**Title:** Epidemiologic evidence on the role of *Lactobacillus iners* in sexually transmitted infections and bacterial vaginosis: a series of systematic reviews and meta-analyses

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**Supplemental methods**

*Search strategy*

Only studies conducted among reproductive-age, nonpregnant, cisgender women were eligible for inclusion. We defined reproductive-age as post-menarchal and pre-menopausal with the bounds of 13-50 years (inclusive). If search results did not report an age-based eligibility criterion nor participant age range, we assumed the study was conducted among reproductive-age women. If authors did not clearly articulate participants’ gender identity, we assumed participants were cisgender women.

Studies of cross-sectional, case-control, cohort, or clinical trial designs were eligible. Cross-sectional studies were eligible because much of the published literature on relationships between vaginal bacteria and sexual health outcomes represents cross-sectional studies. Excluding these studies would have substantially limited the evidence reviewed. Case reports and case series were not eligible.

*Data collection*

Two reviewers (KAC, MDF) used the REDCap surveys to independently record eligible studies’ bibliographic information; study design; study site(s); years of participant enrollment and data collection; study population characteristics; vaginal microbiota characterization method; outcome assessment method; exposure and outcome summary statistics; relevant effect estimate(s) and corresponding measure(s) of uncertainty; and information related to risk of bias assessment.

For studies that did not present an effect estimate of interest but presented sufficient exposure and outcome data to calculate an effect estimate, we calculated effect estimates as follows: prevalence ratios (PR) for cross-sectional studies and cohort studies (when exposure and outcome data were collected at the same time point) that cross-tabulated categorical exposure and outcome data; odds ratios (OR) for case-control studies that cross-tabulated categorical exposure and outcome data; relative abundance ratio of ratios for studies that presented mean *L. iners* and *L. crispatus* relative abundances according to outcome status; and Spearman correlation coefficient differences for studies that presented Spearman correlation coefficients between *L. iners* relative abundance and the outcome and between *L. crispatus* relative abundance and the outcome.

We estimated relative abundance ratio of ratios according to Equation 1. We were unable to estimate confidence intervals for relative abundance ratio of ratios. A relative abundance ratio of ratios >1 indicates *L. iners* enrichment among those with the outcome is greater than *L. crispatus* enrichment among those with the outcome. A relative abundance ratio of ratios <1 indicates *L. crispatus* enrichment among those with the outcome is greater than *L. iners* enrichment among those with the outcome. A relative abundance ratio of ratios of 1 indicates *L. iners* and *L. crispatus* are enriched to the same degree among those with the outcome.

Equation 1:

$\frac{\frac{Mean\left(Lactobacillus iners relative abundance\right)|Outcome present}{Mean\left(Lactobacillus crispatus relative abundance\right)|Outcome present}}{\frac{Mean\left(Lactobacillus iners relative abundance\right)|Outcome absent}{Mean\left(Lactobacillus crispatus relative abundance\right)|Outcome absent}}$

We estimated Spearman correlation coefficient differences according to Equation 2. We were unable to estimate confidence intervals for Spearman correlation coefficient differences. A Spearman correlation coefficient difference >0 indicates *L. iners*-dominated microbiotas are more strongly positively correlated with the outcome than *L. crispatus*-dominated microbiotas. A Spearman correlation coefficient difference >0 indicates *L. crispatus*-dominated microbiotas are more strongly positively correlated with the outcome than *L. iners*-dominated microbiotas. A Spearman correlation coefficient difference of 0 indicates *L. iners*-dominated and *L. crispatus*-dominated microbiotas are correlated with the outcome to the same degree.

Equation 2:

$Spearman correlation coefficient\left(Lactobacillus iners relative abundance, Outcome\right)-Spearman correlation coefficient\left(Lactobacillus crispatus relative abundance, Outcome\right)$

For one study included for any HPV and hrHPV (Reimers et al. 201643s), we estimated odds ratios according to Equation 3. We were unable to estimate confidence intervals for these odds ratios.

Equation 3:

$\frac{OR(Lactobacillus iners-dominated v. Diverse, (hr)HPV v. no HPV)}{OR(Lactobacillus crispatus-dominated v. Diverse,(hr)HPV v. no HPV)} $

*Systematic review* e*vidence synthesis and meta-analysis*

We selected random-effects meta-analysis (RE-MA) (as opposed to fixed-effects meta-analysis) because random-effects meta-analysis assumes that observed effect estimates vary between studies due to real differences in the effect across studies.70s This is appropriate for meta-analyses of associations with the vaginal microbiota with sexual and reproductive health outcomes because there is evidence that these associations vary between populations.3,71s-73s

For each meta-analysis, we constructed a forest plot indicating the included studies; number of participants in each exposure category; number of outcome events in each exposure category; effect estimate and 95% confidence interval (CI); RE-MA weight; RE-MA summary estimate and 95% CI; Cochrane’s Q and p value; and I2. We used the forest and addpoly functions of the metafor package in R for constructing forest plots.39s

*Risk of bias and quality of evidence assessments*

The confounders we considered were vaginal sex (e.g. frequency, number of partners, condom use), hormonal contraception, vaginal washing, race/ethnicity, and variables race/ethnicity may be a proxy for (e.g. socioeconomic status, site/region). We selected these confounders based on expected substantial prevalence/variation of each factor and expected strong confounding. We did not consider recent use of antibiotics or antimycotics as a confounder because many vaginal microbiota studies exclude individuals who recently received either antibiotics or antimycotics. As such, we felt it was a reasonable assumption that the prevalence of recent use of antibiotics or antimycotics would be low enough that any resulting confounding would be small in magnitude. We did not consider co-infection(s) as a confounder because, while co-infection may act as a confounder (co-infection contributing to both exposure and outcome), it likely also acts as a mediator (co-infection as an intermediate between exposure and outcome). We assumed manuscripts would not present sufficiently detailed data to identify whether co-infection was acting as a confounder or mediator, so we did not consider it as a confounder.

As a baseline, we rated risk of bias due to exposure assessment as moderate for all studies due to the compositional nature of marker gene sequencing data, which are, by definition, not well-defined exposures.67s For the BV outcome, we rated risk of bias due to outcome measurement as not enough information for studies that did not provide details on training, quality control, or quality assurance for evaluating BV by Nugent score or Amsel criteria. We used the conservative approach of rating overall risk of bias in each study to be equivalent to its highest-rated domain-specific risk of bias. For example, if all domains other than confounding were rated as moderate, but confounding was rated as critical, we rated overall risk of bias as critical.

The GRADE system applies an initial low quality rating to observational evidence. Quality can be down-rated due to risk of bias, effect estimate inconsistency and imprecision, indirectness, and publication bias. Quality can be up-rated due to large effect, dose response, and if residual confounding increases confidence in effect estimates. We down-rated quality of evidence for indirectness when the majority of studies for an outcome evaluated prevalent outcomes because prevalent outcomes are of less interest to individuals at risk for BV, STI, and cervical dysplasia than incident outcomes. We down-rated quality of evidence for each review for likely publication bias due to insignificant vaginal microbiota*, L. iners*, and/or *L. crispatus* findings being less likely to be published or reported.

*Deviations from registered protocol*

To be eligible, studies had to be conducted among reproductive-age, nonpregnant, cisgender populations. In the registered protocol, we defined reproductive age as 15-50 (inclusive). We adjusted this age range to 13-50 (inclusive) because a single otherwise eligible study included participants as young as 13, and it is reasonable to assume that individuals 13 years old are post- or peri-menarchal and can be considered reproductive age. To clarify, this was one search result across the eight outcomes, and it was for the *Chlamydia trachomatis* review (Tamarelle et al. 202017).

We made the exposure and reference of interest specified in the registered protocol (exposure: *Lactobacillus iners*-dominated microbiota, reference: *Lactobacillus crispatus*-dominated microbiota) our primary exposure and reference of interest. We added a secondary exposure and reference of interest (exposure: *L. iners* relative abundance, reference: *L. crispatus* relative abundance, Supplemental Table 5) because several otherwise eligible studies did not report on *L. iners*- and *L. crispatus*-dominated microbiotas but did report summary measures of *L. iners* and *L. crispatus* relative abundances by outcome status, which reviewers could use to calculate an effect estimate/statistic of interest.

For eligible clinical trials, we included data from baseline prior to intervention receipt instead of including any relevant data from participants randomized to the placebo/standard of care arm of the trial. The primary outcomes of eligible trials were generally not our outcomes of interest, and eligible trials generally did not report on our outcomes of interest among participants randomized to placebo during/at the end of follow-up.

In addition to relative risks (RR), odds ratios (OR), and hazard ratios (HR), we also included prevalence ratios (PR) in meta-analysis for outcomes with at least three eligible studies reporting PRs for the association between *L. iners*, compared to *L. crispatus*, and the outcome of interest.

For otherwise eligible manuscripts that did not present an effect estimate/statistic of interest and did not present sufficient data for reviewers to calculate an effect estimate/statistic of interest, we did not contact authors to obtain data nor did we retrieve publicly available data to calculate an effect estimate/statistic of interest. A total of 57 manuscripts (98 total instances of these 57 manuscripts across the eight outcomes, Supplemental Table 7) were excluded because they did not present an effect estimate/statistic of interest and did not present sufficient data for reviewers to calculate an effect estimate/statistic of interest. We determined it was not feasible to contact authors or retrieve publicly available data to analyze for these 57 studies.

We did not cross-check references for all eligible manuscripts for additional potentially eligible manuscripts due to the nature of our eligibility screening process. We screened full texts for all search results, and eligible manuscripts typically did not report sufficient information in the title or abstract to determine whether they may or may not be eligible. As such, cross-checking references for all eligible manuscripts would have amounted to full-text screenings of all referenced studies (as many as 922 additional manuscripts), which was not feasible.

*Limitations of search strategy*

The results of these systematic reviews and meta-analyses should be interpreted in the context of the search strategy’s limitations. We searched four databases, which excludes relevant publications not indexed in these databases. Only English, full-text, peer-reviewed, original research manuscripts were eligible, which excludes any search results that were unavailable in English as well as evidence from search results that are not original research manuscripts (e.g. conference abstracts). Only studies that were conducted among reproductive age, nonpregnant, cisgender women were eligible, limiting the generalizability of our findings to non-reproductive age populations, pregnant populations, populations of transgender individuals assigned female at birth, and populations of transgender individuals with neovaginas.

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