

## Perspective

# The Human Microbiome: Implications for Health and Disease, Including HIV Infection

*Our increased understanding of the human microbiome has brought insight into the role it plays in health and disease, including HIV infection. Studies have shown that the gut microbiome is less diverse in individuals with HIV infection than in noninfected control subjects. Efforts to modify the microbiome to bolster immune reconstitution in people with HIV infection have so far been unsuccessful. The vaginal microbiome affects risk of HIV acquisition, with *Lactobacillus* dominance being protective compared with vaginosis characterized by larger populations of *Gardnerella*. The vaginal microbiome might also affect efficacy of topical tenofovir disoproxil fumarate preexposure prophylaxis. This article summarizes a presentation by Robert T. Schooley, MD, at the IAS–USA continuing education program held in San Francisco in May 2018.*

**Keywords:** microbiome, HIV, gut, vaginal, *Lactobacillus*, *Gardnerella*

The human microbiome is the collection of organisms that lives in and on us. They live on the skin, in the gastrointestinal (GI) tract, and on virtually every other surface of the body. Each human is composed of approximately 37 trillion cells and we carry some 100 trillion microbiologic organisms. Our increasing knowledge of the microbiome has been made possible through use of metagenomics and the decreasing costs of genetic sequencing, obviating the need to grow microorganisms in culture for identification purposes.

The best handle we have on understanding the microbiome is 16s ribosomal RNA (rRNA). Every microbe has this region of RNA, including portions that are conserved across all of our microbiota, because it is the common feature that allows organisms to translate messenger RNA into protein. Amplification of variable regions permits identification of organisms by families and phyla, for example, by evaluating relationships among sequences.

A number of concepts have evolved to characterize microbial communities. One is the operational taxonomic unit (OTU), a group of organisms that share

greater than 97% DNA sequence commonality in the rRNA gene. This grouping may or may not reflect what has been traditionally considered a species of microorganism. Diversity in microbial communities is described by the metrics of richness (number of OTUs), evenness (relative abundance of different OTUs), dominance (emergence of a single OTU), and diversity index (calculated index of complexity based on richness and evenness).<sup>1</sup> Antibiotic treatment narrows the

**Table.** Terms Describing Diversity in Microbial Communities.<sup>1</sup>

Term	Definition
Richness	Number of OTUs
Evenness	Relative abundance of OTUs
Dominance	Emergence of single OTU
Diversity Index	Calculated index of complexity based on richness and evenness

Abbreviation: OTU is operational taxonomic units.

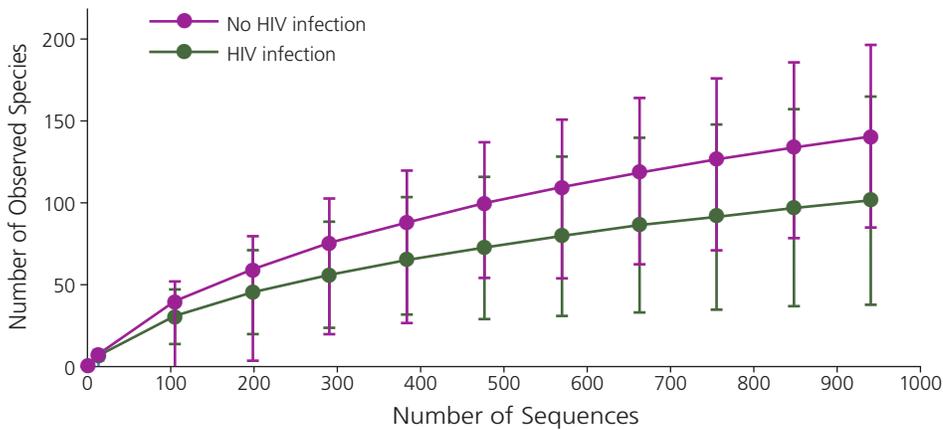
rich microbiome (rich diversity of OTUs) in the human gut. Conditions such as obesity and inflammatory bowel disease are characterized by low diversity, whereas bacterial vaginosis is characterized by an unhealthy high diversity in which lactobacilli that are dominant in the healthy vagina are replaced by a wide variety of other organisms.

The National Institutes of Health Human Microbiome Project has produced a number of insights regarding the microbiome over the past 15 years, beginning with the recognition that each individual is not a single ecosystem but numerous ecosystems with differences among body sites. Further, whereas OTUs in the GI tract are very different from OTUs in the oral cavity in a given individual, OTUs from these sites resemble each other across individuals. In addition, in a given individual, the microbial diversity at particular sites changes over time, although the difference in this regard is not as great as the difference between individuals at a given site.

With regard to the gut microbiome in particular, we have identified a number of key characteristics that affect health and disease. (1) The microbiome is established in early life and differs between infants born by cesarean delivery and by vaginal birth. (2) In murine models, transfer of an “obese” microbiome from obese adult animals to a sterile infant mouse affects the growth characteristics of the mouse for life. (3) The microbiome is drastically altered acutely by antibiotic administration. (4) In farm animals (and mice), antibiotic exposure early in life promotes microbiota associated with high caloric efficiency. (5) There is some evidence that early exposure to antibiotics affects humans the same way. (6) After cessation of antibiotics, the antibiotic “footprint” remains—ie, the microbiome reverts toward but does not reach pre-antibiotic richness and diversity for a prolonged period.

The 4 major phyla in the gut microbiome are: firmicutes—Gram-positive organisms (eg, *Staphylococcus*, *Micrococcus*, *Streptococcus*, and *Lactobacillus*), including sporulating Gram-positive rods; Bacteroidetes—3 large classes of Gram-negative, non-spore forming, anaerobic or aerobic, rod-shaped bacteria (eg, *Bacterioides*); Actinobacteria

Dr Schooley is Professor and Vice Chair of the Department of Medicine at University of California San Diego. He is a member of the Board of Directors of the IAS–USA.



**Figure 1.** Reduced richness of the gut microbiome with HIV infection compared with no HIV infection. Adapted from Mutlu et al.<sup>2</sup>

—primarily Gram-positive bacteria (including those that are acid fast; eg, *Mycobacteria* and *Gardnerella*); and proteobacteria—primarily Gram-negative rods (eg, *Escherichia coli*, *Salmonella*). The firmicutes, in particular, are highly adept at metabolizing food to help us better absorb calories.

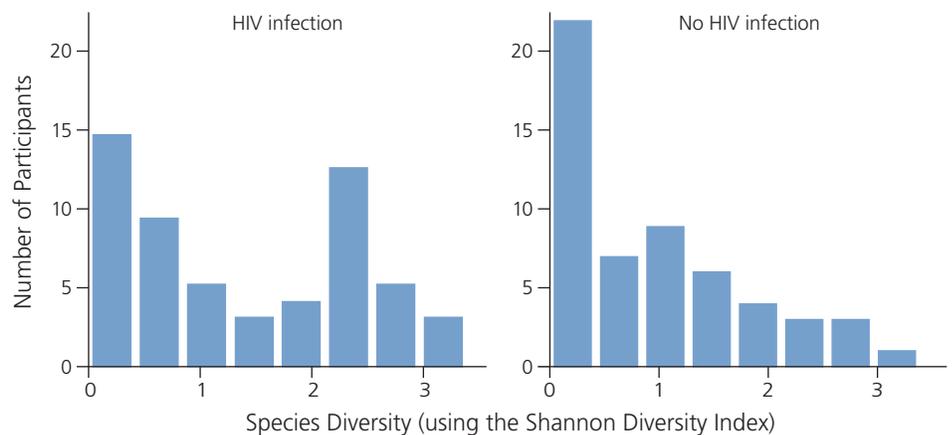
### Gut Microbiome in HIV Infection

An initial study investigating the gut microbiome in HIV infection performed by Mutlu and colleagues demonstrated differences between individuals with and without HIV infection. The study involved 56 samples from individuals with HIV infection (mean CD4+ cell count of 425/ $\mu$ L; plasma HIV-1 RNA level <75 copies/mL in 17 of 21 participants) and 65 samples from 22 age- and sex-matched noninfected control participants undergoing elective colonoscopy (primarily for cancer screening).<sup>2</sup> The microbiome was found to be markedly less diverse in individuals with HIV infection (Figure 1). In addition, the microbiomes of individuals with HIV infection and noninfected control participants contained different bacterial species, with noninfected control participants harboring significantly more *Bacteroides* ( $P = .0002$ ) and Clostridiales-unclassified ( $P = .002$ ), and participants with HIV infection harboring more Enterobacteriaceae-unclassified ( $P = .0002$ ) and *Campylobacter* ( $P = .0034$ ), as well as more fusobacteria and *Prevotella*.

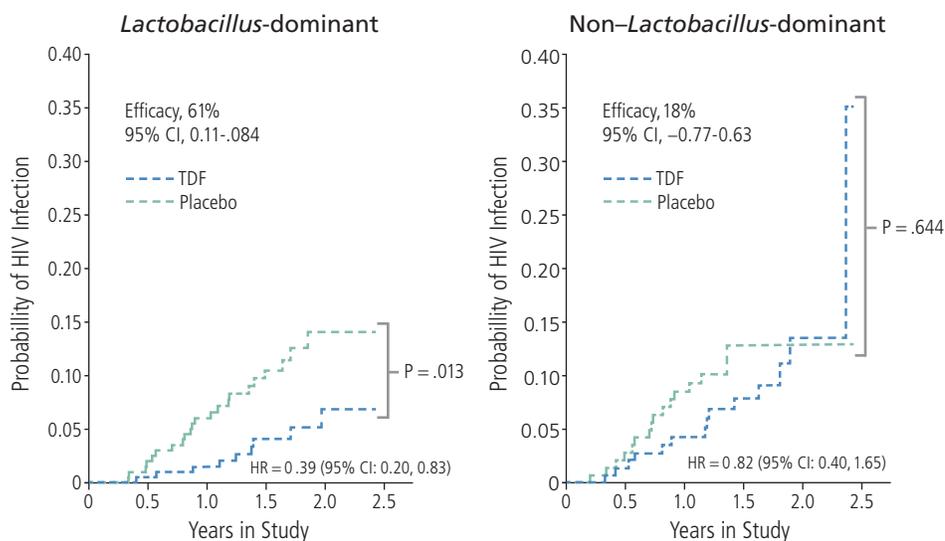
Some of these differences may be due to changes in the innate and adaptive immune response at the level of the gut, one of the first organs attacked during acute HIV infection. Some differences may reflect use of antibiotics in individuals with HIV infection. Studies that have attempted to evaluate the change in microbiome over time as HIV infection progresses suggest that some of the organisms found more frequently in HIV-infected microbiomes are more associated with inflammation and become enriched as immune response declines.

The microbiome is important from the standpoint of maintenance of the gut barrier. A considerable amount of work has been done over the past decade to ascertain whether translocation of gut bacterial components across the GI tract have an impact on

inflammation. The recently reported Promaltia study, performed by Serrano-Villar and colleagues, evaluated whether the gut microbiome could be altered in HIV infection to improve immune reconstitution.<sup>3</sup> In the placebo-controlled study, patients with advanced HIV infection were randomly assigned to receive treatment with a placebo (skim milk) or an immunonutrition mixture of prebiotics, probiotics, oligonutrients, docosahexaenoic acid, eicosapentaenoic acid, gamma linolenic acid, and amino acids for 48 weeks, each in combination with initial antiretroviral therapy. The mixture represented a collation of substances used in prior studies aimed at producing changes in the microbiome. At 48 weeks, there were no significant differences in microbiome diversity between the treatment and placebo groups. Some enrichment of unclassified bacteria from the Lachnospiraceae and Victivallaceae families and depletion of *Blautia* species were observed in the treatment group compared with the placebo group. No differences in inflammatory markers were found between treatment and placebo groups, with no significant differences in changes in CD4+ or CD8+ cell counts being observed between groups over time. Thus, the test of whether or not HIV immune reconstitution can be achieved by changing the microbiome has not really been documented, since the treatment in this study did not change the microbiome. There is more work to be done in this area.



**Figure 2.** Increased risk of HIV infection with greater vaginal bacterial diversity. Adapted from McClelland et al.<sup>5</sup>



**Figure 3.** Effect of the vaginal microbiome on effectiveness of topical tenofovir disoproxil fumarate (TDF) preexposure prophylaxis in *Lactobacillus*-dominant and non-*Lactobacillus*-dominant cell cultures. HR is hazard ratio; CI, confidence interval. Adapted from Klatt et al.<sup>6</sup>

### Vaginal Microbiome in HIV

The vaginal microbiome may be of considerable importance in HIV transmission. The vaginal microbiome tends to be stable over time within individuals. In the healthy state, there is minimal diversity, with dominance of *Lactobacillus*. However, in bacterial vaginosis, there is increased diversity, with dominance of *Gardnerella*. *Lactobacillus* populations are decreased in the context of sexual intercourse (assessed by the presence of prostate specific antigen [PSA] in the sample) and are also decreased in amenorrheic women, but inflammatory mediators (interleukin [IL]-8, IL-12, and MIP-1b) are increased.<sup>4</sup> The presence of inflammatory mediators is also increased in the presence of PSA.

Studies have now shown that risk of HIV acquisition is increased with bacterial vaginosis and increased vaginal bacterial diversity. In a case-control study among African women who had undergone microbiomic studies and were at similar risk of HIV infection, McClelland and colleagues found that participants in the control group who did not acquire HIV infection had greater dominance of *Lactobacillus* in their microbiome than in those who acquired infection.<sup>5</sup> Women who acquired HIV infection had greater

vaginal bacterial diversity in the context of decrease in *Lactobacillus* dominance (Figure 2). These findings provide some evidence that the microbiomic signature characterized by *Lactobacillus* dominance and less diversity is protective against HIV acquisition. The study also found that presence of particular bacterial species was associated with increased risk of acquiring HIV infection. It is of considerable interest whether further studies confirm that particular species are associated with greater transmission risk and whether this might lead to protective measures.

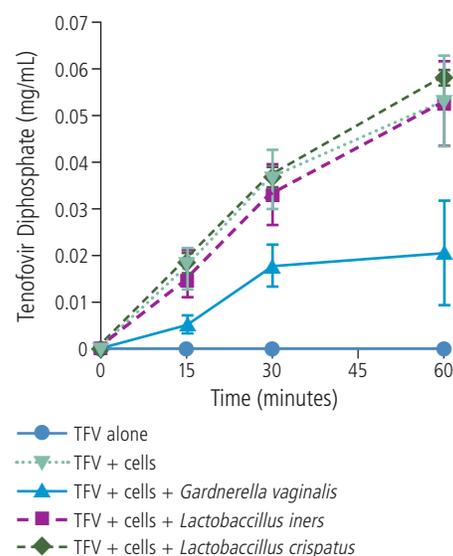
### Vaginal Microbiome and Topical Preexposure Prophylaxis

The efficacy of topical tenofovir disoproxil fumarate (TDF) preexposure prophylaxis in preventing HIV infection has been lower than anticipated in clinical trials, with poor adherence largely being considered the culprit. However, a recent study by Klatt and colleagues in the population of the CAPRISA (Centre for the AIDS Program of Research in South Africa) trial of topical TDF indicates that the vaginal microbiome affects efficacy.<sup>6</sup> In particular, preventive efficacy was 61% ( $P = .013$ ) versus placebo among women with *Lactobacillus*-dominant microbiomes, compared with 18% ( $P = .644$ )

among those without *Lactobacillus* dominance and greater frequency of *Gardnerella* (Figure 3). These investigators found that when TDF was added to cell culture in which *Gardnerella* was grown, the organism degraded tenofovir, metabolizing it to adenine, whereas no such effect was observed when TDF was put into culture containing *Lactobacillus*. These findings indicate that *Gardnerella* metabolizes tenofovir before it can get into the cells and protect the cells from becoming infected with HIV; as shown in Figure 4, the presence of *Gardnerella* prevents cellular uptake of tenofovir and intracellular conversion to the active diphosphate form. Klatt and colleagues have now also reported that dapivirine is also metabolized and degraded in women with the more diverse, non-*Lactobacillus*-dominant microbiomes.<sup>7</sup> There is no evidence that bacterial vaginosis affects the efficacy of oral preexposure prophylaxis.<sup>8</sup>

### Conclusion

Humans live in concert with an immense array of microbial organisms from birth to death. The microbiome differs from body site to body site



**Figure 4.** Depletion of tenofovir (TFV) by *Gardnerella vaginalis* in culture prevents accumulation of intracellular tenofovir diphosphate. No such effect is observed with *Lactobacillus* species. Adapted from Klatt et al.<sup>6</sup>

and from person to person, but is reasonably stable over time unless it is disturbed by antibiotics. Other environmental factors including food and those with whom we associate also have direct effects on the microbiome. The microbiome influences gut metabolism, systemic immunity, and susceptibility to disease. It changes in health and disease, including in HIV infection. We are only now beginning to distinguish the carts from the horses.

Can we alter the microbiome? Yes, but currently only with the elegance of a meat cleaver, as occurs with antibiotic treatment. Antibiotics are the most dramatic way we affect the human microbiome, usually for the worse, as in the case of *Clostridium difficile* overgrowth and *Salmonella* infection. Other approaches to beneficially change the microbiome, including probiotics, prebiotics, fecal transplantation, and bacteriophage therapy (eg, for *C difficile*), are being investigated. 

*Presented by Dr Schooley in May 2018. First draft prepared from transcripts by Matthew Stenger. Reviewed and edited by Dr Schooley in July 2018.*

*Financial affiliations in the past 12 months: Dr Schooley has served as a consultant to CytoDyn, Pfizer, and Vir Biotechnology. He has received grant support from Gilead Sciences, Inc, and holds stock or stock options in Antiva Biosciences and CytoDyn.*

## References

1. Cui L, Morris A, Huang L, et al. The microbiome and the lung. *Ann Am Thorac Soc*. 2014;11 Suppl 4:S227-S232.
2. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. *PLoS Pathog*. 2014;10(2):e1003829.
3. Serrano-Villar S, de LM, Vazquez-Castellanos J, et al. Effects of immunonutrition in advanced HIV disease: a randomized placebo controlled clinical trial (Promaltia Study). *Clin Infect Dis*. 2018;[Epub ahead of print].
4. Jespers V, Kyongo J, Joseph S, et al. A longitudinal analysis of the vaginal microbiota and vaginal immune mediators in

women from sub-Saharan Africa. *Sci Rep*. 2017;7(1):11974.

5. McClelland RS, Lingappa JR, Srinivasan S, et al. Evaluation of the association between the concentrations of key vaginal bacteria and the increased risk of HIV acquisition in African women from five cohorts: a nested case-control study. *Lancet Infect Dis*. 2018;18(5):554-564.
6. Klatt NR, Cheu R, Birse K, et al. Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. *Science*. 2017;356(6341):938-945.
7. Klatt N. The vaginal microbiome and acquisition of HIV infection [CROI Abstract 64]. In Special Issue: Abstracts From the 2018 Conference on Retroviruses and Opportunistic Infections. *Top Antivir Med*. 2018;26(suppl 1):1-483.
8. Heffron R, McClelland RS, Balkus JE, et al. Efficacy of oral pre-exposure prophylaxis (PrEP) for HIV among women with abnormal vaginal microbiota: a post-hoc analysis of the randomised, placebo-controlled Partners PrEP Study. *Lancet HIV*. 2017;4(10):e449-e456.

*Top Antivir Med*. 2018;26(3):75-78.

©2018, IAS–USA. All rights reserved