As is the case for all anatomic sites with a mucosal surface, the vagina may be colonized by a variety of bacteria, fungi, and other potential pathogens. A normal vaginal flora is predominately composed of Lactobacillus species, with low bacterial diversity [1]. Lactobacilli provide significant health benefits to the host by decreasing the vaginal pH through lactic acid production, generating H2O2 and bacteriocins, and stimulating the local immune system [2], all of which serve as barriers to pathogens. Thus, an abnormal vaginal flora may have significant implications for the transmission of human immunodeficiency virus (HIV) and other sexually transmitted infections (STIs). It is therefore unfortunate that, in the real world, a normal (i.e., lactobacilli-predominant) vaginal flora is not the norm.

The bacterial composition of the vaginal flora within a population constitutes a spectrum. In some individuals, H2O2-producing Lactobacillus species predominate, whereas in others, the flora may include an increasing proportion of various bacterial species, such as Gardnerella vaginalis, Mycoplasma hominis, and gram-negative and gram-positive anaerobes, including Prevotella organisms [1, 3]. This spectrum is often quantified numerically by means of the Nugent scoring system, with flora classified as “normal,” “intermediate,” or “bacterial vaginosis” [4]. On the basis of this system, the vaginal flora is actually abnormal (i.e., it is classified as intermediate or bacterial vaginosis) in at least half of women. This abnormal status tends to recur frequently and rapidly in women who have received BV therapy [2]. The prevalence of abnormal vaginal flora is particularly high among women from sub-Saharan Africa [5, 6], where several studies found an association between BV and HIV acquisition [2, 5–7] and where the prevalence of HIV infection is also disproportionately high among women—particularly young women [8]. Of importance, a less abnormal flora may also increase HIV susceptibility, because there is a linear association between the Nugent score and the probability of HIV acquisition [5–7]. Other causes of vaginitis, including infection by Candida albicans and Trichomonas vaginalis, are also common and can cause significant symptoms, with T. vaginalis infection most consistently linked to HIV acquisition [9, 10].

There are several mechanisms by which alterations in vaginal flora and other causes of vaginitis might enhance HIV acquisition. Loss of a lactobacilli-predominant flora is associated with an increased vaginal pH, an absence of H2O2, and a reduction in mucosal levels of secretory leukocyte protease inhibitor, an innate immune factor with anti-HIV activity [2, 11]. Both bacterial vaginosis and intermediate flora have also been associated with substantial local increases in proinflammatory cytokine levels, as have other vaginal infections [12, 13]. These cytokines might directly enhance HIV replication, but because they are produced by activated immune cells, including CD4+ T cells and monocytes/dendritic cells, they are perhaps more importantly a marker for increased numbers of HIV-susceptible target cells in the genital mucosa [14, 15]. Finally, BV may enhance acquisition of herpes simplex virus type 2 (HSV-2), a major cofactor in both the acquisition and secondary transmission of HIV [16], and BV may also increase susceptibility to other STIs, such as T. vaginalis infection, Neisseria gonorrhoeae infection, and Chlamydia trachomatis infection [17–19].
Given that these diverse causes of vaginitis have been linked with HIV acquisition and that BV in particular is extremely prevalent and tends to be recurrent, it makes sense that treatment and/or prevention of vaginitis might be an effective means to reduce HIV acquisition at the population level. However, efforts to control HIV by means of population-based measures that target classic STIs—a similarly logical strategy—have had limited success [20]. It has been hypothesized that the lack of an impact of STI control is related to the reduced role of STIs as drivers of HIV transmission during a mature epidemic [21]. Although a recent meta-analysis demonstrated that STIs remain strongly associated with HIV transmission in this setting [22], the prevention of vaginitis might be a more effective strategy to block HIV transmission in sub-Saharan Africa, because vaginitis is much more common than STIs among women in the general population. Before a clinical trial can test the efficacy of vaginitis prevention, an effective means to prevent vaginitis in an HIV-endemic region is required, particularly since intermittent population-based mass therapy with a cocktail of antibiotics that included metronidazole did not reduce BV rates in a community-based randomized trial in Uganda [23].

The goal of the well-performed randomized, double-blind, placebo-controlled trial reported by McClelland et al. [24] in this issue of the journal was the establishment of such a clinical intervention. In brief, slightly more than 300 HIV-negative female sex workers from Mombasa, Kenya, were randomized at a ratio of 1:1 to receive monthly treatment with metronidazole (2 g) plus fluconazole (150 mg) or to receive metronidazole placebo plus fluconazole placebo. Diagnostic tests and directly observed administration of study medications were performed monthly, and the mean duration of clinical follow-up was 12 months. In the intervention arm, the investigators demonstrated a reduction of ~50% in the incidence of BV, defined on the basis of clinical and Gram stain criteria, and an increase in the rate of colonization by H2O2-producing lactobacilli. There was a strong trend toward a reduced incidence of *T. vaginalis* infection and a weaker trend toward a reduced incidence of vaginal candidiasis, although neither finding was statistically significant, which, in the former case, is possibly explained by an unexpectedly low baseline incidence of *T. vaginalis* infection. Metronidazole therapy was generally well tolerated, although there was an increased rate of nausea in the treatment arm. The statistical analyses performed were appropriate, the placebo and treatment arms were well matched, and the follow-up rates were laudable, given the difficulties inherent in studying such a population.

Where, then, do these results lead us? McClelland and colleagues have clearly demonstrated that monthly metronidazole therapy for the prevention of bacterial vaginosis in a population at high risk for HIV acquisition has an efficacy similar to that of twice weekly intravaginal administration of 0.75% metronidazole gel [25] and, perhaps, has a more convenient administration schedule. This regimen would be appropriate for a clinical trial examining the impact of BV prevention (and, probably, the prevention of *T. vaginalis* infection) as a means of HIV control. However, the addition of fluconazole therapy was poorly effective in preventing vaginal candidiasis, likely because of a suboptimal dosing interval [26] or the induction of secondary candidiasis by metronidazole treatment [25]. Therefore, a clinical trial that examines the effects of improved overall vaginal health (including the prevention of candidiasis) on HIV acquisition, rather than one that focuses on BV (with or without *T. vaginalis* infection), would require an alternative therapeutic regimen.

Two important considerations for a clinical trial will be the effect size of BV on increased HIV acquisition and the possible confounding role of HSV-2 infection. The incidence of BV remained very high in the intervention arm, despite an almost 50% reduction, which means that, in practice, such a clinical trial would either require a very large sample size or a more effective means to prevent BV. Prevalent HSV-2 infection has been associated with increased incidences of bacterial vaginosis [18, 27, 28], *T. vaginalis* infection, other STIs [18], and HIV infection [29]. Furthermore, HSV-2 infection status was generally not tested in earlier studies that linked vaginitis with HIV acquisition [10]. Therefore, the possibility exists that the vaginitis-HIV association may be driven—at least in part—by coinfections with pathogens, such as HSV-2, that will not be prevented by metronidazole therapy. This will need to be considered in the determination of the sample size, inclusion criteria, and/or statistical analyses that are appropriate for the study. However, the study would be an ideal means to explore a promising new HIV prevention strategy and to better elucidate in a controlled fashion the complex interactions between these genital infections. It is debatable whether female sex workers would be an ideal target population for such a trial, given that the HIV population attributable risk of other coinfections, such as HSV-2 infection and other STIs, may be much greater among these individuals than in the wider community.

In summary, we have been slow to define the impact of the most common genital coinfections, specifically vaginitis and HSV-2 infection, on sexual transmission of HIV. These infections have an extremely high prevalence, especially in areas of the world hit hardest by the HIV pandemic, they have a major impact on the immunologic characteristics of the genital tract, and they have been associated with increased HIV acquisition and secondary transmission [30]. Unfortunately, 2 recent trials of HSV-2 suppression in HIV-uninfected women have shown no impact on HIV acquisition [31, 32], although a large study examining the effect of HSV-2 suppression on secondary transmission of HIV from women coin-
References


