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Microbiome

COMMENT

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The role of pharmacomicrobiomics in HIV prevention, treatment, and women's health

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Abstract

In the absence of an effective vaccine or curative treatment for HIV, the global HIV/AIDS epidemic continues despite significant advances in treatment and prevention. Antiretroviral therapy (ART) drugs have transformed HIV from a terminal illness to a manageable chronic condition. Likewise, pre-exposure prophylaxis treatment (PrEP) has dramatically reduced transmission in some of the highest risk populations. However, quality of life and life expectancy in people living with HIV (PWH) still lag significantly behind the general population. The mechanisms that reduce the efficacy of PrEP and ART are multifaceted, but one factor that warrants additional attention is the impact of the microbiome on ART and PrEP efficacy, as well as pharmacokinetics more broadly. In this review, we assess the current state of research on the HIV-associated microbiome, how this impacts treatment efficacy, and how microbiome states can alter HIV susceptibility. We also explore how the mechanisms we propose could extend to the efficacy of other drugs and identify promising areas of research that remain understudied.

Keywords HIV, Microbiome, Pharmacomicrobiomics, ART, PrEP

Background: microbiome and drug metabolism

The human microbiome influences drug availability through direct and indirect mechanisms including absorption, excretion, toxicity, and metabolism. These processes are collectively known as pharmacomicrobiomics [1]. Pharmacomicrobiomics has gained considerable interest due to the therapeutic potential of modulating the microbiome in a personalized medicine approach to interventions such as probiotics, prebiotics, and microbiome transplant.

Bioavailability of a drug refers to the degree and rate at which it becomes accessible in systemic circulation. Bioavailability is dependent on the route of administration, with IV-delivered drugs reaching 100% bioavailability [2]. Oral administration is the most common drug administration route due to its convenience for patients [3], but physiochemical processes can lead to significant differences in the bioavailability of orally administered drugs, which can result in subtherapeutic systemic concentrations and poor efficacy. Several factors affect the bioavailability of drugs including bloodstream absorption from the gut lumen and the rate of first-pass metabolism occurring before the drug reaches systemic circulation including metabolism by the liver, gut epithelium, and gut microbiome [4]. Drug absorption and first-pass metabolism influence a wide range of both drug-specific and patient-specific factors. These factors can ultimately change ARV concentrations. There has been growing interest in determining whether subtherapeutic ARV concentrations in specific tissues is a mechanism of residual HIV replication and chronic immune activation, and therefore, a more complete understanding of the factors affecting ARV drug absorption and first-pass metabolism is critical to ensuring the efficacy of ART. ART effectively stops replication of HIV in peripheral blood; however, viral persistence has been shown in anatomic sites such as the lymph nodes, gastrointestinal

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tract, female reproductive tract (FRT), and central nervous system (CNS) of PWH on long-term ART. One proposed mechanism for this observation is subtherapeutic concentrations of ARVs that lead to lingering HIV replication. Absorption and penetrance of ARVs into these tissues are highly complex and dependent on the physiochemical properties of each ARV, the physiological properties of each tissue, and the activity of influx/efflux drug transporters [5,6]. Lipophilicity, protein binding, molecular size, and ionization properties are all important factors which can have dramatic consequences on ARV penetration and absorption [6]. Differences in blood perfusion in these tissues may also contribute to differences in penetrance. This complexity is supported by the wide range of reported concentrations for individual ARVs across tissues. Poor penetration of ARVs into sanctuary sites like the brain and male genital tract have been linked to the emergence of drug-resistant viruses [7,8]. Likewise, metabolism of ARV drugs by the human microbiome may contribute to subtherapeutic concentration in specific sites. The human microbiome harbors trillions of microorganisms including bacteria, viruses, fungi, and protozoa with an estimated 100–150 times more functional genes than the human genome, including a vast repertoire of metabolic pathways and degradation genes that collectively are able to metabolize nearly any substrate with oxidative potential, a characteristic sometimes called "microbial infallibility." Here, we describe potential mechanisms that the gut and vaginal microbiomes may influence ARV dynamics in people with HIV.

Overview of the gut and vaginal microbiomes in HIV

The pathogenesis of HIV and as it relates to the microbiome

Activated CD4 + T cells are the primary infection target of HIV and are highly concentrated in the gastrointestinal tract and female reproductive tract (FRT) [9–11]. HIV infection depletes CD4 + T cells, including critical Th17 cells which play important immunoregulatory roles in maintaining mucosal homeostasis and are preferentially infected by HIV [12,13]. Inflammation from viral-mediated cell death and loss of immunoregulatory cells combine to cause epithelial damage and increased translocation of microbes and microbial products into systemic circulation, further contributing to inflammation and immune activation [14]. Importantly, this damage is not fully restored with ART, and chronic inflammation and immune activation, driven in part by microbial translocation, lead to the development of inflammatory-mediated serious non-AIDS events including cardiovascular disease and neurocognitive disorders [14–16]. For this reason, understanding the connection between microbial translocation, dysbiosis, and immune activation has been a major interest in HIV research, leading to several clinical trials exploring the use of probiotics to reduce immune activation in PWH with some success [17–19]. Most research so far has been focused on the downstream effects of HIV infection on the gut microbiome, with few studies on other mucosal sites such as the FRT and oral cavity.

In the following sections, we will briefly summarize the current understanding of the microbiome in the gastrointestinal tract and FRT in the context of HIV infection.

The state of the field for the gut microbiome: it is more complicated than originally thought, confounded by many factors

Studies investigating the gut microbiome of PWH frequently yield inconsistent results, likely due to variations in study populations, varying adjustments for confounding factors, and differences in sequencing and bioinformatics techniques [20]. Disentangling the direct effects of HIV infection on the microbiome versus associated effects such as ART regimens and sexual practices has been a challenge. Status as a man who has sex with men (MSM) has only recently been recognized as a significant confounder that may have skewed prior microbiome studies [20,21], and different ART regiments have been shown to have independent effects on the gut microbiome, which agrees with in vitro studies showing antibiotic effects specific to individual ARVs and commensal gut bacteria [22,23]. In two recent meta-analyses, *Zhou* et al. *(2020)* and *Tuddenham* et al. *(2020)* both showed that reduced alpha diversity (intraindividual diversity based on the presence or absence of bacteria) was associated with HIV + status when restricted to women and non-MSM, but not in MSM [24,25]. Furthermore, there was a significant difference in beta diversity (inter-individual diversity based on community similarity) between MSM and non-MSM individuals, with microbiome samples clustering more closely in PCoA by MSM status than HIV status [25].

Similar confounders of the gut microbiome in PWH include sexual practices that have been associated with individual bacterial taxa. For example, previous studies often reported a *Prevotella*-rich and *Bacteroides*-poor gut microbiome associated with PWH; however, it has now been demonstrated that *Prevotella* richness is more associated with MSM status than with HIV [26] and may also be associated with NRTI-based ART regimens [27]. Further, confounders include the association between MSM status and lifestyle differences, with apparent interactions between heavy alcohol use, MSM sexual practices, and HIV progression [28]. The factors driving a *Prevotella*-rich microbiome in MSM remain inconclusive, though

receptive anal intercourse (RSI), increased red meat consumption (specifically in MSM), PrEP use, IV drug use, and a higher incidence of STIs have all been proposed as potential influences but remain challenging to interpret [20]. For example, while red meat consumption in MSM communities is correlated with increased *Prevotella* relative abundance, other studies show that fiber-rich diets are also correlated with increased *Prevotella* abundance in some cases [26,29]. Importantly, although sexual behaviors are a major influence, they alone do not explain all the differences in the gut microbiome of PWH compared to healthy controls, and previous studies have unequivocally demonstrated that HIV alone induces microbial dysbiosis [30]. The effect is further complicated by recent findings that the interactions between HIV, ART, and microbiome are geographically specific [31]. *Rocafort* et al. *(2024)* found that the community differences between HIV uninfected and PWH were specific to individual geographic populations and to treatment status. In their cohort, all PWH from the USA and PWH on ART from Botswana saw decreases in *Faecalibacterium prausnitzii* compared to HIV-uninfected participants. However, *F. prausnitzii* was increased in PWH from Uganda, while *Prevotella* was decreased [31]. This result is quite surprising since *F. prausnitzii* is one of the bacteria most consistently associated with reduced inflammation [32] while *Prevotella* is consistently associated with increased inflammation [33].

Clearly, despite considerable effort spent on profiling the HIV-associated gut microbiota, a consensus microbiome signature differentiating PWH from HIV-uninfected individuals remains elusive [24]. The composition of the gut microbiome is highly dynamic and influenced by myriad factors, making it difficult to find a unique signature associated with HIV infection alone. However, it is clear that PWH consistently have dysbiosis-associated immune activation, elevated inflammatory cytokine production, and HIV reservoirs, regardless of how other factors (e.g., MSM, ART, HIV, or diet) may contribute to the dysbiosis [30,34]. For example, the relative abundance of *Subdoligranulum* and *Coprococcus comes* was shown to be elevated in PWH with low CD4 counts (< 350 cells/mm^3) and also positively correlated with activated CD8 + T cells [35]. Furthermore, *Li* et al. *(2019)* showed that fecal microbiome transplant with stool from MSM into gnotobiotic mice resulted in increased CD4 + and CD8 + T-cell activation compared to stool from non-MSM [36]. In vitro experiments have strengthened these correlations by demonstrating that bacterial strains and communities from PWH can induce various degrees of cytokine production, T-cell activation, and neutrophil survival [21,37]. The capacity for bacteria to induce these changes appears to be strain specific as shown by *Zhang* et al. *(2023)* who demonstrated that *Prevotella copri* strains in healthy controls and PWH were genetically distinct and had different associations with cytokine production, possibly due to differences in their peptide profiles [30].

The state of the field for the vaginal microbiome: lack of studies on downstream effects of HIV and more focused on susceptibility and BV

While there has been a great deal of research on the gut microbiome after HIV infection, there has been remarkably little on the vaginal microbiome. Most HIV-vaginal microbiome studies have focused on susceptibility to HIV infection [38–40], demonstrating a strong link between the abundance of bacterial vaginosis-associated bacteria (BVAB) and increased risk of HIV-1 infection [41–48]. Since bacterial vaginosis (BV) is a common condition and is usually defined by changes in vaginal microbial community profile, it is challenging to disentangle the effect of HIV on the vaginal microbiome from BV itself. Is an increase in vaginal diversity caused by HIV, or is it co-occurring or preexisting BV? Indeed, the distinction is made more complex by the established science demonstrating that BV is a risk factor for HIV infection [46,49,50]. However, there is some evidence that HIV infection either triggers BV or causes similar symptoms. This relationship is best exemplified in an analysis that assessed a cohort of pregnant participants with and without HIV [51]. They showed that HIV was associated with inflammation, spontaneous preterm birth, and increased metagenomic diversity in the absence of other BV symptoms, an important criterion since each of these effects is also independently associated with BV in HIV-uninfected women. The inherently semantic distinctions between BV and other conditions that cause concurrent microbiome shifts and BV-like symptoms make it very challenging to pinpoint HIV-associated vaginal microbiome changes. However, the effect of BV on HIV susceptibility has received considerable attention and consistently demonstrates increased susceptibility in women with BV.

Gut and vaginal microbiomes and susceptibility to HIV-1 infection

HIV susceptibility is influenced by the composition and function of gut and vaginal microbiota and microbiota-produced metabolites. There are clear differences between common gut and vaginal taxa and differences in the gut and vaginal community dynamics. The role of bacterial diversity is one of the most important differences to note. The optimal vaginal community is dominated by just a few species of *Lactobacillus*, and increasing microbial diversity is associated with increased inflammation, *Gardnerella* and *Prevotella* being notable examples of

nonoptimal bacteria. In the gut, increasing diversity is generally associated with decreased inflammation, and inflammatory conditions including arthritis and inflammatory bowel disease are correlated with lower diversity and increased dominance of a small number of taxa [33,38,52]. There is also evidence of cross talk between gut and vaginal microbiota with correlations between increased gut inflammation and higher vaginal microbiome diversity and inflammation [53]. Critically, studies have shown that individuals with a higher abundance of pro-inflammatory gut or vaginal bacteria, including microbes from the *Prevotella*, *Gardnerella*, *Escherichia*, *Closterium*, *Enterococcus*, and *Proteus* genera, have increased susceptibility to HIV infection in both men and women [36,54–56].

However, the associations between individual taxa and HIV risk are inconsistent, ostensibly because of the considerable intra-genera diversity of commensal microbes. Many existing microbiome studies use 16S rRNA metaamplicon sequencing methods that infer taxonomic identity from a ~ 300-bp region of the 16S rRNA gene sequence. 16S rRNA sequencing can only reliably identify taxa down to the genus level and therefore miss important differences between bacterial species and strains. Bacterial strain variation combined with interpersonal differences in microbiome dynamics results in conflicting findings, with the same genus or species being associated with both positive and negative disease outcomes. In addition, behavioral analysis is markedly missing in most microbiome studies and may further obfuscate factors leading to microbial dysbiosis.

Despite those caveats, it seems clear that inflammatory bacteria disrupt the intestinal mucosal barrier and promote systemic inflammation directly and by mechanisms such as over-activating neutrophils that exacerbate inflammation and any existing disruptions to the mucosal barrier, potentially facilitating viral entry and replication [21]. The role of diet and lifestyle in promoting specific taxa is still difficult to disentangle, but the correlation between inflammation and bacteria like *Prevotella* is quite strong [33]. This inflammatory state can also lead to the recruitment of CD4 + T cells, the primary targets of HIV, into the gut mucosa, increasing the pool of susceptible cells [57]. Additionally, the presence or absence of certain metabolites, such as short-chain fatty acids (SCFAs), can modulate immune responses. Lower levels of beneficial SCFAs, like butyrate, typically produced by commensal bacteria such as *Faecalibacterium* [58,59], have been associated with increased inflammation and therefore HIV susceptibility and morbidity [60,61]. This altered gut microbiota profile and metabolite production can compromise the immune system's ability to mount an effective response against HIV, thereby enhancing vulnerability to the virus. Similarly, the altered gut environment can impact CD8 + T-cell function [62–65], potentially diminishing their ability to control HIV replication (Fig. 1).

Vaginal specific HIV-microbiome dynamics

Vaginal microbiome and HIV susceptibility research focuses almost exclusively on PrEP efficacy. Early studies assessing the efficacy of PrEP for women produced widely varying results. Initially, inconsistent efficacy was attributed to differing levels of PrEP adherence [66]. However, an alternative or complementary explanation was that PrEP drug metabolism by vaginal bacteria, especially nonoptimal bacteria, caused decreased efficacy [47,48]. Follow-up studies showed that controlling for vaginal microbiome eliminated most of the inconsistency between PrEP studies. *Velloza* and *Heffron* provide a thorough overview of the developments that lead to the BV-PrEP metabolism connection [66]. The correlation between BV, reduced PrEP efficacy, and metabolism of PrEP drugs by anaerobic vaginal microbes was formalized and confirmed in seminal papers by the Klatt research group highlighting the exact nature of PrEP efficacy [67], the effect of BV on HIV risk [48], and the first direct evidence of microbial metabolism of PrEP drugs [47,48]. However, the role of the vaginal microbiome in metabolizing PrEP and thereby reducing its efficacy is complicated by findings indicating that the efficacy of orally administered PrEP is not significantly altered by the vaginal microbiome composition since it may circumvent the vaginal microbiome [68].

Beyond the effect of the vaginal microbiome and BV on PrEP efficacy, the vaginal microbiome plays a critical role in modulating HIV susceptibility, with the dominance of *Lactobacillus* species being particularly protective. An optimal vaginal microbiome, characterized by high levels of *Lactobacillus* relative to gram-negative anaerobes, helps maintain a low vaginal pH and produces antimicrobial compounds including lactic acid and hydrogen peroxide, which may also inhibit HIV replication and reduce the risk of infection [69–71]. Conversely, a depletion of *Lactobacillus* and an overgrowth of other nonoptimal bacterial taxa, such as *Gardnerella* or *Fannyhessea* (formerly *Atopobium*), lead to a higher pH and an environment more conducive to HIV infection. These nonoptimal bacterial communities can increase inflammation and compromise the integrity of the mucosal barrier, thereby enhancing the likelihood of viral transmission [72,73]. This inflammatory state can also increase the number CD4 + T cells in the vaginal mucosa, increasing the pool of target cells for HIV. Clearly, the vaginal microbiome's influence on local immune responses plays a significant role; a balanced microbiome promotes a more

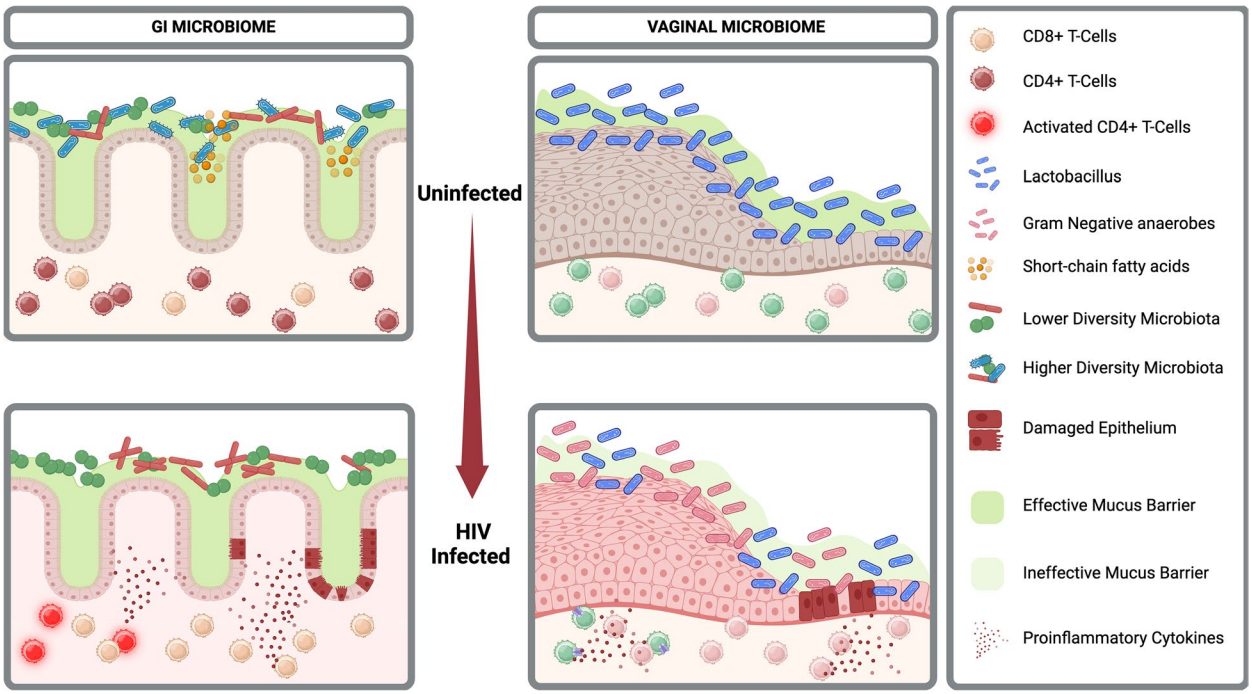

Fig. 1 A simplified schematic of HIV-induced microbiome changes in the GI and FRT. In the GI microbiome (left), the uninfected state is characterized by higher microbial diversity including short-chain fatty acid producers, reduced immune activation, and higher barrier integrity. On the right (FRT), the uninfected vaginal microbiome is characterized by lower microbial diversity, reduced immune activation, and higher barrier integrity including an effective mucus structure. Created in Biorender

robust mucosal immune defense, including the effective function of CD8 + T cells, whereas dysbiosis can lead to immune activation and increased target cells for HIV, further exacerbating susceptibility [34,74]. The dominant effect of BV-related inflammation on PrEP efficacy can be seen in clinical trials as well. Research shows that that even with high regimen adherence to vaginal tenofovir gel, women with elevated levels of key cytokines including IL-6, IL-10, and TNF-α had no protection against HIV infection compared to 75% efficacy in women without cervicovaginal inflammation [75].

Mechanisms of gut microbiome influences on ARV bioavailability and efficacy

Gut bacteria have direct and indirect mechanisms for modulating the bioavailability and absorption of drugs. Microbial metabolism is a direct mechanism that can reduce the luminal concentrations of drugs before they are able to be absorbed and, in some instances, can significantly impact the efficacy of the drug. Studies investigating the ability of the gut microbiome to metabolize drugs often culture bacterial strains or whole stool communities in liquid culture media containing the drug of interest and measure its concentration over time using mass spectrometry techniques. A limited number of these studies have screened microbial activity against HIV antivirals or drug boosters. *Javdan* et al. *(2020)* performed a screen for microbiome-derived metabolism of 575 drugs, including 16 HIV antivirals, against a polymicrobial community of gut bacteria isolated from a human subject's stool sample. Out of the 16 ARVs tested, which included several of the current antivirals in use such as emtricitabine and raltegravir, only zidovudine was determined to be significantly metabolized. Similarly, *Zimmermann* et al. *(2019)* screened 76 human gut bacterial strains for metabolic activity against 271 drugs, including 3 HIV antivirals/boosters (abacavir sulfate, nevirapine, and ritonavir) and found that nevirapine was significantly depleted by 7 different bacterial strains, and ritonavir was depleted by 19 different strains. Gut bacteria can also impact luminal drug concentrations by direct drug sequestration internally without metabolically transforming them, known as bioaccumulation. *Klünemann* et al. *(2021)* investigated the depletion of 15 drugs, including one HIV antiviral (tenofovir), by 25 strains of gut bacteria in vitro and found that over half of the bacteria-drug depletion interactions were due to bioaccumulation [76].

These studies collectively provide evidence that gut bacteria can metabolize and deplete specific HIV antiviral drugs in vitro; however, whether this effect is clinically relevant remains to be determined. Interindividual variability in plasma trough concentrations of ARVs has been

previously described and varies significantly between different drugs [77–82], leading to both subtherapeutic and toxic concentrations, which has supported the use of therapeutic drug monitoring in PWH [81]. Factors including drug adherence, food intake, drug interactions, and genetic variations in drug-metabolizing enzymes (DMEs) are often attributed to inter- and intraindividual variation of ARV concentrations, though the impact of the gut microbiome is seldom considered. Studies correlating in vivo gut bacterial relative abundances with ARV concentrations have so far found no association between the two. *Dubé* et al. *(2018)* found that long-term PrEP use in healthy MSM resulted in an increased abundance of Erysipelotrichaceae and decreased *Streptococcus*; however, the relative abundance of these bacteria was not correlated with tenofovir diphosphate blood concentrations [83]. In addition, *Haaland* et al. *(2018)* found no correlation between PrEP concentrations in rectal secretions, rectal biopsies, and PBMCs with bacterial abundances or microbial composition [84]. Notably, both these studies utilized rectal swabs for measuring bacterial abundances which may not be representative of the microbiome in the small intestine which is the major site of oral drug absorption [85]. It is possible that for rapidly absorbed drugs, microbial metabolism in the gut may not affect systemic drug concentraitons [86]. However, given the importance of maintaining optimal ARV concentrations in PWH, it is vital to continue this research to better understand the influence of the gut microbiome.

Modulation of drug transport proteins

Another mechanism gut bacteria could impact ARV concentrations is through the modulation of drug transport proteins such as ATP-binding cassette (ABC) transporters, P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) which are active on many ARVs [87]. These efflux proteins can pump ARVs out of cells targeted by HIV, or out of sites where target cells are located, reducing their intracellular concentrations and effectiveness. TAF, for example, is a substrate for P-gp and BCRP, and intracellular concentrations of tenofovir diphosphate (TFG-DP) have been shown to decrease when coadministered with rifampicin, attributed to the induction of P-gp [87,88]. Dose adjustments of several ARVs, including TAF and dolutegravir, are recommended when coadministered with rifamycin or other drugs known to be strong inducers or inhibitors of P-gp [87]. Furthermore, pharmacogenomics studies have found that single-nucleotide polymorphisms (SNPs) in drug transporter proteins are associated with kidney dysfunction, plasma ARV concentrations, and virological failure for PWH on ART [89]. *Thompson* et al. *(2019)* utilized mass spectrometry imaging (MSI) to visualize the distribution of six ARVs in gut tissue sections across three species and found that raltegravir colocalized with P-gp in the macaque ileum and was predominantly found on the luminal surface of the mucosa, suggesting that P-gp may be a barrier to entry into the gut tissue for raltegravir - a known P-gp substrate [90, 91]. They also found that 50–60% of CD3 + cells did not colocalize with detectable ARVs, and that up to 90% of HIV/SHIV RNA was found where there was little to no exposure to ARVs [90], suggesting that there may be pockets in the gastrointestinal tract where reduced ARV exposure contributes to low-level HIV replication. *Minuesa* et al. *(2016)* further showed that in vitro overexpression of P-gp in CD4 + T cells resulted in decreased intracellular raltegravir concentrations and found that HIV-1 viraemia correlated with P-gp activity [92]. These studies confirm that the distribution and concentration of ARVs across tissues and target HIV cells are influenced by the activity of drug transport proteins. A better understanding of the factors that regulate their activity is therefore important for optimal efficacy of ARVs.

A primary function of drug transport proteins, especially ABC transporters, is the protection of cells through efflux of xenobiotics, which includes microbial products produced by the gut microbiome [93]. One of the first established connections between the gut microbiome and drug transporter proteins was the unexpected development of a mouse colitis model upon knocking out P-gp/ABCB1 (*mdr1* − / −) [93,94]. Mice with this knockout are observed to spontaneously develop colitis, though only when conventionally housed with an intact gut microbiome [93]. Germ-free mice, or those kept on antibiotics, do not develop colitis, which has led researchers to hypothesize that reduced activity of P-gp leads to the buildup of microbial products in the intestinal epithelium leading to inflammation [93] and suspect that the gut microbiome may regulate the expression of drug transport proteins [95,96]. However, there has been relatively little investigation into how the gut microbiome may regulate the expression of drug transport proteins and the potential downstream consequences for therapeutics. In one recent study, *Whyte-Allman* et al. *(2021)* showed that in vitro activation of CD4 + T cells by an HIV pseudotype virus ($pHIV_{NL4-3}$) resulted in significantly elevated expression of ABC transporters including P-gp, BCRP, and multidrug resistance-associated protein-1 (MRP1) and suggested that this increased expression may result in reduced penetration of ARVs [97]. Given that gut bacteria enriched in PWH have also repeatedly been shown to activate CD4 + T cells *in vitro* [37], further investigation into whether this contributes to ARV pharmacodynamics through drug efflux pump regulation is warranted.

Metabolites produced by the gut microbiome may also regulate drug efflux pumps and influence systemic drug

levels. In a mouse model studying the gut microbiome's impact on tacrolimus pharmacokinetics, *Degraeve* et al. *(2023)* found that antibiotic treatment significantly lowered tacrolimus serum levels compared to untreated mice [98]. Antibiotic-treated mice showed higher P-gp expression in the small intestine, which was inversely correlated with tacrolimus levels. In vitro, fecal water from untreated mice significantly reduced P-gp expression in Caco-2 cells compared to a PBS control, whereas fecal water from antibiotic-treated mice significantly increased P-gp expression. This finding led the authors to conclude that microbial metabolites from untreated mice likely inhibit P-gp expression, and the loss of these metabolites through antibiotic treatment results in increased P-gp expression and efflux of tacrolimus out of the intestine, reducing serum tacrolimus concentrations.

In summary, there are several lines of evidence that drug transporter proteins may play a role in suboptimal ARV concentrations that could contribute to lingering HIV replication in PWH on ART. However, it is unknown if this is mammalian cells, bacteria cells, or a combination thereof. Therefore, understanding the factors that contribute to the expression and function of drug transporter proteins is a crucial aspect to the effectiveness of ART and development of precision medicine for PWH.

GI microbiome and pharmacogenomics

Closely related to the regulation of transporters by the microbiome is the broader intersection between the microbiome and pharmacogenomics. Pharmacogenomics assess how individual variations in host genes and the epigenome impact pharmacodynamics and pharmacokinetics. Gut microbiome-pharmacogenomic interactions may play pivotal roles in the efficacy and metabolism of HIV drugs. As discussed above, gut microbes alter ART bioavailability and are implicated in altered therapeutic outcomes [89,92,99], and those effects are compounded by pharmacogenomics. Polymorphisms in the cytochrome p450 gene (CYP450), especially the CYP2B6 variant, are a prime example. Single-nucleotide polymorphisms (SNPs) in CYP450 alter the metabolism ART drugs including efavirenz [100–102]. The resulting changes in plasma concentrations among patients add additional challenges to finding an optimal HIV suppression regimen. Conversely, adverse drug reactions (ADRs) are decreased when precision methods are used to account for patient's genetic background [103]. This is demonstrated by the success of SNP screening for abacavir dosing which can be subject to toxicity from hypersensitivity by a mutation in the HLA-B gene. Screening people for the HLA-B*57:01 point mutation has resulted in significant decreases in treatment associated ADRs [104–106].

Microbes can also impact pharmacogenomics directly by modifying host epigenetics including DNA methylation patterns and the expression of noncoding RNAs [107,108]. GI microbes including *Bifidobacterium* and *Lactobacillus* produce butyrate which can inhibit histone deacetylases (HDACs) [109,110]. This inhibition leads to increased acetylation of histones, altering gene expression patterns which may impact both drug metabolism and HIV reservoirs [111]. Additionally, the microbial metabolite 2-hydroxyglutarate is linked to DNA methyltransferase activity through alterations in TET-1 expression, ultimately affecting methylation and expression of genes including dioxygenase and HIF-1 [112,113]. Pharmacogenomics is a relatively new field, and limited work has been done to explicitly connect host pharmacogenomics to the microbiome in the context of HIV. Combining pharmacogenomics with microbiome analysis is highly complex, but this field represents an untapped opportunity to increase HIV treatment efficacy.

GI microbiome drug metabolism potential — reservoirs and inflammation

Initiation of ART in treatment-naïve individuals results in suppression of viral infection and partial immune recovery. However, persistence of integrated proviruses in long-living memory CD4+T cells can lead to the development of an HIV viral reservoir which can rebound when ART is stopped, even after many years of uninterrupted treatment and viral suppression [11,13]. The HIV reservoir is typically defined as cells which carry replication-competent proviruses that can cause new infections upon ART interruption Therefore, although ART is very effective at controlling viremia and preventing new infections, it is not a cure for HIV. Currently, the mechanisms of action for all ART drugs only prevent the infection of new cells, and they do not prevent transcription of HIV RNA or translation of HIV proteins in already infected cells, which is likely to contribute to chronic inflammation in PWH, a condition reciprocally connected to the microbiome. The role of the microbiome in the development and persistence of HIV reservoirs is an unexplored area that could help identify additional interventions to reduce the incidence of poor viral control stemming from HIV reservoir replication.

Vaginal microbiome, drug metabolism, and PrEP efficacy

Drug metabolism and the vaginal microbiome

The vaginal microbiota exists in a complex niche space with considerable variability in environmental conditions including pH, nutrient availability, mucus production,

and mucosal immune factors [73]. Despite this heterogeneity, many women have a stable microbiome dominated by one of a few species of *Lactobacillus* [114,115]. However, as many as 30–50% of women globally have disrupted vaginal microbiomes that transition from a state dominated by optimal bacteria to a state dominated by nonoptimal bacteria [116,117]. Women with nonoptimal vaginal microbiomes often experience symptoms that are diagnosed as bacterial vaginosis (BV), and the bacteria associated with bacterial vaginosis (BV-associated bacteria, BVAB) have been implicated in morbidities beyond the symptoms of BV [118–121]. One of the most serious morbidities associated with BV is HIV transmission. Increased HIV transmission is likely a multifaceted effect of BV, and several questions remain unconfirmed and understudied. One key mechanism that has not received enough attention is altered drug efficacy for women on PrEP. The effect of BVAB on PrEP efficacy could be mediated in several ways. The most direct effect is metabolism of PrEP drugs by BVAB. While this possibility has been addressed, additional research is needed to elucidate the full role of BVAB in HIV susceptibility and drug metabolism (Fig. 1).

In the first detailed investigation of the effect of bacterial on PrEP efficacy, *Klatt* et al. (2017) showed that women with *Gardnerella vaginalis*-dominated vaginal microbiomes using tenofovir gel were nearly three times more likely to be infected by HIV than women with *Lactobacillus*-dominated vaginal microbiomes [48]. They also showed that *G. vaginalis* metabolized tenofovir more rapidly than *Lactobacillus* in culture, providing a clear mechanism for decreased efficacy. The Klatt research group expanded on these results with another study demonstrating in vitro metabolism of tenofovir and dapivirine by cervical microbiomes with lower than 50% *Lactobacillus*. They also demonstrated that reduced ARV uptake in CD4+T cells (Jurkat) cultured with *G. vaginalis* vs *Lactobacillus* and increased infection rates for CD4+T cells relative to tenofovir and dapivirine degradation rate, but not tenofovir alafenamide [47]. However, others report a lower impact of BV on oral PrEP efficacy. *Heffron* et al. (2017) used Nugent scoring to assess the effect of the vaginal microbiome on PrEP efficacy [68]. Nugent scoring is based on microscopic assessment of a gram-stained slide preparation of a vaginal swab. Samples are scored from 0 to 10 based on the morphology of microbes present, with scores above 7 indicative of low *Lactobacillus* abundance and BV-type microbiome [122]. They found no significant difference in HIV risk between Nugent groups of 0–3, 4–6, and 7–10 in women on a once daily oral tenofovir PrEP regimen [68]. Two other studies by *Thurman* et al. (2019, 2022) also returned findings of no significant differences in PrEP efficacy based on the vaginal microbiome [123,124]. However, *Hillier* et al. (CROI, 2017) demonstrated that both plasma and cervical tenofovir level are modulated by the ratio of *G. vaginalis* to *Lactobacillus* in the vagina [125]. These results highlight the complex dynamics of vaginal microbiome drug metabolism. Two differences in these crucial studies are particularly important. First, the vaginal microbiome was molecularly characterized by the Klatt Group for their studies, whereas the Heffron Group used clinical BV diagnostics. Thus, the differences reported could be the result of differences in participant classification. Second, the Klatt Group assessed a vaginal gel PrEP formulation, while *Heffron* et al. assessed an oral formulation of PrEP. This difference could also alter drug metabolism dynamics. *Cheu* et al. (2020) also found a significant difference in metabolism between tenofovir and its perorally administered prodrug, tenofovir alafenamide, a finding that could help explain the different outcomes [47]. Collectively, the differences in these studies reveal the continued need to better understand the role the vaginal microbiome plays in drug metabolism. The vaginal microbiome and BVAB cultures are clearly implicated in drug metabolism, but the clinical and public health implications of vaginal microbiome drug metabolism are complex, and further studies to better understand the impact of the vaginal microbiome in HIV transmission and overall health are critically needed.

Mucosal dynamics affecting HIV transmission

There is growing evidence indicating that FRT microbiota influences drug efficacy and HIV susceptibility through mechanisms beyond direct PrEP drug metabolism, including the modification of cervical mucosa viscosity and mesh size [50,126,127]. The cervical mucus, comprising a complex mixture of mucins, glycolipids, and other proteins, plays a critical role in the diffusion and bioavailability of pharmaceuticals as well as the physical exclusion of viral particles from mucosal cell populations. Interactions between the microbiota and the chemical components of cervicovaginal mucosa can alter the physical properties of the mucus, potentially impacting viral and small molecule penetrance.

Nunn et al. (2015) demonstrated that the presence of *Lactobacillus crispatus* in the cervical mucus significantly increased the viscosity of the mucus. This increased viscosity was shown to increase HIV particle trapping, suggesting a protective role for *L. crispatus* in preventing HIV transmission. Conversely, the presence of *G. vaginalis* was associated with a decrease in mucus viscosity with increased 100-nm particle migration and decreased HIV-1 trapping [127]. *Borgdorff* et al. (2016) observed similar effects. They revealed BVAB, *L. crispatus*, *and Lactobacillus iners*-dominated microbiomes

each altered the mucosal proteome, inflammasome, and barrier function [73]. Similarly, *Akiyama* et al. (2019) found that changes in the mucosal environment caused by the microbiota influenced the efficacy of hormonal contraceptives that rely on modulating cervical mucosal viscosity. Specifically, the presence of *Lactobacillus* species was associated with a more stable mucosal barrier, which improved the consistency of contraceptive hormone absorption and effectiveness. On the other hand, dysbiosis and the presence of pathogenic bacteria led to increased mucosal permeability, reducing the stability and effectiveness of contraceptive methods [128].

Microbiome-modulated drug transporters

Some evidence suggests that the vaginal microbiota can affect intracellular drug concentrations by modulating the expression of transporter genes responsible for the regulation of drug influx, efflux, and substrate-specific transport across cellular membranes. The differential expression of these transporters, induced by microbial activity, could significantly influence the pharmacokinetics and pharmacodynamics of a wide range of drug classes in addition to ARVs.

Transporter expression in the FRT has been confirmed by staining, PCR, and RNA sequencing [95]. However, direct measures of transporter effects on ARVs or PrEP are lacking and would help to better understand the potential impact of drug metabolism by bacteria in both the FRT and the gastrointestinal tract.

Grammen et al. *(2014)* used a rabbit model system to identify the presence of P-gp, BCRP, and MRP-2 efflux transporters in cervical and vaginal epithelial cells. They confirmed that upregulation of those efflux transporters reduced cellular concentrations of ARVs including darunavir, saquinavir, and maraviroc, suggesting a reduced therapeutic efficacy [129]. Surprisingly, no research has directly assessed vaginal efflux expression in PWH or the effect of the cervicovaginal microbiome on efflux expression. Future studies should address this critical gap in the literature.

Vaginal microbiome and pharmacogenomics

As with the GI system, the dynamic of microbe-transporter interactions merges with the microbiome and pharmacogenomics. Pharmacogenomics as a field holds significant implications for personalized medicine, HIV treatment, and prevention [130–132]. As previously discussed, the vaginal microbiome influences local immune responses and the integrity of the mucosal barrier, which are critical in HIV susceptibility [125,127]. Pharmacogenomics connects the host genome to these microbial effects by examining genetic variations that alter individual responses to drugs, including ART [132]. Microbial and pharmacogenomic effects on drug metabolism and efficacy may act synergistically or antagonistically, necessitating a wholistic approach when tailoring personalized treatments for PWH.

As established in the previous section, vaginal microbiota can concurrently modulate local drug efficacy and directly metabolize PrEP drugs. In the context of pharmacogenomics, many of the systemic processes relevant in the GI tract are also important in the FRT. Decreased PrEP or ART levels due to CYP450 SNPs are also important in women's specific prevention [102], and there are additional processes that uniquely impact women. Vaginal bacteria have been shown to express beta-glucuronidase and sulfatases which could deconjugate drug metabolites in the vaginal mucosa, potentially reducing the local efficacy of topical PrEP applications and increasing HIV transmission [133,134]. The dual influence of the microbiome and pharmacogenomics underscores the need for personalized medicine approaches that consider both genetic makeup and microbial composition on combination.

The vaginal microbiome also impacts epigenetics including DNA methylation and the expression of noncoding RNAs. These alterations then modulate host gene expression relevant to HIV infection. As referenced above, a *Lactobacillus*-dominated microbiome is associated with optimal FRT mucosal function [120]. Conversely, nonoptimal conditions characterized by an overgrowth of anaerobic bacteria highlighted by *G. vaginalis* and *Atopobium vaginae* are associated with hypomethylation of DNA and altered expression of microRNAs including miR-223 and miR-146a [135,136]. These changes can upregulate receptors like CCR5 and CXCR4 on host cells, increasing HIV susceptibility [137]. Further understanding these microbial and epigenetic interactions is crucial for efficacious HIV prevention and treatment strategies based on individual microbiome and genetic profiles.

Drug sequestration by the vaginal microbiome

To date, no in vivo research has directly measured potential ARV or PrEP sequestration analogous to the sequestration observed in the gut, although sequestration of antibiotics has been demonstrated [138]. However, given the presence of transporters in vaginal microbes and the sequestration dynamics by gut microbes, it seems probable that ARV drug sequestration happens to some degree in the FRT. Additionally, tenofovir cellular internalization was observed by *Cheu* et al. (2020), making sequestration plausible [47].

Drug metabolism by microbiome — examples beyond HIV

While our focus has been the effect of the microbiome on HIV, ART, and PrEP, the processes that we highlight are not exclusively relevant to HIV. Long-term use of

immune suppressive drugs in transplant recipients, serotonin selective reuptake inhibiting (SSRIs) drugs to treat depression, and chronic use of and exposure to antibacterial drugs could be influenced by microbial metabolism, sequestration, transporter effects, and mucosal changes. Our current understanding of microbial metabolism of tacrolimus, digoxin, and other transplant immune suppression drugs has been extensively reviewed [98,139,140]. However, given the research we highlight above, the interaction between HIV, the microbiome, and SSRI metabolism warrants additional research, particularly since PWH are more likely to be proscribed SSRIs that the general population.

Antidepressants, particularly SSRIs, are commonly prescribed to treat depression, which is prevalent in PWH. The efficacy of SSRIs is influenced by various factors, including the gut microbiome [141]. Recent studies have suggested that there is a reciprocal relationship between the microbiome and SSRIs, with bacterial potentially modifying efficacy, and SSRIs impacting the microbiome [141–143]. Depression is a significant comorbidity in PWH, with a prevalence rate substantially higher than in the general population. The interplay between HIV infection, ART, and the gut microbiome complicates the management of depression in this group. PWH also often exhibit dysbiosis, characterized by reduced microbial diversity or altered bacterial composition. Dysbiosis can exacerbate inflammation and disrupt gut-brain communication, contributing to the pathophysiology of depression [144,145]. This altered gut microbiota in PWH may impact the metabolism of ARVs or PrEP, potentially leading to suboptimal therapeutic outcomes. The interaction between gut bacteria and antidepressants may result in either reduced efficacy or increased adverse effects. The metabolism of antidepressants by the gut microbiome is a crucial factor in the treatment of depression, especially in PWH. Furthermore, the role of antidepressant use on vaginal microbial communities in PWH has not been reported. Understanding the specific interactions between bacterial taxa and SSRIs can inform more effective and personalized treatment strategies. Further research is needed to elucidate these complex relationships and to develop interventions that consider both microbiome composition and the unique challenges faced by PWH.

Antibiotics — degradation by nontarget organisms

Relatively little research has focused directly on the potential of antibiotic metabolism by target or nontarget microbes as a mechanism for antibiotic resistance or decreased antibiotic efficacy. Antibiotic resistance has deservedly received considerable attention in for many years as the problem of antibiotic resistance has grown to epidemic levels. Antibiotic exposure is increased in PWH due to frequent coinfection with other STIs like *Neisseria gonorrhoeae* and syphilis [146]. The cumulative effect is high rates of antibiotic resistance not just in STIs but in commensal or opportunistic microbiota like *Staphylococcus. aureus* [147]. Independent of HIV infection, *Deng* et al. suggested that part of the reason that the vaginal microbiome might be resistant to metronidazole is because the CRISPR-cas9 system is increased in some strains of *Gardnerella* that are associated with treatment-resistant BV [148], providing a potential mechanism for rapid development of resistance and transport genes. Those results align with ordinary differential equation (ODE) modeling of drug uptake by *Gardnerella* and *L. iners*, suggesting that metronidazole drug metabolism by *G. vaginalis* and *L. iners* is a potential mechanism for high rates of BV recurrence and the associated increase in HIV transmission in women with BV [149]. Given that PWH and people at high risk for HIV tend to also have high incidents of STIs and antibiotic treatment, assessing the role of antibiotics on microbial populations (both GI an FRT) as well as drug metabolism outcomes is extremely important [150].

Conclusions: what does this mean for HIV?

Understanding the interindividual factors that determine drug efficacy in PWH is paramount considering that these individuals must take ART indefinitely. Furthermore, the increased risk of comorbidities that arises from HIV infection means that PWH are also more likely to be on other medications. This review highlights our current understanding of interactions between HIV and the microbiome, with particular emphasis on the role of the microbiome in decreasing the availability of ART and PrEP drugs. The HIV-microbiome literature is complex and dynamic, with the ongoing development if the field exemplified by the discovery that *Prevotella* is not primarily associated with HIV but rather with the MSM communities that are disproportionately recruited in HIV studies. That observation plus the lack of female representation in many HIV studies demonstrates again the critical need for more equitable research in the HIV field, with increased focus on the inclusion of women in study populations and additional basic research on aspects of HIV transmission specific to women's health. By reviewing the complex interplay between the microbiome and ART efficacy, we also highlighted the promise of personalized medicine. We showed that in the context of HIV, the potential of microbiome modulation through probiotics or fecal microbiome transplants is particularly relevant. Lastly, this review shows again that incorporating microbiome information in HIV prevention and treatment strategies is crucial for continued progress in

the field. As additional research is added in the fields of pharmacomicrobiomics and integrated with pharmacogenomics, treatments that target patients' individual risks of drug metabolism and systemic alteration by microbiota could greatly improve outcomes for PWH.

Abbreviations

PrEP	Pre-exposure prophylaxis
GI	Gastrointestinal
PWH	People with HIV
ART	Antiretroviral therapy
ARV	Antiretroviral
FRT	Female reproductive tract
MSM	Men who have sex with men
BV	Bacterial vaginosis
BVAB	Bacterial vaginosis-associated bacteria
SCFA	Short-chain fatty acids
ODE	Ordinary differential equations
SSRI	Selective serotonin reuptake inhibitor
DME	Drug-metabolizing enzymes
CNS	Central nervous system
ABC	ATP-binding cassette
P-gp	P-glycoprotein
BCRP	Breast cancer resistance protein

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