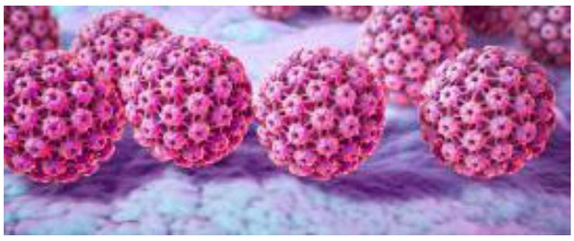
Driehoeksverhouding vaginale microbiomen, HPV en baarmoederhalskanker

"The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis"
Norenhag J. et al. (British Journal of Obstetrics and Gynaecology), 25 juni 2019

Een cervicale dysplasie en baarmoederhalskanker kunnen het gevolg zijn van een langdurige infectie met een humaanpapillomavirus met een verhoogd risico. Recente studies wijzen op een mogelijk verband tussen de samenstelling van vaginale microbiomen, een HPV-infectie en de evolutie naar een dysplasie en kanker.



Bij de meerderheid van de vrouwen bestaat het vaginaal microbioom uit **lactobacillen die zorgen voor een omgeving die beschermt** tegen de ontwikkeling van exogene bacteriën en virussen door de productie van melkzuur, bacteriocines en biosurfactanten. **Bij veranderingen van het vaginale microbioom door bacteriële vaginose verdwijnen de lactobacillen** om plaats te maken voor anaerobe bacteriën. Soms is er zelfs een verband met bepaalde zwangerschapscomplicaties en bekkenbodeminfecties.

In de meeste gevallen geneest een HPV-infectie, de meeste voorkomende overdraagbare seksuele infectie, spontaan na enkele maanden. De evolutie naar een **dysplasie en kanker is het gevolg van een persisterende infectie met een hoogrisico-HPV-virus**, al zijn de oorzaken van deze langdurige infectie niet goed gekend. Gynaecologische, seksuele en fertiliteitsrisicofactoren en tabaksverslaving werden in kaart gebracht, maar ook die risicofactoren met een link naar immuunaandoeningen (hiv).

Microbioomveranderingen verhogen de kans op een HPV-infectie, de lange duur van de infectie **of de snelheid van de evolutie naar een dysplasie**. Een review heeft geprobeerd om een antwoord op deze vragen te formuleren. De review omvatte 11 studies die vanaf 2000 werden gepubliceerd. Tien ervan hebben het verband tussen vaginaal microbioom + HPV-infectie onderzocht. Vier zochten een link op tussen vaginaal microbioom + dysplasie en kanker.

Microbiomen en HPV

Zo'n 55% van de met HPV-besmette vrouwen en 49% van de vrouwen die drager zijn van een **persisterende hoogrisico HPV-infectie hadden microbiomen met weinig lactobacillen**, terwijl microbiomen met weinig lactobacillen voorkwamen bij 38% van de vrouwen die niet met HPV waren besmet en bij 37% van de vrouwen zonder een langdurige HPV-infectie.

In vergelijking met microbiomen die rijk zijn aan *L. crispatus* (klasse I) kwamen microbiomen met weinig lactobacillen (klasse IV) meer voor bij HPV-infecties (OR: 4,73; CI 95%, 2,06-10,86). In vergelijking met microbiomen die rijk zijn aan *L. crispatus* kwamen microbiomen met veel *L. iners* (klasse III) meer voor bij HPV-infecties (OR: 3,22; CI 95%, 1,39-7,47).

Microbiomen, dysplasieën en kanker

Microbiomen met weinig lactobacillen (OR: 2,78; CI 95%; 1,50-5,16) of gedomineerd door *L. iners* (OR: 1,95; CI 95%; 1,07-3,56) kwamen meer voor bij een cervixletsel, in de meest brede zin van de term, gaande van een squameuze intra-epitheale laesie tot kanker, in vergelijking met microbiomen gedomineerd door *L. crispatus*.

Er was een verband tussen microbiomen met weinig lactobacillen, gedomineerd door Prevotelle, Atopobium en Gardnerella (klasse IV) en een persisterende hoogrisico-HPV-infectie, terwijl microbiomen die gedomineerd zijn door *L. crispatus* (klasse I) eerder in verband stonden met negativatie en/of opruiming van HPV.

Deze **resultaten wijzen op een verband tussen types van microbiomen en infecties met het humaanpapillomavirus, of met geassocieerde cervixletsels**.

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Systematic review

The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis

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Background Persistent infection with high-risk human papillomavirus can lead to cervical dysplasia and cancer. Recent studies have suggested associations between the composition of the vaginal microbiota, infection with human papillomavirus (HPV) and progression to cervical dysplasia and cancer.

Objective To assess how specific cervico-vaginal microbiota compositions are associated with HPV infection, cervical dysplasia and cancer, we conducted a systematic review and network meta-analysis (registered in PROSPERO: CRD42018112862).

Search strategy PubMed, Web of science, Embase and Cochrane database.

Selection criteria All original studies describing at least two community state types of bacteria (CST), based on molecular techniques enabling identification of bacteria, and reporting the association with HPV infection, cervical dysplasia and/or cervical cancer.

Data collection and analysis For the meta-analysis, a network map was constructed to provide an overview of the network relationships and to assess how many studies provided direct evidence for the different vaginal microbiota compositions and HPV, cervical dysplasia or cancer. Thereafter, the consistency of the model was assessed, and forest plots were constructed to pool and summarise the available evidence, presenting odds ratios and 95% confidence intervals.

Main results Vaginal microbiota dominated by non-*Lactobacilli* species or *Lactobacillus iners* were associated with three to five times higher odds of any prevalent HPV and two to three times higher for high-risk HPV and dysplasia/cervical cancer compared with *Lactobacillus crispatus*.

Conclusions These findings suggest an association between certain bacterial community types of the vaginal microbiota and HPV infection and HPV-related disease. This may be useful for guiding treatment options or serve as biomarkers for HPV-related disease.

Keywords cervical cancer, dysplasia, *Lactobacilli*, microbiome, vaginal microbiota.

Tweetable abstract This network meta-analysis suggests an association between different vaginal bacterial community types and the risk of HPV.

Linked article This article is commented on by K Njoku and EJ Crosbie. To view this mini commentary visit https://doi.org/10.1111/1471-0528.15867.

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Introduction

The vaginal microbiota in most women consist mainly of *Lactobacillus* species, which create a low pH environment protecting against both exogenous bacteria and viruses by producing lactic acid, bacteriocins and biosurfactants.[1,2]

Vaginal microbiota disturbances can cause bacterial vaginosis, characterised by a depletion of lactobacilli and overgrowth of non-*Lactobacillus* microbes, typically anaerobic bacteria.[3] Bacterial vaginosis has been associated with numerous reproductive health problems including pelvic inflammatory disease and adverse obstetric outcomes.[4,5]

Most studies of the vaginal microbiota previously relied on Amsel criteria (clinical settings) or Gram-stain Nugent

PROSPERO registration number: CRD42018112862.

scoring (research settings).[6,7] New culture-independent molecular techniques, such as sequencing, have enabled further characterisation of the vaginal microbiota, pointing to different major 'community state type' (CST), defined by the relative abundance and diversity of the identified species. These CSTs are broadly divided in *Lactobacillus*-dominated CST (e.g. *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jensenii*) and non-*Lactobacillus*-dominated CST with low number of *Lactobacillus* spp. and an increased diversity of anaerobic bacteria, or a mixture of aerobic and (facultative) anaerobic bacteria.[8–10] Some studies also subdivide non-*Lactobacillus* CSTs into several CSTs based on the dominant anaerobe or aerobe, but without a consistent pattern across studies.[11].

Several relatively small studies have suggested that there is an association between changes in the composition of the vaginal microbiota and infection with human papillomavirus (HPV), whereas previous meta-analyses did not distinguish between specific species.[3,12,13]

Human papillomavirus is one of the most common sexually transmitted infections; however, most infections clinically resolve after a few months. Persistent infection with high-risk HPV (hrHPV) can lead to dysplasia and cervical cancer,[14,15] which is the fourth most common cancer in women worldwide. Most deaths due to cervical cancer occur in low-and middle-income countries with limited access to screening and health care,[16] but even in high-income countries the related morbidity remains considerable.

It is still unclear why some hrHPV infections resolve clinically while others persist and cause dysplasia and even cervical cancer. Several risk factors including smoking, sexual and reproductive factors, and immunosuppression such as HIV infection have been identified.[15] Women with a certain specific composition of the vaginal microbiota may be more prone to acquire HPV or show a more rapid dysplasia progression and therefore require closer follow up and/or more advanced treatment.

Therefore, the objective of our current systematic review and network meta-analysis is to examine if different specific compositions of the vaginal microbiota (defined by molecular techniques) are associated with HPV infection, cervical dysplasia and cervical cancer.

Methods

We conducted a systematic review according to the PRISMA guidelines incorporating network meta-analysis[17] and the study protocol, which was registered in PROSPERO (CRD42018112862). Our aim was to see how the cervico-vaginal microbiota is associated with HPV infection, persistent infection and severity of dysplasia based on cross-sectional and longitudinal studies. Different terminology has been used by the individual studies in this review to describe the bacterial clusters. In this systematic review and meta-analysis, we use the term 'community-state type' (CST), defined by the relative abundance and diversity of the identified species. Our hypothesis was that some CSTs are more frequently associated with HPV infection and some CSTs can be associated with cervical dysplasia and more advanced stages of disease.

Search strategy and selection criteria

We included original studies describing at least two CSTs, or the equivalent bacteria in the vaginal microbiota based on molecular techniques enabling identification of bacterial CSTs and reporting the association with HPV infection, cervical dysplasia and/or cervical cancer in humans, enabling risk assessment at a patient level (not only species level). Conference abstracts were excluded, as well as studies only based on microscopy or clinical diagnoses of bacterial vaginosis.

The search was conducted using PubMed, Web of science, Embase and Cochrane database and was last updated 4 December 2018 (see Supplementary material, Table S1). The time-period was restricted to articles published after the year 2000 because current diagnostic techniques were introduced relatively recently. We also retrieved articles from the reference lists and citations of the selected articles (so-called backward and forward citation tracking).

The literature was selected in duplicate by two researchers (JN, NB) based on the eligibility criteria mentioned above. The final selection of studies was agreed upon by all authors. If two or more studies presented the same cohort or overlapping cohorts, we discussed which study to include (preferably the most recent with the most comprehensive reporting and largest sample size).

Data extraction

Data extraction included study characteristics (author, year, country, study period), population characteristics (including demographics, recruitment procedure) and information on CSTs or equivalent in relation to prevalent, incident and/or persistent HPV (any HPV or hrHPV), as well as cervical dysplasia (based on cytology and/or histology). Data used for the meta-analysis were extracted in triplicate by three researchers to ensure quality (JN, JD, NB), and meta-analyses were only conducted if at least three studies reported the outcome. To enable sufficient power we grouped the CSTs into the following five categories (if feasible) based on the dominant species: *L. crispatus*, *L. gasseri*, *L. iners*, 'low *Lactobacilli*' (based on the categorisation and cut-offs in the included studies, defined as non-*Lactobacillus*-dominated CSTs with an increased diversity of anaerobic or a mixture of aerobe and, facultative anaerobe bacteria) and 'other lactobacilli' (i.e. *Lactobacillus gallinarum*, *Lactobacillus salivarius*, *Lactobacillus parvus*,

Lactobacillus johnsonii, *Lactobacillus fermentum*, *L. jensenii*, *Lactobacillus brevis*). CSTs that could not be transformed into these groups were omitted from the analysis. Quality assessment was conducted using the Agency for Healthcare Research and Quality quality assessment for cross-sectional studies and was conducted in duplicate (JN, NB).[18]

Statistical analyses

The cumulative proportions of the different CSTs in each study were pooled and weighted, and presented as percentages. To enable direct and indirect comparisons between all CSTs, we used a fixed network meta-analysis approach,[19] and a classic pairwise meta-analysis to validate the results and explore heterogeneity (see Supplementary material, Appendix S1).

Patient involvement

None, because study was based on published literature.

Results

Our systematic search resulted in 11 eligible included articles (Figure 1), of which the general characteristics and quality assessment are described in the Supplementary material (Tables S2 and S3).[8,9,20–28] Reasons for exclusion of the articles excluded after full text screening are reported in the Supplementary material (Appendix S2), with 15 studies lacking essential data for inclusion in the network meta-analysis.[29–43]

Three studies were based on asymptomatic women either recruited for the study or attending screening,[8,22,25] and three were based on women belonging to a risk group (female sex workers, recruitment from an HIV testing centre, African/Caribbean and other black women identified as a risk group).[21,24,27] The remaining five studies were based on women that were chosen from referrals to colposcopy or from other studies for being HPV-positive or having cervical dysplasia.[9,20,23,26,28] Two of the studies were conducted in North America,[8,27] one study in South America,[20] two in Europe,[9,23] three in Africa,[21,22,24] and three in Asia.[25,26,28]. The number of individuals included in the studies varied from 29 to 278, and the age of the women included ranged between 18 and 65 years old.

Of all 11 studies, ten investigated the association between the vaginal microbiota and HPV,[8,9,20–25,27,44] and four

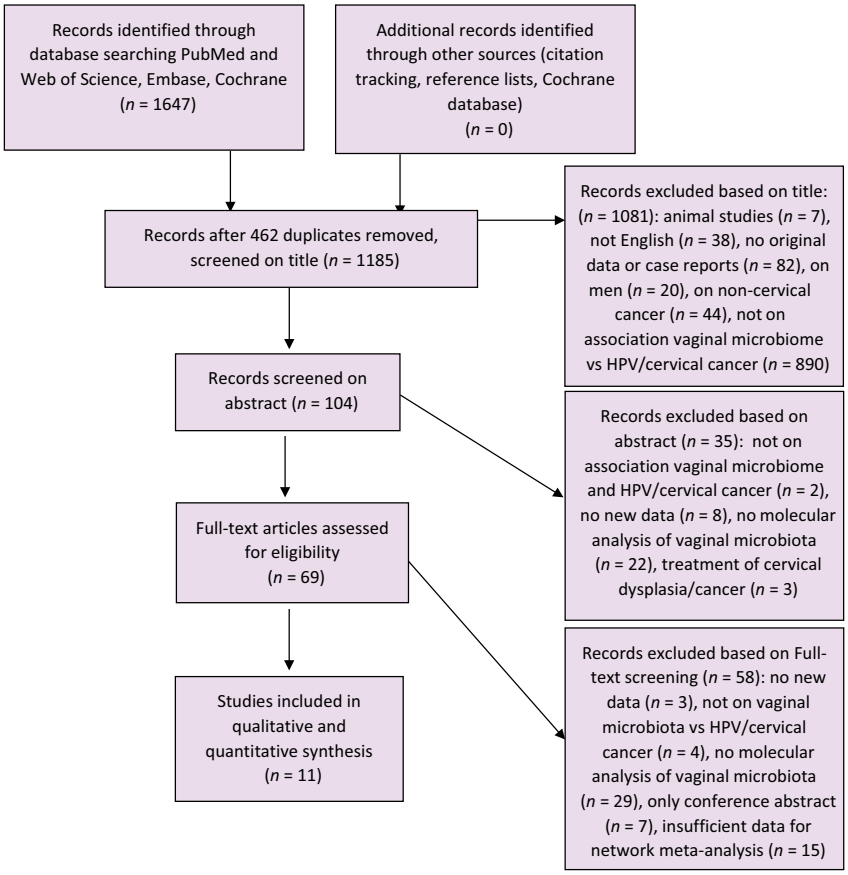

Figure 1. PRISMA flowchart of the systematic literature selection.

investigated the association between vaginal microbiota and dysplasia/cancer.[9,20,26,44] Eight studies used 16S rRNA analysis for characterising the vaginal microbiota.[8,9,20,22,23,26–28] Two studies used microarray,[21,24] and one study used polymerase chain reaction/denaturing gradient gel electrophoresis.[25]

CST/types of bacteria

The number of CSTs found in the studies ranged between four and eight. All found CSTs dominated by *L. iners*, and ten of the studies found CSTs dominated by *L. crispatus*. All but one of the studies found at least one CST dominated by anaerobic bacteria or with a paucity of lactobacilli.[24] Four of the studies kept the non-*Lactobacillus* dominated CSTs as one,[9,23,27,28] whereas six studies also divided the non-*Lactobacillus* CST further into species-specific CSTs.[8,20–22,25,26] For the meta-analysis we divided the different CSTs into the following groups, depending on the dominant species, as presented in the studies included in this review; *L. crispatus*, *L. iners*, *L. gasseri*, 'low lactobacilli', and 'other *Lactobacillus* spp.' (i.e. *L. gallinarum*, *L. salivarius*, *L. parvus*, *L. johnsonii*, *L. fermentum*, *L. jensenii*, *L. brevis*).

Microbiota and HPV: proportions

Ten studies presented data on the vaginal microbiota and its association with prevalent HPV,[8,9,20–25,27,28] with four studies reporting on any HPV infection,[8,21,22,27] and eight on hrHPV infection only.[9,20–25,28] Incident HPV[8] and HPV persistence[23] were each only reported in one study, and therefore not assessed by means of meta-analysis.

Figure 2 shows the weighted proportion of different CSTs to assess the prevalence of CSTs in relation to HPV-positive or HPV-negative study participants. CST dominated by 'low *Lactobacillus*' species was found among 55% of women positive for any HPV and among 49% of women positive for hrHPV. 'Low lactobacillus'-dominated CST was found among 38% of those negative for any HPV and among 37% of those negative for hrHPV. CSTs dominated by *L. crispatus* or *L. iners* were found among 44 and 43% of those positive for any HPV or hrHPV, respectively, and *L. crispatus* or *L. iners* were found among 58 and 54% of those negative for any HPV or hrHPV, respectively.

Microbiota and HPV: meta-analyses

The network map of any HPV infection (Figure 3) shows that estimations for direct associations are available

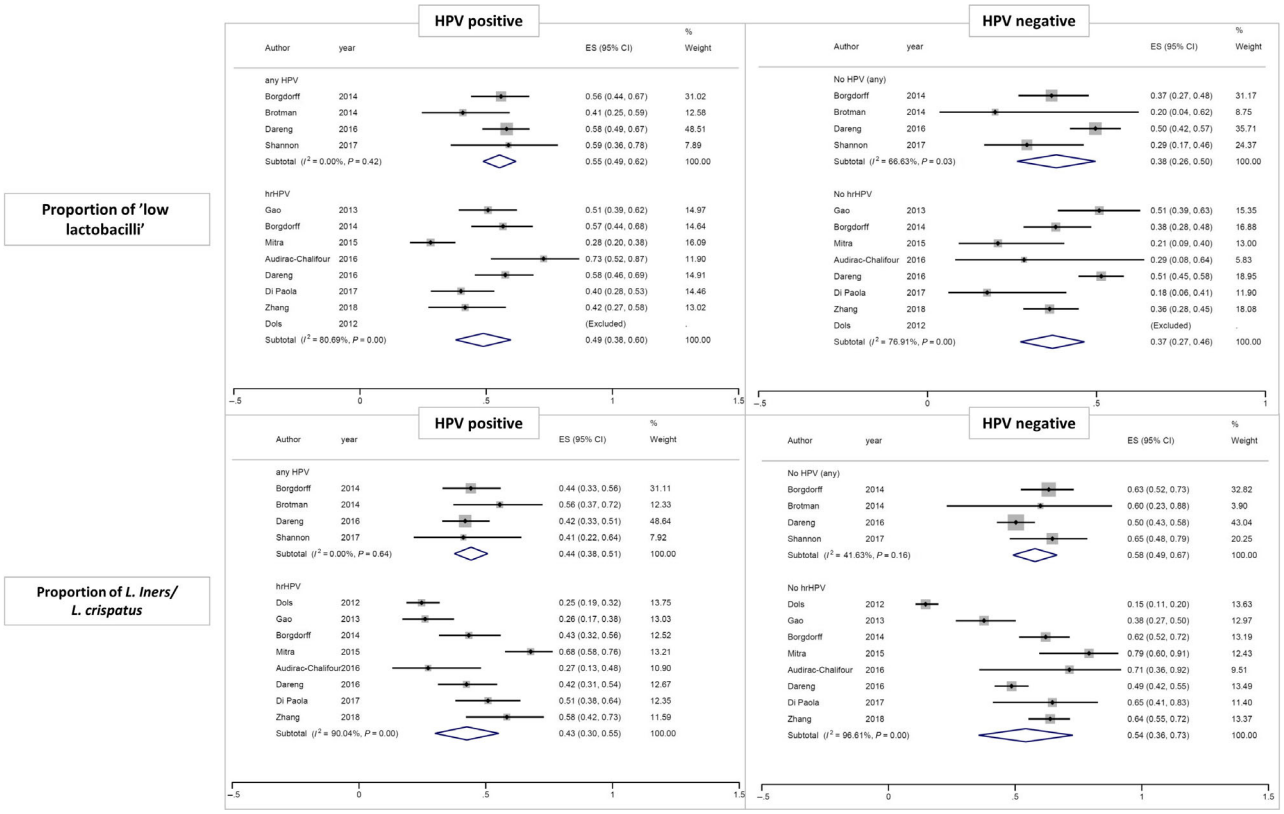

Figure 2. Weighted pooled proportion of vaginal microbiota defined as 'low lactobacilli' or *Lactobacillus iners* or *Lactobacillus crispatus* (combined) by human papillomavirus (HPV) status of all women.

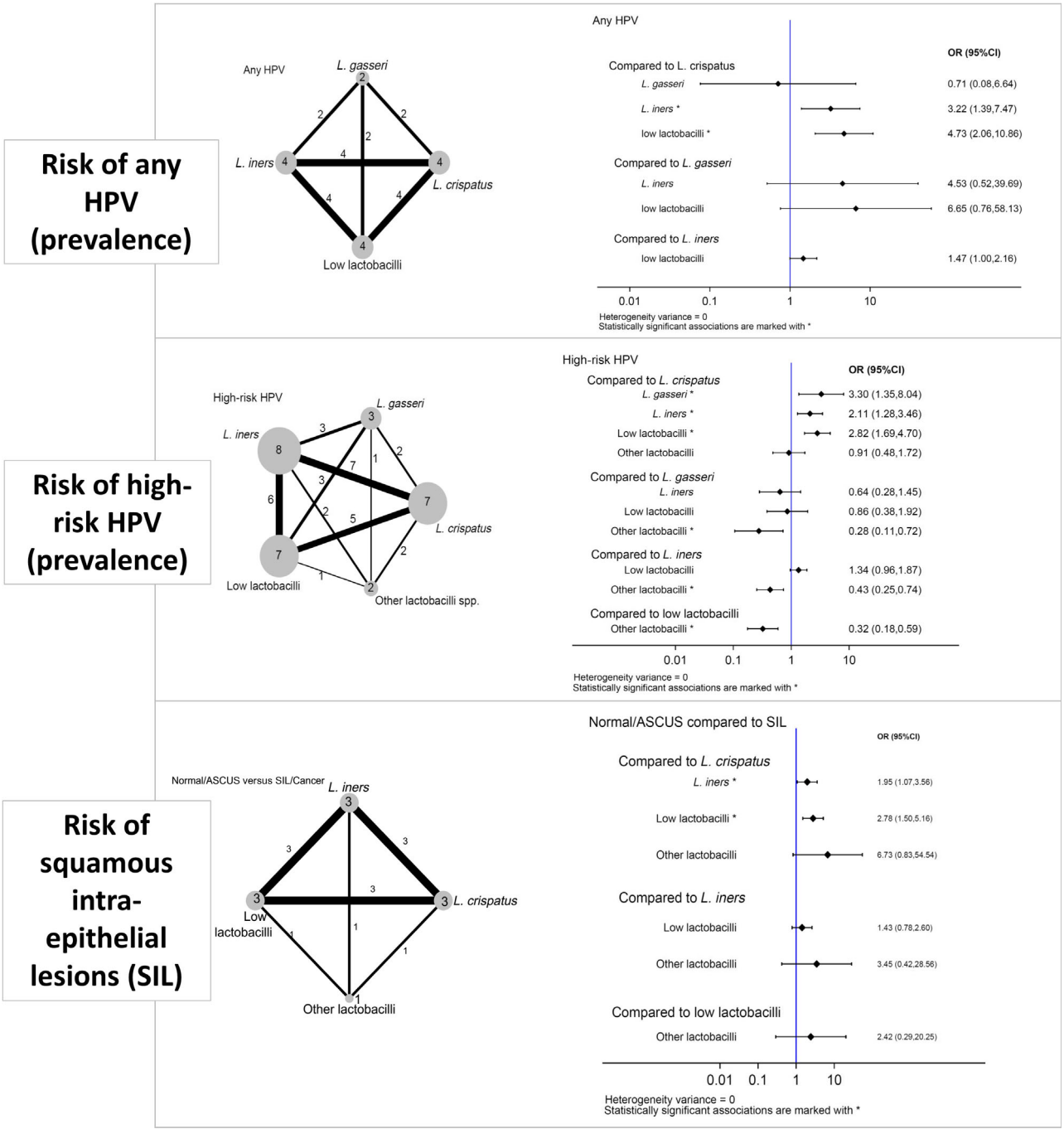

Figure 3. Network maps and forest plots showing the association between the vaginal microbiota compositions and the risk of any prevalent HPV, high-risk HPV or cervical dysplasia and cancer. Network map: the larger the dots, the more studies report on the vaginal microbiota composition mentioned (also indicated by the number within the dot). The thicker the lines, the more studies report on the association between these two vaginal microbiota composition group (also indicated by the number next to the lines). ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; L, *Lactobacillus*; OR, odds ratio; SIL, squamous intraepithelial lesion.

between all four CSTs (insufficient information available on the 'other lactobacilli' CST). The overall test of inconsistency ($P = 0.785$) and loop consistency ($P > 0.50$) for all tests confirmed that consistency assumption can be accepted (data not shown). 'Low lactobacilli'-dominated CST showed the strongest association with any HPV, with odds ratio (OR) 4.73 (95% CI 2.06–10.86) compared with *L. crispatus* (Figure 3). *Lactobacillus iners* also showed

higher odds of any HPV compared with *L. crispatus* (OR 3.22 (95% CI 1.39–7.47). The ranking of CSTs showed that the 'low lactobacilli' group had the highest risk of any HPV with a probability of 92.4% (data not shown).

For hrHPV infection, the loop consistency test was < 0.05 ($P = 0.028$) for the comparison between *L. crispatus* and *L. gasseri*, indicating some inconsistency when comparing direct and indirect estimates (data not shown); however, this did not affect the overall model (Figure 3). One study was excluded from the analysis as it combined *L. crispatus* and *L. iners* in one group.[28] The analyses on hrHPV show that *L. gasseri* had the highest odds for hrHPV compared with *L. crispatus* (OR 3.30, 95% CI 1.35–8.04) (Figure 3), followed by 'low-lactobacilli' CST (OR 2.82, 95% CI 1.69–4.70) and *L. iners* (OR 2.11, 95% CI 1.28–3.46). CST with 'other *Lactobacillus* spp.' showed the lowest odds of prevalent hrHPV compared with *L. gasseri* (OR 0.28, 95% CI 0.11–0.72) 'low lactobacilli' (OR 0.32, 95% CI 0.18–0.59) and *L. iners* (OR 0.43, 95% CI 0.25–0.74). The ranking of CSTs showed that *L. gasseri* and 'low lactobacilli' had the highest risk of hrHPV with a probability of, respectively, 64.2 and 34.6% (Data not shown).

Heterogeneity variance was low in all analyses. The classic pairwise fixed meta-analysis for prevalent hrHPV (see Supplementary material, Table S4) confirms the higher risk among women presenting with *L. iners* compared with *L. crispatus* (OR 1.31, 95% CI 1.00–1.73); and the lower risk for other lactobacilli compared with *L. iners* (OR 0.73, 95% CI 0.55–0.97), with low statistical heterogeneity ($I^2 = 0$). The other comparisons did not reach statistical significance. A sensitivity analysis was conducted, excluding the study using polymerase chain reaction/denaturing gradient gel electrophoresis, which provided similar results (see Supplementary material, Figure S1).

Dysplasia and cancer

Four studies presented sufficient data for meta-analysis on the microbiota and dysplasia and/or cancer.[9,20,26,28] They all used different terminology when comparing different stages of dysplasia. The Mexican study divided the study participants into HPV-positive/negative non-cervical lesions, squamous intraepithelial lesions (SIL) and cervical cancer.[20] The study from the United Kingdom had the following subdivisions of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and cancer.[9] The Chinese study compared normal cytology and cervical intraepithelial neoplasia stage 1 (CIN1) with CIN2+,[28] and the South Korean study compared normal or ASCUS cytology to CIN 1–3 as one group.[26]

Given the differences in terminology among the studies involved we therefore compared normal/ASCUS with SIL/cancer, for which three studies presented sufficient data for the analysis, with direct estimations available between all CSTs (Figure 3). The overall test of inconsistency ($P = 0.922$) and loop consistency ($P > 0.60$) for all tests confirmed that consistency assumption can be accepted (data not shown). Both 'low lactobacilli'-dominated CST (OR 2.78, 95% CI 1.50–5.16) and *L. iners*-dominated CST (OR 1.95, 95% CI 1.07–3.56) had an association to SIL/cancer compared with *L. crispatus*-dominated CST (Figure 3). The ranking of the CSTs indicated that the 'low lactobacilli' had the highest risk of SIL/cancer with a probability of 77.4% (data not shown).

We also compared normal/ASCUS with HSIL/cancer. LSIL was categorised as normal in two studies,[9,28] and in the other two studies it was categorised as HSIL/cancer.[20,26] The analysis showed no significant associations (see Supplementary material, Figure S2).

Progression, remission

Two studies looked at disease progression and remission in relation to vaginal microbiota. Both studies found CSTs dominated by anaerobic bacteria such as *Prevotella*, *Atopobium and Gardnerella* associated with HPV persistence.[8,23] Both studies also found *L. crispatus*-dominated CST associated with HPV negativity and/or clearance.[8,23] One of the studies found *L. gasseri* associated with the fastest clearance rate.[8] The studies did not provide sufficient data for a meta-analysis.

Discussion

Main findings

Community state types dominated by 'low lactobacilli' or *L. iners* were associated with higher HPV prevalence when compared with *L. crispatus*, both when restricting to hrHPV and when assessing any HPV type; with the same associations shown for dysplasia and cancer,

Strengths and limitations

To our knowledge, this is the first meta-analysis to examine if different specific CSTs, defined by molecular techniques, are associated with HPV infection, cervical dysplasia and cervical cancer. This study is based on a thorough systematic literature search and uses a novel yet valid approach in the microbiome field to combine different CSTs to each other by means of a network meta-analysis, enabling a more objective and overall quantification of the risk of different vaginal microbiota compositions. The statistical heterogeneity was low in all (network and pairwise) meta-analyses, indicating that despite methodological and clinical differences, the main findings appear to be robust; although the effect size will need to be confirmed in future studies.

There are a number of limitations to this review. The studies in this review used a number of different methods to collect and analyse the microbiota samples. Two studies used microarray techniques,[21,24] which are not fully quantitative, and cannot detect new species that are not included beforehand on the microarray.[21] Differences in method, as well as differences in target region of 16S rRNA gene analysis or in sequencing methods could produce discrepancies among the results (in particular considering the number of taxa and abundances), and contribute to the methodological heterogeneity.[45] Yet, given the bimodal categorisation handled in the network meta-analyses, the impact of these differences should be minimal.

The number and composition of the CSTs differed between the studies, especially with regard to the non-*Lactobacillus*-dominated CSTs. Some of these differences relate to different analytical methods but also to differences in study population. The research area is fairly new and has not yet reached consensus on the preferred categories and methods.[11] As mentioned, the 'low lactobacilli' CST in this meta-analysis was based on previous categorisation made by the individual studies which makes this CST very heterogeneous. The findings on *L. gasseri* should be interpreted with caution because of the low number of individuals ($n = 4$ in any HPV analysis, and $n = 33$ in hrHPV analysis) and heterogeneity, with contradictory findings in the individual studies. One of the longitudinal studies furthermore reported *L. gasseri* associated with the fastest clearance rate of HPV infection.[8]

When studying the association between the microbiota and dysplasia and/or cancer, the included studies used different terminology for different stages of disease, which made the meta-analysis more difficult. Furthermore, one study grouped CIN 1–3 together, whereas it would be more relevant from a clinical perspective to have the cut-off at CIN2+.[26] There were relatively small sample sizes, ranging from 29 to 430, with < 100 individuals in five studies.

Interpretation

Our findings are in line with most studies suggesting a greater microbiota diversity and a lower abundance of lactobacilli among the HPV-positive women,[37,38,40] and an association between *L. iners* and CIN,[38] or even CIN2+.[39] Yet, several studies were too small to reach statistical significance or did not distinguish between different *Lactobacillus*-dominated CSTs. These findings are also in line with our previous meta-analysis, assessing the association between bacterial vaginosis (broadly defined) and HPV incidence and persistence.[3] This study was restricted to longitudinal studies to eliminate the risk of reverse causation and supports the hypothesis of a relatively stable vaginal microbiome affecting HPV acquisition and persistence, and not the HPV causing major changes in the vaginal microbiome.[3] This strengthened our current approach including cross-sectional data, to obtain sufficient power for species-specific analyses restricting to molecular studies.

The ability of lactobacilli to lower pH has been previously discussed, and a previous study found a 10% lower risk of HPV detection in individuals with a vaginal pH < 5.0 than in those with a pH > 5.0.[46] Our findings also suggest that different lactobacilli may have different roles. Some studies suggest that the two isoforms of lactic acid might differ in their microbial effects, because D-lactic acid but not L-lactic acid levels have been inversely associated with the ability of HIV to transverse cervicovaginal mucus, and *L. crispatus* produces both isoforms, whereas *L. iners* produces only L-lactic acid.[40,47,48] Hydrogen peroxide, produced by *L. crispatus* but not *L. iners*, was also suggested,[9,40] yet its in vivo antimicrobial role has been questioned, which could be seen as a marker for vaginal *Lactobacillus* strains that have other beneficial properties.[49] Both these explanations correspond well with our findings that a microbiota dominated by *L. crispatus* has a lower risk, and *L. iners* a higher risk of HPV, SIL and cancer.

Some of the included studies discussed the possibility of certain bacterial species, such as *Fusobacterium*, being oncogenic and promoting the development of dysplasia.[9,20] This is in line with previous studies of the gut microbiome and the association between *Fusobacterium* and colorectal cancer.[50–52] *Gardnerella vaginalis* and *Atopobium vaginae* were also proposed as molecular markers, as both could be involved in forming biofilm that may contribute to viral persistence.[23,53–55]

The number and composition of CSTs in this review varied between the studies. A systematic review from 2014 found that among 17 articles that used a clustering technique to characterise the vaginal microbiota, three to nine clusters were described.[11] Most common were CSTs dominated by *L. iners* and *L. crispatus*, whereas *L. jensenii*, *L. gasseri* or *G. vaginalis* were less common, and all studies identified at least one cluster that contained mixtures of anaerobes with or without *Lactobacillus* spp.[11]

Finally, differences in study population are likely to influence the vaginal microbiota, which could explain why there are differences in bacterial clusters presented in the studies. Differences between ethnic groups could be due to genetically determined differences between hosts, such as differences in innate and adaptive immune systems, the composition and quantity of vaginal secretions, and ligands on epithelial cell surfaces, among others.[10,22,56,57]

It is possible that a certain composition of the vaginal microbiota could make an individual more susceptible to acquiring an HPV infection, as well as creating the conditions for the infection to persist and progress to dysplasia and subsequently cancer. The possibility of having certain bacteria as biological markers is interesting from a clinical

perspective as it is possible that women with a certain composition of the vaginal microbiota could require more frequent check ups or another treatment approach.

Conclusion

Our findings suggest a more bacterial community type-specific involvement in HPV acquisition and progression to cancer as opposed to only differentiating between a *Lactobacillus*-dominated vaginal microbiota or not. In the future, more longitudinal studies as well as standardised methods for sampling and analyses are needed to firmly establish causal relationships and the extent of effect.

Disclosure of interests

None declared. Completed disclosure of interest forms are available to view online as supporting information.

Contribution to authorship

JN and NB designed the study and JD, MO, HV and LE provided important feedback on the proposed study design. JN and NB conducted the systematic literature search and quality assessment; NB conducted the meta-analyses, and the results were interpreted by all authors (JN, JD, MO, HV, LE, NB). JN drafted the initial manuscript, which was thoroughly reviewed for important intellectual content and revised by all authors (JD, MO, HV, LE, NB). All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Details of ethics approval

Not applicable.

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None.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Sensitivity network meta-analysis (network map and forest plot) to assess the association between cervical microbiota and high-risk human papillomavirus (hrHPV) detection, excluding one study using a different molecular approach to assess the vaginal microbiota.

Figure S2. Network meta-analysis (network map and forest plot) to assess the association between the vaginal microbiota and the risk of high-grade squamous cell intraepithelial lesions.

Table S1. Systematic literature selection – search strategy.

Table S2. Characteristics of the individual studies.

Table S3. Quality assessment of all 11 included studies by means of the AHRQ quality assessment for cross-sectional studies (AHRQ, Agency for healthcare research and quality).

Table S4. Classic 'pairwise' meta-analysis to assess the risk of high-risk human papillomavirus (prevalence) according to the different vaginal microbiota compositions, presenting both random and fixed effects models.

Appendix S1. Supplementary methods – network meta-analysis.

Appendix S2. Articles excluded after full text screening with reasons for exclusion. ■

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