Risk of Bacterial Vaginosis Among Women With Herpes Simplex Virus Type 2 Infection: A Systematic Review and Meta-analysis

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Background. Bacterial vaginosis (BV) is a perturbation of vaginal flora characterized by reduced levels of lactobacilli and concomitant overgrowth of anaerobic bacterial species. BV is highly prevalent and associated with multiple adverse outcomes, including enhanced human immunodeficiency virus transmission. Because recent reports reveal that herpes simplex virus type 2 (HSV-2) infection may increase BV risk, we initiated a systematic review and meta-analysis of the link between HSV-2 infection and BV.

Methods. We searched the MEDLINE, EMBASE, and CENTRAL databases to identify articles posted before 1 December 2014. Two screeners independently reviewed the titles and abstracts of all identified articles, reviewed the full text of articles deemed potentially eligible, and extracted data from 14 cross-sectional and 3 prospective studies. Using random-effects models, we computed separate pooled estimates for cross-sectional and prospective studies.

Results. The pooled odds ratio for cross-sectional studies was 1.60 (95% confidence interval, 1.32–1.94). Stronger support for the causal effect of HSV-2 infection on BV risk was revealed by the summary relative risk for the prospective studies, which was 1.55 (95% confidence interval, 1.30–1.84), with minimal heterogeneity ($I^2 = 0$).

Conclusions. These analyses imply that HSV-2 infection is an important BV risk factor. Pharmacologic HSV-2 suppression may reduce BV incidence and BV-associated adverse events.

Keywords. bacterial vaginosis; herpes simplex virus type 2; meta-analysis; systematic review.

Bacterial vaginosis (BV), a perturbation of vaginal flora characterized by reduced levels of lactobacilli and overgrowth of Gardnerella, Prevotella, Mobiluncus, and other anaerobic bacterial species, affects nearly 1 in 3 reproductive-age women worldwide [1]. Clinically, BV is diagnosed by a constellation of signs and symptoms that include malodorous vaginal discharge, increased vaginal pH, and microscopic identification of vaginal epithelial cells dotted with adherent coccobacilli (ie, clue cells) (Amsel criteria) [2]. For research purposes, BV is frequently diagnosed by a Gram stain–based evaluation of vaginal bacterial morphotypes, quantified using a 10-point scoring system (Nugent score) [3]. BV is associated with numerous adverse outcomes, including increased human immunodeficiency virus (HIV) transmission and, among pregnant women, spontaneous abortion and premature delivery [1, 4, 5]. Although not a conventional sexually transmitted infection (STI), BV is associated with sexual activity and occurs more often in women with greater numbers of sex partners and higher frequency of intercourse [6]. Other BV risk factors include nonuse of condoms, introduction of a new sex partner, history of STIs, and douching [7–10]. The strongest predictor of BV in US women is nonwhite race. Even after adjustment for other BV risk factors [11], BV prevalence in the United States remains highly dependent on race (52% and 23% in black and white women, respectively) [12]. This racial disparity suggests that an unknown risk factor (or factors) for
BV is more common in black women than in white women. One possible risk factor is genital herpes. Genital herpes is most commonly caused by infection with herpes simplex virus type 2 (HSV-2), which has a seroprevalence of 48% in black women, compared with 16% in white women [13]. Because newer evidence indicates that HSV-2 infection promotes loss of normal, lactobacillus-dominated vaginal flora [14, 15], we conducted a systematic review and meta-analysis to evaluate whether HSV-2 infection is a risk factor for BV.

METHODS

We included published reports on women who were BV-negative at baseline in longitudinal studies (observational studies or randomized trials), which measured incident BV diagnosed via Amsel criteria or Nugent score, among women who were HSV-2 seropositive (diagnosed via type-specific serological assays), compared with incident BV in women who were HSV-2 seronegative. We also included published, cross-sectional reports on the association between HSV-2 serostatus (using type-specific serological assays) and prevalent BV (determined on the basis of the Amsel criteria or Nugent score), comparing BV prevalence among HSV-2–infected women with BV prevalence in women without HSV-2 infection.

Search Strategy

We initially identified studies by searching the electronic databases MEDLINE via PubMed, EMBASE, and CENTRAL (Cochrane Central Register of Controlled Trials) for articles posted between 1 January 1983 (the year the Amsel criteria were introduced) and 1 December 2014 (see Supplementary Materials for full search terms) [2]. Two authors (A. E. and A. N. T.) independently reviewed the titles and abstracts of all resulting articles. Any article that appeared potentially relevant by review of the title and abstract was retrieved for full-text review to assess meta-analysis eligibility. To be eligible, studies had to report a relevant measure of association for the prospective or cross-sectional association between HSV-2 infection and BV or include the data necessary to permit its calculation. We did not restrict inclusion by women’s age, HIV status, pregnancy status, or other potentially influential covariates (eg, sexual behavior and race), but we made note of these variables during data extraction. We included studies in which BV status had been assessed using the Amsel criteria or Nugent scoring. Because a substantial minority of genital herpes cases are caused by HSV-1 rather than HSV-2, we included only those studies in which HSV-2 infection was evaluated by type-specific serologic assays [16]. We separately examined prospective studies in which HSV-2 infection status was established at the study start and incident BV was assessed later in follow-up and cross-sectional studies in which HSV-2 and BV status were concomitantly measured. We hand-searched reference lists of included studies, but we did not include conference abstracts or other reports not published in the peer-reviewed literature.

Data Abstraction

Two authors (A. E. and A. N. T.) independently extracted relevant data from the selected studies into a spreadsheet, using a piloted, standardized form, including: (1) participant characteristics (ie, age, HIV status, and population [eg, general population or sex workers]), (2) study setting (ie, country, and community based or clinic based), (3) method of BV diagnosis (ie, Amsel criteria vs Nugent score), (4) method of HSV-2 diagnosis, (5) study size, (6) study design (ie, cross-sectional or prospective), (7) frequency of BV evaluation, (8) confounders included in adjusted models, (9) measure of association (ie, hazard ratio [HR], odds ratio [OR], prevalence ratio [PR], risk ratio, or incidence rate ratio [IRR]) and 95% confidence intervals (CIs), and (10) funding source. Any inconsistencies in extracted data were resolved by discussion between the 2 reviewers.

Quality Assessment

We used the Newcastle-Ottawa quality assessment scale to assess the risk of bias for each study [17]. The scale comprises 3 assessment categories: selection, comparability, and outcome. The scale includes 4 items to evaluate selection: (1) representativeness of the exposed cohort, (2) selection of the nonexposed cohort, (3) ascertainment of the exposure, and (4) demonstration that the outcome of interest was not present at start of study. In our review, studies were classified as representative on the basis of both eligibility criteria and participation rate. We used 2 items to gauge comparability: whether the study controlled for (a) sexual behavior (including at least one measure of sexual frequency/partnerships or condom use), and (b) key patient characteristics including HIV (if both HIV-positive and HIV-negative women were eligible to participate) and race (if the study population was multi-racial). We assessed the final Newcastle-Ottawa evaluation category, outcome, by examining the measurement approach to detect the outcome (BV via Nugent score or Amsel criteria), the length of follow-up (at least 1 month, with HSV-2 and BV assessed at baseline and at least 1 additional time point), and the adequacy of the duration of follow-up (at least 80% retention).

Data Analysis

We analyzed the data using Stata 13 (Stata, College Station, Texas). We ran separate analyses for the prospective and cross-sectional studies. We used DerSimonian–Laird random effects models, which allow for between-study heterogeneity, to produce summary estimates. CIs were calculated using the Woolf method. Heterogeneity was assessed using $I^2$ [18]. Among the prospective studies ($n = 3$), 1 reported a HR [14], 1 reported an IRR [15], and 1 reported a risk ratio [19]. Similar to the approach used by others (such as Atashili et al [20]), because these estimates are all from multiplicative-scale models, we combined them with no
additional transformations [21], and the pooled estimate of the prospective studies is reported as a summary relative risk. The key measure of association for all included cross-sectional studies was the OR, and the pooled estimate is a summary OR. We evaluated publication bias in the cross-sectional studies by using a funnel plot and the Begg and Egger tests [22, 23].

Sensitivity Analyses
To assess the robustness of our findings, we performed 5 sensitivity or subgroup analyses on the pooled estimate of cross-sectional studies. First, we used influence analyses to estimate the impact of each study on the summary estimate. Studies were individually removed for subsequent recalculation of a pooled estimate with a 95% CI; individual studies were deemed excessively influential if the magnitude of the recalculated summary estimate fell outside the 95% CI of summary estimates in which it was included [24]. Second, we stratified the results by study population (ie, clinic vs community based) to determine whether the association between HSV-2 and BV varied by population type. Third, we included only studies that provided adjusted estimates of association, to determine whether confounding had affected the pooled estimate in the main analysis. Fourth, we restricted our analysis to studies found to be of higher quality according to the Newcastle-Ottawa assessment scale. Finally, because the Amsel criteria are less sensitive than the Nugent score for BV diagnosis (70% vs 89%) [25], we restricted our evaluation to the studies that used the Nugent score, to examine the impact of diagnostic sensitivity on our main analysis.

RESULTS
The results of this systematic review and meta-analysis are presented according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines [26].

Characteristics of Eligible Studies
The initial MEDLINE, EMBASE, and CENTRAL searches identified 404 studies; 66 were duplicates, and 225 were excluded for nonrelevance to the research question, following review of their title and abstract (Figure 1). We retrieved full-text articles for the remaining 113 publications and excluded a further 96 articles that did not report a relevant measure of association or include the data necessary to calculate one. From the 17 remaining articles, 1 prospective study also presented a relevant cross-sectional estimate of the HSV-2–BV association using baseline data, and 1 cross-sectional study provided 2 estimates from different populations. Ultimately, from the 17 eligible studies, we extracted 19 measures of association [14, 15, 19, 27–40]: 16 were cross-sectional estimates, and 3 were prospective estimates. The association between BV and HSV-2 was a primary outcome in all but 1 included studies.

In the 3 prospective estimates [14, 15, 19], the mean time for follow-up ranged from 8.5 months to 2.1 years, with follow-up visits occurring every 3, 4, or 6 months. The population and location of these studies also varied: Kaul et al [15] enrolled 443 female sex workers in Kenya, Cherpes et al [19] evaluated 773 sexually active women in 3 US clinics, and Nagot et al [14] included 273 female sex workers in Burkina Faso (Table 1).

**Figure 1.** Flow of information through the different phases of the systematic review. Abbreviations: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; OR, odds ratio.
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Design</th>
<th>Population (All Women)</th>
<th>HIV Status; Age</th>
<th>Setting</th>
<th>No.</th>
<th>Adjustment Variables</th>
<th>Measure of Effect</th>
<th>Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allsworth et al [27]</td>
<td>US</td>
<td>Cross-sectional</td>
<td>Nationally representative</td>
<td>Positive and negative; 20–49 y</td>
<td>Community</td>
<td>2326</td>
<td>Race/ethnicity, no. of sex partners in past year, douching</td>
<td>OR</td>
<td>1.3 (1.1–1.6)</td>
</tr>
<tr>
<td>Chawla et al [28]</td>
<td>India</td>
<td>Cross-sectional</td>
<td>Urban slum and middle class colony</td>
<td>Negative; 15–49 y</td>
<td>Community</td>
<td>120</td>
<td>Unadjusted</td>
<td>OR</td>
<td>0.6 (1.3–3.0)</td>
</tr>
<tr>
<td>Cherpes et al [29]</td>
<td>US</td>
<td>Cross-sectional</td>
<td>General</td>
<td>Not tested; 18–30 y</td>
<td>Clinic</td>
<td>773</td>
<td>Race, age, smoking, douching, ≥5 male sex partners, uncircumcised partner, history of BV, gonorrhea, trichomoniasis, group B streptococcal infection</td>
<td>OR</td>
<td>2.2 (1.5–3.2)</td>
</tr>
<tr>
<td>Evans et al [30]</td>
<td>United Kingdom</td>
<td>Cross-sectional</td>
<td>General</td>
<td>Negative, but positive women were eligible; 14–66 y</td>
<td>Clinic</td>
<td>520</td>
<td>Race, age, class, smoking, contraception, cotache, no. of sex partners (past year and total), anal sex, sex with foreign partner, no nonregular sex partners, genital herpes, candidiasis, history of genital herpes or PID, yeast</td>
<td>OR</td>
<td>3.1 (9.9–10.5)</td>
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<td>Kapiga et al [31]</td>
<td>Tanzania</td>
<td>Cross-sectional</td>
<td>Bar and hotel workers</td>
<td>Positive and negative; 18–55 y</td>
<td>Community</td>
<td>268</td>
<td>Religion, trichomoniasis, chlamydia, syphilis</td>
<td>OR</td>
<td>1.8 (1.0–3.3)</td>
</tr>
<tr>
<td>Kaul et al [15]</td>
<td>Kenya</td>
<td>Cross-sectional</td>
<td>Sex workers</td>
<td>Negative; 18–52 y</td>
<td>Community</td>
<td>443</td>
<td>Unadjusted</td>
<td>OR</td>
<td>0.6 (1.4–1.0)</td>
</tr>
<tr>
<td>Kirakaya-Samadoulougou et al [32] *</td>
<td>Burkina Faso</td>
<td>Cross-sectional</td>
<td>Pregnant</td>
<td>Positive and negative; 15–49 y</td>
<td>Clinic</td>
<td>2019</td>
<td>Age, marital status, contraceptive use, HIV infection, trichomoniasis</td>
<td>OR</td>
<td>1.6 (1.0–2.6)</td>
</tr>
<tr>
<td>Kirakaya-Samadoulougou et al [32] *</td>
<td>Burkina Faso</td>
<td>Cross-sectional</td>
<td>General</td>
<td>Positive and negative; 15–49 y</td>
<td>Community</td>
<td>883</td>
<td>Age, marital status, contraceptive use, HIV infection, trichomoniasis</td>
<td>OR</td>
<td>3.7 (1.9–6.3)</td>
</tr>
<tr>
<td>Kirakaya-Samadoulougou et al [33]</td>
<td>Burkina Faso</td>
<td>Cross-sectional</td>
<td>Pregnant</td>
<td>Positive and negative; 14–49 y</td>
<td>Clinic</td>
<td>2133</td>
<td>Polygamous status, province, parity, history of abortion, trichomoniasis, candidiasis, HIV infection, syphilis</td>
<td>OR</td>
<td>1.6 (1.1–2.6)</td>
</tr>
<tr>
<td>Madhivanan et al [34]</td>
<td>India</td>
<td>Cross-sectional</td>
<td>General</td>
<td>Not tested; 15–30 y</td>
<td>Clinic</td>
<td>863</td>
<td>Age, religion, sex partner uses alcohol, sex under influence of alcohol, tubal ligation, ever had oral sex, trichomoniasis</td>
<td>OR</td>
<td>1.5 (0.9–2.4)</td>
</tr>
<tr>
<td>Masese et al [40]</td>
<td>Kenya</td>
<td>Cross-sectional</td>
<td>Sex workers</td>
<td>Negative; 18–50 y</td>
<td>Community</td>
<td>406</td>
<td>Age</td>
<td>OR</td>
<td>1.3 (1.1–1.6)</td>
</tr>
<tr>
<td>Msuya et al [39]</td>
<td>Tanzania</td>
<td>Cross-sectional</td>
<td>General</td>
<td>Positive and negative; ≥18 y</td>
<td>Clinic</td>
<td>382</td>
<td>Age, age of sexual debut, no. of sex partners, parity, history of STD, history of abortion, clinical signs, syphilis, HIV infection</td>
<td>OR</td>
<td>1.3 (0.7–2.3)</td>
</tr>
<tr>
<td>Riedner et al [35]</td>
<td>Tanzania</td>
<td>Cross-sectional</td>
<td>Bar workers</td>
<td>Positive and negative; 16–39 y</td>
<td>Community</td>
<td>600</td>
<td>Examination round, HIV infection, workplace, duration of work as bar worker, change of residence ≤12 mo before enrollment</td>
<td>OR</td>
<td>1.0 (0.3–3.6)</td>
</tr>
<tr>
<td>Uma et al [37]</td>
<td>India</td>
<td>Cross-sectional</td>
<td>Sex workers</td>
<td>Positive and negative; 18–40 y</td>
<td>Community</td>
<td>582</td>
<td>Unadjusted</td>
<td>OR</td>
<td>1.9 (1.3–2.7)</td>
</tr>
<tr>
<td>Uma et al [38]</td>
<td>India</td>
<td>Cross-sectional</td>
<td>Low income</td>
<td>Positive and negative; 18–40 y</td>
<td>Community</td>
<td>487</td>
<td>Unadjusted</td>
<td>OR</td>
<td>2.3 (1.4–3.9)</td>
</tr>
<tr>
<td>Wang et al [38]</td>
<td>China</td>
<td>Cross-sectional</td>
<td>Sex workers</td>
<td>Positive and negative; ≥18 y</td>
<td>Community</td>
<td>345</td>
<td>Age, duration of work as female sex worker, oral or vaginal sex with the last client, HIV infection</td>
<td>OR</td>
<td>5.6 (1.2–26.9)</td>
</tr>
<tr>
<td>Cherpes et al [14]</td>
<td>US</td>
<td>Prospective</td>
<td>General</td>
<td>Not tested; 18–30 y</td>
<td>Clinic</td>
<td>773</td>
<td>Race, smoking, vaginal sex in past 4 months, vaginal sex after anal sex, uncircumcised partner, lack of lactobacilli detected at prior visit, lack of H2O2-producing lactobacilli detected at prior visit</td>
<td>HR</td>
<td>1.7 (1.3–2.3)</td>
</tr>
<tr>
<td>Kaul et al [15]</td>
<td>Kenya</td>
<td>Prospective</td>
<td>Sex workers</td>
<td>Negative; 18–52 y</td>
<td>Community</td>
<td>443</td>
<td>Sexual risk taking, antibiotic use</td>
<td>IRR</td>
<td>1.4 (1.1–1.8)</td>
</tr>
<tr>
<td>Nagot et al [19]</td>
<td>Burkina Faso</td>
<td>Prospective</td>
<td>Sex workers</td>
<td>Positive and negative; 16–54 y</td>
<td>Community</td>
<td>273</td>
<td>No. of sex partners in preceding week, douching, hormonal contraception, trichomoniasis, HIV infection</td>
<td>RR</td>
<td>1.7 (1.1–2.7)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; IRR, incidence rate ratio; OR, odds ratio; PID, pelvic inflammatory disease; RR, relative risk.

* The study by Kaul et al [15] contributed both a cross-sectional estimate and a prospective estimate to the meta-analysis. The study by Kirakaya-Samadoulougou et al [32] contributed 2 distinct estimates (one from a clinical setting and another from a community setting) to the cross-sectional meta-analysis. As such these studies each appear twice here.

* The overall design of these studies was prospective, but the estimates extracted for meta-analysis were cross-sectional, so these studies are categorized here as cross-sectional.
Among the 16 cross-sectional estimates, 4 were from studies conducted in India, 3 each were conducted in Burkina Faso and Tanzania, 2 each were conducted in the United States and Kenya, and 1 each was conducted in the United Kingdom and China. Only 2 studies were explicit about the inclusion of pregnant women [32, 33], while the others either excluded pregnant women or did not specify the pregnancy status of participants. The 4 estimates from studies that enrolled participants in the United States or United Kingdom had participants from varied racial backgrounds. One of these was a nationally representative US sample [27], and the remaining 3 included predominantly white women (with 70% [14], 73% [29], and 79% [30] of the participants self-identifying as non-Hispanic white). All other studies were conducted in Africa, Southeast Asia, or East Asia, where participants were more likely to be from a single race group. One of these was a nationally representative US sample [27], and the remaining 3 included predominantly white women (with 70% [14], 73% [29], and 79% [30] of the participants self-identifying as non-Hispanic white). All other studies were conducted in Africa, Southeast Asia, or East Asia, where participants were more likely to be from a single race group. Eleven of the cross-sectional estimates allowed for enrollment of HIV-positive women [27, 28, 30–33, 36–39], with HIV prevalences ranging from 0% [28] to 67% [35]. Two studies expressly excluded HIV-positive women [15, 40], and the remaining 2 [29, 34] either did not assess or did not describe assessment of HIV status.

Of the 16 cross-sectional estimates, 12 controlled for a variety of demographic, sexual, and reproductive factors, with considerable variation across studies in approaches to control confounding (Table 1). For the remaining 4 estimates, we computed unadjusted ORs by using the published tabular data. All 3 prospective estimates were presented with adjustment for a range of variables: Kaul et al stratified by sexual risk taking and antibiotic use [15]; Cherpes et al adjusted for race, smoking status, vaginal intercourse in the past 4 months, vaginal intercourse after anal intercourse, male sex partner circumcision status, and an intermediate Nugent score or lack of H2O2-producing lactobacilli at the prior visit [14]; and Nagot et al adjusted for number of sex partners in the preceding week, douching, hormonal contraception, HIV status, and Trichomonas vaginalis coinfection [19].

**BV Diagnosis**

Most of the included studies diagnosed BV on the basis of the Nugent score, with only 4 relying on the Amsel criteria [30, 31, 38, 39]. All 3 prospective estimates used the Nugent score for BV diagnosis.

**Cross-sectional Associations Between HSV-2 and BV**

The 16 cross-sectional estimates showed moderate to high heterogeneity ($I^2 = 64\%$), ranging from an OR implying that HSV-2 infection was associated with a reduced BV prevalence (0.6;
<table>
<thead>
<tr>
<th>Study</th>
<th>Representativeness of the Exposed Cohort</th>
<th>Selection of Nonexposed Cohort</th>
<th>Exposure Ascertainment</th>
<th>Absence of Outcome at Baseline</th>
<th>Adjusted for Sexual Behavior</th>
<th>Accounted for HIV and Race</th>
<th>Outcome Assessment</th>
<th>Length of Follow-up</th>
<th>Adequacy of Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allsworth et al [27]</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Chawla et al [28]</td>
<td>+</td>
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<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Cherpes et al [29]</td>
<td>−</td>
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<td>Evans et al [30]</td>
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<td>Kapiga et al [31]</td>
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<tr>
<td>Kaul et al [15] (cross-sectional)a</td>
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<tr>
<td>Kirakaya-Samadoulougou et al [32] (clinic)a</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Kirakaya-Samadoulougou et al [32] (community)a</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Kirakaya-Samadoulougou [33]</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<tr>
<td>Madhivanan et al [34]</td>
<td>−</td>
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<tr>
<td>Masese et al [40]</td>
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<td>Msuya et al [39]</td>
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<tr>
<td>Uma et al [37]</td>
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<td>Uma et al [36]</td>
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<td>Wang et al [38]</td>
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The Newcastle-Ottawa scale includes the following quality-assessment criteria: (1) representativeness of cohort: was detailed eligibility criteria and information on participation rates provided? (2) selection of the nonexposed cohort: were these individuals selected from the same study population? (3) exposure ascertainment: was herpes simplex virus type 2 (HSV-2) diagnosed by type-specific serological assay? (4) absence of outcome at baseline: were participants confirmed to be BV negative at enrollment (prospective studies only)? (5) adjusted for sexual behavior: did analysis control for at least 1 measure of sexual behavior, including sexual frequency, sex partnerships, or condom use? (6) accounted for HIV and race: did analysis measure key population characteristics including HIV (if both HIV-positive and HIV-negative women were eligible for the study) and race (if the study population was multiracial)? (7) assessment of outcome: was BV diagnosed by the Amsel criteria or Nugent score? (8) length of follow-up: were participants followed for at least 1 month, with HSV-2 and BV both assessed at baseline and at least 1 follow-up visit (prospective studies only)? and (9) adequacy of follow-up: did at least 80% of participants return for at least 1 follow-up visit (prospective studies only)? Cross-sectional studies could receive a maximum of 6 pluses, and prospective studies could receive up to 9 pluses.

Abbreviations: BV, bacterial vaginosis; HIV, human immunodeficiency virus; HSV, herpes simplex virus; +, criterion was met; −, criterion was not met.

* The study by Kaul et al [15] contributed both a cross-sectional estimate and a prospective estimate to the meta-analysis. The study by Kirakaya-Samadoulougou et al [32] contributed 2 distinct estimates (one from a clinical setting and another from a community setting) to the cross-sectional meta-analysis. As such these studies each appear twice here.
95% CI, 1.1–3.0) [28] to an OR suggesting that BV prevalence was >5 times as high in HSV-2–positive women, compared with HSV-2–negative women (5.6; 95% CI, 1.2–26.9) [38]. The pooled analysis of the cross-sectional studies yielded a summary OR of 1.60 (95% CI, 1.32–1.94; Figure 2), indicating that the odds of prevalent BV was 60% greater among HSV-2–positive women, compared with HSV-2–negative women.

Quality Assessment
In general, the quality of included cross-sectional estimates was moderate to high, as assessed using the Newcastle-Ottawa scale (Table 2). Nine cross-sectional estimates met at least 5 of the specified criteria (out of 6 items relevant to cross-sectional studies) and were included in the sensitivity analysis restricted to higher-quality studies [27, 30–32, 35, 38–40]. All but 3 of the cross-sectional estimates [28, 34, 35] were deemed to have a low risk of bias stemming from their funding, as the source was acknowledged or the authors stated that they had no conflicts of interest.

Publication Bias
The funnel plot of cross-sectional estimates suggests that publication bias did not meaningfully affect the summary estimate (Figure 3). The Begg test \((P = .82)\) and the Egger test \((P = .23)\) similarly demonstrate that publication bias was unlikely to have had a significant impact on the cross-sectional analysis.

Sensitivity Analyses
The influence analysis assessed the impact of each cross-sectional estimate on the pooled estimate [24]. For each study that was removed, the resulting estimate remained within the CI of the original pooled estimate, suggesting that no study was excessively influential. Summary estimates in the influence

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**Figure 3.** Funnel plot with pseudo 95% confidence limits.

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**Figure 4.** Influence analysis of cross-sectional studies. The solid lines at 1.60, 1.32, and 1.94 represent the primary pooled estimate and 95% confidence interval (CI) for all cross-sectional studies. The open circles indicate the pooled estimate when the named study is omitted and the corresponding lines are the upper and lower limits of the 95% CI. The study by Kaul et al [15] also contributed a prospective estimate to the meta-analysis, as shown in Table 1. The study by Kirakaya-Samadoulougou et al [32] contributed 2 distinct estimates (one from a clinical setting and another from a community setting) to the cross-sectional meta-analysis and therefore appears twice here.
analysis ranged from 1.51 to 1.69, values qualitatively similar to the primary pooled estimate of 1.60 (Figure 4).

Four subgroup analyses were also performed with the cross-sectional data. For the 6 clinic-based estimates, the pooled OR was 1.72 (95% CI, 1.41–2.11; $I^2 = 0\%$), similar in magnitude to the pooled OR for the 10 community-based estimates (1.55; 95% CI, 1.18–2.05; $I^2 = 74\%$). The difference in $I^2$ by population indicates that the heterogeneity in the overall pooled estimate is driven by variability in the community-based studies. Inclusion of only adjusted estimates in the cross-sectional analysis eliminated 4 reports, but the summary OR in this sensitivity analysis (1.65; 95% CI, 1.37–1.99; $I^2 = 51\%$) was also comparable to that in the primary analysis. Results of the sensitivity analysis restricted to higher quality cross-sectional studies only (n = 9) were also very similar to those of the primary analysis (1.61; 95% CI, 1.28–2.02; $I^2 = 54\%$). Restricting the evaluation to estimates using the Nugent score for BV diagnosis excluded 4 reports, but the summary OR (1.55; 95% CI, 1.25–1.92; $I^2 = 70\%$) was again very similar to that from the primary analysis.

Prospective Associations Between HSV-2 and BV

Despite the considerable variability in the studies generating the 3 prospective estimates, including population, sample size, control for confounding, and other factors, the adjusted estimates were highly consistent, with an $I^2$ of 0% (Figure 5). Each demonstrated that HSV-2–infected women were at increased risk for incident BV, with comparable values of 1.4 (95% CI, 1.1–1.8) in the study by Kaul et al, 1.7 (95% CI, 1.3–2.3) in the study by Cherpes et al, and 1.7 (95% CI, 1.1–2.7) in the study by Nagot et al (Table 1). Pooled analysis of these estimates with a random effects model yielded a summary estimate of 1.55 (95% CI, 1.30–1.84; Figure 5). In other words, compared with HSV-2–negative women, HSV-2–infected women had 55% increased risk of BV acquisition.

All 3 estimates were judged to be of higher quality, based on satisfying at least 7 of the 9 Newcastle-Ottawa scale criteria applicable to prospective studies. All 3 also had low risk of bias due to funding source or stated conflicts of interest (Table 2).

**DISCUSSION**

In this systematic review and meta-analysis, HSV-2 infection was significantly associated with increased BV prevalence and incidence. Moreover, despite variations in study populations, BV diagnosis methods, and confounder adjustment, multiple sensitivity analyses confirmed the robustness of these findings.

Despite the consistency in our findings, some limitations persist when combining estimates to create summary pooled measures. While heterogeneity overall was much greater in the cross-sectional compared to the prospective estimates, the sensitivity analysis of cross-sectional estimates stratified by population type suggested that the community-based studies were considerably more heterogeneous than the clinic-based studies. This finding is not surprising, as diverse populations were recruited in the community-based projects: 6 enrolled sex workers, and 4 enrolled from the general population. Despite these differences, our influence analysis confirmed that no study exerted excessive influence on the pooled estimate.

A greater number of prospective estimates in the meta-analysis would have generated a more precise pooled estimate and would have permitted additional sensitivity analyses regarding the robustness of our main findings. Although an assessment of publication bias is not possible with only 3 prospective reports, the extremely low heterogeneity and highly comparable measures of association, despite differences in population and analytic approaches, argue against the presence of significant bias in the pooled prospective estimate. Publication bias also appears not
to have impacted the summary estimate of the cross-sectional studies. Of course, limitations in the design, conduct, measurement, and analysis of the included reports may have adversely affected the overall quality of the pooled estimates. For example, one challenge in BV-related research is that many women cycle between BV and normal flora on a daily or weekly basis [41]. The absence of BV at enrollment into a prospective study assessing the effect of HSV-2 infection on BV risk, while methodologically necessary to measure BV incidence, does not exclude the possibility that some participants had BV that resolved prior to enrollment. Prevalent BV may even lead to an increased risk of HSV-2 acquisition [29], underscoring the complexity in assessing the temporal association between these 2 highly prevalent vaginal infections.

While the quality of included studies was generally moderate to high, we also identified limitations in some studies by using the Newcastle-Ottawa scale. For example, approximately half the studies did not meet the criterion for representativeness of the exposed cohort, typically because the authors failed to provide data about participation rates, which could reveal how many potential research subjects were screened and deemed ineligible for the research or how many individuals who were found to be eligible went on to enroll in the study. Yet despite variations in study quality, both the sensitivity analysis restricted to the higher-quality projects and the subgroup analysis presenting separate estimates for clinic- and community-based studies confirmed that the variability across studies did not have a large impact on the summary estimate.

Our findings are also limited because of the design of our review. We chose not to include unpublished findings presented at conferences (the so-called gray literature). No approach exists to systematically search the unpublished literature. In addition, analyses presented in conference abstracts are (by requirement) brief, providing considerably less opportunity to assess quality or to extract all relevant information about the population and analytic methods. Unpublished findings may remain unpublished because they are of lower quality than published studies, and their inclusion in a systematic review may introduce bias [42]. We also chose to limit this review to English-language-only publications. While this restriction may have led to the exclusion of potentially relevant findings, prior research illustrates that excluding non–English-language reports does not lead to significant differences in the summary estimates reported in meta-analyses [43]. We did allow for non–English-language abstracts and titles in our search strategies, and only 3 potentially relevant articles were identified. We also did not contact study authors for clarification or missing information, which in theory could have increased the number of studies included in the review. However, we were generally able to find information missing from a given paper by consulting previously published papers from the same authors on the same study population. Given that we searched 3 databases (MEDLINE, EMBASE, and CENTRAL) and hand-searched reference lists of included studies, we suggest that the likelihood is low that our summary estimates are biased because of missing eligible studies.

Our findings of a significant association between HSV-2 infection and BV are further supported by additional research not directly contributing to our meta-analysis calculations. As examples, active genital shedding of HSV-2 is associated with increased BV prevalence [44]. Compared with HSV-2-negative women, HSV-2-positive women have reduced vaginal colonization with H2O2-producing lactobacilli [45]. Finally, women with HSV-2 are more likely to lose healthy vaginal lactobacilli and less likely to successfully respond to standard antimicrobial BV therapy [44,46]. After antimicrobial therapy, BV is expected to recur in about 10% of women overall within 1 month, in 30% within 3 months, and in 50% within 1 year [47,48]. However, among HSV-2–infected women, BV recurrence rates within 1 month of treatment appear to approach 90% [46].

Our findings offer new indication that HSV-2 infection creates a vaginal environment that promotes loss of vaginal lactobacilli or overgrowth of abnormal flora. Moreover, as the fundamental characteristic of HSV-2 infection is intermittent viral reactivation and epithelial shedding, we hypothesize that local host responses to replicating virus are responsible for creating a vaginal environment that is inhospitable to healthy flora and therefore are an underappreciated but important risk factor for incident BV. If our hypothesis is correct, daily HSV-2 suppressive therapy (a safe, available, and affordable treatment option) may help normalize vaginal flora, reduce BV incidence, and improve the efficacy of antimicrobial BV therapy among HSV-2–infected women. Testing this hypothesis through primary research or secondary use of existing data from HSV-2 suppression trials may uncover a secondary prevention strategy that could reduce the significant racial disparities in BV prevalence and reduce the incidence of BV-associated adverse events.

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


