

Evaluation of Health Disparity in Bacterial Vaginosis and the Implications for HIV-1 Acquisition in African American Women

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Keywords

BV, inflammation, pathogenesis, race, stress, vaginal epithelium

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Submission October 6, 2015;
accepted January 7, 2016.

Citation

Donald J. Alcendor. Evaluation of health disparity in bacterial vaginosis and the implications for HIV-1 acquisition in African American women. *Am J Reprod Immunol* 2016; 76: 99–107

doi:10.1111/aji.12497

Introduction

Bacterial vaginosis (BV) is a common vaginal disorder in women first reported by Gardner and Dukes in 1955.¹ In women of childbearing age, BV is the most common cause of vaginitis and has also been associated with fetal loss, chorioamnionitis, cervicitis, endometritis, urinary tract infections, cervical intraepithelial neoplasia, pelvic inflammatory disease (PID), preterm labor, and delivery of low birth weight infants.^{2–5} BV occurs in nearly 29% of 14- to 49-year-old women in the United States with an approximate 50% infection rate in African American women and higher infection rates for women in sub-Saharan Africa.^{6,7} African American women are also disproportionately impacted by HIV/AIDS with infection rates more than 20× the rate for white

There is a health disparity for both bacterial vaginosis (BV) and human immunodeficiency virus type 1 (HIV-1) infection in African American women that may be linked. The evidence that BV predisposes women to higher risk for HIV infection is well documented. The underlying mechanisms to support the epidemiological connections will require further investigations. This review explores the risk factors for BV disease with implications for HIV-1 acquisition in the context of race as a potential driver of the 20-fold increase in HIV-1 acquisition for African American women compared to white women. Specifically, it explores (i) disparities for BV in African American women, (ii) racial disparity for HIV-1 acquisition in African American women, (iii) common factors associated with BV and HIV acquisition in African American women, and (iv) potential mechanisms of the enhancement of HIV-1 transmission by BV.

females and account for 66% of all new AIDS cases in women.⁸ In recent years, BV has been significantly associated with an increased incidence of HIV infection.^{9–13} Analyses performed by Atashili et al.¹⁴ suggested that BV increases the risk for HIV-1 acquisition by 60%. This review explores the epidemiologic synergy between the two infections as well as the biologic basis for this synergy.

Racial disparity for BV in African American women

Black and Mexican American women have significantly higher rates of BV with prevalence rates of 51.6% and 32.1%, respectively, than white, non-Hispanic women (23.2%).⁵ The prevalence of BV has been associated with several demographic and behavioral factors that include race, age, the number

of sexual partners, the use of hormonal contraceptives, menopausal status, smoking, alcohol consumption, and douching.¹⁵ Although vaginal douching is more common among African American and Caribbean women than among white women, this practice appears to be independent of other behavioral factors.¹⁶

Bacterial vaginosis is characterized by a loss of vaginal lactobacilli usually found in healthy women, followed by an overgrowth of a mixed vaginal flora consisting mainly of anaerobic and facultative aerobic bacteria that includes *Gardnerella vaginalis* and *Mycoplasma hominis*, as well as *Atopobium*, *Mobiluncus*, *Bacteroides*, *Prevotella*, and *Peptostreptococcus* species.^{17,18} The microbiome in BV (BV-associated bacteria) is very diverse, and the studies suggested that it varies with ethnicity. Deep sequencing analysis of BV-associated bacteria (BVAB) has been performed. Srinivasan et al. used deep sequencing of the 16S rRNA gene from BV-associated bacteria from 220 women with and without BV coupled with species-level taxonomic identification to determine the associations between the presence of individual bacterial species and clinical diagnostic characteristics of BV. They found that several BV-associated bacteria showed race-dependent prevalence when analyzed in separate groups by BV status.¹⁹ In their final analysis, 28 bacterial taxa were significantly associated with race and included specific *Leptotrichia* and *Atopobium* species.¹⁹ These bacteria were more prevalent in African American women without BV than in white women without BV.

Additionally, African American women's vaginal microflora was dominated by *Lactobacillus iners* and white women's by *L. crispatus*. This is thought to be significant because women with high levels of *L. crispatus* generally have low vaginal pH, whereas women with high levels of *L. iners* have both high and low vaginal pH.²⁰ The presence of *L. crispatus* has been associated with healthy vaginal microenvironments. The authors suggested that this may contribute to the increased incidence of BV in African American women, but it remains inconclusive.²⁰ The predominance of *L. crispatus* among a minority of women of African descent exhibits low inflammatory states in the cervicovaginal mucosa; however, the majority of these women have diverse cervicovaginal bacterial communities with a low abundance of lactobacilli²¹. This would argue that women of African descent are more likely to be colonized by diverse communities of genital bacteria that support

a high inflammatory state. In the study performed by Anahtar et al.²¹ in young South African women with BV, only 37% of participants had lactobacillus-dominant cervicovaginal bacterial communities. By comparison, studies in developed countries showed 90% and 62% with lactobacillus-dominant cervicovaginal bacterial communities among black and white women, respectively.^{22,23}

These findings suggested that there are differences in vaginal bacteria profiles in women of African descent from developing countries compared to women of African descent from developed countries. They also found that among the South African women with lactobacillus-dominant cervicovaginal communities, 77% were colonized with *Lactobacillus iners*.²¹ *L. iners* is unique among lactobacilli in its ability to survive in diverse cervicovaginal bacterial communities.²⁴ Surprisingly, 45% of the 63% women in the cohort with no lactobacillus showed dominance with *Gardnerella*. *Prevotella* species were found at a level of 10% in all cohorts studied.²¹ This would suggest that *Gardnerella* is a dominant cervicovaginal species in women of African descent with BV.

A report by Royce et al.²⁵ evaluating vaginal flora and vaginal pH of 842 women at 24–29 weeks of gestation, found that vaginal pH and vaginal flora differed significantly by race/ethnicity; African Americans were more likely to have a vaginal pH > or = 4.5, no lactobacilli, small gram-variable and gram-negative rods, and *Mobiluncus* bacterial species compared to white women after controlling for sociodemographics, sexual activity, sexually transmitted diseases, health behavior, and sexual hygiene. In a report by Friscella et al.²⁶ that examined 13,917 women with BV from largely low-income backgrounds enrolled during routine pre-natal visits from 23 to 26 weeks of gestation, no significant difference in vaginal pH level between black and white women was found after controlling for differences in Gram stain score, age, and study site. The relationship between vaginal pH, race, and BV prevalence is conflicting and will require further study. However, colonization with specific types of *Lactobacillus* strains has been shown to influence vaginal pH as well as CO₂-level exposure when measuring the vaginal pH.²⁷

Racial disparity for HIV-1 acquisition in African American women

In the United States, women represent 25% of all persons living with HIV/AIDS.²⁸ African American

women are disproportionately impacted by HIV/AIDS with infection rates more than 20× the rate of their white female counterparts.²⁹ In 2014, the CDC projected that 1 in 32 African American women in the United States will be diagnosed with HIV at some point in their lifetime.³⁰ In 2010, African American teens and young adults represented 57% of all new infections in that age group.³¹ HIV infection is a leading cause of death among African American women aged 25–44.³² In the United States, older African women represent one of the fastest growing populations newly infected with HIV.³³ Older African women infected with HIV are an emerging population that will increase over time due to the use of antiviral therapy.³³

The U.S. criminal justice system has the highest incarceration rate in the world.³⁴ African American women are disproportionately impacted by the U.S. criminal justice system.³⁵ African American women are 7 times more likely to be incarcerated in their lifetime than white women.³⁶ Incarcerated women have higher rates of HIV and sexually transmitted infections (STI) than the general population.³⁷ In 2010, 1.9% of incarcerated adult women in the United States were HIV positive, which is 13 times the rate of adult women in the general population (0.15%).³⁸ There are approximately 23,000 HIV-infected adult women released from correctional institutions annually in the United States with the majority of being African American women.³⁹

Common factors associated with BV and HIV acquisition in African American women

There is a disproportionately high prevalence of herpes simplex virus type 2 (HSV-2) infection among African American women.^{40–42} In a study carried out by Alsworth et al.⁴³ African American women had an HSV-2 seroprevalence of 48% compared to 16% for white women. It is well documented that HSV-2 infection is associated with a threefold increased risk for HIV-1 acquisition in women.⁴⁴ This increased risk is present even in the absence of HSV-2 virus replication.⁴⁵ The vaginal mucosa is known to retain CCR5+ CD4+ T cells, as well as infiltrating plasmacytoid and myeloid dendritic cells (DCs), even after HSV-2 lesions heal and in the presence of acyclovir therapy.^{46,47} The persistence of HIV target cells in the vaginal mucosa could help to explain the increased risk for HIV transmission in HSV-2-positive individuals.

Recent studies showed that HSV-2 infection promotes a reduction in normal vaginal lactobacilli that could contribute to BV acquisition.^{48,49} Esber et al.⁵⁰ performed a systematic review and meta-analysis of the link between HSV-2 infection and BV and found that HSV-2 is an important risk factor for BV acquisition and that pharmacologic HSV-2 suppression may reduce BV incidence and BV-associated adverse events.

Recent studies have shown a higher incidence of BV and HIV infection among women with vitamin D deficiency.^{51–53} It has been suggested that African American women and women with heavily pigmented skin may be more prone to develop vitamin D deficiencies because of photoprotection by higher levels of melanin in the skin which prevents the skin from absorbing sunlight needed for vitamin D production.⁵⁴ However, the exact mechanistic role of melanin in vitamin D synthesis remains unclear.⁵⁴ In a substudy of the Women's Interagency HIV Study (WIHS), vitamin D deficiency was independently associated with BV among HIV-infected women compared to HIV-uninfected women.⁵⁵ The authors concluded that vitamin D deficiency was significantly associated with BV among HIV-infected women in the cohort and that the mechanistic role of vitamin D deficiency in BV disease and HIV-1 acquisition requires further examination.⁵⁵ The authors suggested that the prevalence of vitamin D deficiency in African American women could explain, in part, the racial disparity associated with BV disease. Other studies have found that risk for BV in association with vitamin D deficiency differs by pregnancy status.⁵⁶ A study performed by Turner et al., examined the ability of vitamin D and metronidazole therapy to reduce BV recurrence. Their findings revealed that short-term, high-dose vitamin D supplementation did not reduce BV recurrence.⁵⁷ However, previous studies examined BV prevalence rather than the recurrence of BV. These authors stated that it is currently unknown whether sufficient vitamin D supplementation can prevent the initial development of BV.⁵⁷ The effects of vitamin D supplementation on BV recurrence may require more than a 24-week vitamin D supplementation and metronidazole therapy regimen as performed in the study by Turner et al. Existing data supporting vitamin D and BV prevalence also focused on pregnant women and may suggest that vitamin D effects on BV prevalence in pregnant women are different because of the unique

conditions of pregnancy including hormone levels and immune factors.^{58,59}

One study suggested that the male partner's race is a risk factor for BV disease, supporting the notion that BV is sexually transmitted and could help explain the disparity of BV in African American women.⁶⁰ Klebanoff et al.⁶⁰ showed that BV occurred in 12.8% of 906 sexually active intervals compared to white women of 24.8% of sexually active intervals when the woman reported an African American partner and 10.7% when all partners were white. The small size of this study is a limitation and the results are controversial because the association with race of the male partner BV prevalence could be directly linked to having a previous partner with BV. Taken together, these findings will require further investigation.

Poverty that leads to poor educational opportunities, housing insecurity, and the lack of access to care are some of the common problems for African American women at risk for both BV and HIV acquisition.^{61,62} Studies that examined BV acquisition among socioeconomically disadvantaged women suggested that women with low socioeconomic status are at an increased risk for BV acquisition.^{63,64} These women are more likely to be exposed to sexually transmitted infections and participate in unhealthy behaviors like cigarette smoking which has also been associated with the increased risk for BV.^{65,66}

Exposure to chronic stress can predispose individuals to disease by the impairment of immune function, leading to the overproduction of glucocorticoid hormones and catecholamines.⁶⁷ Stress-related immune dysfunction has been associated with poor responsiveness to vaccines, increased risks for upper respiratory tract infections, impaired wound healing, and progression of HIV infection.^{68,69} Studies reported that African American women are exposed to higher levels of psychological and physiological stressors than white women and that increased chronic stress is associated with the increased risk for BV.⁷⁰⁻⁷² In a study by Culhane et al.⁷³ after adjusting for maternal age, race, education, parity, douching, the number of sexual partners, sexual practices, and the use of illicit drugs, women with high psychological stress were 2.2 times more likely to develop BV. In addition, stressors that include perceived racial discrimination, poverty, poor housing, and exposure to crime over a life course have also been associated with the increased risk for BV.⁷⁴

Potential mechanisms of enhancement of HIV-1 transmission by BV

Klebanoff et al.⁷⁵ reported that the presence and level of lactobacilli in the vaginal fluid could influence heterosexual transmission of HIV via the bacterium's release of hydrogen peroxide that results in the inactivation of HIV. Therefore, women with no or low levels of hydrogen peroxide-producing vaginal lactobacilli would be at an increased risk for HIV-1 infection.⁷⁶ Interactions between interleukin receptor 2 (IL-1R2) and Toll-like receptor 4 (TLR4) have been shown to be associated with proinflammatory cervical cytokine concentrations. A report by Rkycman et al.⁷⁷ examining single nucleotide polymorphisms (SNPs) in cytokine genes associated with cervical cytokine levels in African American and white women found significant gene-gene interactions between IL-1R2 rs485127 and two SNPs in TLR4 (rs1554973 and rs7856729) and with IL-1 β . Differences were observed in allele frequencies between African Americans and white women. These gene-gene interactions likely impact regulatory mechanisms involving cervical cytokine concentrations that can be influenced under conditions like BV.⁷⁸ This heightened proinflammatory state of the cervical epithelium could result in the increased recruitment of target cell populations and subsequent increased risks for HIV-1 acquisition.

Bacterial vaginosis enhances HIV-1 replication and leads to the increased vaginal shedding of HIV.⁷⁹ The bacterial flora in BV has been shown to induce HIV-1 transcriptional activation and replication by stimulating and recruiting HIV-infected cells.⁸⁰ BV bacterial lysates have been shown to increase the transcriptional activity of HIV⁸¹, and our research suggested that the persistence of BV-associated bacteria may affect the structural integrity of the vaginal epithelium.⁸¹ This in turn would allow HIV to pass between vaginal epithelial cells, which otherwise prevent the virus' ability to gain access to submucosal CD4⁺ T cells, the virus' preferred host cell type. The persistent presence of BV bacteria could heighten the inflammatory state of the vaginal epithelium, resulting in the recruitment of cells permissive for HIV infection.

When squamous vaginal epithelial cells (VK2) are exposed with both macrophage and T-cell tropic strains of HIV, no significant evidence for direct infection is observed even at high multiplicities of infection. This finding is not surprising because VK2

cells do not have receptors or coreceptors for HIV. In our studies, we observed upregulation of TNF- α , IL-6, IL-8, and IL-1 β just 1 hr post-exposure to a pure culture of the BV-associated bacteria *G. vaginalis*⁸¹ (Marrs and Alcendor, 2012, Fig. 1). *G. vaginalis* infection also decreased the expression levels of tight junction proteins ZO-1 and ZO-2, with no significant change in adherens junction protein expression levels (Alcendor et al., personal communications, Fig. 1). After characterization of the expression profile of vaginal epithelial cells exposed to *G. vaginalis*, Marrs et al.⁸¹ observed that the cytoskeletal protein vimentin was upregulated within 18 hrs post-exposure (Fig. 1). Vimentin is a class III cytoskeletal intermediate filament protein that supports cell strength and tissue integrity. Vimentin has also been shown to interact with the ibeA gene product of *E. coli* K1, a known virulence factor associated with the binding and invasion of *E. coli* K1 into human brain microvascular endothe-

rial cells.⁸² Marrs et al.⁸¹ performed transmission electron microscopy and confocal microscopy experiments, which showed the internalization of *G. vaginalis* by vaginal epithelial cells (VECs). These tight junction and cytoskeletal protein modifications could represent a potential mechanism for bacterial-mediated uptake and internalization by vaginal epithelial cells. The ability of *G. vaginalis* to be taken up and internalized by VECs could contribute to the persistence of the bacteria in the vaginal epithelium. A report by Fichorova et al.⁸³ showed the intracellular presence of *Atopobium vaginae* and *Prevotella bivia* in vaginal epithelial cells, which supports the notion that many vaginal bacteria have an intracellular presence in BV.

Internalization would allow these bacteria to survive metronidazole and clindamycin therapy, the current standard of care for BV, thus contributing to the high rate of recurrence of BV in some women.^{84,85} Moreover, the intracellular presence of

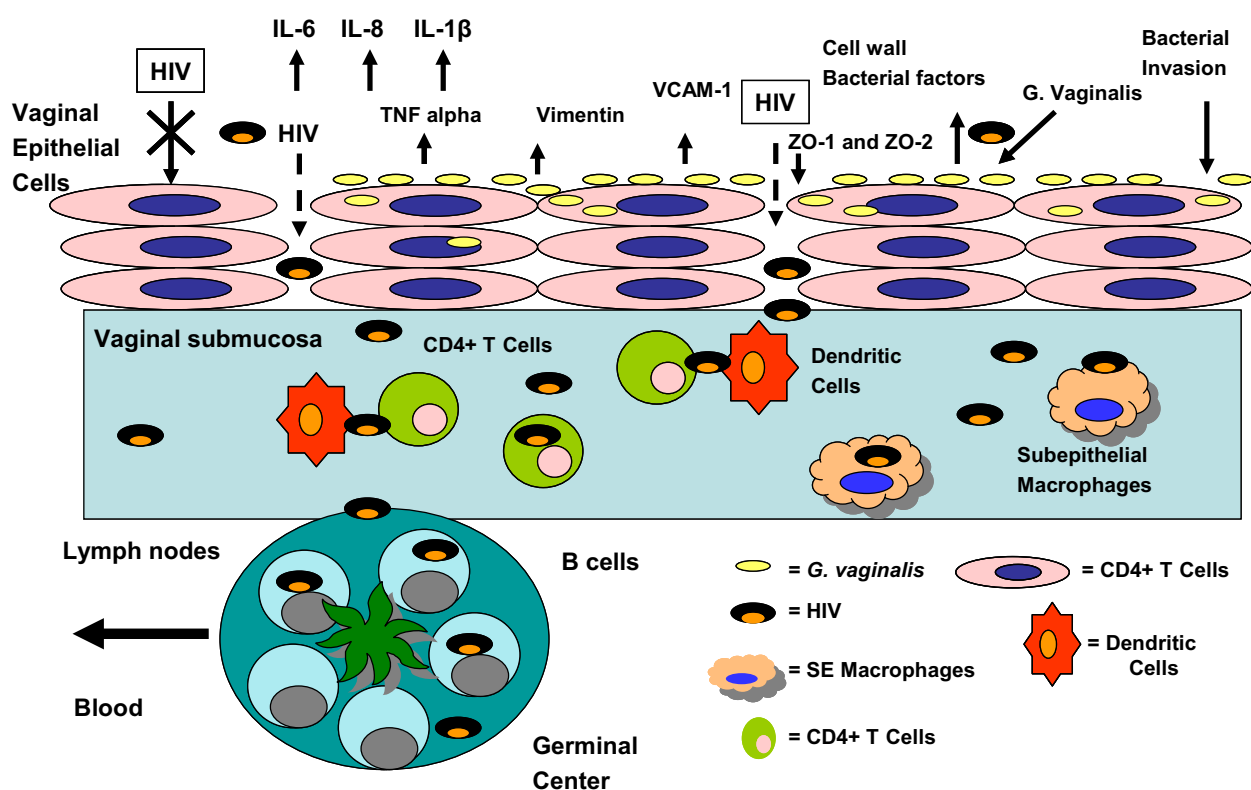


Fig. 1 Exposure of vaginal epithelial cells to *Gardnerella vaginalis* results in upregulation of proinflammatory cytokines, namely IL-6, IL-8, TNF- α , and IL-1 β . Vimentin is upregulated after *G. vaginalis* exposure. Upregulation of vimentin influences uptake and internalization of bacteria. Tight junction proteins (TJ) ZO1 and ZO2 are downregulated in vaginal epithelial cells exposed to *G. vaginalis*. Downregulation of TJ proteins could result in HIV passing in between cells (paracellular transport) in route to the vaginal submucosa where subepithelial T cells and macrophages reside and are highly permissive for HIV infection. Trafficking of infected T cells by resident lymph nodes would facilitate HIV dissemination via the blood.

G. vaginalis, *A. vaginae*, and *P. bivia* in the genital tract could allow these organisms to evade immune responses. A persistent intracellular presence of these bacteria would stimulate cytokine cascades, heightening the inflammatory state of the vaginal epithelium and making it more susceptible to infections like HIV-1, a virus known to benefit from the inflammatory recruitment of CD4+ T cells. In addition, downregulation of tight junction proteins could facilitate the paracellular transport of HIV into the vaginal submucosa (Fig. 1). This would provide a mechanism to help explain increased HIV-1 transmission rates in BV-positive women. Additional studies are needed to provide direct evidence for tight junction disruption of the vaginal epithelium by *G. vaginalis* or other BV-related bacteria. Fig. 1 shows vaginal epithelial cells after exposure to *G. vaginalis* as a prototype BV-associated bacteria, but similar mechanisms may be applicable to other BV bacteria such as *Atopobium vaginae* and *Prevotella bivia*.

G. vaginalis is thought to be necessary for the establishment of BV, but alone is not sufficient to produce BV. Other bacterial species, including *Atopobium vaginae* and *Prevotella bivia*, are found in BV.⁸³ In a study by Mitchell et al.⁸⁶ they detected lower levels of alpha defensins 1–3 in pregnant black and Hispanic women with BV compared to white women with BV. They also determined that increased concentrations of *Atopobium vaginae*, BVAB1, and BVAB2 were associated with lower levels of human beta defensin-3 (HBD3), but not human beta defensin 2 (HBD2) or alpha defensins 1–3.⁸⁶ Human beta defensins have been shown to be protective against HIV-1 infection.^{87–89} Herrera et al.⁹⁰ showed HIV inactivation and a reduction in HIV transepithelial transmission by human beta defensin-3 in adult oral epithelial cells.

Conclusions

Going forward, the epidemiological trends for African American women at risk for BV and HIV are discouraging. Therefore, targeted interventions that will reduce the incidence of vaginal infections, treat dietary deficiencies, enhance social support structures, and reduce poverty and its downstream effects on at-risk communities will likely reduce the incidence of BV and HIV acquisition in African American women. Understanding abnormal vaginal bacteria/host interactions in vaginal epithelium in women of different ethnic backgrounds will allow us to deter-

mine the underlying mechanisms involving vaginal bacterial species, their link to BV pathogenesis, and its contribution to increased risk for HIV-1 sexual transmission. With the well-documented disparity in HIV incidence between black and white women, there may also be a genetic basis for BV occurrence in black women disproportionate to women of other ethnic backgrounds. This in turn would also increase the risk of HIV-1 transmission in African American women. BV is a treatable condition. Increased screening for BV and its subsequent treatment could greatly reduce the burden of HIV and AIDS among black women. Long-term results from these studies may also provide direction for therapeutic BV/HIV strategies resulting in changes in health policy to reduce the burden of HIV-related health disparities.

Acknowledgements

I would like to acknowledge Jane Schwebke for reviewing the manuscript and Jared Elzey of the Meharry Research Concierge Services (supported by NIH grants U54MD007593 and UL1TR000445) for editing and comments. D.J.A. was supported by the Meharry Translational Research Center (NIH U54 MD0007593-07); Tennessee Center for AIDS Research (NIH P30 AI110527).

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