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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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Abstract: Although *Lactobacillus crispatus*–dominated vaginal microbiotas are thought to protect against bacterial vaginosis (BV) and sexually transmitted infections, the role of *Lactobacillus iners*–dominated microbiotas is less clear. To better understand the impact of *L. iners* on common cervicovaginal infections, we conducted systematic reviews of the associations between *L. iners* compared with *L. crispatus* and 8 outcomes: *Chlamydia trachomatis* (Ct), BV, human papillomavirus, cervical dysplasia, human immunodeficiency virus, genital herpes, *Trichomonas vaginalis*, and *Neisseria gonorrhoeae*. On April 30, 2021, we searched PubMed, Embase, Cochrane Library, and Web of Science for epidemiologic studies of reproductive-age, nonpregnant, cisgender women that used marker gene sequencing to characterize vaginal microbiota composition and presented an effect estimate for the association between *L. iners*, compared with *L. crispatus*, and outcomes of interest. For outcomes with ≥3 eligible results presenting the same form of effect estimate, we conducted random-effects meta-analysis. The review protocol was registered prospectively (PROSPERO CRD42020214775). Six Ct studies were included in meta-analysis, which showed *L. iners*–dominated microbiotas were associated with 3.4-fold higher odds of Ct compared with *L. crispatus*–dominated microbiotas (95% confidence interval, 2.1–5.4). Three BV studies were included in meta-analysis, which indicated *L. iners*–dominated microbiotas were associated with 2.1-fold higher prevalence of BV compared with *L. crispatus*–dominated microbiotas (95% confidence interval, 0.9–4.9). Evidence was too sparse to perform meta-analysis for the remaining outcomes. *L. iners*–dominated vaginal microbiotas may be suboptimal compared with *L. crispatus*–dominated microbiotas for BV and Ct. These reviews highlight evidence gaps regarding the remaining outcomes and opportunities to improve epidemiologic rigor in vaginal microbiome science.

The World Health Organization recognizes sexual health as essential to overall health and wellbeing, and achieving sexual health depends on access to comprehensive sexual health care.[1] As a modifiable risk factor, the vaginal microbiota and its role in sexual health outcomes is a promising avenue for developing novel interventions to promote sexual health, which may become essential components of sexual health care in the future. Across populations, the vaginal microbiota of reproductive-age individuals is often dominated by *Lactobacillus iners* or *Lactobacillus crispatus*.[2–8] *L. crispatus*–dominated vaginal microbiotas are widely thought to protect against adverse sexual health outcomes including cervicovaginal infections and cervical disease.[9–12] Recent efforts to define optimal vaginal microbiota composition concluded that *L. crispatus* dominance is optimal.[13–16] The role of *L. iners*–dominated microbiotas in modifying susceptibility to cervicovaginal infection and cervical disease is less clear,[9–12] and it is often omitted from discussions of what constitutes an optimal vaginal microbiota.[13–16] When included, it is considered suboptimal as *L. iners* dominance has been associated with increased burden of sexually transmitted infections (STIs) and bacterial vaginosis (BV) when compared with *L. crispatus* dominance, but reduced burden when compared with diverse, BV-like microbiotas.[2,3,17–24]

As *L. iners* is considered one of the most prevalent and abundant vaginal bacterial species,[10,12] understanding its influence on cervicovaginal infections and cervical disease relative to *L. crispatus* is urgently needed to better understand the etiology of adverse outcomes and develop effective prevention and treatment strategies. Much of the interventional work in this area has focused on improving BV treatment efficacy; however, there is increasing interest in developing and evaluating methods to (re)establish an optimal microbiota after BV treatment or other exogenous pressures that impact vaginal microbiota composition.[18,25–30,31s–33s] Better understanding the relative benefits and risks of *L. iners* and *L. crispatus* dominance as they relate to human immunodeficiency virus (HIV), STIs, BV, and cervical disease can inform the development of these approaches to (re)establish an optimal microbiota. To this end, we conducted a series of systematic reviews and meta-analyses of the associations of

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L. iners–dominated vaginal microbiotas with common cervicovaginal infections and cervical disease to evaluate the state of epidemiologic evidence regarding *L. iners* and these outcomes.

MATERIALS AND METHODS

We conducted 8 systematic reviews of studies evaluating associations between *L. iners*, compared with *L. crispatus*, and genital *C. trachomatis* (Ct) infection, BV, cervical human papillomavirus (HPV) detection, cervical dysplasia, HIV infection, genital herpes simplex virus type-2 (HSV-2) infection, *Trichomonas vaginalis* (Tv) infection, and genital *N. gonorrhoeae* (Ng) infection (outcome definitions in Supplemental Table 1, http://links.lww.com/OLQ/A878). We prospectively registered the reviews in PROSPERO (CRD42020214775 https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=214775). We conducted the reviews according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 and Conducting Systematic Reviews and Meta-Analyses of Observational Studies of Etiology guidelines (PRISMA checklists in Supplemental Tables 2–3, http://links.lww.com/OLQ/A879, http://links.lww.com/OLQ/A880).[34s,35s]

Search Strategy

On April 30, 2021, we searched PubMed, Embase, Cochrane Library, and Web of Science (search terms in Supplemental Table 4, http://links.lww.com/OLQ/A881). Studies were eligible for inclusion in systematic reviews if they were conducted among reproductive-age, nonpregnant, cisgender women; used marker gene (e.g., 16S rRNA gene, *cpn60*) amplicon sequencing to characterize vaginal microbiota composition; and presented an effect estimate for the association between *L. iners*, compared with *L. crispatus*, and the outcome of interest, or presented data which reviewers could use to calculate an effect estimate (exposure definitions in Supplemental Table 5, http://links.lww.com/OLQ/A882). Studies of cross-sectional, case-control, cohort, or clinical trial designs were eligible. For clinical trials, only data from baseline before intervention were included. Only English, full-text, peer-reviewed, original research manuscripts were eligible. Eligibility was not restricted by publication year. Additional details are provided in Supplemental Methods (http://links.lww.com/OLQ/A886).

Data Collection

We exported all search results for an outcome to a Zotero library (one library per outcome) and manually deduplicated results. Two reviewers (K.A.C., M.D.F.) independently reviewed full texts of all deduplicated results to determine eligibility. Reviewers settled discrepancies by consensus, or by consulting a third reviewer (J.E.B.) when consensus was not reached.

Two reviewers (K.A.C., M.D.F.) independently tracked eligibility decisions and recorded key characteristics of study design, exposure and outcome measurement, and effect estimates with a REDCap survey specifically designed for each outcome (Supplemental Methods, http://links.lww.com/OLQ/A886, lists data recorded). If relevant data were not found in the main text, reviewers examined supplemental materials. If any of these data were missing or unclear, we considered them "not reported." 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 appropriate (details in Table 2 footnotes, Supplemental Methods, http://links.lww.com/OLQ/A886).

Systematic Review Evidence Synthesis and Meta-Analysis

For BV, we synthesized evidence according to whether BV was assessed using Nugent score or Amsel criteria.[36s,37s] All studies using Nugent score defined BV as Nugent score ≥7. All studies using Amsel criteria defined BV as ≥3 Amsel criteria present. For HPV, we synthesized evidence according to whether the outcome included detection of any HPV type or was restricted to detection of high-risk HPV (hrHPV) types. We included multiple effect estimates from eligible studies reporting both HPV outcomes. For the remaining outcomes, we synthesized evidence from all included studies.

Studies included in systematic reviews were eligible for meta-analysis if they presented a prevalence ratio (PR), odds ratio (OR), relative risk, or hazard ratio (HR) for the association between *L. iners*, compared with *L. crispatus*, and the outcome, or if reviewers were able to estimate one of these measures based on data provided in the publication. For each outcome, we conducted random-effects meta-analysis (RE-MA) if there were ≥3 eligible studies that presented the same form of effect estimate. We evaluated heterogeneity in study findings using Cochrane's Q and the I^2 statistic.[38s] We used the rma.mv function (study as the random effect) of the metafor package (version 3.0–2 throughout) in R (version 4.0.4 throughout) for conducting meta-analyses and estimating Cochrane's Q, and we used code provided on the metafor package website for estimating I^2.[39s,40s] For each meta-analysis, we constructed a forest plot using the forest and addpoly functions of the metafor package in R (details in Supplemental Methods, http://links.lww.com/OLQ/A886).[39s] Because of the few studies being included in meta-analyses, we did not explore potential

TABLE 1. PRISMA Diagram Summary for Each Systematic Review

	Ct		BV		HPV		Cervical Dysplasia		HIV		Genital HSV-2		Tv		Ng	
Review Step	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Total deduplicated results reviewed*	42	100	320	100	52	100	39	100	144	100	16	100	22	100	36	100
Excluded because not peer-reviewed original research manuscript†	17	40	125	39	23	44	15	38	84	58	8	50	13	59	18	50
Excluded because wrong methods‡	12	29	133	42	16	31	15	38	32	22	2	13	4	18	10	28
Excluded because no (data to calculate) effect estimate	6	14	54	17	8	15	7	18	25	17	5	31	4	18	8	22
Studies included in review	7	17	8	3	5	10	2	5	3	2	1	6	1	5	0	0
Reports of included studies§	7	—	8	—	8	—	2	—	3	—	1	—	1	—	0	—
Reports included in meta-analysis§	6	—	3	—	0	—	0	—	0	—	0	—	0	—	0	—

*Results from searches of Cochrane Library, Embase, PubMed, and Web of Science.

†Reasons include full text not retrieved, full text not found in English, not peer-reviewed article (e.g., conference abstract), and peer-reviewed review/commentary.

‡Reasons include in vitro/in silico/animal studies only, wrong study population, and did not use marker gene sequencing to characterize the vaginal microbiota.

§Percentages not provided because reports of studies and studies may have different denominators.

TABLE 2. Summary of Findings for Each Systematic Review

Author Year Country	Study Design*	N Total (N Contributed to Estimate)†	Duration Between Exposure and Outcome Assessment‡	16S rRNA Gene Hypervariable Region(s)	*L. iners*–Dominated Communities, n (%)§
C. trachomatis					
van der Veer, 2017[21] Netherlands	Cross-sectional	93 (93)	Prevalent outcome	V3–V4	32 (34)
Balle, 2018[22] South Africa	Cross-sectional	72 (72) participants, 144 (144) observations	Prevalent outcome	V4	40 (28)
Tamarelle, 2018[5] France	Cross-sectional	132 (132)	Prevalent outcome	V3–V4	51 (39)
van Houdt, 2018[19] Netherlands	Case-control	115 (115)	1 y, incident outcome	V3–V4	38 (33)
Filardo, 2019[23] Italy	Case control	138 (100)	Prevalent outcome	V4	34 (25)
Tamarelle, 2020[17] United States	Case control	240 (240)	Prevalent outcome	V3–V4	NR
Ceccarani and Foschi,*** 2019[44s] Italy	Cross-sectional	79 (41)	Prevalent outcome	V3–V4	Mean RA 20%
BV					
Ravel, 2012[24] United States	Cross-sectional	148 (58)	Prevalent outcome	V1–V2	26 (18)
Balle, 2018[22] South Africa	Cross-sectional	72 (NR) participants, 144 (67) observations	Prevalent outcome	V4	40 (28)
Haahr, 2019[6] Denmark	Cross-sectional	120 (100)	Prevalent outcome	V4	51 (43)
Mehta, 2015[45s] United States	Cross-sectional	64 (64) participants, 581 (581) observations	Prevalent outcome	V1–V2	Mean RA 38%
Campisciano, 2017[46s] Italy	Cross-sectional	96 (69)	Prevalent outcome	V1–V3	Mean RA 29%
Ceccarani and Foschi,*** 2019[44s] Italy	Cross-sectional	79 (41)	Prevalent outcome	V3–V4	Mean RA 20%
Ravel, 2011[2] United States	Cross-sectional	394 (394)	Prevalent outcome	V1–V2	135 (34)
Smidt, 2015[47s] Estonia	Cross-sectional	21 (21)	Prevalent outcome	V6	Detected 52%
Any HPV					
Brotman, 2014[3] United States	Cohort	32 (32) participants, 930 (930) observations	3–4 days, incident outcome	V1–V2	13 participants (41), observations NR
Reimers, 2016[43s] United States	Cross-sectional	64 (64) participants, 398 (398) observations	Prevalent outcome	V1–V2	NR
Onywera, 2019[7] South Africa	Cross-sectional	62 (27)	Prevalent outcome	V4	24 (39)
Borgogna, 2020[48s] United States	Cross-sectional	39 (20)	Prevalent outcome	V1–V3	8 (24)
hrHPV					
Reimers, 2016[43s] United States	Cross-sectional	64 (64) participants, 398 (398) observations	Prevalent outcome	V1–V2	NR
Onywera, 2019[7] South Africa	Cross-sectional	62 (27)	Prevalent outcome	V4	24 (39)
Berggrund, 2020[49s] Sweden	Case control	96 (60)	Prevalent outcome	V2–V4, V6–V9	30 (31)
Borgogna, 2020[48s] United States	Cross-sectional	39 (20)	Prevalent outcome	V1–V3	8 (24)
Cervical dysplasia					
Onywera, 2019[7] South Africa	Cross-sectional	62 (27)	Prevalent outcome	V4	24 (39)
Berggrund, 2020[49s] Sweden	Case-control	96 (60)	5 mo, incident outcome	V2–V4, V6–V9	30 (31)

L. crispatus–Dominated Communities, n (%)[¶]	Outcome Assessment	Outcome Among *L. iners*–Dominated, n (%)[‖]	Outcome Among *L. crispatus*–Dominated, n (%)**	Estimate Definition[††]	Estimate [CI][‡‡]
22 (24)	NAAT, cervicovaginal swab	21 (66)	6 (27)	aOR[§§]	4.20 [1.20–15.40]
27 (19)	NAAT, vulvovaginal swab	NR	NR	OR	2.50 [0.56–13.60]
49 (37)	NAAT, cervicovaginal swab	11 (22)	4 (8)	OR	3.09 [0.91–10.49]
43 (37)	NAAT, cervicovaginal swab	42 (prevalence of *L. iners*–dominated microbiotas among those with Ct)	30 (prevalence of *L. crispatus*–dominated microbiotas among those with Ct)	maOR[¶¶]	2.58 [1.01–6.61]
66 (48)	NR	36 (prevalence of *L. iners*–dominated microbiotas among those with Ct)	26 (prevalence of *L. crispatus*–dominated microbiotas among those with Ct)	mOR[‖‖]	3.92 [1.50–10.23]
NR	NAAT, vaginal swab	NR	NR	OR	4.51 [1.41–16.42]
Mean RA 26%	NAAT, vaginal swab	Mean RA 28%	Mean RA 28%	RA ratio of ratios	3.01
32 (22)	Nugent score[†††]	3 (12)	1 (3)	PR	3.69 [0.41–33.43]
27 (19)	Nugent score[†††]	11 (27)	3 (11)	PR	2.45 [0.76–8.05]
49 (41)	Nugent score[†††]	4 (8)	3 (6)	PR	1.28 [0.30–5.43]
Mean RA 4%	Amsel criteria[‡‡‡]	Mean RA 58%	Mean RA 2%	RA ratio of ratios	5.61
Mean RA 22%	Nugent score[†††]	Mean RA 15%	Mean RA 6%	RA ratio of ratios	1.76
Mean RA 26%	Amsel criteria[‡‡‡]	Mean RA 11%	Mean RA 5%	RA ratio of ratios	5.92
101 (27)	Nugent score[†††]	13 (10)	0 (0)	Spearman correlation difference	0.21
Detected 76%	Nugent score[†††]	NR	NR	Spearman correlation difference	−0.04
5 participants (16), observations NR	NAAT, vaginal swab	NR (72)	NR (45)	aTRR[§§§]	1.79 [0.71–4.51]
NR	NAAT, cervicovaginal lavage	NR	NR	aOR[¶¶¶]	1.90
3 (5) *Lactobacillus* sp. dominated	NAAT, cervical cytobrush	11 (46)	2 (67)	PR	0.69 [0.28–1.71]
12 (36)	NAAT, vaginal swab	4 (50)	6 (50)	PR	1.00 [0.41–2.45]
NR	NAAT, cervicovaginal lavage[‖‖‖]	NR	NR	aOR[¶¶¶]	4.18
3 (5) *Lactobacillus* sp.–dominated	NAAT, cervical cytobrush****	8 (33)	2 (67)	PR	0.50 [0.19–1.33]
30 (31) *Lactobacillus* sp.–dominated	NAAT, cervical cytobrush[††††]	33 (prevalence of *L. iners*–dominated microbiotas among those with hrHPV)	25 (prevalence of *L. crispatus*–dominated microbiotas among those with hrHPV)	mOR[‡‡‡‡]	2.04 [0.71–5.89]
12 (36)	NAAT, vaginal swab[§§§§]	3 (38)	1 (8)	PR	4.50 [0.56–35.98]
3 (5) *Lactobacillus* sp.–dominated	Cervical cytology, cervical cytobrush	HSIL: 1 (4)	HSIL: 1 (33)	PR	0.13 [0.01–1.52]
30 (31) *Lactobacillus* sp.–dominated	Cervical histology, cervical biopsy	29 (prevalence of *L. iners*–dominated microbiotas among those with CIN2+)	34 (prevalence of *L. crispatus*–dominated microbiotas among those with CIN2+)	mOR[‡‡‡‡]	0.76 [0.27–2.13]

(Continued next page)

TABLE 2. *(Continued)*

Author Year Country	Study Design*	N Total (N Contributed to Estimate)†	Duration Between Exposure and Outcome Assessment‡	16S rRNA Gene Hypervariable Region(s)	*L. iners*–Dominated Communities, n (%)§
HIV					
Spear, 2011[50s] United States	Case control	46 (46)	Prevalent outcome	V1–V2	Mean RA 24%
Mehta, 2015[45s] United States	Case control	64 (64) participants, 581 (581) observations	Prevalent outcome	V1–V2	Mean RA 38%
Gosmann, 2017[20] South Africa	Cohort	236 (236)	11 mo, incident outcome	V4	75 (32)
Genital HSV-2					
Mehta, 2020[8] Kenya	Cross-sectional	231 (117)	Prevalent outcome	V3–V4	97 (42)
Trichomonas vaginalis					
Brotman, 2012[51s] United States	Cross-sectional	394 (240)	Prevalent outcome	V1–V2	135 (34)

*Design of the analysis used in the systematic review and meta-analysis (as applicable). Not necessarily the same as the original study's design.

†N total refers to the number of participants or observations with vaginal microbiota and outcome data. N contributed to estimate refers to the number of participants or observations whose data were used in generating the effect estimate.

‡Not applicable for cross-sectional studies, case-control studies that collected exposure and outcome data at a single time point (e.g., Filardo et al. 2019), or cohort studies from which we used exposure and outcome data collected at baseline (e.g., Ravel et al. 2012).

§N and prevalence of *L. iners*–dominated vaginal microbiotas, unless otherwise noted.

¶N and prevalence of *L. crispatus*–dominated vaginal microbiotas, unless otherwise noted.

‖N outcome events and outcome prevalence among *L. iners*–dominated vaginal microbiotas, unless otherwise noted.

**N outcome events and outcome prevalence among *L. crispatus*–dominated vaginal microbiotas, unless otherwise noted.

††RA ratio of ratios were calculated as: $\frac{Mean(Lactobacillus\ iners\ RA)|Outcome\ present}{Mean(Lactobacillus\ crispatus\ RA)|Outcome\ present} \Big/ \frac{Mean(Lactobacillus\ iners\ RA)|Outcome\ absent}{Mean(Lactobacillus\ crispatus\ RA)|Outcome\ absent}$

Spearman correlation coefficient differences were calculated as:

$Spearman\ correlation\ coefficient(Lactobacillus\ iners\ RA, Outcome) - Spearman\ correlation\ coefficient(Lactobacillus\ crispatus\ RA, Outcome)$

‡‡We were not able to estimate confidence intervals for RA ratio of ratios or Spearman correlation coefficient differences. We were not able to estimate confidence intervals for Reimers et al. 2016 effect estimates for any HPV and hrHPV because these ORs were estimated as: $\frac{OR(Lactobacillus\ iners\text{–}dominated\ v.Diverse, HPV\ v.no\ HPV)}{OR(Lactobacillus\ crispatus\text{–}dominated\ v.Diverse, HPV\ v.no\ HPV)}$

§§Adjusted for age (≤21 years, >21 years), type of last sex partner (steady, nonsteady).

¶¶Matched on age, ethnicity. Adjusted for relationship status (living together, living apart, single, unknown).

‖‖Matched on age, race.

***Cofirst authors.

†††BV defined as Nugent score ≥7. Non-BV defined as Nugent score ≤6.

‡‡‡BV defined as ≥3 Amsel criteria present. Non-BV defined as ≤2 Amsel criteria present.

§§§Adjusted for normalized menstrual cycle, age (<30 years, 30–39 years, ≥40 years), hormonal contraception (none, nonintrauterine device [IUD] hormonal contraception, IUD hormonal contraception), study phase (regular douching practices phase vs douching cessation intervention phase), vaginal sex in the day before vaginal swab collection (time-varying; no vaginal sex, vaginal sex with condom, condomless vaginal sex).

¶¶¶Adjusted for vaginal pH (continuous).

‖‖‖hrHPV types evaluated included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

****hrHPV types evaluated included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.

††††hrHPV types evaluated included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59.

‡‡‡‡Matched on age.

§§§§hrHPV types evaluated included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68.

¶¶¶¶Matched at the index visit for age, smoking status, sexual activity (frequency and number of partners), male condom and contraceptive use.

N, number; aOR, adjusted odds ratio; NR, not reported ; maOR, matched adjusted odds ratio; mOR, matched odds ratio; RA, relative abundance; aTRR, adjusted transition rate ratio; HSIL, high-grade squamous intraepithelial lesion; CIN2+, cervical intraepithelial neoplasia grade 2+; mRA ratio of ratios, matched relative abundance ratio of ratios.

L. crispatus–Dominated Communities, n (%)[¶]	Outcome Assessment	Outcome Among *L. iners*–Dominated, n (%)[‖]	Outcome Among *L. crispatus*–Dominated, n (%)**	Estimate Definition[††]	Estimate [CI][‡‡]
Mean RA 11%	Serology	Mean RA 21%	Mean RA 11%	mRA ratio of ratios[¶¶¶]	0.53
Mean RA 4%	Serology	Mean RA 51%	Mean RA 4%	mRA ratio of ratios[¶¶¶]	2.78
23 (10)	NAAT, blood	9 (12)	0 (0)	HR	3.84 [0.86–17.18]
20 (9)	Serology	51 (53)	6 (30)	PR	1.75 [0.87–3.51]
105 (27)	NAAT, cervicovaginal swab	2 (1)	1 (1)	PR	1.56 [0.08–93.14]

causes of heterogeneity, conduct sensitivity analyses to assess summary estimate robustness, or assess publication bias.

Risk of Bias and Quality of Evidence Assessments

Two reviewers (K.A.C., M.D.F.) independently used a standardized instrument developed for observational studies of etiology to assess risk of bias in included studies.[41s] Reviewers settled discrepancies by consensus, or by consulting a third reviewer (J.E.B.) when consensus was not reached. The instrument uses signaling question to assess risk of bias in 6 domains: confounding, selection bias, exposure measurement, outcome measurement, missing data, and selection of reported results (details in Table 3 footnotes, Supplemental Methods (http://links.lww.com/OLQ/A886); signaling questions in Supplemental Table 6, http://links.lww.com/OLQ/A883). Signaling question responses are used to rate risk of bias in each domain as low, moderate, serious, critical (highest level), or not enough information to assess. 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. Reviewers settled discrepancies in domain- and study-level risk of bias by consensus. One reviewer (K.A.C.) used the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) system to assess the quality of evidence included for each outcome (details in Table 4 footnotes, Supplemental Methods, http://links.lww.com/OLQ/A886).[42s]

RESULTS

The number of search results reviewed, excluded, and included for each outcome are presented in Table 1 (PRISMA diagrams in Supplemental Figs. 1–8, http://links.lww.com/OLQ/A887). Deduplicated search results reviewed ranged from 16 for HSV-2 to 320 for BV. For each outcome, ≤8 studies were eligible for inclusion in systematic reviews, and 38–59% of search results were excluded because they were not peer-reviewed original research manuscripts; 13–42% because they did not use marker gene sequencing to characterize the vaginal microbiota of reproductive-age, nonpregnant, cisgender women; and 14–31% because they did not present a relevant effect estimate and reviewers were unable to calculate one (details in Supplemental Table 7, http://links.lww.com/OLQ/A884, http://links.lww.com/OLQ/A885).

Table 2 summarizes key features and effect estimates from each included study. Tables 3 and 4 present risk of bias and quality of evidence assessments, respectively. We begin by presenting results for Ct and BV as these were the only outcomes for which meta-analyses were performed. Next, we present HPV, hrHPV, and cervical dysplasia together, as these outcomes are etiologically related. We then present HIV and finish with 1 subsection for HSV-2 and Tv because these reviews included few studies. No eligible studies were identified that reported on the relationship between *L. iners* and Ng. Throughout the results, we were unable to estimate confidence intervals (CIs) for relative abundance ratios of ratios, Spearman correlation differences, or ORs from Reimers et al.[43s] (details in Table 2 footnotes, Supplemental Methods, http://links.lww.com/OLQ/A886). Throughout the results, enrichment refers to the degree to which *L. iners* relative abundance exceeds *L. crispatus* relative abundance for an outcome status (ratio of *L. iners* to *L. crispatus* relative abundances, details in Supplemental Methods, http://links.lww.com/OLQ/A886).

Chlamydia trachomatis

Seven studies were included in the Ct review. Six studies evaluated vaginal microbiota composition and Ct cross-sectionally; the seventh evaluated vaginal microbiota composition 1 year before Ct.[19] Six of 7 used nucleic acid amplification tests (NAATs) to evaluate Ct; the seventh did not report Ct testing method.[23] Five were conducted in Europe, 1 in South Africa, and 1 in the United States. Six studies were included in meta-analysis,[5,17,19,21–23] which represented data from >402 participants and >109 Ct infections (one study did not report the number of participants with *L. iners*–dominated or *L. crispatus*–dominated microbiotas,[17] 2 studies did not report the number of Ct infections among participants with *L. iners*–dominated or *L. crispatus*–dominated microbiotas[17,22]). The summary OR of 3.38 (95% CI, 2.12–5.40; Fig. 1) indicates that individuals with *L. iners*–dominated microbiotas had 3.4-fold higher odds of Ct than individuals with *L. crispatus*–dominated microbiotas. For the remaining study, we estimated a relative abundance ratio of ratios of 3.01, indicating that *L. iners* enrichment during prevalent Ct is 3-fold greater than *L. crispatus* enrichment.[44s] We found 6 of the 7 studies to be at serious risk of bias due to confounding, and we rated overall quality of evidence as very low due to risk of bias, indirectness (6 studies evaluated prevalent Ct), and likely publication bias.

Bacterial Vaginosis

Eight studies were included in the BV review, all of which evaluated vaginal microbiota composition and BV cross-sectionally. Four were conducted in Europe, 3 in the United States, and1 in South Africa. Three studies were included in meta-analysis.[6,22,24] These studies evaluated BV by Nugent score and represented data from 225 participants and 25 BV events. The summary PR of 2.10 (95% CI 0.90–4.88; Fig. 2) indicates that individuals with *L. iners*–dominated microbiotas had twice the prevalence of BV compared with individuals with *L. crispatus*–dominated microbiotas.

For 3 additional studies, we estimated relative abundance ratio of ratios indicating *L. iners* enrichment during prevalent Amsel BV is approximately 5.75-fold greater than *L. crispatus* enrichment,[44s,45s] whereas *L. iners* enrichment during prevalent Nugent BV is 1.76-fold greater than *L. crispatus* enrichment.[46s] The remaining 2 studies evaluated BV using Nugent score. For one of these studies, we estimated a Spearman correlation difference of 0.21,[2] suggesting *L. iners* relative abundance is more strongly positively correlated with Nugent score than *L. crispatus* relative abundance. For the second of these studies, we estimated a Spearman correlation difference of −0.04,[47s] suggesting *L. iners* and *L. crispatus* relative abundances have similar correlations with Nugent score. We found 7 of the 8 studies to be at serious or critical risk of bias due to confounding, and 6 of 8 did not provide enough information to evaluate risk of bias due to outcome measurement. We rated overall quality of evidence as very low. We down-rated quality of evidence due to risk of bias, indirectness (studies evaluated prevalent BV), imprecision, and likely publication bias. We up-rated quality of evidence based on evidence of a dose-response relationship with all relative abundance ratio of ratios >1.[44s–46s]

HPV, hrHPV, Cervical Dysplasia

Eight reports from 5 studies were eligible for inclusion in the HPV review, 4 of which included any HPV as the outcome,[3,7,43s,48s] and the remaining 4 focused on hrHPV.[7,43s,48s,49s] All 4 HPV studies tested for HPV using NAAT, and 3 evaluated vaginal microbiota composition and HPV cross-sectionally. The fourth study evaluated vaginal microbiota composition and HPV detection twice-weekly for 16 weeks and examined the relationship between microbiota composition at a given time point and HPV detection 3–4 days later.[3] Three studies were conducted in the United States and 1 in South Africa. The single longitudinal study presented a transition rate ratio of 1.79 (95% CI 0.71–4.51),[3] and we estimated an OR of 1.90 for a cross-sectional study.[43s] Both estimates suggest individuals with *L. iners*–dominated microbiotas are at 80% to 90% higher risk of HPV detection than individuals with *L. crispatus*–dominated microbiotas. The 2 remaining cross-sectional studies presented PRs of 1.00 (95% CI,

TABLE 3. Summary of Risk of Bias Assessment for Each Review

Study	Confounding*	Selection Bias	Exposure Measurement†	Outcome Measurement‡	Missing Data	Selection of Reported Results	Overall
C. trachomatis							
van der Veer, 2017[21]	Serious	Low	Moderate	Low	Low	Low	Serious
Balle, 2018[22]	Moderate	Low	Moderate	Low	Moderate	Low	Moderate
Tamarelle, 2018[5]	Serious	Low	Moderate	Low	Moderate	Low	Serious
van Houdt, 2018[19]	Serious	Low	Moderate	Low	Moderate	Low	Serious
Filardo, 2019[23]	Serious	Low	Moderate	Moderate	Low	Low	Serious
Tamarelle, 2020[17]	Serious	Moderate	Moderate	Moderate	Moderate	Low	Serious
Ceccarani and Foschi,§ 2019[44s]	Serious	Low	Serious	Low	Low	Low	Serious
BV							
Ravel, 2012[24]	Serious	Moderate	Moderate	Low	Serious	Moderate	Serious
Balle, 2018[22]	Serious	Low	Moderate	Not enough information	Low	Low	Serious-to-critical¶
Haahr, 2019[6]	Moderate	Moderate	Moderate	Not enough information	Moderate	Low	Moderate-to-critical‖
Mehta, 2015[45s]	Critical	Serious	Serious	Moderate	Moderate	Low	Critical
Campisciano, 2017[46s]	Serious	Low	Serious	Not enough information	Low	Low	Serious-to-critical¶
Ceccarani and Foschi,§ 2019[44s]	Serious	Low	Serious	Not enough information	Low	Low	Serious-to-critical¶
Ravel, 2011[2]	Serious	Low	Moderate	Not enough information	Low	Moderate	Serious-to-critical¶
Smidt, 2015[47s]	Serious	Low	Moderate	Not enough information	Serious	Low	Serious-to-critical¶
Any HPV							
Brotman, 2014[3]	Moderate	Low	Moderate	Moderate	Serious	Low	Serious
Reimers, 2016[43s]	Serious	Serious	Moderate	Moderate	Low	Moderate	Serious
Onywera, 2019[7]	Serious	Low	Moderate	Low	Moderate	Low	Serious
Borgogna, 2020[48s]	Serious	Not enough information	Moderate	Moderate	Moderate	Low	Serious-to-critical**
hrHPV							
Reimers, 2016[43s]	Serious	Serious	Moderate	Moderate	Low	Moderate	Serious
Onywera, 2019[7]	Serious	Low	Moderate	Low	Moderate	Low	Serious
Berggrund, 2020[49s]	Serious	Serious	Moderate	Serious	Low	Low	Serious
Borgogna, 2020[48s]	Serious	Not enough information	Moderate	Moderate	Moderate	Low	Serious-to-critical**
Cervical dysplasia							
Onywera, 2019[7]	Serious	Low	Moderate	Low	Moderate	Low	Serious
Berggrund, 2020[49s]	Serious	Serious	Moderate	Serious	Low	Low	Serious
HIV							
Spear, 2011[50s]	Serious	Moderate	Moderate	Low	Low	Low	Serious
Mehta, 2015[45s]	Serious	Serious	Serious	Low	Moderate	Low	Serious
Gosmann, 2017[20]	Serious	Low	Moderate	Low	Low	Low	Serious
Genital HSV-2							
Mehta, 2020[8]	Serious	Low	Moderate	Low	Moderate	Moderate	Serious
Trichomonas vaginalis							
Brotman, 2012[51s]	Serious	Low	Moderate	Low	Low	Low	Serious
Total							
Total serious+, N (%)††	27 (90)	8 (27)	5 (17)	8 (27)	3 (10)	0 (0)	29 (97)

*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).

†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.

‡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.

§Cofirst authors.

¶Rated as serious-to-critical because the highest-rated domain-specific risk of bias was serious, but there was not enough information to evaluate risk bias due to outcome measurement. It is possible we would have rated risk bias due to outcome measurement and overall risk of bias as critical with more information to evaluate risk bias due to outcome measurement.

‖Rated as moderate-to-critical because the highest-rated domain-specific risk of bias was moderate, but there was not enough information to evaluate risk bias due to outcome measurement. It is possible we would have rated risk bias due to outcome measurement and overall risk of bias as critical with more information to evaluate risk bias due to outcome measurement.

**Rated as serious-to-critical because the highest-rated domain-specific risk of bias was serious, but there was not enough information to evaluate risk selection bias. It is possible we would have rated risk of selection bias and overall risk of bias as critical with more information to evaluate risk of selection bias.

††Sum and proportion of studies rated as moderate-to-critical, serious, serious-to-critical, critical, or not enough information in each bias domain and overall. Studies that contributed to multiple reviews were counted for each review in which they were rated as serious+ because risk of bias was assessed for each review separately.

TABLE 4. Summary of Quality of Evidence Assessment for Each Review

Review	Risk of Bias	Inconsistency	Indirectness*	Imprecision	Publication Bias†	Dose Response	Overall‡
C. trachomatis	−1 serious	−0	−1 serious	−0	−1 serious	+0	−1 very low
BV	−2 very serious	−0	−2 very serious	−1 serious	−1 serious	+1	−3 very low
Any HPV	−2 very serious	−1 serious	−1 serious	−1 serious	−1 serious	+0	−4 very low
hrHPV	−2 very serious	−2 very serious	−1 serious	−2 very serious	−1 serious	+0	−6 very low
Cervical dysplasia	−1 serious	−0	−0	−1 serious	−1 serious	+0	−1 very low
HIV	−1 serious	−1 serious	−0	−2 very serious	−1 serious	+0	−3 very low
Genital HSV-2	−1 serious	NA	−1 serious	−1 serious	−1 serious	+0	−2 very low
Trichomonas vaginalis	−1 serious	NA	−1 serious	−2 very serious	−1 serious	+0	−3 very low

*We downrated 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 downrated quality of evidence for each review for likely publication bias due to insignificant vaginal microbiota, *L. iners*, and *L. crispatus* findings being less likely to be published or reported.

‡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. Large effect, plausible residual confounding in favor of observed are effect not included because all reviews were rated as +0.

0.41–2.45)[48s] and 0.69 (95% CI, 0.28–1.71),[7] indicating null-to-inverse associations. No meta-analysis was performed because the studies did not present the same form of effect estimate. We rated 3 studies to be at serious risk of bias due to confounding, and we rated overall quality of evidence as very low due to risk of bias, inconsistency, indirectness (cross-sectional studies evaluated prevalent HPV), imprecision, and likely publication bias.

All 4 hrHPV studies used NAAT to test for hrHPV, and all characterized the vaginal microbiota and hrHPV cross-sectionally (Table 2 footnotes list hrHPV types evaluated). Two were conducted in the United States, 1 in South Africa, and 1 in Sweden. One study presented an OR of 2.04 (95% CI, 0.71–5.89),[49s] and for a second study we estimated an OR of 4.18,[43s] suggesting *L. iners*–dominated microbiotas are associated with 2- to 4-fold higher odds of prevalent hrHPV than *L. crispatus*–dominated microbiotas. The remaining 2 studies presented PRs of 4.50 (95% CI 0.56–35.98)[48s] and 0.50 (95% CI 0.19–1.33),[7] providing conflicting evidence regarding hrHPV prevalence. No meta-analysis was performed because the studies did not present the same form of effect estimate. We found all 4 studies to be at serious risk of bias due to confounding, and 2 to be at serious risk of selection bias with a third not providing enough information to evaluate risk of selection bias. We rated overall quality of evidence as very low due to risk of bias, inconsistency, indirectness (studies assessed prevalent hrHPV), imprecision, and likely publication bias.

Two studies were included in the cervical dysplasia review; one used cytology[7] and one used histology[49s] to evaluate cervical disease. A cross-sectional study conducted in South Africa classified cervical dysplasia as high-grade squamous intraepithelial lesions and presented a PR of 0.13 (95% CI, 0.01–1.52).[7] A case-control study conducted in Sweden characterized the microbiota 5 months before evaluating cervical intraepithelial neoplasia grade 2+ and reported an OR of 0.76 (95% CI, 0.27–2.13).[49s] Both studies suggest *L. iners*–dominated microbiotas are associated with the absence of cervical dysplasia. We found both studies to be at serious risk of bias due to confounding, and we rated overall quality of evidence as very low due to risk of bias, imprecision, and likely publication bias.

HIV

Three studies were included in the HIV review. Two of the studies were case-control in design, conducted in the United States, and characterized vaginal microbiota composition and tested for HIV by serology cross-sectionally. We estimated relative abundance ratio of ratios of 2.78[45s] and 0.53[50s] for these studies, providing conflicting evidence on the relative enrichment of *L. iners* and *L. crispatus* among individuals living with HIV. The third study was a prospective cohort study conducted in South Africa. Vaginal microbiota composition was evaluated at baseline and HIV testing by NAAT occurred twice-weekly during 11 months of follow-up. The authors presented a HR of 3.84 (95% CI, 0.86–17.18), indicating individuals with *L. iners*–dominated microbiotas had nearly 4-fold higher risk of acquiring HIV than individuals with *L. crispatus*–dominated microbiotas.[20] No meta-analysis was performed because the studies did not present the same form of effect estimate. We found all 3 studies to be at serious risk of bias due to confounding, and we rated overall quality of evidence as very low due to risk of bias, inconsistency (cross-sectional studies assessed prevalent HIV), imprecision, and likely publication bias.

Genital HSV-2, *Trichomonas vaginalis*

Here we present results for HSV-2 and Tv because these reviews each included 1 study. The HSV-2 study was cross-sectional

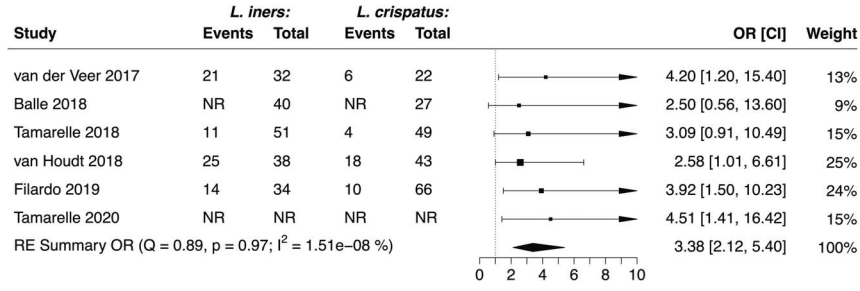

Figure 1. *C. trachomatis* REs meta-analysis forest plot. *L. iners* heading refers to genital chlamydial infection events among and total number of participants with a *L. iners*–dominated vaginal microbiota. *L. crispatus* heading refers to genital chlamydial infection events among and total number of participants with a *L. crispatus*–dominated vaginal microbiota.NR, not reported; RE, random effects.

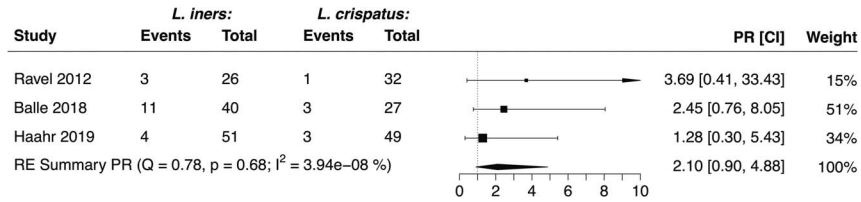

Figure 2. BV REs meta-analysis forest plot. *L. iners* heading refers to BV events among and total number of participants with a *L. iners*–dominated vaginal microbiota. *L. crispatus* heading refers to BV events among and total number of participants with a *L. crispatus*–dominated vaginal microbiota. PR, prevalence ratio.

and conducted in Kenya. We estimated a PR of 1.75 (95% CI 0.87–3.51), suggesting individuals with *L. iners*–dominated microbiotas have 75% higher prevalence of genital HSV-2 than individuals with *L. crispatus*–dominated microbiotas.[8] The Tv study was cross-sectional, conducted in the USA, and evaluated Tv by NAAT. We estimated a PR of 1.56 (95% CI 0.08–93.14), which, given the confidence interval, does not provide evidence of an association in either direction.[51s] We found both studies to be at high risk of bias due to confounding.

DISCUSSION

The Ct and BV evidence reviewed here and their meta-analyses indicate *L. iners*–dominated microbiotas may be suboptimal compared with *L. crispatus*–dominated microbiotas, suggesting *L. iners* dominance may confer risk of acquiring Ct or developing BV. These results should be interpreted with caution as the meta-analyzed studies are at serious risk of bias and represent very-low-quality evidence. These systematic reviews also highlight the dearth and low quality of epidemiologic evidence on the role of *L. iners* in sexual health outcomes. Based on the evidence reviewed, it is challenging, if not impossible, to draw conclusions regarding relationships between *L. iners* and (hr)HPV, cervical dysplasia, HIV, genital HSV-2, Tv, or Ng. Claims regarding the relative benefits or risks of *L. iners* and *L. crispatus* as they relate to these outcomes should be interpreted with caution as epidemiologic evidence is limited, conflicting, largely cross-sectional, and at serious risk of bias.

Despite limitations of the epidemiologic evidence, Ct findings are consistent with genomic and in vitro evidence. All 7 Ct studies and the RE-MA estimate indicate *L. iners* is associated with Ct presence or acquisition while *L. crispatus* is associated with its absence. Six of 7 studies assessed vaginal microbiota composition and Ct cross-sectionally.[5,17,21–23,44s] The *L. iners* genome contains unique stress response genes, enabling specific, directed stress responses to a wider array of environmental conditions than in other lactobacilli and contributing to *L. iners*' unique ability to persist during cervicovaginal infections.[52s] The seventh study assessed microbiota composition 1 year before Ct.[19] It is unlikely that the vaginal microbiota at a given time point has direct impact on STI acquisition 1 year later; however, in vitro evidence suggests *L. iners* may have less capacity to prevent Ct acquisition than *L. crispatus*. *L. iners* lacks genes for D-lactic acid (LA) production and produces very little D-LA *in vitro*.[52s–56s] In a 3-dimensional cervical epithelial cell model, pretreatment with *L. crispatus* cell-free supernatant (CFS) reduced Ct infectivity 10-fold more than pretreating with *L. iners* CFS.[56s] This difference appears to be driven by differences in D-LA concentrations: *L. iners* CFS supplemented with D-LA achieved similar reductions in infectivity as *L. crispatus* CFS.[56s] As the D-LA isomer is more potent than L-LA,[57s] differences in D-LA concentration and anti-chlamydial activity are likely maintained between *L. crispatus*–dominated and *L. iners*–dominated vaginal microbiotas *in vivo*.

Genomic, in vitro, and in silico studies also support the BV findings. All 8 BV studies assessed vaginal microbiota composition and BV cross-sectionally, and 7 studies,[2,6,22,24,44s–46s] and the RE-MA estimate indicate *L. iners* is associated with BV presence whereas *L. crispatus* is associated with its absence. It should be noted that some degree of the association between *L. iners* and Nugent BV can be attributed to measurement error in Nugent scoring. Unlike other lactobacilli, *L. iners* stains Gram-negative to Gram-variable and exhibits pleiomorphic cell morphology with many cells being short rods.[58s,59s] As such, *L. iners* may be misscored as *Gardnerella*, artificially inflating Nugent scores. All included studies that evaluated BV by Nugent score categorized scores as non-BV (Nugent score ≤6) or BV (Nugent score ≥7).[2,6,22,24,46s,47s] In this case, we expect *L. iners* misscoring to result in misclassification of non-BV samples as BV samples for communities with high relative abundance of *L. iners* in which virtually all *L. iners* is misscored, or for mixed communities containing some proportion of *L. iners* in which misscoring inflates Nugent score from 6 to 7. Five of 6 included studies that evaluated Nugent BV did not report on Nugent score training or quality assurance/control, so we cannot infer the degree to which *L. iners* misscoring biased observed associations.[2,6,22,46s,47s]

Measurement error aside, evidence indicates that *L. iners* may contribute to BV development via reduced antagonism of BV-associated taxa, and that *L. iners* may persist during BV to a greater degree than other lactobacilli. As *L. iners* lacks genes for D-LA production,[52s,53s] it likely exhibits little antibacterial activity against BV-associated taxa in vivo. In addition, *L. iners* produces inerolysin, a cytolysin unique among lactobacilli and related to the *Gardnerella* virulence factor vaginolysin.[60s,61s] Inerolysin is most expressed and active at pH characteristic of BV (4.5–6), and *L. iners* may use inerolysin to obtain nutrients from the host in a commensal manner during BV.[52s,53s,60s–62s] Finally, in coculture with cervical epithelial cells, adherent *L. iners* is not displaced by *Gardnerella*, and *L. iners* enhances *Gardnerella* adhesion.[63s] These features suggest *L. iners* is uniquely well poised to persist during BV and transitions in and out of BV. A recent *in silico*, validated mathematical model corroborates this hypothesis. Over 3-month intervals, *L. iners*–dominated microbiotas shifted to diverse, BV-like communities 32% of the time.[64s] BV-like communities likewise transitioned to *L. iners*–dominated communities 20% of the time; they rarely transitioned to *L. crispatus*–dominated communities (1–2%).[64s]

The BV and Ct findings support ongoing work to identify novel *L. iners*–related therapeutic targets to promote *L. crispatus* dominance. Recent work demonstrated that vaginal lactobacilli lack canonical cysteine biosynthesis pathways, and *L. iners* also lacks transport systems for exogenous cysteine uptake.[18] *L. iners* required exogenous L-cystine to synthesize cysteine, and cystine uptake inhibitors caused species-specific growth inhibition of *L. iners*.[18] In mock communities containing *L. iners*, *L. crispatus*, and BV-associated taxa, treatment with cystine uptake inhibitors and metronidazole reduced BV-associated taxon abundances and

favored expansion of *L. crispatus* over *L. iners*.[18] This work is in early stages, but cystine uptake inhibitors may hold promise as a means to shift vaginal microbiota composition toward an optimal state, potentially contributing to reduced BV and Ct incidence.

The third key finding of these reviews relates to the rigor of epidemiologic studies of the vaginal microbiota and sexual health outcomes. We downrated quality of evidence for all but 2 outcomes due to indirectness given the substantial proportion of studies that collected exposure and outcome data cross-sectionally (Table 4). Prevalent outcomes are not of particular interest to individuals at risk for the outcome, and they provide limited epidemiologic evidence that may be subject to reverse causation and length-biased sampling.[65s] Future studies should use truly longitudinal study designs in which exposure data are collected before outcome data, only incident outcomes are considered, and the interval between exposure and outcome measurement is informed by the outcome's natural history and the timeframe on which the vaginal microbiota is expected to influence the outcome. Longitudinal designs are the only designs that provide epidemiologic evidence regarding temporal relationships between vaginal bacteria (antecedent exposures) and BV, STIs, or cervical dysplasia (subsequent outcomes). Such temporal evidence is relevant to the etiology and prevention of adverse outcomes, which is of interest to individuals at risk for those outcomes.

In addition to temporality concerns, the vast majority of studies included across the outcomes were at serious to critical risk of bias due to confounding (27 of 30; Table 3). This is alarming, especially considering 18 of these 27 studies were unadjusted and unmatched.[2,5,7,8,17,20,22,24,44s–48s,51s] We expect the confounders we considered (Table 3 footnotes, Supplemental Methods, http://links.lww.com/OLQ/A886) to have concordant relationships with our exposure (*L. iners*–dominated vs *L. crispatus*–dominated microbiotas) and outcomes (BV, STI, cervical dysplasia) of interest, so we generally expect uncontrolled confounding due to these factors to bias effect estimates upward (away from 0).[66s] The unadjusted and unmatched effect estimates included in these reviews likely overestimate true associations, distorting our understanding of how *L. iners* may influence sexual health outcomes. Elements of study design and data analysis (e.g., identifying confounders a priori, enrolling sufficient participants to adjust for confounders, adjusting for confounders) can mitigate the effects of confounding and generate less-biased effect estimates. These estimates may yield more accurate understanding of *L. iners'* influence on sexual health outcomes and more precisely guide future mechanistic and interventional research.

These systematic reviews and meta-analyses should be interpreted in the context of the search strategy's limitations (additional limitations in Supplemental Methods, http://links.lww.com/OLQ/A886). Only studies that used marker gene sequencing were eligible for inclusion, which excluded studies that targeted *L. iners* and *L. crispatus* by quantitative polymerase chain reaction. As marker gene sequencing relative abundance data are compositional and semi-quantitative, this excluded all truly quantitative data regarding the associations of interest.[67s] Further, marker gene sequencing typically precludes sub-species taxonomic assignments. Inter-strain diversity has been documented for *L. iners* and *L. crispatus*, but we were unable to examine whether associations of interest varied across strains.[53s,68s,69s]

We conducted a series of systematic reviews and meta-analyses to evaluate the state of epidemiologic evidence regarding the role of *L. iners* in 8 sexual health outcomes. Our findings indicate *L. iners*–dominated vaginal microbiotas may be suboptimal compared with *L. crispatus*–dominated vaginal microbiotas for Ct and BV, which is consistent with prior research. Evidence was sparse for (hr)HPV, cervical dysplasia, HIV, genital HSV-2, Tv, and Ng. Additional epidemiologic and mechanistic studies are needed to further elucidate the role of *L. iners* and identify targets for novel interventions to prevent and treat adverse sexual health outcomes. This research holds great promise as demonstrated by recent work that identified cystine uptake inhibitors as a candidate to promote *L. crispatus* dominance over *L. iners* dominance.[18]

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For further references, please see "Supplemental References," http://links.lww.com/OLQ/A886.