, 66-74, 81-85, 89. Oncogenic subtypes (IARC) separate	Low and high risk combined in analyses	Not reported at baseline nor assessed in study	n.a.	n.a.	64	64	2-11 per person (median 6) tested for both HPV and bacterial dysbiosis	semi-annual
14	Hybrid capture 2 assay	High risk (13): 16, 18, 31, 33, 35, 39, 45, 51, 52,56,58, 59, 68	Only high risk in analyses	Not reported at baseline	Bethesda	Colposcopy	55	55 (+17 control samples)	2 per person	1 year
15	PCR (Multimetrix, Progen Biotechnik)	Combined (24): high risk (18): 16, 18, 26, 31, 33, 35, 39, 45, 51-53, 56, 58, 59, 66, 68,73, 82; low risk (6): 6, 11, 43, 43, 44, 70.	Low and high risk combined in analyses	Not reported at baseline or during follow-up	Bethesda	n.a.	329	329	6 per person	0, 2, 12, 24, 36, 72 months
16	PCR (Roche Linear Array)	Combined (37): high risk (14): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69.	Low and high risk combined in analyses	Not reported at baseline nor assessed in study	n.a.	n.a.	23	23	2 per person	6 months

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(continued)

APPENDIX 3
(*Continued*)

	AM	AN	AO	AP
1	**Total follow-up**	**Participation ratio/ coverage**	**How are STIs/*Candida* handled?**	**Risk of bias**
2	3 years	20% of invited women	STI's assessed yet not adjusted for in relevant outcomes	high
3	max. 4 years	24% of 821 attending clinic were HPV+ and therefore eligible	analyses adjusted for presence other STIs	moderate
4	n.r.	n.r.	analyses adjusted for presence other STIs	low
5	4 years (mean)	n.r.	If other STIs or candida: excluded from analyses	high
6	5.6 years (mean)	n.r.	Not assessed	moderate
7	4.8 years (mean)	79% of 1559 women were HPV+ and therefore eligible	analyses adjusted for presence other STIs	low
8	1.2 years (median)	n.r.	*Candida*/*Trichomonas* collected, relevant outcomes not adjusted.	moderate
9	7.5 years (max)	n.r.	Reports results separately for *Candida*+/-, interaction with *Trichomonas* assessed.	low
10	6-12 months	n.r.	Not assessed	high
11	12-28 months	33% of orginal 2529 women provided follow-up sample	If tested for Chlamydia 3 months before: excluded. Other STIs assessed but adjusted estimates not reported for relevant outcomes	high
12	16 weeks	32/39 with complete follow-up data (82%)	Not assessed	moderate
13	6.9 years (mean)	n.r.	If STIs at baseline: excluded	low
14	1 year	55/1029 were HPV+ at baseline (5.3%)	Not assessed	high
15	6 years	n.r.	Not assessed	moderate
16	6 months	n.r.	Excluded if current STI	high

AIDS, acquired immunodeficiency syndrome; *ASCUS*, atypical squqmous cells of undetermined origin; *CIN*, cervical intraepithelial neoplasia; *CST*, community state type; *HPV*, human papilloma virus; *HSIL*, high-grade squamous intraepithelial lesions; *KOPAC*, Dutch alternative classification of cytological changes in cervical smears; *LEEP*, loop electrosurical excision procedure; *LSIL*, low-grade squamous intreaepithelial lesions; *n.a.*, not applicable; *n.r.*, not reported; *Pap smear*, Papanicolaou test; *PCR*, polymerase chain reaction; *RNA*, ribonucleic acid; *SIL*, squamous intreaepithelial lesions; *STI*, sexually transmitted infection; *UK*, United Kingdom; *USA*, United States of America.

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APPENDIX 4

Quality assessment of all 15 eligible studies included in the qualitative analyses

	Mao et al 2003[30]	Samoff et al 2005[34]	Watts et al 2005[36]	Engberts et al 2007[25]	Lehtovirta et al 2008[29]	Moscicki et al 2010[31]	Denslow et al 2011[23]	King et al 2011[28]	Guo et al 2012[26]	Oakeshott et al 2012[32]	Brotman et al 2014[22]	Reimers et al 2016[33]	Di Paola et al 2017[24]	Kero et al 2017[27]	Shannon et al 2017[35]
Indicator															
Prospective	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+
Representative sample	−	-	+	+	+	+	+	+	+	−	−	+	−	+	−
Gram stain or PCR	−	+	+	−	−	−	−	+	−	+	+	+	+	+	+
Adequately powered	−	−	+	+	+	+	−	+	+	−	−	−	−	+	−
Control for confounders	±	−	±	−	±	+	±	+	−	+	−	±	−	−	−
Serial assessment	+	+	+	−	+	+	−	+	−	−	+	+	−	+	−
Intermittent treatment	±	±	±	−	±	−	±	±	−	−	±	±	−	−	−
Loss to follow-up	−	−	+	+	+	+	+	+	+	−	−	+	+	−	+
Quality score (numerical)	3/8	4/8	7/8	3/8	5/8	6/8	4/8	7.5/8	3/8	3/8	3.5/8	6/8	3/8	5/8	3/8
Risk of bias (categorical)	High	Moderate	Low	High	Moderate	Low	Moderate	Low	High	High	Moderate	Low	High	Moderate	High

PCR, polymerase chain reaction.

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APPENDIX 5
Articles excluded with reasons, based on full text assessment

In total 33 studies were excluded after screening of the full text.

- Not longitudinal or the necessary data were incomplete, n = 23.[1–22]
- No data on HPV, n = 1.[23]
- Not on the association between bacterial vaginosis and HPV, n = 2.[24,25]
- On role of bacterial vaginosis in regression/progression of cervical cancer, n = 4.[26–29]
- Conference abstracts, n = 3.[30–32]

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REFERENCES

1. Audirac-Chalifour A, Torres-Poveda K, Bahena-Roman M, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. PloS One 2016;11: e0153274.

2. Borgdorff H, Tsivtsivadze E, Verhelst R, et al. Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. ISME J 2014;8:1781–93.

3. Castle PE, Hillier SL, Rabe LK, et al. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomarkers Prev 2001;10: 1021–7.

4. Dareng EO, Ma B, Famooto AO, et al. Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. Epidemiol Infect 2016;144:123–37.

5. Gao W, Weng J, Gao Y, Chen X. Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect Dis* 10 2013;13:271.

6. Hadjimichael O, Janerich D, Lowell DM, Meigs JW, Merino MJ, Schwartz PE. Histologic and clinical characteristics associated with rapidly progressive invasive cervical cancer: a preliminary report from the Yale Cancer Control Research Unit. Yale J Biol Med 1989;62: 345–50.

7. Kiseki H, Tsukahara Y, Tajima N, Tanaka A, Horimoto A, Hashimura N. Influence of co-infection complicated with human papillomavirus on cervical intraepithelial neoplasia development in patients with atypical squamous cells of undetermined significance. J Infect Chemother 2017;23:814–9.

8. Laniewski P, Barnes D, Goulder A, et al. Linking cervicovaginal immune signatures, HPV and microbiota composition in cervical carcinogenesis in non-Hispanic and Hispanic women. Scie Rep 2018;8:7593.

9. Lee JE, Lee S, Lee H, et al. Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PloS One 2013;8:e63514.

10. Maria de Castro-Sobrinho J, Helena Rabelo-Santos S, Ribeiro Fugueiredo-Alves R, et al. Bacterial vaginosis and inflammatory response showed association with severity of cervical neoplasia in HPV-positive women. Diagn Cytopathol 2017;45:474–6.

11. Marks MA, Gupta S, Liaw KL, et al. Prevalence and correlates of HPV among women attending family-planning clinics in Thailand. BMC Infect Dis 2015;15:159.

12. Mitra A, MacIntyre DA, Lee YS, et al. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. Sci Rep 2015;5:16865.

13. Mukanyangezi MF, Sengpiel V, Manzi O, et al. Screening for human papillomavirus, cervical cytological abnormalities and associated risk factors in HIV-positive and HIV-negative women in Rwanda. HIV Med 2018;19:152–66.

14. Oh HY, Kim BS, Seo SS, et al. The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. Clin Microbiol Infect 2015;21:674.e671–9.

15. Piyathilake CJ, Ollberding NJ, Kumar R, Macaluso M, Alvarez RD, Morrow CD. Cervical microbiota associated with higher grade cervical intraepithelial neoplasia in women infected with high-risk human papillomaviruses. Cancer Prev Res (Philadelphia, Pa 2016;9:357–66.

16. Shaw E, Ramanakumar AV, El-Zein M, et al. Reproductive and genital health and risk of cervical human papillomavirus infection: results from the Ludwig-McGill cohort study. BMC Infect Dis 2016;16:116.

17. Silva C, Almeida EC, Cobo Ede C, Zeferino VF, Murta EF, Etchebehere RM. A retrospective study on cervical intraepithelial lesions of low-grade and undetermined significance: evolution, associated factors and cytohistological correlation. Sao Paulo Med J 2014;132:92–6.

18. Swanepoel PJ, Michelow P, Du Plessis R, et al. Cervical squamous intraepithelial lesions and associated cervical infections in an HIV-positive population in Rural Mpumalanga, South Africa. Cytopathology 2013;24:264–71.

19. Walker S, Hamilton W. Risk of cervical cancer in symptomatic women aged >= 40 in primary care: a case-control study using electronic records. Eur J Cancer Care 2017;26.

20. Wang Z, Wang J, Fan J, et al. Risk factors for cervical intraepithelial neoplasia and cervical cancer in Chinese women: large study in Jiexiu, Shanxi Province, China. J Cancer 2017;8: 924–32.

21. Vieira-Baptista P, Lima-Silva J, Tavares S, Beires J, Donders G. Cytolytic vaginosis does not have an impact on human papilloma virus (HPV) infection and cervical dysplasia. J Lower Genit Tract Dis 2017;21:S27–8.

22. Zhang D, Li T, Chen L, Zhang X, Zhao G, Liu Z. Epidemiological investigation of the relationship between common lower genital tract infections and high-risk human papillomavirus infections among women in Beijing, China. PloS One 2017;12(5):e0178033.

23. Donders GGG, Vieira-Baptista P. Bacterial vaginosis and inflammatory response showed association with severity of cervical neoplasia in HPV-positive women. Diagn Cytopathol 2017;45:472–3.

24. Fennema JSA, Vanameijden EJC, Coutinho RA, Vandenhoek A. HIV, sexually-transmitted diseases and gynecological disorders in women—increased risk for genital herpes and warts among HIV-infected prostitutes in Amsterdam. AIDS (London, England) 1995;9:1071–8.

25. Wessman M, Thorsteinsson K, Jensen JS, et al. Bacterial vaginosis, human papilloma virus and herpes viridae do not predict vaginal HIV RNA shedding in women living with HIV in Denmark. BMC Infect Dis 2017;17:376.

26. Frega A, Lavini G, Guarino A, et al. Cervical carcinogenesis, bacterial vaginosis,

HPV-mRNA test and relapse of CIN2+ after loop electrosurgical excision procedure (LEEP). Eur Rev Med Pharmacol Sci 2017;21:2504–11.

27. Mitra A, MacIntyre D, Lai J, et al. The vaginal microbiota after excisional treatment for cervical intraepithelial neoplasia. J Lower Genit Tract Dis 2017;21:S1.

28. Song SH, Lee JK, Oh MJ, Hur JY, Park YK, Saw HS. Risk factors for the progression or persistence of untreated mild dysplasia of the uterine cervix. Int J Gynecol Cancer 2006;16:1608–13.

29. Wang PC, Song JH. The correlation between vaginal microecological changes and HPV outcome in patients with cervical lesions in the Inner Mongolia area of China. Int J Clin Exp Med 2017;10:5711–20.

30. Mitra A, MacIntyre DA, Lee YS, et al. The vaginal microbiome of women with cervical intraepithelial neoplasia. Int J Gynecol Cancer 2015;25:976–7.

31. Nikolova N, Nikolova T, Vojnika A, Bina A, Micevska M. Bacterial vaginosis and HPV persistence. Int J Gynecol Cancer 2014;24:815.

32. Wojciech WKK, Kotarski JKJ, Barczynski BBB, Warchol WWW, Wolun-Cholewa M, Gozdzicka-Jozefiak A. A metagenomicapproch to characteriztion of the cervix microbiome during HPV dependent carcinogenesis. Int J Gynecol Cancer 2015;25:848.