

Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies

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Objectives: To assess and summarize the published literature on the extent to which bacterial vaginosis may increase the risk of HIV acquisition.

Design: Meta-analysis of published studies.

Methods: Medline and other electronic databases were systematically searched for eligible publications. The association between bacterial vaginosis and incident HIV was separately analyzed from that between bacterial vaginosis and prevalent HIV. The latter was further analyzed, stratified by bacterial vaginosis diagnostic method, HIV risk profile of the study population, and whether or not adjusted estimates were presented.

Results: Twenty-three eligible publications were identified, including a total of 30 739 women. Bacterial vaginosis was associated with an increased risk of HIV acquisition in HIV-incidence studies (relative risk = 1.6, 95% confidence interval: 1.2, 2.1). All but one of 21 HIV-prevalence studies reported estimates above the null. The latter results were heterogeneous and showed some evidence of funnel plot asymmetry, precluding the estimation of a single summary measure. The association between bacterial vaginosis and HIV in prevalence studies appeared stronger for women without high-risk sexual behavior.

Conclusion: Bacterial vaginosis was consistently associated with an increased risk of HIV infection. High bacterial vaginosis prevalence may result in a high number of HIV infections being attributable to bacterial vaginosis. More prospective studies are needed to accurately evaluate the role of bacterial vaginosis in HIV acquisition in low-risk versus high-risk women. Furthermore, randomized clinical trials may be worth considering to determine the effect of bacterial vaginosis control measures on HIV acquisition.

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Introduction

Bacterial vaginosis is the most frequent type of vaginitis in women of reproductive age [1–3]. Bacterial vaginosis is an imbalance in the ecology of the normal vaginal flora [4] that is characterized by the depletion of lactobacilli [3] and the proliferation of anaerobic bacteria such as *Gardnerella vaginalis*, *Morbillincus species*, *Prevotella species*, *Mycoplasma hominis* and the recently identified *Atopobium vaginae* [2,5–7]. It most often manifests clinically as a

vaginal pH of more than 4.5, the presence of thin whitish homogenous vaginal discharge, the detection of ‘clue’ cells and the presence of an amine odor after the addition of 10% of potassium hydroxide [8,9]. Bacterial vaginosis has been shown to increase the risk of adverse gynecological and obstetrical outcomes such as preterm delivery [10,11], pelvic inflammatory disease (PID) and upper genital tract infections [12–14]. However, the effect of bacterial vaginosis on the risk of HIV infection in women has not been clearly quantified.

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The magnitude of the association between bacterial vaginosis and HIV has varied in epidemiological studies, ranging from the absence of any association [15] to a near four-fold odds of being HIV infected among bacterial vaginosis-positive women compared with bacterial vaginosis-negative women [16]. Bacterial vaginosis is estimated to be the most prevalent vaginal infection particularly in countries with high HIV prevalence [2]. If bacterial vaginosis is confirmed to increase the risk of HIV infection, the treatment of bacterial vaginosis could be a meaningful intervention to prevent HIV acquisition. In a 2001 review on the role of sexually transmitted diseases in HIV acquisition, Rottingen *et al.* [17] estimated that bacterial vaginosis was associated with a 40% increase in the risk of HIV, based on an analysis of two studies. Obtaining a precise and updated estimate of the strength of the association between bacterial vaginosis and HIV from published studies could be useful in predicting the potential impact of the control of bacterial vaginosis on HIV incidence rates in a population. This prediction could also be more accurate if factors that modify the strength of the bacterial vaginosis and HIV association were identified.

This article aims to systematically review all published studies on the association between bacterial vaginosis and HIV infection. Estimates of the association between bacterial vaginosis and HIV are presented for both HIV incidence and prevalence studies and analyzed for potential modification factors, publication bias, and heterogeneity of study results.

Methods

Search strategy

Medline was searched for peer-reviewed publications on the association between bacterial vaginosis and HIV using the following terms: ('Vaginosis, Bacterial' [MeSH] or 'bacterial vaginosis' or ('Bacterial Infections' [MeSH] and ('Vaginitis' [MeSH] or vagina*)) or 'Gardnerella' [MeSH]) and (HIV or 'HIV Seropositivity' [MeSH] or 'HIV' [MeSH] or 'HIV Infections' [MeSH]). This search yielded 281 published articles. The Web of Science, Popline and the National Library of Medicine (NLM) Gateway databases were further searched using the keywords 'bacterial vaginosis and HIV'. These searches yielded 193, 83 and 166 publications, respectively, but no additional articles were located beyond the Medline search. Articles were included in this meta-analysis if the magnitude of the association between bacterial vaginosis and HIV was presented or could be calculated from the information provided in the article, and if there was a clear description of the diagnostic methods used for ascertaining both bacterial vaginosis and HIV infections. When there was evidence of multiple publications of the

same study over time, only the article with the largest sample size was included. Although non-English articles were eligible for inclusion, none were found. Conference abstracts and other unpublished articles were excluded, as these could not be systematically reviewed.

Data abstraction and analysis

For each study, the following information was extracted: author(s), year of publication, study site, year(s) during which study was conducted, study design (HIV incidence or prevalence), HIV risk of study population (low or high), bacterial vaginosis diagnostic criteria, HIV ascertainment method, mean or median age of participants, crude and adjusted measures of the association between bacterial vaginosis and HIV, and variables for which measures were adjusted. Appropriate measures of association were calculated, when possible, for studies that did not present them.

HIV incidence studies were defined as those that recruited HIV-negative women and prospectively measured incident HIV infection. HIV prevalence studies were those that assessed bacterial vaginosis and HIV status at the same time. Study populations with HIV-risk factors, such as female sex workers (FSWs), sexually transmitted infection (STI) clinic attendees and regular partners of HIV-infected men were classified as 'high HIV-risk groups'. Women without any of these characteristics were classified as being in a low HIV-risk group. Bacterial vaginosis diagnostic criteria included the Nugent's scoring system [18], Amsel clinical criteria [8], and modifications of Amsel criteria. In the Nugent's scoring system (bacterial vaginosis when score ≥ 7), Gram-stained vaginal smears were assessed for the average number of bacterial morphotypes seen per oil immersion field with *Lactobacilli* being scored from 0 to 4, *Gardnerella/Bacteroides* spp from 0 to 4 and curved gram-variable rods 0–2. Amsel criteria define bacterial vaginosis as the presence of any three of the following characteristics: abnormal vaginal discharge, vaginal pH more than 4.5, presence of clue cells, and/or positive amine test with release of fishy odor on addition of 10% potassium hydroxide to vaginal secretions. Modifications of the Amsel criteria included diagnosing bacterial vaginosis only when all four of the above elements of criteria are present or when only two of the four elements are present. Bacterial vaginosis prevalence at baseline or bacterial vaginosis prevalence in controls of case-control studies was recorded as an estimate of bacterial vaginosis prevalence in the study population.

All abstracted data were double checked to confirm accuracy. Separate meta-analyses were conducted for HIV incidence studies and HIV prevalence studies (STATA version 8; STATA Corp., College Station, Texas, USA). Study estimates, relative risks (RR), or prevalence odds ratios (POR), and corresponding 95% confidence intervals (95% CIs) were plotted for each analysis. Adjusted

estimates, when reported, were preferentially used in analyses. For studies that reported estimates using two different methods of bacterial vaginosis diagnosis, the estimate based on the Nugent's score was used because the definition of bacterial vaginosis using this score was more consistent across studies than the use of Amsel diagnostic criteria. Reported estimates were examined for publication bias graphically, using funnel plots, and statistically using the test of Begg and Mazumdar [19], the test of Egger *et al.* [19], and Duval and Tweedie's 'trim and fill' imputation method [19]. Homogeneity test *P* values were computed from Cochran's *Q*-test statistic [19]. To explore sources of heterogeneity, HIV prevalence studies were stratified by HIV-risk group, bacterial vaginosis diagnostic criteria and whether there was any adjustment for confounders or not. Random-effect meta-regression models, using restricted maximum likelihood to estimate the among-study variance [20], were also conducted to assess the association of average study estimates with study characteristics. Bacterial vaginosis diagnostic method, HIV-risk group and a variable indicating the use of adjusted or unadjusted estimates were the independent variables in the models. The impact of each estimate on the summary estimate was explored using influence analyses [14].

Results

Study characteristics

Twenty-three eligible articles were identified altogether including 30 739 women. These reported 29 different estimates of the association between bacterial vaginosis and HIV for 25 study populations (two articles each reported estimates for two distinct study populations [16,21], and four other articles each reported estimates using two different methods of bacterial vaginosis diagnosis [15,22–24]). Four of the 25 study populations were followed prospectively in Kenya [25], Malawi [21] and South Africa [24] to assess incident HIV infection. The remaining 21 studies were studies of prevalent HIV (Table 1) [15,16,22,23,26–41]. Five of these were conducted in the USA, two in Thailand and the remainder in sub-Saharan Africa.

Bacterial vaginosis diagnosis and prevalence

Bacterial vaginosis was diagnosed either using clinical criteria only (in 13 out of 25 study populations), Nugent's score only (in eight out of 25 study populations) or both clinical and Nugent's score (in four out of 25 study populations). Bacterial vaginosis prevalence in these studies ranged from 11.1% in women aged 20–35 years in the USA to 70.0% in STI symptomatic women in South Africa. The pooled bacterial vaginosis prevalence was 33%. Bacterial vaginosis prevalence was consistently higher using Nugent's criteria as opposed to clinical criteria in all four studies [15,22–24] that used both bacterial vaginosis diagnostic methods.

Bacterial vaginosis-HIV association

HIV incidence studies

There was little evidence of funnel plot asymmetry (Begg $P=1.0$, Egger $P=0.2$) or heterogeneity in the study estimates ($P=0.7$, Fig. 1). Bacterial vaginosis was associated with an increased risk of HIV acquisition in HIV incidence studies (Fig. 1) (RR = 1.61; 95% CI: 1.21, 2.13).

HIV prevalence studies

Funnel plot tests gave some evidence of asymmetry (Begg $P=0.3$, Egger $P=0.06$). In the 'trim and fill' analysis, when a random-effect model was used, one hypothetically missing result was imputed. When a fixed-effect model was used, nine hypothetically missing results were imputed (Fig. 2).

HIV seroprevalence was higher in bacterial vaginosis-positive women in all but one study [15,16,22,23,26–41] (Fig. 3). The POR estimates from these prevalence studies were highly heterogeneous ($P<0.0005$) and ranged from 0.77 to 3.70. As shown in Table 2, this heterogeneity persisted even within strata defined by each of HIV-risk group, bacterial vaginosis diagnostic method, or estimate adjustment. POR estimates, in HIV low-risk groups, tended to be higher than that in HIV high-risk groups. It is worth noting that all but one [27] of the studies classified as low-risk group were studies of pregnant women. In a multivariate meta-regression for prevalence studies, controlling for differences in bacterial vaginosis diagnostic method and adjusted versus non-adjusted estimates, the POR in the low HIV-risk group was still higher, 1.43-fold that in the high HIV-risk group (95% CI: 0.94, 2.17). There was little evidence that bacterial vaginosis diagnostic criterion (comparing Nugent to clinical criterion) was associated with the magnitude of the association as the ratio of PORs, accounting for variations due to HIV-risk groups and adjusted versus nonadjusted estimates, was 0.88 (95% CI: 0.61, 1.26). Similarly, there was little evidence that estimate adjustment was a substantial source of heterogeneity as the ratio of PORs, comparing adjusted with unadjusted estimates, accounting for variations due to HIV-risk groups and bacterial vaginosis diagnostic method, was 1.14 (95% CI: 0.76, 1.74).

Influence analyses

The impact of each study on the summary estimate was evaluated by successively omitting each of the studies and obtaining a summary for all the other studies. For HIV incidence studies, the RR varied little, ranging from 1.58 [after excluding the study by Taha *et al.* (1998) [21]] to 1.93 [after excluding the study by Martin *et al.* (1999) [25]]. The POR estimate from the study by Greenblatt *et al.* (1999 [23]), by far the most precise of the estimates from the prevalence studies, was highly influential. Although the homogeneity test *P* value remained less than 0.0005 upon removing this result, it substantially influenced the

Table 1. Summary of studies of the association between bacterial vaginosis and HIV (published on or before October 2005).

References (country)	Study year	Participants	N	Age ^a (range)	BV diagnosis	BV (%) ^b	Age	Factors adjusted for in analysis ^c		
								Sex partners	SES	Condom use
HIV incidence studies										
Taha <i>et al.</i> [21] (Malawi) ^A	1990–1993	HIV-pregnant women	1196	–	Amsel criteria	30.0	Y	Y	Y	Y
Taha <i>et al.</i> [21] (Malawi) ^B	1990–1993	HIV-women in postpartum	1169	–	Amsel criteria	30.0	Y	Y	Y	Y
Martin <i>et al.</i> [25] (Kenya) ^d	1993–1997	FSW	779	(18–48)	Nugent's score ≥ 7	40.0	n	Y	n	n
Myer <i>et al.</i> [24] (South Africa)	2000–2002	Women screened for cervical cancer	410	(35–65)	I. Amsel criteria	15.7	Y	Y	Y	Y
HIV prevalence studies										
Cohen <i>et al.</i> [15] (Thailand) ^d	1992	FSW at STI clinic	140	24 (15–49)	II. Nugent's score ≥ 7	62.4	Y	Y	n	Y
Meda <i>et al.</i> [30] (Burkina Faso) ^d	1992	Symptomatic STI patients	220	27	I. Amine test, pH > 4.5, clue cells II. Nugent's score ≥ 7	39.3	n	n	n	n
Sewankambo <i>et al.</i> [27] (Uganda) ^C	1994–1995	Population-based sample	4718	(15–59)	'clue' cells, abundant Gram negative flora	20.0	n	n	n	n
Rugpao <i>et al.</i> [31] (Thailand) ^d	1992–1996	Female partners of HIV+ male blood donors	481	27	Nugent's score ≥ 7 All four elements of Amsel's definition	50.9	Y	Y	n	Y
Royce <i>et al.</i> [32] (USA) ^D	1995–1997	Pregnant women	616	–	Nugent's score ≥ 7	20.9	n	n	n	n
Taha <i>et al.</i> [16] (Malawi)	1990	HIV-pregnant women	6677	–	Amsel criteria	31.1	Y	Y	n	Y
Taha <i>et al.</i> [16] (Malawi)	1993	HIV-pregnant women	2449	–	Amsel criteria	27.0	Y	Y	n	Y
Fonck <i>et al.</i> [33] (Kenya) ^d	1998	FSW	256	32 (18–57)	Nugent's score ≥ 7	47.3	n	n	n	n
Fonck <i>et al.</i> [26] (Kenya) ^d	1996–1997	STI clinic attendees	324	26 (14–49)	pH > 4.5, Amine test, clue cells	19.0	n	n	n	Y
Fonck <i>et al.</i> [34] (Kenya) ^d	1998–1999	FSW	532	30 (22–38)	Nugent's score ≥ 7	39.7	n	n	n	n
Mbizvo <i>et al.</i> [28] (Zimbabwe) ^d	1999	Clinic attendees	386	27 (16–49)	Amsel criteria	36.4	n	n	n	Y
Warren <i>et al.</i> [22] (USA) ^d	1993–1995	Matched HIV+/- women	1288	35 (16–55)	I. Amsel criteria	34.9	n	Y	n	Y
Kapiga <i>et al.</i> [35] (Tanzania) ^{A,d}	2000	Bar/hotel workers	268	28 (16–55)	II. Nugent's score ≥ 7	46.2	Y	Y	n	n
Moodley <i>et al.</i> [36] (South Africa) ^d	1999–2000	STI-symptomatic women in a clinic	598	24 (17–70)	Amsel criteria	26.1	n	n	n	n
Msuya <i>et al.</i> [37] (Tanzania)	1999	Clinic attendees	382	27 (16–46)	Amsel criteria	34.0	n	n	n	Y
Riedner <i>et al.</i> [29] (Tanzania) ^d	2000	Bar/hotel workers	600	25	Nugent's score ≥ 7	40.2	Y	n	n	n
Sagay <i>et al.</i> [38] (Nigeria)	2002–2003	Pregnant women	2931	28 (<19-> 40)	Amsel criteria	17.8	Y	n	n	Y
Demba <i>et al.</i> [39] (Gambia) ^d	2000	STI clinic attendees	210	28 (18–50)	Nugent's score ≥ 7	47.6	n	n	n	n
Cu-uvvin <i>et al.</i> [40] (USA) ^{E,Fd}	1993–1995	Matched HIV+/- women	1285	35 (16–55)	Amsel criteria	33.0	n	Y	n	n
Greenblatt <i>et al.</i> [23] (USA) ^{A,Fd}	1994–1995	Matched HIV+/- women	2521	36 (16–73)	I. Amsel criteria	18.5	n	n	n	n
Helfgott <i>et al.</i> [41] (USA) ^{Fd}	1995–1997	HIV+/- women from STI and FP clinics	303	27 (20–35)	II. Nugent's score ≥ 7 Amsel criteria	42.3	n	n	n	n

BV, bacterial vaginosis; FP, family planning; FSW, female sex workers; N, number of participants; SES, socioeconomic status; STI, sexually transmitted infections. The significance of superscripts (A,B,C,D,E,F) are as follows. A, relative risk (RR) or odds ratio (OR) comparing BV to no BV estimated from data presented in article; B, Only OR comparing BV to absence of any Amsel criterion presented in article; C, moderate BV (score 7–8) and severe BV (score 9–10) were combined; D, HIV status self-reported on a standardized telephone interview; E, HIV status determined from participants' medical records; F, studies that used a case-control design.

^amean or median age in years.

^bBV prevalence.

^cY, adjusted for; n, not adjusted for.

^dStudy populations categorized as 'High HIV-risk' in this analysis.

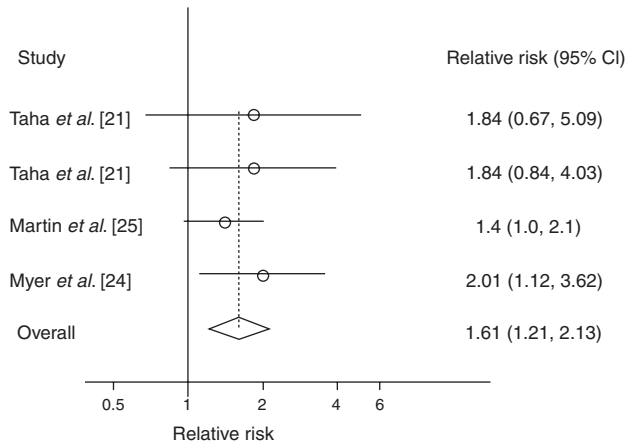


Fig. 1. Forest plot of relative risk estimates of incident HIV infection by bacterial vaginosis status, stratified by HIV-risk group. Studies are identified by the references. The horizontal lines represent the 95% confidence intervals (CI). Overall heterogeneity $P=0.7$.

symmetry of the funnel plot. When this result was removed, the symmetry tests produced $P=0.2$ (Begg) and $P=0.6$ (Egger); the ‘trim and fill’ analysis imputed just one hypothetically missing result, regardless of whether a fixed effect or random-effect model was used.

Sensitivity analyses

Sensitivity analyses were conducted to determine the impact of the decision to systematically choose Nugent’s score results over clinical criteria for bacterial vaginosis

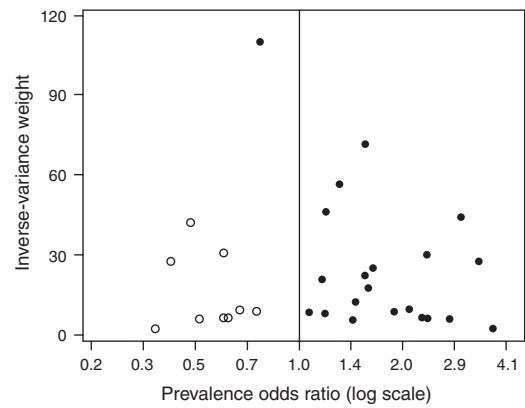


Fig. 2. Funnel plot of estimates of the association of prevalent HIV infection with bacterial vaginosis. The full circles represent real study estimates, whereas the blank circles are the imputed estimates from a ‘trim and fill’ analysis.

diagnosis for studies with both results available. These sensitivity analyses were conducted by repeating the analyses after replacing, for studies that used two diagnostic methods, estimates based on Nugent’s scores with those based on clinical criteria. The sensitivity of the effect estimate to this choice was found to be robust with an overall RR in HIV incidence studies of 1.47 (95% CI: 1.10, 1.95). Heterogeneity in prevalence studies remained unchanged with the POR estimate in low HIV-risk groups of 2.30 (95% CI: 1.68, 3.15) still being higher compared with the POR in high HIV-risk groups of 1.53 (95% CI: 1.29, 1.82).

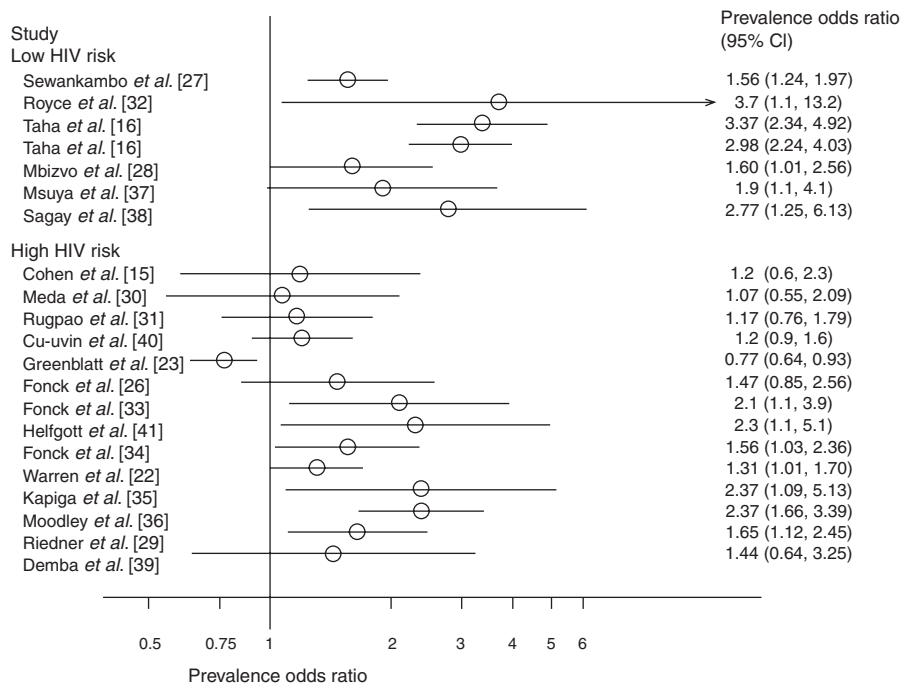


Fig. 3. Forest plot of estimates of the association of prevalent HIV infection with bacterial vaginosis, stratified by HIV risk. Studies are identified by the references. The horizontal lines represent the 95% confidence intervals (CI). Heterogeneity P value in low HIV-risk group = 0.002. Heterogeneity P value in high HIV-risk group < 0.0001. Overall heterogeneity $P < 0.0001$.

Table 2. Summary of analysis stratified by study characteristics.

Characteristic	N	Summary		Within-stratum heterogeneity <i>P</i> value ^a	Funnel plot <i>P</i> values ^b	Meta-regression ratio of odds ratios (heterogeneity <i>P</i> values) ^c	
		OR	95% CI				
Study design	HIV prevalence	21	1.69	1.36, 2.10	0.00	0.29; 0.06	1 ^d
	HIV incidence	4	1.61	1.21, 2.13	0.74	1.00; 0.24	1.02 (0.95)
BV diagnosis ^e	Clinical	11	1.93	1.45, 2.57	0.00	0.44; 0.95	1 ^d
	Nugent	10	1.47	1.11, 1.94	0.00	0.86; 0.12	0.75 (0.12)
HIV risk group ^e	Low	7	2.30	1.68, 3.15	0.00	0.44; 0.02	1 ^d
	High	14	1.44	1.15, 1.80	0.00	0.54; 0.52	0.62 (0.01)
Adjustment ^e	Unadjusted	7	1.33	0.90, 1.97	0.00	0.06; 0.28	1 ^d
	Adjusted	14	1.90	1.53, 2.35	0.00	0.76; 0.16	1.44 (0.05)

BV, bacterial vaginosis; CI, confidence interval; *N*, number of estimates; OR, odds ratio.

^aHeterogeneity *P* values for all studies within each level of study characteristics.

^bBegg's test *P* value (continuity corrected); Egger's test *P* value.

^cRatio of odds ratios (between-stratum heterogeneity *P* values) from meta-regression.

^dReferent category.

^eOnly for HIV prevalence studies.

Discussion

This is an updated systematic review and meta-analysis of the association between bacterial vaginosis and HIV infection. Overall bacterial vaginosis prevalence was high in several populations of women studied, with prevalence rates as high as 70%. Our analyses of HIV incidence studies indicate that bacterial vaginosis increases the risk of HIV acquisition by approximately 60% (95% CI 21–113%). This was slightly higher than that reported (40%) in a previous review of two studies [17]. Studies of HIV prevalence tended to find higher HIV prevalence in women with bacterial vaginosis. However, these prevalence study estimates were heterogeneous and had evidence of funnel plot asymmetry.

The bacterial vaginosis–HIV association tended to be weaker in high HIV-risk groups, though the small number of prospective studies limited the confirmation of this trend in HIV incidence studies. A weaker association in high-risk women may possibly be due to a depletion of susceptibility to HIV resulting from women in high-risk groups having a greater risk of acquiring HIV from causes other than bacterial vaginosis. Once HIV infected, they are no longer at risk of acquiring HIV attributable to bacterial vaginosis, thus reducing the effect of bacterial vaginosis in this group. More data from prospective cohorts are needed to better examine the heterogeneity, by HIV-risk group, in the effects of bacterial vaginosis on the risk of acquiring HIV. This information could be helpful in identifying specific sub-populations, with a stronger association between bacterial vaginosis and HIV, in whom to target bacterial vaginosis control measures.

Bacterial vaginosis results in several changes in the vaginal flora that provide biological plausibility for an increased risk of HIV acquisition in bacterial vaginosis-positive women. Bacterial vaginosis is associated with a depletion of hydrogen peroxide-producing lactobacilli that may

reduce vaginal defense against microorganisms including HIV [42,43]. Higher vaginal pH (>4.5) that occurs with bacterial vaginosis may also increase the availability of vaginal HIV target cells by increasing CD4 lymphocyte activation and multiplication [44]. High vaginal pH may also increase the adherence and survival of HIV [21]. Bacterial vaginosis has also been associated with a reduction in vaginal fluid levels of secretory leukocyte protease inhibitor (SLPI) [5], which has been shown to block HIV infection *in vitro* [45]. Finally, by increasing intravaginal levels of interleukin-10, bacterial vaginosis may increase the susceptibility of macrophages to HIV [46]. These changes, combined with the difficulties of successfully eradicating bacterial vaginosis [47], may explain the increased risk observed in most epidemiology studies.

Some methodological limitations to this review need to be considered. The first are concerns on whether a meta-analysis of observational studies can effectively control for confounding and bias [19]. An attempt at reducing these was made by the preferential use of adjusted estimates in the estimation of summary measures. Meta-regression also revealed little difference between the adjusted and unadjusted estimates used in the final analysis. The second limitation has to do with the relatively few prospective studies included in this analysis. The restricted number of HIV incidence studies prohibited any sub-group analysis. However, this did not appear to be necessary as there was no heterogeneity among the estimates from these studies. The limited number of studies also prohibited any reliable analysis of other potential sources of heterogeneity, such as pregnancy or age. More prospective studies are needed to accurately evaluate the causal association between bacterial vaginosis and HIV. Third, this review was limited to that of published studies. This had little impact on the estimate from HIV incidence studies as there was no evidence of funnel plot asymmetry. The impact of publication bias on HIV prevalence studies was, however, unclear because of the heterogeneity in the estimates and

discrepancies in the results of the various methods of assessing publication bias (Begg's versus Egger's methods; Duval and Tweedie's 'trim and fill' random versus fixed models). The funnel plot of the POR estimates was unusual in that the one estimate that was by far the most precise (Greenblatt *et al.* 1999 [23]) fell well outside the range of the other estimates. Given the pronounced heterogeneity among all POR results, this result was not given a very high weight when the trim and fill analysis was conducted using a random-effect model. With a fixed-effect model, however, the exceedingly high inverse-variance weight assigned to this estimate caused the trim and fill analysis to suggest publication bias so profound that one-third of all prevalence results are unreported, all of them on the reduced-prevalence side of the null. Although some publication bias in that direction might have occurred, we are not inclined to believe that it could have been great. In any event, the prevalence results were much too heterogeneous to warrant aggregating them to produce a single, summary estimate. We found nothing obvious about the Greenblatt *et al.* (1999 [23]) study that should have caused it to produce an estimate so unlike the remainder of the literature. It was one of five studies conducted in the United States and one of four US studies designed to include sizable proportions of HIV-positive and HIV-negative women. Yet it was the only one to produce an inverse association. We are inclined to consider its departure from the main thrust of the literature an unexplained anomaly.

Limitations in the original studies included in this meta-analysis could also impact our estimates. With bacterial vaginosis being a time-dependent condition, prospective studies of HIV incidence are susceptible to misclassification in the definition of bacterial vaginosis status resulting from the use of either bacterial vaginosis status at enrollment or bacterial vaginosis status at prior visit as indicators of bacterial vaginosis status immediately preceding HIV acquisition. Misclassification could also result from false positive or false negative diagnosis of bacterial vaginosis using either clinical or bacteriologic criteria. Both of these mechanisms of misclassification are expected to be nondifferential and thus lead to more conservative estimates of the effect of bacterial vaginosis on HIV acquisition. Original studies of HIV prevalence by bacterial vaginosis status (using case-control or cross-sectional designs), in addition to the aforementioned susceptibility to misclassifying bacterial vaginosis status, could also be subject to selection bias when HIV cases are enrolled from high sexual-risk populations in whom bacterial vaginosis is more frequent. If not controlled, such a bias would result in an overestimate of the association between bacterial vaginosis and HIV. However, we do not think this may have been substantial as most studies did control for at least one indicator of sexual risk, thus attenuating the impact of selection bias. Finally, all the observational studies included in this analysis are subject to residual confounding, which could result in an underestimate or overestimate of the

magnitude of the association between bacterial vaginosis and HIV.

Despite these limitations, this review was strengthened by an extensive search of published literature using multiple databases and references of identified publications. Furthermore, by separating HIV incidence studies from HIV prevalence studies, the effect of bacterial vaginosis on incident HIV was separately analyzed. This distinction is important as studies of incident HIV are not liable to reverse causation bias that would result from HIV-infected women being more likely to acquire bacterial vaginosis. The analysis of sources of heterogeneity also allowed us to identify HIV-risk group, and not method of bacterial vaginosis diagnosis, as an important source of heterogeneity in prevalence study results. Finally, we refrained from using summary estimates in the presence of heterogeneity. It has been argued that even when a random-effect model is used to obtain a summary estimate, the latter is not always conservative and is potentially misleading, if interpreted as an average effect [48].

The high prevalence of bacterial vaginosis in certain populations (particularly, those most impacted by the HIV pandemic) implies that notwithstanding the relatively modest effect of bacterial vaginosis on HIV infection, a high proportion of HIV infection could be attributable to bacterial vaginosis. In a population of women having a bacterial vaginosis prevalence of 30%, with a RR of 1.6, the population attributable risk proportion (PARP), the proportion of HIV in a population that is attributable to bacterial vaginosis, is estimated at 15%. Although other STIs have been shown to increase the risk of HIV infection with a higher RR in the order of 2–5 [49], the relatively lower prevalence of these STIs as seen in some of the studies included in this analysis [26–29] may result in similar proportions of HIV infection being attributable to these STIs as to bacterial vaginosis.

The potential impact of bacterial vaginosis could also be expressed in the number of women who need to have bacterial vaginosis for each additional case of HIV. This depends on the baseline risk of HIV among women without bacterial vaginosis. For instance, with a 2.0% baseline risk of HIV seroconversion among bacterial vaginosis-negative women [21], a RR of 1.6 would correspond to an absolute risk increase of approximately 1.2%, or about one additional case of HIV for every 80–90 women with bacterial vaginosis. These data suggest that greater attention needs to be given to bacterial vaginosis in the global fight against HIV infection. Randomized clinical trials (RCT) to determine the effect of bacterial vaginosis control measures on HIV acquisition may be worth considering. A previous RCT of the effects of mass treatment of STIs on HIV conducted in Rakai (Uganda) used a single dose of oral metronidazole 2 g and found no effect on HIV acquisition [50]. However, although 2 g of metronidazole can cause short-term remission, it is not the

recommended treatment [51,52], thus limiting the inference that can be made on the effect of bacterial vaginosis treatment from the Rakai study. Future RCTs assessing this effect will need to use the recommended treatment regimen with a longer duration associated with lower recurrence rates. In addition to the need to evaluate the potential of bacterial vaginosis treatment to prevent HIV acquisition and transmission, a better understanding of its risk factors and determinants of bacterial vaginosis recurrence is required.

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