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Review

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Vaginal pharmacomicrobiomics modulates risk of persistent and recurrent bacterial vaginosis

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Bacterial vaginosis (BV) is the most commonly diagnosed vaginal infection in women of reproductive age, with most patients unaware that they have BV due to its asymptomatic nature. BV is a dysbiotic condition defined by a deviation from the healthy *Lactobacillus* dominance to a polymicrobial anaerobic bacterial community that increases the risk of sexually transmitted infections and adverse reproductive outcomes, including spontaneous preterm birth. The increasing number of infectious agents in BV, biofilm persistence and antibiotic resistance in the vaginal canal hinder effective treatments with antibiotics leading to consistent recurrence of BV in many women (30–70%). Like in the gut, these vaginal drug-microbiome interactions termed pharmacomicrobiomics could alter drug disposition, mechanism of action, and toxicity that reduce the efficacy of antibiotics and increase the risk of persistent and recurrent BV and its sequelae. For instance, both vaginal epithelial and bacterial cells co-exist and possess enzymes that metabolize antibiotics, and transporter proteins that expel drugs and toxins, rendering them ineffective. Despite significant progress on pharmacomicrobiomics in the gut, little is known about this phenomenon in the vaginal microenvironment, which harbors a consequential microbiota and a major source of infection and antibiotic resistance. Therefore, to improve therapeutic outcomes and reduce the rate of persistent/recurrent BV and infection-associated preterm birth, we present an overview of the evidence pertaining to the effect of vaginal microbiome-drug interactions and efficacy of antibiotics against recurrent BV. We also highlight plausible mechanistic underpinnings of these interactions and implications for treatment modalities to combat infection-associated preterm birth.

Bacterial vaginosis (BV) is the commonest vaginal infection in women of reproductive age[1,2] with most patients unaware that they have BV due to its asymptomatic nature[3]. Bacterial vaginosis represents an infectious milieu that has posed severe health risks in women of reproductive age, particularly during pregnancy[4,5]. BV patients may not develop any symptoms but are at risk of serious reproductive health issues later, with limited therapeutic options[3–5]. BV is a dysregulated microbiota condition defined by a deviation from the optimal *Lactobacillus* dominance to a polymicrobial anaerobic bacterial community that increases the risk of sexually transmitted infection (STI) and adverse reproductive outcomes, including pelvic inflammatory disease, preeclampsia, and preterm birth[1,6–9]. BV rates vary from 30% in the US[2,3,10–12] to more than 50% in sub-Saharan Africa[5,8,10,13] and significantly impact the individual's quality of life[2,14–17]. Furthermore, it is estimated that the cost of BV is US $4.8 billion annually[2]. There is no known single causative agent of BV[18,19], hence, it is diagnosed microbiologically using the Nugent Score, which is rarely performed at the point of care because it requires trained personnel, more time, and other paraphernalia[20,21]. BV can also be diagnosed clinically by identification of at least 3 Amsel criteria: characteristic homogeneous milk-like vaginal discharge, "fishy" odor, >20% clue cells, and pH >4.5[22,23]; and more recently, by nucleic acid amplification tests (NAATs)[20,24,25]. However, routine screening for BV in asymptomatic women is not recommended[26–28], as treatment does not mitigate adverse obstetric/reproductive outcomes, especially infection-associated preterm birth[29–36].

Recommended treatment for BV involves antibiotic therapy with metronidazole or clindamycin[27,37–39], with a 1- to 4-week cure rate of

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55%–90%[40–43]. However, 30%–70% of women experience BV recurrence within 6 months after antibiotic treatment[41,44]. Tinidazole, another recommended antibiotic[27], has a recurrence rate of 20%–40% within 1–2 months after treatment for 7 days[45]. These high recurrence rates are due to the persistence of protective bacterial biofilm[46–53], and the development of antibiotic resistance within the biofilm and vaginal canal[46,54]. Continuous exchange of pathogenic bacterial vaginosis-associated bacteria (BVAB) between sexual partners pre/post-treatment[55,56], failure to re-establish an optimal lactobacilliary vaginal microbiome, vaginal colonization by extravaginal reservoirs of BVAB, and patient non-adherence to multidose therapy also contribute to recurrent BV[27]. Consequently, another antibiotic with a longer serum half-life, secnidazole, which has similar antibiotic activity as metronidazole and tinidazole but spares lactobacilli, has been used to treat BV[57–59]. While there is an intense search and application of alternative treatment regimens for recurrent BV[7,25,27,41,60,61], the effect of vaginal microbiome variations or microbiome-drug interactions on the effectiveness of antibiotics against the condition has received very limited attention. This has stimulated our interest in reviewing and conceptualizing the concept of pharmacomicrobiomics in the context of persistent and recurrent BV, which significantly contributes to the inefficiency of antibiotics in the treatment and prevention of infection-associated preterm birth[29,30,35,36].

Pharmacomicrobiomics was initially defined as the effect of microbiome variations on drug disposition, action, and toxicity[62,63]. It has progressed to an emerging discipline that explores the interaction between drugs and microbes[64,65] to expand the scope of precision medicine[63]. That is the human microbiome from various anatomic sites such as skin, mouth, nose, and lungs, gut, and vagina[66] may improve or hinder the efficacy of drugs and personalized therapeutics, especially drugs administered directly to these body sites[65]. In addition to interindividual variations in the microbiome, there are intraindividual variations, which could be spatial, temporal, seasonal, developmental, hormonal, dietary, or drug-dependent[67,68]. As expected, the concept of pharmacomicrobiomics is exemplified in the impact of gut microbiota on cardiovascular drugs, chemotherapeutic agents, and natural products, including plant xenobiotics and dietary supplements[63]. This includes facilitating drug efficacy, abrogating and compromising drug effects, and mediating toxicity by modulating host response to drugs[64]. Research has highlighted the importance of improving therapeutic outcomes through personalized therapy, especially in cancer treatment[69]. Although the mechanisms and genetic basis for the microbiome-drug interactions remain largely elusive, mechanisms associated with some drugs, including acetaminophen[70], digoxin[71], and cyclophosphamide[72], have been unraveled. However, the data growth rate in the PharmacoMicrobiomics portal (http://www.pharmacomicrobiomics.org), a publicly available web resource initiated to collate available literature for microbiome interactions and classify them[73], is hampered by the number of curators and published drug–microbiome interactions. There have been efforts to integrate the available data with biochemical pathways involved in the drug–microbiome interactions from the SEED[74] and KEGG[75] databases. Plans to directly link the interactions to existing pharmacogenomics databases such as PharmGKB[76], CTD[77,78], and PACdb[79], as well as human microbiome sequence databases have also been initiated[80,81].

Despite the significant progress made in studying drug–microbiome interactions in the gut[70,82–88], little is known about this phenomenon in the vaginal microenvironment[63], which harbors about the third most populous microbiome in the body (albeit derived from/crosstalk with the gut microbiota)[89–91], a major source of infection and route of drug administration. Moreover, the observation that overexpression of a DNA repair protein (RecA) in *Bacteroides fragilis*, a gut and vaginal commensal bacteria[89], increases resistance to metronidazole[86,87] is thought-provoking. Oral metronidazole is an ineffective long-term treatment for recurrent BV as it only temporarily reinstates healthy vaginal microbiota in patients with recurrent BV[6,40,60,92,93]. High abundance of *Prevotella* prior to treatment and *Gardnerella* immediately after treatment is associated with an increased risk of BV recurrence[94]. Since the vagina and gut harbor similar bacterial species, though with different and sometimes opposite functional characteristics[89], we hypothesize that the vaginal microbiome can also alter drug disposition, mechanism of action, and toxicity. This could contribute to antibiotic resistance and failure of antibiotic therapy in treating recurrent BV and reducing adverse obstetric/reproductive outcomes associated with genital tract infections. Therefore, to improve the therapeutic outcomes, reduce the rate of recurrent BV, and alleviate the resultant adverse obstetric/reproductive outcomes, available evidence on the effect vaginal microbiome-drug interactions or pharmacomicrobiomics on the efficacy of antibiotics against recurrent BV was reviewed. Possible mechanistic underpinnings of these interactions were also suggested.

Assessment of pharmacomicrobiomics in women's vaginal health

Although pharmacomicrobiomics has been extensively explored in the gut microbiome, some important drug–microbiome interactions have been reported in the vaginal space[63]. An important question that has not been answered is the presence of confounding viral and bacterial infections in the female reproductive tract. One such discovery is the metabolism of the anti-HIV drug, tenofovir (TFV), by *Gardnerella vaginalis*. TFV reduced HIV incidence by only 18% in African women with *G. vaginalis*-dominated (BV-like) microbiota and 61% in women with *Lactobacillus*-dominant microbiota[95]. *G. vaginalis* and *Prevotella* spp. metabolized TFV, thereby decreasing its bioavailability in women with a *Lactobacillus*-deficient and *G. vaginalis*-dominated microbiota[95]. With the 3-fold reduction in the efficacy of TFV in the group with *G. vaginalis*-dominated microbiota, the authors concluded that having a *Lactobacillus*-dominant vaginal microbiota was associated with higher preexposure efficacy of vaginal 1% TFV gel[95]. These findings were sequel to the observations of varying concentrations of TFV in the vagina of a South African cohort[96]. Lower preexposure efficacy was observed in the women with low vaginal TFV concentrations[96].

Another evidence of a link between vaginal microbiota and microbicide efficacy of systemically administered drugs was reported in a predominantly African American population of HIV-infected women[97]. The authors tested the hypothesis that the concentration of antiretroviral drugs would be increased in *Lactobacillus*-dominated vaginal microbiota[97]. The study reports that the female genital tract/plasma ratios of ritonavir-boosted atazanavir (ATV) and TFV were lower (<half) in women with low- (*Lactobacillus*-dominant) and high- (*Lactobacillus*-deficient and high BV) microbial community diversity compared to women with intermediate-diversity (lower *Lactobacillus* proportion). Another drug, emtricitabine (FTC, pKa 2.65), showed similar results for low- vs intermediate-diversity community types but not for high- vs intermediate-diversity[97]. Because the systemic distribution of drugs into different body compartments is influenced by both host-specific and drug-specific factors[98,99], the authors opined that a dysbiotic female genital tract characterized by increased microbial diversity and BV is capable of increasing local pH and altering other factors that can determine the movement of drugs across the female genital tract compartment[97].

The healthy *Lactobacillus*-dominant vagina is naturally acidic with a pH of 3.5–4.5[4,100], which increases during infection[4,99]. By "ion trapping", acidic drugs such as ATV (pKa of 4.7)[101,102] and tenofovir disoproxil fumarate (TDF, pKa 3.75)[103] may be ionized and rendered ineffective by a more basic BV-like vaginal pH. On the other hand, these drugs are more likely to remain unionized, more lipid soluble, and better absorbed in a *Lactobacillus*-dominant vaginal environment with acidic pH[99,104]. For instance, TVF uptake by human cells is reduced with increased vaginal pH[104]. In addition to metabolizing TFV to adenine[95], *G. vaginalis* inhibited TFV endocytosis by human cells through the release of adenine[104]. T-cell uptake of TFV in the vaginal space is reduced with increased extracellular pH to 6.5–8.2, as observed in BV[104,105]. As pH increases from 4.5 to 7.5 and 8.2, uptake of TFV by T cells decreased by >50%, whereas TDF uptake increased by the same margin[104]. T-cell uptake of TFV may be determined by changes in vaginal microbiota and pH, which could contribute to inconsistent drug efficacy in subpopulations, including adolescents or BV-

positive individuals[105]. Another study demonstrated faster TDF permeation of vaginal tissues at pH 5.0 but decreased absorption at pH 3.8 due to partial ionization of the drug[103]. This ion trapping effect and the ability of *G. vaginalis* and other anaerobes to metabolize TFV may contribute to TFV's decreased efficacy in reducing the incidence of HIV in the studies by Klatt et al.[95] and Donahue, Carlson et al.[97]. The observation of similar concentrations of ATV and TFV in individuals with *Lactobacillus*-dominant and *Lactobacillus*-deficient microbiotas[97] supports the assertion that specific microbes may also alter the movement of drugs across the genital tract by altering local drug transporters in a pH-dependent or independent manner[97,106]. For instance, unlike *L. jensenii* and *L. iners*, which have been linked to dysbiosis, BV, and preterm birth[107–111], *L. crispatus* takes up TFV by an organic anion transporter (OAT)-dependent mechanism and reduces extracellular concentration of TFV by >75%[104,105]. However, vaginal drug pharmacokinetics is complicated by the ability of some strains of *L. crispatus* to transport and metabolize TFV actively[104].

The effect of vaginal pH on drug efficacy has also been demonstrated in labor induction for term or preterm birth. Vaginal pH >4.5 (range, 4.0–6.0) was a positive factor for the effectiveness of dinoprostone (pKa = 4.9)[112–114], but pH <5 did not have any effect when misoprostol (pKa = 14.68)[115,116] was administered. Misoprostol (PGE_1) and dinoprostone (PGE_2) are used to induce labor - uterine contraction and cervical ripening, and dilation[112–116]. Moreover, based on observations from the gut microbiota, there are speculations that vaginal microbiota could indirectly influence the efficacy of drugs by altering the host drug metabolism and producing bacterial metabolites that compete with the drug receptors[117] (Table 1). This is supported by the plausible microbiological metabolism of nicotine observed in the vagina of women with BV-like microbiota[118]. Nicotine (pKa 8.0)[119] is concentrated in the acidic (pH 4.0–4.6)[11] vaginal environment, whereas women with *Lactobacillus*-deficient vaginal microbiota (pH 5.3) show lower levels of nicotine[118]. Another metabolite (hippurate) derived from toluene, a by-product of cigarette smoke, is a known substrate for *G. vaginalis*[120,121], a BV-associated bacteria[4,23,122]. Hippurate was reduced in women with *Lactobacillus*-deficient vaginal microbiota compared to those with *Lactobacillus*-dominant microbiota, and increased in non-smokers over smokers suggesting microbial (especially *Gardnerella*) utilization (hydrolysis) of hippurate[118,120]. Although larger confirmatory longitudinal studies are required, smoking may alter the vaginal environment[123] to be conducive to the proliferation of *G. vaginalis*[118,124,125], and smoking cessation could facilitate the reduction of recurrent BV[54,124].

Taken together, vaginal microbiota may influence pharmacokinetics and contribute to inconsistent efficacy of locally administered drugs through mechanisms highlighted in Table 1[104,106]. In the context of BV and other STIs including HIV, suboptimal drug exposure associated with certain vaginal microbiota communities could be responsible for poor therapeutic responses to treatment in some women[97]. Hence, selection/utilization of drugs that are less prone to environmental factors, such as certain prodrugs, may lead to more consistent drug efficacy[104].

Vaginal pharmacomicrobiomics and risk of recurrent bacterial vaginosis

Host-microbiome interplay shapes the vaginal microecosystem[126]. The host vaginal epithelium and microbiota coexist in a mutually beneficial relationship that supports a lactobacilliary community with low diversity[4,126]. Under the influence of estrogen, the host cells provide glycogen that is metabolized to lactic acid by *Lactobacillus* species to lower the vaginal pH and prevent growth of other potentially harmful bacteria[4,126]. Reproductive hormones regulate the community composition and population of the vaginal microbiome during the menstrual cycle and pregnancy[127,128], and this directly impacts medicated vaginal inserts or pessaries[65]. Alterations in the vaginal microbiota towards communities with increased diversity and BV increase vaginal pH that can alter other local factors, which could impact the movement and metabolism of drugs in the female genital tract[97]. The gut microbiota has shown resilience by recovering to a stable but distinct community structure after disruption by antibiotic therapy. For instance, 2 years after clindamycin treatment, gut *Bacteroides* clonal diversity decreased significantly with the development of clindamycin-resistant clones[129]. *B. fragilis* also mounts resistance against metronidazole by overexpression of RecA[86,87], *nim* genes (nitroreductases), multidrug efflux pumps (BmeR-ABC5) that are capable of pumping metronidazole out, and deficiency of ferrous iron transporter feoAB[130]. Lower levels of iron cause the deficiency of feoAB, which leads to decreased electron-mediated metronidazole activation[130]. *B. fragilis* is also associated with BV[4,5] and could contribute to metronidazole resistance by mechanisms similar to those in the gut. Additionally, *Gardnerella* spp., which usually lays the foundation for biofilm formation[5,25,50,52,53,131,132], also scavenges iron from the vaginal milieu, an action that deprives other bacterial species of iron[133–135]. Although, whether this also leads to decreased electron-mediated metronidazole activation in the vagina is unclear, an adherent *Gardnerella* spp. biofilm persist on the vaginal epithelium following metronidazole treatment[53]. Like *B. fragilis* in the gut, *G. vaginalis* may decrease metronidazole activation in the vagina by decreasing the level of iron necessary for its activation. Similar combination therapy with metronidazole, clarithromycin, and omeprazole significantly altered the microbial community for about 4 years, with the persistence of high levels of the macrolide resistance gene erm(B)[136].

Another bacteria that shows synergism with *Gardnerella* spp. to form BV biofilms is *Prevotella*[25]. *Gardnerella*[137,138] and *Prevotella*[139–141] produce sialidase that degrades the cervical mucus to facilitate bacterial attachment to the vaginal epithelium and formation of biofilms[5,137,142–144] characteristic of BV[49], and probably contribute to treatment failure and recurrent BV[47]. This is by acting as a barrier to inhibit antibiotic penetration and expression of efflux pumps[145]. To support these assertions, a recent Melbourne study reported that *Prevotella* and *Gardnerella* contributed to treatment failure and high rates of recurrent BV following first-line antibiotic therapy – metronidazole and clindamycin[94]. A high abundance of *Gardnerella* post-treatment is associated with a high risk of recurrent BV[94], while a sustained cure is associated with a low relative abundance of *Gardnerella* after treatment[146–148]. Metronidazole and clindamycin have limited ability to dislodge established multispecies *Gardnerella* biofilms[149–152]. During BV infection, *Gardnerella* and *Prevotella* are early colonizers of the vaginal environment and are able to evade host immune surveillance by sialidase production while establishing the biofilm that sets the stage for secondary colonizers, including *Fannyhessea vaginae*[49,153–155], *Sneathia* spp. and other BVAB that are more potent triggers of host immune response to BV[25]. The poor performance of metronidazole and clindamycin against BV biofilms has triggered the clamor for antibiofilm agents to effectively treat BV[47].

Table 1 | Pharmacokinetic actions of vaginal microbiota

Factor	Mechanism and effect	References
pH and Ion trapping	Reduce/increase drug lipophilicity and absorption	11,112–116,118
Drug metabolism	Enzymatic (hydrolysis, reduction, etc.) inactivation of metabolites and drugs	95,96,104,117,118,120,165,176,193,201
Competitive inhibition	Bacterial metabolites bind to drug receptors, inhibiting uptake by human cells	104,117
Binding and/or intracellular accumulation	Bacteria binds irreversibly to drugs, resulting in reduced drug activity	104
Drug transport	Uptake and efflux of drugs from bacterial cells	104,105,159,160,165,169,171–178,201

Factors that contribute to bacterial colonization and infection of host tissues

Efflux pumps and transporter proteins

The expression of factors such as efflux pumps on vaginal epithelial and bacterial cells capable of expelling drugs and drug metabolites, thereby reducing their efficacy, requires further investigation[156,157] (Fig. 1). Several ATP-binding cassette (ABC), solute carrier (SLC), and solute carrier organic anion (SLCO) transporters play crucial roles in maintaining a healthy lactobacilliary vaginal microbiota and in the pharmacokinetics of many drugs, including antiretroviral drugs[156–158] (Table 2). However, studies on their roles in recurrent BV that can induce infectious spontaneous preterm birth are limited. As they regulate the entry (SLC and SLCO) and exit (ABC)[156] of substances into the vaginal environment, disruption of vaginal transporter protein activity can lead to dysbiosis increasing the risk of infections, including BV[156,159,160]. Vaginal epithelial cells promote luminal acidification during acute bacterial infection via a TLR4-dependent pathway by up-regulating the expression of Na^+/H^+ exchanger-1 (NHE1) that pumps protons into the lumen[161]. ABCs such as P-gp, MRP4, and BCRP have been identified in the epithelial and vascular endothelial cells of human, mouse, and macaque cervicovaginal canal[156,157,162] (Table 2). These are post-absorptive factors that could cause poor and variable absorption of drugs, such as the P-gp-mediated ATZ efflux from the gastrointestinal tract[163]. The microvilli on vaginal epithelial cells also contain concentrative nucleoside transporter 3 (CNT3), which could shuttle nucleoside analogs delivered to the vaginal tract from the epithelium to immune cells in lower layers of the tract[164]. Nucleoside analogs such as acyclovir are useful for treating and preventing viral STIs, and they need to accumulate within the cells; hence, the importance of CNT3 in their delivery, absorption, distribution, and efficacy[160,164].

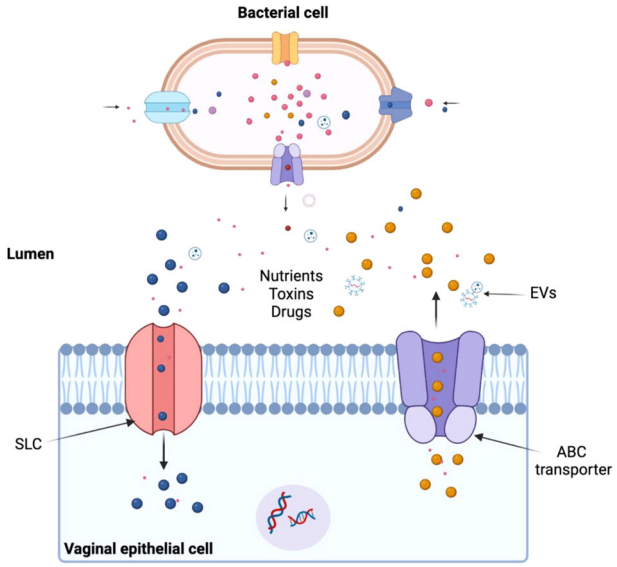

Fig. 1 | Efflux and uptake transporter proteins in the vaginal microenvironment. Both bacteria and human epithelial cells possess transporter proteins with which they take up and expel nutrients, toxins, and drugs. The cells also secrete extracellular vesicles that can also convey the transporter proteins, which can be exchanged between the host and bacterial cells. Together, these mechanisms can influence the state of the microbiota (healthy or dysbiotic) and drug efficacy, leading to antimicrobial resistance that is associated with recurrent bacterial vaginosis and a high incidence of infection-associated preterm birth. ABC ATP-binding cassette transporters, EVs extracellular vesicles, SLC solute carrier. Created in BioRender. https://BioRender.com/j51k900).

Bacterial transporter proteins

Bacteria can display resistance to multiple drugs and other cytotoxic agents that they have never encountered[165]. This is multidrug resistance (MDR) that has posed a major challenge to drug-based clinical treatment[165], including recurrent BV. Transporter proteins are also found on bacterial cell membranes, where they are important virulent factors involved in nutrient uptake and secretion of toxins and antimicrobial agents[159,160,165–168] (Fig. 1). Vaginal bacteria utilize transporter proteins to acquire nutrients such as sugars, glycogen, and lipids released by vaginal epithelial cells[167,169,170]. The jostle for nutrients may induce the expression of unique transporter proteins by certain bacteria to promote their dominance in the microbiota[169]. The bacterial membrane transport system is driven by ATP, proton motive force, and phosphoenolpyruvate (PEP); and includes ABC transporters, major facilitator superfamily (MFS) proton symporters, sodium solute symporters (SSS), enzyme II integral membrane subunits of the bacterial PEP-dependent phosphotransferase system (PTS)[169]. For example, vaginal microbiota dominated by lactobacilli (particularly *L. crispatus*) exhibit higher expression of glutamate, putrescine/spermidine, zinc, manganese, phosphate, and phosphonate transporters with which they promote their growth, protect themselves against oxygen toxigenicity, lower intracellular pH, and maintain acid tolerance to the detriment of their competitors[171]. *G. vaginalis*, a renowned BVAB,[4] bears up to 7 members of the ABC superfamily on its membrane[159]. Efflux pumps and ABC transporters used for antimicrobial resistance[172] were upregulated in *G. vaginalis* biofilms, e.g., genes encoding NLPA lipoprotein involved in ABC transporters[173]. *G. vaginalis* also has other efflux pumps, including MFS transporter, sulfonate ABC transporter, osmotic enzyme, and cobalt ABC transporter[174]. The high levels of lactic acid and hydrogen peroxide in *Lactobacillus*-dominant vaginal microbiota compels *G. vaginalis* to expend enormous energy to excrete these two antimicrobial molecules at the expense of its growth and proliferation. Hence, it is unable to form biofilms[174]. Metronidazole-resistant strains of *Gardnerella* (e.g., JNFY3, JNFY4, JNFY14, JNFY17, and JNFY28) possess an ABC-type multidrug transport system (YadH) and 5-methylcytosine-specific restriction endonuclease

Table 2 | Examples of efflux and uptake transporters identified in human cervicovaginal tissues

Efflux transporters	Uptake transporters	
ATP-binding cassette (ABC)	**Solute carrier (SLC)**	**Solute carrier organic anion (SLCO)**
P-glycoprotein (P-gp)[156,157]	Sodium/hydrogen exchanger-1 (NHE1/SLC9A1)[161]	Organic anion transporter (OAT)-2[156]
Multidrug resistance protein (MRP) – MRP1, MRP4, MRP5, MRP7[156,157]	Monocarboxylate transporter-1 (MCT-1/SLC16A1)[218], MCT-4/SLC16A3[158]	Organic anion transporting polypeptides (OATP): OATP-D/SLCO3A1, OATP-E/ SLCO4A1[156]
Breast cancer resistance protein (BCRP)[156,157]	Organic cation transporter-2 (OCT2/SLC22A2), -3 (OCT3/SLC22A3)[156]	
ATP-binding cassette (ABC) sub-family G member 1 (ABCG1)[158]	Concentrative nucleoside transporter 3 (CNT3/SLC28A2)[164]	
	Equilibrative nucleoside transporter (ENT1/SLC29A1)[156]	
	Nucleotide sugar transporter family (SLC35E4)[158]	

Table 3 | Role of efflux pumps in drug–microbiome interactions

Action	Mechanism	References
Antibiotic resistance	Active expulsion of multiple classes of antibiotics from bacterial cells	182–185
Virulence factor secretion	Export virulence factors, including adhesins, toxins, and other proteins crucial for the colonization of host cells	219,220
Biofilm formation	Essential for persistent infections	221
Quorum sensing	Modulates the intracellular concentration of quorum-sensing signals	220
Bacterial survival and host adaptation	Exports toxic environmental compounds encountered within the host and maintains cellular homeostasis	219,222

(McrA)[175], that excrete and hydrolyze the methyl-nitryl group of metronidazole, respectively[165,176]. Similarly, *Chlamydia trachomatis*[177] and group B *Streptococcus*[178] also carry immunogenic ABC transporter proteins[160].

Drug metabolizing enzymes

The ectocervix and vagina also express large amounts of drug metabolizing enzymes, including Phase I cytochrome P450 (CYP1A1, CYP1B1, CYP2C8, CYP3A4) and the Phase II UDP-glucuronosyltransferases (UGT1A1, -1A4, -1A7, -1A8, -1A10, UGT2B4, -2B15, -2B17) that metabolize antiretroviral and other drugs[156,179–181]. These enzymes metabolize the drugs and, in conjunction with the transporters, reduce microbicide exposure and efficacy in the cervicovaginal environment[156]. However, their roles in the inefficacy of the antibiotic treatments for recurrent BV and infection-associated preterm birth administered both vaginally and systemically is yet to be elucidated.

Taken together, we hypothesize that the transporters and enzymes in the lower genital tract and bacterial cell membranes may alter the tissue-to-lumen efflux and lumen-to-tissue drug distribution of both vaginally and systemically administered antimicrobial drugs. The mutualistic interactions between the bacterial species and host cells in the reproductive tract may include the temporal exchange of these drug efflux/transporter proteins, rendering the ecosystem resistant to both toxins and therapeutic agents. Such interactions may reduce drug efficacy, which could contribute to the inefficacy of current treatment for BV, high rates of recurrent BV, and poor performance of antibiotics in reducing the incidence of infectious preterm birth.

From the above evidence, efflux pumps, including ABC transporters, contribute to bacterial colonization and infection of host tissues, as well as multidrug resistance by actively expelling various antibiotics and metabolites from bacterial cells[182] (Fig. 1, Table 1). These transporters can recognize and export multiple classes of antibiotics, including β-lactams, macrolides, and aminoglycosides[182–184]. Interestingly, this broad substrate range can lead to cross-resistance to multiple antibiotics and even some disinfectants[185]. Table 3 summarizes the role of efflux pumps in drug–microbiome interactions that require more exploration in the lower genital tract – vaginal epithelial cells, ectocervix and endocervix.

Discussion and future perspectives

As with other (including systemic) routes of drug administration, the efficacy of vaginally administered drugs depends on intrinsic factors of the vagina, including microbial composition, pH, fluid composition, viscosity, enzymatic metabolism, clearance, amongst others[99]. In order to prevent persistent and recurrent BV, there is a need to identify suitable microbicide agents[186] as well as pharmaceutical vehicles/strategies that can improve the performance of drugs for better protection against such infections[187–190]. A few studies have demonstrated that the composition of the vaginal microbiota can alter the pH and metabolize anti-HIV drugs[95,97,103–105], thereby reducing the efficacy of the drugs. However, these crucial drug-microbiota interactions have not been extensively explored in BV, which is not only the most common genital tract infection of reproductive-age women[1,2] and a risk factor for adverse reproductive outcomes[4,5,9,23,89,191,192], but has an alarmingly high recurrence rate after antibiotic therapy[41,44,45].

We hypothesize that the efficacy of recommended antibiotics for treating BV may be reduced by vaginal microbiota-associated factors including pH and metabolism, leading to antibiotic resistance. Metronidazole resistance mechanisms exhibited by anaerobes, including *Gardnerella* spp., *Bacteroides* spp., *Fusobacterium* spp., *Mobiluncus* spp.[4,5,23,193–197] include deletion, inactivation of genes including nitroreductase activity, drug inactivation, decreased drug uptake, increased efflux, altering drug targets, modulating DNA repair system, and increasing activity of oxygen-consuming enzymes[198,199]. Two bona fide BVAB (*Bacteroides* and *Prevotella*) that are highly resistant to metronidazole[4,5,23,193,200] alter pyruvate fermentation, and so do not activate the prodrug[86,198,199]. Particularly, *B. fragilis* possesses *nim* genes (*nimA-nimG*) that encode 5-nitroimidazole reductase enzymes that convert metronidazole to a non-toxic compound[193]. *B. fragilis* also mounts resistance to clindamycin by modifying the 23S RNA using N^6-methyltransferases encoded by the *ermB*, *ermF*, and *ermG genes*, as well as expelling the antibiotic using efflux pumps encoded by the *msrSA* and *mefA* genes[193,201]. Others, like *G. vaginalis*, possess endonucleases that hydrolyze the methyl-nitryl group from metronidazole[165,175,176]. Formation of biofilms and horizontal transfer of resistant genes[202] among the constituent bacterial species produce a combined and more formidable antibiotic resistance that is responsible for the high recurrence of BV[203]. Insufficient drug exposure in the lower genital tract induced via the aforementioned mechanisms, amongst others, has led to suboptimal therapeutic outcomes. Consequently, some researchers administered a maintenance therapy to suppress recurrent BV and recorded low clinical recurrence of BV during the period patients used the treatment. This included a high dose of intravaginal metronidazole - 750 mg twice weekly for 3 months with additional follow-up for 3 months[204]. However, recurrence was high after cessation of suppression therapy[204].

Because the pharmacokinetics of antibiotics for BV and bacterial STIs (which are often subclinical) is not often extensively assessed in clinical settings, the aforementioned drug-microbiota interactions have also contributed to the poor performance of antibiotic therapy in reducing the incidence of infection-associated spontaneous preterm birth, even when initiated earlier in gestation[29,30,35,36]. Therefore, to improve therapeutic outcomes and attenuate drug adverse effects, several strategies to selectively exploit microbiota were indicated. These include administration of probiotics (viable health-promoting microbial species), prebiotics (non-digestible compounds that selectively enhance the growth/activity of beneficial bacteria), synbiotics (a mixture of probiotics and prebiotics with improved effect compared to the sum of the two agents), postbiotics (nonviable but biological active microbial products or metabolites), and antibiotics in support of conventional treatments[69]. The gastrointestinal tract has benefited immensely from these therapeutic strategies[69,205–207], a gesture that is practicable but underexplored in the genital tract.

Recently, a meta-analysis of 35 randomized controlled trials (RCTs), comprising 3751 patients, reported a significantly increased cure rate and reduced recurrence rate of BV when probiotics (*L. crispatus*, *L. gasseri*, *L. rhamnosus*, *Bifidobacterium,* etc.) are used as an adjuvant treatment with antibiotics (mostly metronidazole and clindamycin) compared to antibiotics alone[208]. Furthermore, postbiotics such as lactic acid, exopolysaccharides (EPS), and S-layer proteins produced by certain probiotic strains prevent colonization by harmful bacteria[209] and reduce the rate of BV[210]. Newer alternative therapies such as bacteriophage-encoded endolysins (PM-477) have also shown high selectivity and effectiveness in eliminating *Gardnerella*, both in polymicrobial biofilms of BV patients and

cultures of isolated strains, while sparing beneficial bacteria[211]. These therapeutic and preventative strategies hold great promise in reducing persistent and recurrent BV and require more comprehensive investigations.

There is also a need to study the feasibility of employing transporter and enzyme inhibitors to enhance drug exposure in the genital tract in relation to persistent and recurrent BV and prevention of infection-associated spontaneous preterm birth. The transporter proteins are differentially expressed across the lower reproductive tract. For instance, P-gp and MRP4 are predominantly localized in the cervicovaginal epithelial and vascular endothelial cells, while BCRP was predominantly found in the vascular endothelial cells[157]. This implies that different regions of the lower genital tract may require different levels of drug concentration for successful treatment and inhibition of transmission of infection[157]. The balance between uptake (SLC) and efflux (ABC) transporters in the cervicovaginal space may also be crucial to BV that is unresponsive to antimicrobial therapy. There could also be interindividual and intraindividual variability in cervicovaginal drug concentration and efficacy due to race, age, menstrual stage, and contraception choice-dependent changes in the expression and activity of transporters and enzymes[156]. As this may influence the pharmacokinetic profile and efficacy of administered drugs[156], future experimental and clinical studies should account for such factors. Lastly, since bacterial and human cells can exchange extracellular vesicles and their cargo[212], the exchange of uptake and efflux transporters between these cells is plausible. The implications of such interactions for the propagation of genital tract infection, multidrug resistance in the genital tract, and general maintenance of reproductive health deserve attention. Therefore, future studies should employ experimental strategies such as vaginal organ-on-chip models[213–215] that can simulate host-microbiota-drug interactions as in vivo, combined with fluorescence in situ hybridization (FISH) susceptibility testing to determine optimal therapy choice as it permits unique efficacy assessment of individually adjusted topical therapy without microbial isolation[216]. The findings from such in vitro experiments can be validated by well-designed clinical studies such as longitudinal vaginal metagenomic-metabolomic profiling[217] to investigate pharmacomicrobiomics mechanisms including pH and ion trapping, efflux/uptake, enzymatic inactivation, etc., in the context of persistent/recurrent BV.

In conclusion, various strategies that could improve antibiotic efficacy in the context of persistent/recurrent BV, which has overwhelmed recommended antibiotic therapy and increased the burden of spontaneous preterm birth, have been highlighted in this review. Through the concept of pharmacomicrobiomics or drug-microbe interactions, the gut has benefited from some of these interventions, giving credence to their translatability to maintaining genital tract eubiosis and reducing the incidence of persistent and recurrent bacterial vaginosis and infection-associated spontaneous preterm birth by designing new drugs, delivery systems, and dosing strategies.

Data availability

No datasets were generated or analyzed during the current study.

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Author contributions

E.A. and the co-authors discussed the concept. E.A. designed the study and performed the initial literature search, and produced the first draft of the manuscript, which was reviewed and edited by M.T., A.K., L.R., B.T., S.S., and R.M. All authors have read and agreed to the submission of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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