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Towards a deeper understanding of the vaginal microbiota

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The human vaginal microbiota is a critical determinant of vaginal health. These communities live in close association with the vaginal epithelium and rely on host tissues for resources. Although often dominated by lactobacilli, the vaginal microbiota is also frequently composed of a collection of facultative and obligate anaerobes. The prevalence of these communities with a paucity of *Lactobacillus* species varies among women, and epidemiological studies have associated them with an increased risk of adverse health outcomes. The mechanisms that drive these associations have yet to be described in detail, with few studies establishing causative relationships. Here, we review our current understanding of the vaginal microbiota and its connection with host health. We centre our discussion around the biology of the vaginal microbiota when *Lactobacillus* species are dominant versus when they are not, including host factors that are implicated in shaping these microbial communities and the resulting adverse health outcomes. We discuss current approaches to modulate the vaginal microbiota, including probiotics and vaginal microbiome transplants, and argue that new model systems of the cervicovaginal environment that incorporate the vaginal microbiota are needed to progress from association to mechanism and this will prove invaluable for future research.

The microbial communities that inhabit the human vagina are unique. Unlike the relatively diverse and even communities found at other body sites[1], the vaginal microbiota of reproductive-age cisgender women is often dominated by single species of *Lactobacillus*[2–4]. This *Lactobacillus*-dominant configuration was first reported in 1892 by Donderlein[5] and has long been considered to be a hallmark of vaginal health[6–9]. The production of lactic acid as a fermentation end-product by *Lactobacillus* spp. lowers vaginal pH (~4.0) and is thought to constrain the growth of many pathogenic microorganisms[10,11] and have a beneficial effect on the host epithelium, such as immune modulation[12,13]. However, around 25% of women in North America have communities that are not dominated by *Lactobacillus* spp. and are instead composed of a more proportionally even collection of obligate and facultative anaerobes (for example, species in the genera *Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, *Megasphaera* and *Peptoniphilus*)[2,4,14–16]. Women with these microbial communities are often diagnosed with bacterial vaginosis (BV), a common vaginal condition poorly characterized as a dysbiosis of the vaginal microbiota[17,18]. Many of these women do not report experiencing adverse vaginal symptoms (for example, odour and discharge), and appear to be otherwise healthy following gynaecological examination[2,14,19,20]. Epidemiological studies have linked the presence of these non-*Lactobacillus*-dominant communities with increased risk for adverse health outcomes, including sexually transmitted infection (STI) acquisition[21–24] and spontaneous preterm birth[25–35], which indicates that such communities may be less protective and hence non-optimal[36]. The mechanistic underpinnings of these epidemiological associations have yet to be described in detail. Here, we discuss our current understanding of the vaginal microbiota, how these communities interact with host tissues and propose the next steps on the path towards a deeper understanding of their relationship to health.

This Review focuses on the vaginal microbiota of cisgender female individuals, primarily of reproductive age. A brief discussion on the vaginal microbiota of premenarchal girls and postmenopausal women is included and highlights gaps in our knowledge of these age groups. We know comparably little about the vaginal microbiota of other individuals with a vagina, including transgender individuals. This topic was reviewed recently[37]. More study is needed to comprehensively characterize these microbial communities and their relationships with health.

Composition of the vaginal microbiota

Advances in molecular biology and DNA sequencing have enabled the high-throughput characterization of the taxonomic composition of the vaginal microbiota[2,38]. Composition is often established through the sequencing of 16S rRNA gene amplicons, although others have sequenced *cpn60* gene amplicons[39] or used a battery of taxon-specific quantitative polymerase chain reaction assays[40]. Although the bulk of these data describe the vaginal microbiota of women of reproductive age from North America, a growing number of studies have reported data from women from other reg ions[15,24,41–47]. Most reproductive-age women have a vaginal microbiota where the taxonomic composition resembles one of a limited number of configurations termed community state types (CSTs; also referred to as vaginotypes or cervicotypes, see also ref. [36]). These configurations can be represented by five CSTs, four of which are dominated by single species of *Lactobacillus* (CST I–*Lactobacillus crispatus*, CST II–*Lactobacillus gasseri*, CST III–*Lactobacillus iners* and CST V–*Lactobacillus jensenii*). A fifth configuration, CST IV, represents the more proportionally even collection of facultative and obligate anaerobes. The phylotypes common to CST IV include, among others, *Gardnerella*, *Atopobium*, *Prevotella*, *Candidatus* Lachnocurva vaginae (formerly known as BVAB1 (ref. [48])), *Sneathia*, *Peptoniphilus*, *Finegoldia* and *Megasphaera*[38,49]. These are largely fastidious bacteria that are either difficult to cultivate or as yet uncultivatable (for example, *Ca.* Lachnocurva vaginae[48]). The CSTs I, III and IV are the most prevalent, with around 90% of reproductive-age women having these CSTs[2]. Larger studies have used finer resolution

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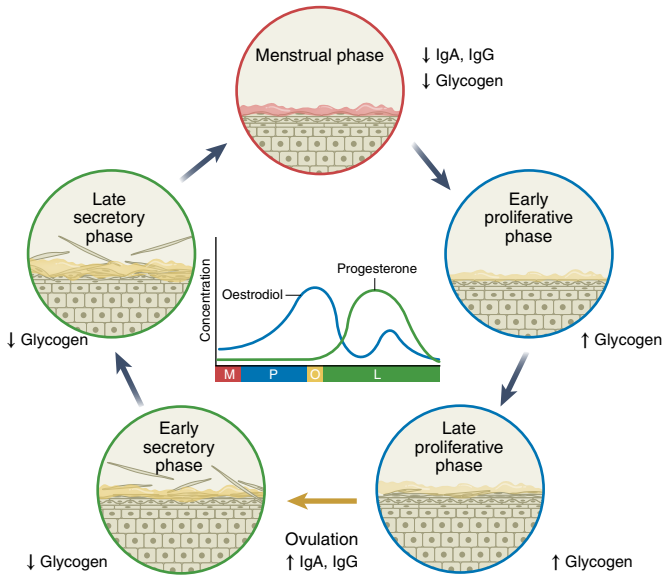

Fig. 1 | Effect of the menstrual cycle on the vaginal microenvironment. During the menstrual phase (M; red), blood and the shed functional layer of the uterine endometrium flow through the vagina. During the subsequent proliferative phase (P; blue), higher oestradiol levels promote the growth and maturation of the vaginal epithelium. The mucus is thinner during this stage, which is thought to facilitate sperm penetration. Following ovulation (O; orange), progesterone levels rise during the secretory phase (L; green), which halts the growth and maturation of the epithelium. Superficial cells of the epithelium are shed, and the protective mucus layer is thicker.

classification schemes that split the five CSTs into subtypes[38], most of which distinguish between variations of CST IV and describe uncommon communities (for example, communities dominated by *Bifidobacterium* or *Streptococcus*). Although the CST approach does simplify community composition, it continues to be an important framework for the study of the vaginal microbiota.

The term 'community state type' was originally meant to convey its representation of the taxonomic composition at a single time point[2]. This distinction is important because the vaginal microbiota of some women varies, including shifts in CST[50,51]. Changes in composition are sometimes explicable, occurring at the onset of menstruation or following unprotected vaginal intercourse. Menstruation is accompanied by biophysical and hormonal fluctuations that affect host physiology and therefore the microbial communities present. Unprotected vaginal intercourse introduces semen into the vagina, an alkaline substance that temporarily raises vaginal pH[52], and has the potential to bring new microbial species and strains into the community from the penile microbiota[53]. Other changes in the vaginal microbiota cannot be obviously attributed to a specific factor and may be the result of fluctuations in host physiology, competitive interactions between members of the community, bacteriophage activity, ecological drift or some other mechanisms[54]. The vaginal microbiota of some individuals, however, do not demonstrate temporal variation and instead maintain their community composition over several menstrual cycles[50]. It is unclear whether this stability is a property of the microbiota, host physiology or a combination of the two. Understanding the factors that drive temporal variation in the vaginal microbiota will be critical in the development of strategies to modulate these communities.

The vaginal microenvironment

The oestrogenized vaginal epithelium consists of several squamous layers, with a superficial outermost layer overlying an intermediate, parabasal and basal layer beneath[55] (Fig. 1). The upper layer is composed of flattened, dead cells that have undergone cornification, which offers a physical protective barrier[56]. This barrier also serves as an immune junction separate from that of the cervix. Although immune cells are present at the transformation zone of the cervix[57], vaginal mucosal tissue harbours few T cells and antigen-presenting cells under normal conditions but displays increased numbers in response to inflammatory triggers. In addition, the vaginal mucosal immune profile fluctuates with hormonal cycles, such that the highest levels of immunoglobulin A (IgA) and IgG are present immediately before ovulation, with lower levels at the time of menses[58].

The vaginal epithelium itself also responds to hormonal fluctuations, undergoing cyclic proliferation throughout the menstrual cycle with a peak at ovulation (Fig. 1), although changes are not as drastic as those of the uterus[59]. The vaginal epithelium is coated in a cervical mucus layer that is subject to regulation by hormonal fluctuation, with progesterone-associated thickening seen in the peri-ovulatory phase[60]. Although the vagina does not produce its own mucus, cervical mucus is produced in high enough abundance to flow down and coat the vaginal epithelium[61]. The mucus is composed primarily of proteins, lipids, water and glycoproteins referred to as mucins[62,63]. Every mucin is rich in sequences of repeating serine and threonine residues, with the repeat regions serving as the location for O-linked glycosylation chains composed of *N*-acetylgalactosamine, galactose and *N*-actylglucosamine and capped with fucose or sialic acid[64,65]. These glycosylation chains play a key role in mucin function, and alterations to these patterns are associated with several adverse health conditions, including spontaneous preterm birth[66]. Mucins are hypothesized to play a protective role in the vaginal epithelium[67,68] and may serve as a source of nutrition for the vaginal microbiota[69,70]. Mucin levels vary throughout the menstrual cycle; for instance, the amount of MUC5B peaks mid-cycle at ovulation[71] and is accompanied by an increase in the glycosylation of several mucins[72]. Glycogen made by the vaginal epithelium is also thought to be a nutrient source for vaginal bacteria[73,74]. Vaginal epithelial cells, in particular, contain an overabundance of glycogen relative to other epithelial tissues[75]. Higher concentrations of free glycogen are associated with lower levels of progesterone[76], whereas concentrations of intracellular glycogen are associated with higher levels of oestrogen[77]. Levels of both free and intracellular glycogen fluctuate throughout the menstrual cycle.

Many characteristics of vaginal physiology are altered following hormonal changes associated with the onset of menopause. The predominant cell type of the parabasal layer changes from stratum spinosum to predominantly basophilic stratum granulosum with clear cell nuclei[78,79]. Cycles of epithelial cell proliferation no longer occur due to the reduction in circulating oestrogen levels, and vaginal atrophy is common[80]. Moreover, there are decreases in cervical mucus production[81] and changes in mucus composition[82] concomitant with the decline in oestrogen and testosterone levels observed in this period. Levels of free and intracellular glycogen also decline[83]. In addition, an increase in vaginal pH to ≥4.7 is one of the more sensitive markers of menopause[84]. Altogether, these changes contribute to a vastly different microenvironment for the microorganisms that reside in the vagina. These differences are thought to be responsible for menopause-associated changes in vaginal microbiota composition[85] and the genitourinary syndrome of menopause[86]. Hormonal replacement therapy is often used to treat genitourinary syndrome of menopause, and this may in turn affect the vaginal microbiota through its effect on the vaginal microenvironment.

Lactobacillus spp. and the vaginal microbiota

It is well accepted that a vaginal microbiota dominated by *Lactobacillus* spp. offers a greater degree of protection to their host compared with a more diverse microbiota. Recent work has highlighted that populations of lactobacilli are typically not composed

of a single strain and display a substantial amount of intraspecies diversity[87]. Considering the continual supply of new mutants originating from each genetic background[88], these populations might best be thought of as clouds of related genotypes rather than single entities. This intraspecies diversity could be a critical determinant of community stability by buffering the dominant *Lactobacillus* population against perturbations[89]. There is consensus that the *Lactobacillus* spp. common to the human vagina are probably not equivalent with respect to their positive impacts on the host. Communities dominated by *L. crispatus* are thought to offer the most protective benefits whereas those dominated by *L. iners* offer the least. *L. jensenii* and *L. gasseri* may be equivalent to *L. crispatus* as they are more similar to this species in their metabolic capabilities than to *L. iners*[90], but their rarity impedes the investigation of their relationships to host health. Many hypotheses exist to explain the associations between *Lactobacillus* dominance and vaginal health, and they have varying degrees of evidential support[9,91,92]. In this section, we review our current understanding of the mechanistic explanations for these associations and discuss the ecology of the vaginal microbiome when *Lactobacillus* spp. are abundant. We focus our discussion on the biology of *L. crispatus* in the vaginal microenvironment of women of reproductive age (Fig. 2), followed by a brief examination of *L. iners* and how it differs from the other vaginal lactobacilli.

L. crispatus is a Gram-positive, facultative, anaerobic bacterium that produces both the L-lactic and D-lactic acid isomers as its primary fermentation end-products[93] (Fig. 2). Although originally thought to lack the intrinsic ability to degrade glycogen without the help of host amylases[94,95], studies have now confirmed and described this metabolic capability in *L. crispatus*, including the identification of PulA homologues[96–98]. As the human vaginal epithelium is rich in glycogen[99], *L. crispatus* probably derives the majority of its carbon and energy through the fermentation of glycogen, converting it ultimately into lactic acid. Lactic-acid production lowers vaginal pH, often to levels less than pH 4.2 (refs. [11,38]), and this acidification of the vaginal microenvironment is one hypothesized means by which *L. crispatus* benefits the host. In vitro studies have demonstrated that acidic conditions can preclude or inhibit the growth of less beneficial bacterial species, including *Gardnerella*, *Prevotella*, *Mobiluncus* and *Escherichia coli*[100–102]. Lactic acid may also have direct effects on host tissues by modulating the immune system and gene expression. For example, D-lactic acid, which is produced by *L. crispatus* (and *L. gasseri* and *L. jensenii*) but not *L. iners*[90,103], has been associated with the differential expression of immune factors by host tissues[104,105]. Meanwhile, another study[13] found that the ionization status of lactic acid, which is a function of pH, had a larger impact on its ability to suppress inflammation than the isomer form. Lactic acid more readily diffuses through epithelial cell membranes when in the non-ionized form[106]. It is clear that the relationship between lactic acid and vaginal health is multifaceted and its effects extend beyond lowering vaginal pH.

There are other mechanisms by which *L. crispatus* is thought to exert beneficial effects on vaginal health. *L. crispatus* (and *L. gasseri* and *L. jensenii*) have long been known to produce hydrogen peroxide (H_2O_2) in the presence of oxygen[107] (Fig. 2). It was thought that their production of H_2O_2 served to inhibit the growth of anaerobic bacteria in the vaginal microenvironment[108,109]. Observational studies found associations between the presence of H_2O_2-producing lactobacilli and vaginal health[7,109,110]. We now know that only *L. iners* does not produce H_2O_2 (ref. [3]), which confounds this observation with other factors that distinguish *L. iners* from the other lactobacilli[103,111]. In vitro studies have shown that H_2O_2 produced by lactobacilli can inhibit the growth of many of these less beneficial bacteria[112], although *Gardnerella* spp. seem to have the capability to resist H_2O_2 (ref. [113]). These studies do not necessarily have relevance to the in vivo production of H_2O_2 by *Lactobacillus* spp. The reactions require molecular oxygen, which is probably rare in the microaerobic vaginal microenvironment where oxygen (O_2) concentrations are one-tenth to one-fifth of that of atmospheric concentrations[114]. Furthermore, any H_2O_2 that is produced can be quenched through reactions with various non-microbial components of vaginal fluid[115]. If H_2O_2 production does play an inhibitory role in the vaginal microenvironment, it is probably limited to localized interactions between the lactobacilli and their competitors. *L. crispatus* and other vaginal lactobacilli may also have other means of inhibiting the growth of competitors, including the production of bacteriocins[116,117].

In addition to its thick cell wall, *L. crispatus* produces a proteinaceous outer surface layer called the S-layer[118,119] (Fig. 2). The S-layer, and its associated proteins, is thought to contribute to the ability of this species to adhere to host cells[118,119] and to its immunomodulatory capabilities[120,121]. The adherence of *L. crispatus* to vaginal epithelial cells is thought to block adhesion of pathogens[122,123], although the role of adhesion to a rapidly shedding vaginal epithelium is unclear. Vaginal microbiota that are dominated by *L. crispatus* have been associated with lowered vaginal inflammation[27,124], although a complete mechanistic explanation of the immunomodulatory capacity of the species has not been described. It is likely that proteins in the S-layer contribute. Efforts to further characterize the biology of *L. crispatus* and many other vaginal bacteria have been severely hampered by a lack of tools to manipulate the genetics of the species. Methods to generate targeted gene knockout mutants of these species will prove critical in future research.

A final aspect of *L. crispatus* biology that is often overlooked but may be relevant to vaginal health is the dominance of *L. crispatus* in the vaginal microbiota and therefore the low proportion of other bacteria. *L. crispatus*, and the other vaginal *Lactobacillus* spp., can dominate the vaginal niche, often accounting for 99% of the sequences in 16S rRNA gene amplicon data[2,38]. Some women also have *L. crispatus* as the main species over several menstrual cycles, which indicates that the dominance of these populations can be relatively stable[50]. By dominating the vaginal niche, *L. crispatus* reduces and precludes the growth of other, potentially harmful, bacteria. This concept, termed 'pathogen resistance', is certainly a benefit provided by a *L. crispatus*-dominant vaginal microbiota[125]. Ecological theory predicts that a more complex community utilizes more resources in an environment than a simple community due to the non-overlapping portions of the niches of the constituents[126]. A community that mostly comprises a single species should therefore not exploit the vaginal environment to the same extent as the more proportionally even CST IV community. For example, *L. crispatus* is not predicted to be a substantial degrader of host protective mucus, as it is not capable of removing terminal sialic acid and fucose residues from mucin glycosylation chains[127,128]. This is in contrast to some of the other non-*Lactobacillus* spp. that are capable of these metabolic feats[127–132]. *L. crispatus* can therefore preserve this critical barrier that protects the vaginal epithelium. Moreover, *L. crispatus* does not produce a cytolysin that would allow it to liberate resources through the lysis of host cells[133,134], and it does not appear to be capable of producing many of the biogenic amines thought to be responsible for vaginal odour[135] (for example, trimethylamine or cadaverine).

L. iners is perhaps the most common vaginal bacteria and is unique among the *Lactobacillus* spp.[2,38]. The species was first identified as the vaginal lactobacilli that did not produce H_2O_2 (refs. [108,110]). Compared with other vaginal *Lactobacillus* spp., *L. iners* has a smaller genome[103,111], produces a cytolysin[133] and does not produce the D-isomer of lactic acid[90,104]. Its relevance to vaginal health has been a topic of much discussion[136]. The dominance of *L. iners* in the vaginal microbiota is associated with low vaginal pH (<4.5) due to its production of L-lactic acid as a fermentation end-product[2,38]. Longitudinal studies have also found that communities dominated

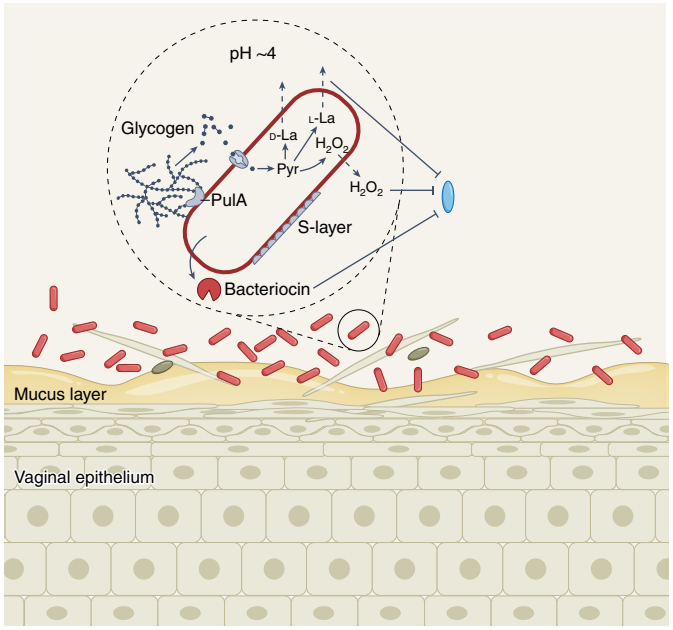

Fig. 2 | The biology of *L. crispatus* in the vaginal microbiota. When lactobacilli (red rods) dominate the vaginal microbiota, less beneficial bacteria (blue rod) are lower in abundance. *Lactobacillus* spp. produce PulA, a glycogen-degrading enzyme that generates smaller glucose polymers that are then imported into the cell and fermented via pyruvate (Pyr), which produces lactic acid isomers (D-La and L-La). This acidifies the microenvironment to a pH of <4. Glycogen-breakdown products can also be used to produce H_2O_2. Growth of less beneficial bacteria is suppressed by the low vaginal pH and bacterial products, such as lactic acid, bacteriocins and H_2O_2. The production of D-lactic acid and S-layer proteins can modulate host immune function in an anti-inflammatory manner.

by *L. iners* are less stable than those dominated by other lactobacilli, and often transition to CST IV, which may contribute to its limited association with vaginal health[50,137]. In line with this, *L. iners* is sometimes found in low-to-moderate relative abundance in CST IV communities[2,38,108,110]. This species can vary its gene expression when found within CST IV communities, including higher expression of its cytolysin[138,139]. These results suggest that the impact of *L. iners* on vaginal health may depend on the composition of the community. Although more research is needed to define the relationship between *L. iners* and vaginal health, all indications are that *L. iners* offers fewer benefits to its host than *L. crispatus*, or the other vaginal lactobacilli, although strain-level variations might modulate these benefits. One study[140] has indicated that metabolic differences between *L. iners* and the other vaginal lactobacilli could be leveraged to selectively inhibit *L. iners*.

The vaginal microbiota when *Lactobacillus* does not dominate

Many women have a vaginal microbiota that is composed of other facultative and obligate anaerobic bacteria[2,4,14–16] (Fig. 3). These communities are associated with a higher vaginal pH (>4.5) and with symptoms such as abnormal discharge and/or odour, although many are asymptomatic[2,14,19,20]. It is estimated that between 23% and 29% of women of reproductive age have BV[17,18], which is diagnosed on the basis of a high vaginal pH, a paucity of *Lactobacillus* spp., an increased abundance of odorific biogenic amines and the presence of clue cells (shed vaginal epithelial cells coated in bacteria)[141]. In research settings, BV is typically identified using a Gram-stain procedure that produces a Nugent score[142]. Standard-of-care treatment for BV includes the use of metronidazole (topical or systemic) or clindamycin (topical)[143], but treatment often fails to produce a lasting resolution of the condition[144,145]. The connections between BV and CST IV are clear: both are defined by a lack of lactobacilli and a higher vaginal pH. However, CST IV communities are not always associated with vaginal symptoms, and this is often described as asymptomatic BV. The question of whether to treat remains controversial, as epidemiological studies have linked asymptotic BV with an increased risk of adverse health outcomes[146]. Understanding which, if any, CST IV communities do not cause vaginal symptoms and/or do not increase risk of adverse health outcomes will go a long way towards understanding when treatment is necessary.

Similar to lactobacilli, host-produced glycogen is likely to be a major source of carbon and energy for CST IV bacteria. *Gardnerella* and many of the other species common to CST IV have genes associated with glycogen degradation[97,139,147]. Expression levels of predicted glycogen-debranching enzymes are high in these communities and similar to that observed in communities in which *Lactobacillus* spp. are dominant[139]. Studies have shown a positive association between levels of free glycogen in vaginal fluid and *Lactobacillus* spp. dominance[74,148]; however, we argue that this does not conflict with the observation that CST IV bacteria also utilize glycogen. The CST IV vaginal microbiota, which is often higher in bacterial load and more diverse, might simply consume more of the host-produced glycogen. The species common to CST IV have at least two other metabolic capabilities that probably allow them to access more host-produced resources (Fig. 3). First, various *Gardnerella* spp. and *Prevotella* spp. can produce sialidase and fucosidase enzymes that can degrade mucin glycan chains[127–132]. Second, *Gardnerella* (and other species) produce a cholesterol-dependent cytolysin that can lyse epithelial cells, thereby liberating their intracellular contents for use by the microbiota[134,149,150]. Damage to the vaginal epithelium probably activates proinflammatory signalling pathways, drawing leukocytes to the area[151]. These two metabolic feats—mucin degradation and host cell lysis—might synergistically act to damage the vaginal epithelium; that is, removing the mucin layer would give the cytolysin better access to epithelial cells. Although mature vaginal epithelium cells are regularly shed, the CST IV microbiota is probably capable of actively depleting the vaginal epithelium (Fig. 3). Consistent with this hypothesis is the observation that women with symptomatic BV experience higher cell shedding, whereas those with asymptomatic BV shed fewer but more immature epithelial cells[152]. We argue that these results indicate that the vaginal epithelium of some women with CST IV microbiota is damaged and might require repair before a *Lactobacillus*-dominant microbiota can re-establish. This hypothesis may explain the frequency of recurrence following treatment of BV.

The metabolic activities of the microorganisms that constitute the CST IV vaginal microbiota also affect the vaginal metabolome. A prominent example is that these communities are associated with an increased abundance of biogenic amines, including putrescine, cadaverine and tyramine[135,153] (Fig. 3). Biogenic amines are hypothesized to explain the connection between BV and vaginal odour. However, their role in the vaginal microenvironment probably extends beyond this symptom. Production of biogenic amines is a mechanism of acid tolerance that could be necessary for these bacteria to survive in the vagina[154]. Moreover, several biogenic amines can either increase the lag time or decrease the growth rate of the vaginal lactobacilli, which suggests that they may drive the establishment and maintenance of the CST IV microbiota[155]. *Gardnerella* is not thought to be a primary producer of these metabolites; species within the *Prevotella*, *Mobiluncus*, *Dialister*, *Parvimonas*, *Megasphaera* and *Peptostreptococcus* genera are instead suspected to be responsible[135]. The metabolic pathways that microorganisms use to produce biogenic amines are generally not well characterized, so other bacteria could be involved in their generation. For example, it is not known how trimethylamine, the compound thought to be responsible for the fishy odour symptom of BV, is produced in the vagina. *Mobiluncus* spp. are capable of producing trimethylamine[156]

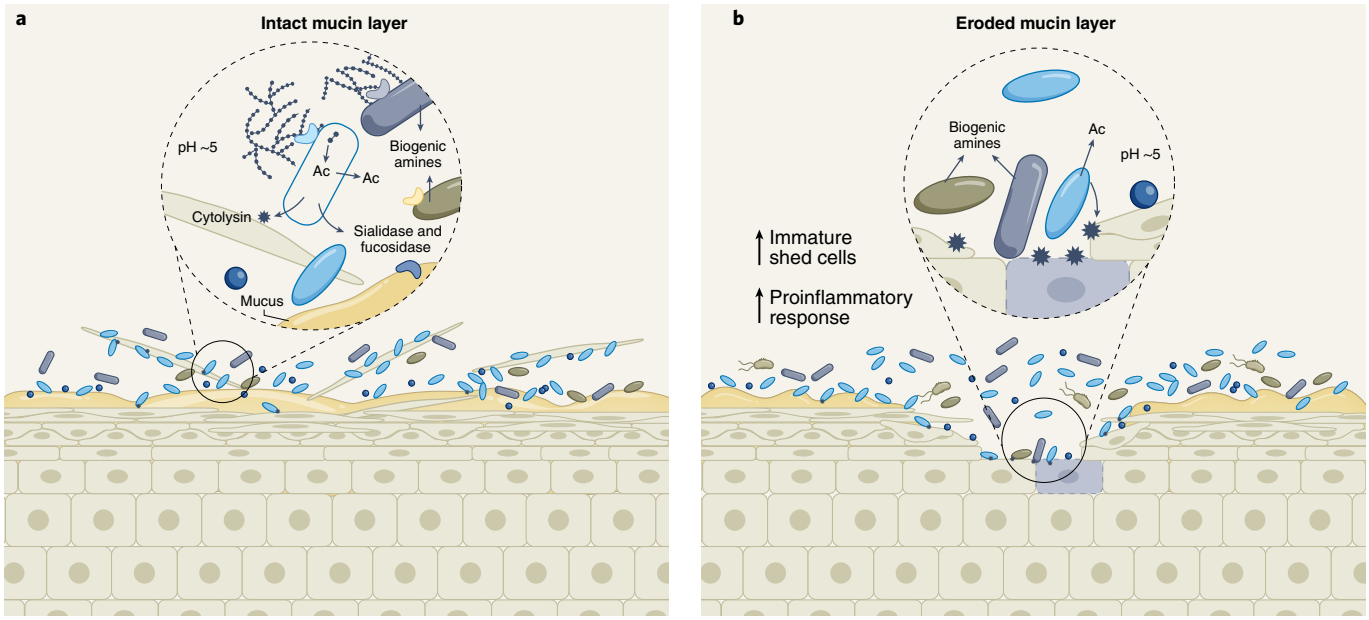

Fig. 3 | The CST IV vaginal microbiota. a, The CST IV vaginal microbiota is composed of a more even collection of *Gardnerella* (blue rods), *Prevotella* (green-yellow rods), *Atopobium* (purple circles), *Ca.* Lachnocurva vaginae (brown, flagellated) and is associated with a higher pH (>4.5). These bacteria produce biogenic amines that raise the vaginal pH, affect host physiology and inhibit the growth of *Lactobacillus* spp. These species can break down glycogen produced by the host to produce acetate (Ac), for example, using sialidase and fucosidase enzymes. Production of cytolysin enzymes by *Gardnerella* and other species enable the community to liberate more resources through the lysis of host cells. **b**, A subset of CST IV communities has the potential to degrade host mucin glycochains owing to their ability to produce sialidase and fucosidase enzymes. If the mucus layer is degraded faster than it can be replenished, the integrity of the protective mucus layer might become compromised, which exposes the vaginal epithelium to further damaged by cytolytic activity.

but it seems unlikely that this is the only source, as these bacteria are not common in the vaginal microbiota.

It is important to also recognize that the CST IV microbiota is not monolithic. A unifying characteristic of these communities is that they are not dominated by lactobacilli, but their composition can take a number of forms. Although the presence of *Gardnerella*, *Atopobium* and various *Prevotella* spp. is a common motif, some women have CST IV communities that also include high proportions of *Ca.* Lachnocurva vaginae, *Sneathia*, *Mobiluncus* and even *L. iners*[38,40,157]. It could be that a subset of species common to CST IV are responsible for the majority of its association with adverse health outcomes or that these associations could be strengthened by looking at subtypes of these communities. Compositional characterizations of the vaginal microbiota have largely been derived from 16S rRNA gene amplicon survey data, which has probably underestimated the diversity within CST IV communities. *Gardnerella vaginalis*, for example, has long been known to be a diverse species[158] and has recently been split into multiple genomospecies[159]. Most women who are colonized by *Gardnerella* have several of these species in their vaginal microbiota[87,158]. Over the years, many genomic and in vitro phenotypic comparisons of *Gardnerella* strains have been conducted, some of which suggest that there is variation in pathogenic potential within *Gardnerella* (for example, not all *Gardnerella* genomes encode a known sialidase)[158,160–162]. Shotgun metagenomic studies are necessary to disentangle diversity within *Gardnerella* and many of the other species common to CST IV communities. Disentangling the diversity of CST IV will be crucial for resolving the connection between these communities and vaginal health and will lead to improved targeted treatments.

Host factors that affect vaginal microbiota composition

Early epidemiology studies have reported that vaginal microbiota composition varies depending on the ethnicity or race of the woman. Some studies found that Black women in North America and Europe were less likely to have a vaginal microbiota dominated by *Lactobacillus* than white women in these populations[2,14]. For example, in a study of 396 women in North America, 10.3% of those who identified as white or of European descent had a CST IV vaginal microbiota compared with 40.4% of those who identified as Black or African American[2]. Another study identified a subtype of CST IV, defined by the presence of *Ca.* Lachnocurva vaginae, that was not prevalent in women in North America who identified as Asian[38]. Given that race is a social construct, the factors that drive these differences are multifaceted and it has been hypothesized that socioeconomic, cultural, genetic and/or behavioural factors, as well as inequalities in healthcare, are responsible[163]. However, it is important to note that these differences have largely not been found to extend within CSTs. The taxonomic composition of a vaginal microbiota assigned to CST IV, or any other for that matter, does not appear to depend on race or ethnicity. One exception is that *Prevotella* spp. may be more abundant in CST IV communities from women in populations from Africa[24,42]. An in-depth comparison of women from Africa and women with African ancestry living on other continents is necessary to confirm this observation.

Moreover, it is important to recognize and discuss the concordance in the composition of the human vaginal microbiota among women of reproductive age from around the world. *L. iners*, *L. crispatus* and *G. vaginalis* are three of the most prevalent bacterial species in the vaginal microbiota of women from every population examined thus far, including populations from North America[38], South America[164], Europe[165], Africa[24,45] and Asia[15,41]. A study of Amerindian women living a pre-agricultural lifestyle found that their vaginal microbiota was commonly composed of *L. iners* or *G. vaginalis*, but *L. crispatus* was less prevalent than in other populations[47]. All indications are that the taxonomic composition of the vaginal microbiota is a shared distinguishing trait of humanity. *Lactobacillus* spp. do not dominate the vaginal microbiota of any other known mammal[166], and many bacterial species common to the

Table 1 | Epidemiological associations between the composition of the vaginal microbiota and vaginal health

Outcomes	Summary of findings	Refs.
STI acquisition (including HIV, gonorrhoea, chlamydia, trichomonas, HSV, HPV and syphilis)	Results of studies vary, especially depending on the STI; the presence or increased relative abundance of *Lactobacillus* spp. is generally associated with decreased risk; BV, a CST IV vaginal microbiota, and particular BV-associated phylotypes are associated with increased risk	21,42,23,24,179,180,182–184,190–194
Vulvovaginal candidiasis	Results of studies vary, with one finding no evidence for differences in vaginal microbiota of women with and without recurrent vulvovaginal candidiasis but another suggesting that risk of symptomatic candidiasis may be higher for a *Lactobacillus*-dominant community	197,198
UTIs	UTIs were more common among women with vaginal *E. coli* colonization and without H_2O_2-producing *Lactobacillus*	195
PID	Higher growth of several BV-associated bacteria was associated with increased risk for PID, whereas there was no association between carriage of non-BV-associated bacteria and PID risk	200–202
Preterm delivery	Results of studies vary; increased relative abundance of *Lactobacillus* spp. has generally been shown to be associated with decreased risk; BV, a CST IV vaginal microbiota, and particular BV-associated phylotypes may be associated with increased risk	26–28,32,34,203,29,205,206,226

vaginal microbiota of humans have not been identified in the vaginal microbiota of other mammals, including non-human primates. *Gardnerella* spp. have been identified in rhesus macaques, but less frequently than in humans and at lower relative abundance[167]. It remains to be seen whether these *Gardnerella* spp. are distinct from those found in humans. The driving factors that underlie the development of the unique vaginal microbiota in humans are unknown.

Age is also known to affect the vaginal microbiota. Less is known about the communities that reside in the vagina during and before puberty or during and following menopause. This lack of knowledge should not be interpreted as a reflection of the relative importance of the vaginal microbiota to health in these populations. For example, the vaginal microbiota is thought to play a role in urinary tract infections (UTIs) during childhood, which afflict 3–7% of premenarchal girls[168,169]. For postmenopausal women, the vaginal microbiota is thought to contribute to atrophic vaginitis and associated sexual dysfunction[170,171]. Both premenarchal girls and postmenopausal women are less likely to have communities dominated by *Lactobacillus* spp., although their composition is also distinct from the CST IV communities commonly found in women of reproductive age[172,173]. One commonality between these two age groups is their propensity to have lower levels of circulating oestrogen than women of reproductive age[99]. Low oestrogen levels are thought to result in a thinner vaginal epithelium that is not as rich in glycogen[77]. It could be that without this glycogen, the environment is less conducive for the growth of lactobacilli and other species common to the vaginal microbiota of women of reproductive age. The number of bacteria in these communities is typically several logs lower than that found in women of reproductive age, which could be driven by lower nutrient levels[174]. Additional studies are needed to define the relationship between the vaginal microbiota and health in these age groups (see ref. [175] for more in-depth discussion on the menopause and the vaginal microbiota).

Although often overlooked, the vaginal microbiota of premenarchal girls is of particular interest as it may influence the future composition of these communities. At birth, neonatal oestrogen levels are high due to the circulating hormones of their mother. However, oestrogen levels decline during the first weeks of life and normally remain low until the initiation of puberty[176]. A recent study[177] examined the vaginal microbiota of 4–6-year-old girls from China and found that the communities were composed of a diverse collection of *Peptoniphilus*, *Porphyromonas*, *Prevotella*, *Pseudomonas* and *E. coli* species. The timing of the transition towards a vaginal microbiota that resembles that at reproductive age is not well characterized. One study[178] reported that the vaginal microbiota of 10–13-year old girls resembled that of a woman of reproductive age before their first menses, which indicates that the transition must happen earlier in life. If we generalize the results of these two studies, we can posit that the transition must occur between the age of 6 and 12 years. Oestrogen levels begin to rise during this time frame, which indicates that it may be a driving force behind the transition. The source of the species that gain dominance in the vaginal microbiota during reproductive ages (for example, *L. crispatus*, *L. iners* and *G. vaginalis*) is also not clear. It could be that these species are vertically transmitted from mother to offspring during the birthing process or early in life. Under this scenario, the species would need to persist in the vagina throughout early childhood and then increase in abundance during adolescence. However, the vaginal microbiota might experience more frequent influxes of new strains and species through another mechanism, and transmission happens later in life.

The vaginal microbiota and adverse health outcomes

Results from epidemiological studies have described associations between the composition of the vaginal microbiota and adverse health outcomes (Table 1). In this section, we will refer to a community with a lower proportion or abundance of *Lactobacillus* and a higher proportion or abundance of facultative and obligate anaerobes (for example, *Gardnerella*, *Prevotella*, *Atopobium* and *Sneathia*) as a 'non-optimal vaginal microbiota'. Note that this definition includes women with asymptotic and symptomatic BV. There is strong and consistent evidence from longitudinal studies linking this non-optimal microbiota to an increased risk of acquiring and transmitting human immunodeficiency virus (HIV)[42,23,24,179,180]. Similar associations have been identified between these communities and an increased risk for acquiring other STIs, including gonorrhoea, chlamydia, trichomonas, herpes simplex virus 2 (HSV2) or syphilis[21,181–184]. The non-optimal microbiota has also been linked to both incidence and prevalence of human papillomavirus (HPV), as well as the associated development and progression of cervical intraepithelial neoplasia and increased risk for cervical cancer[185–190]. Again though, there are contrary reports[191–194]. The composition of the vaginal microbiota has also been associated with an increased risk for non-sexually transmitted infections, including UTIs[195,196], vulvovaginal candidiasis[197–199] and pelvic inflammatory disease (PID)[200–202]. There is evidence to support an association between the composition

Box 1 | Model systems to study the vaginal microbiota

A major obstacle in vaginal microbiome research is a lack of suitable animal and cell culture model systems. These model systems are needed to investigate and test mechanistic hypotheses generated through observational studies of the vaginal microbiota. Unfortunately, the uniqueness of the human vaginal microenvironment and the human vaginal microbiota means that routinely used animal model systems lack relevance. Mouse models, which have proven useful for investigations of the intestinal tract microbiota[227,228], have also been used in studies of the vaginal microbiota[229–231]. However, because these animals do not naturally have a vaginal microbiota that resembles that of humans, it is difficult to interpret whether results are generalizable to humans. Animal models are more frequently used in STI research[232–235], but these studies have historically, and unfortunately, not considered the role of the microbiota in the host–pathogen interaction. Two-dimensional and three-dimensional cell culture models have been developed and used in vaginal microbiome research, including cellular hydrogels[236,237], self-assembled organoids[238,239] and microfluidic organ-on-a-chip models[240]. Notably, microfluidic organ-on-a-chip models offer the ability to place cells within defined geometries, can reproduce key microenvironment conditions and can be maintained for long durations. They also allow the integration of immune cells, the use of hormonal control and the application of relevant mechanical forces[241]. An ideal organ-on-a-chip model would include cervical and vaginal tissues with a transition zone between them. The vaginal epithelium should be stratified in multiple layers and should shed superficial cells that contain glycogen stores; and vaginal mucus, either produced by the cervical tissue or supplied from an external source, should coat the vaginal tissue. The application of spatial transcriptomics to such a model system would allow the researcher to characterize the local host response to the microbiota and would be critical for the multi-layered vaginal epithelium[242,243]. The development and use of such a model would be a major breakthrough for vaginal microbiota research and will enable the testing of mechanistic hypotheses.

of the vaginal microbiota and reproductive health, including risk for spontaneous preterm birth[32,203–205]. Studies that utilized sequence-based methodologies have found associations between specific vaginal bacteria, and bacterial community structures and preterm birth, spontaneous preterm birth and preterm premature rupture of fetal membranes; however, results were heterogeneous across studies, with some finding no association[26–28,34,29,206,207].

Despite the volume of work that has established associations between the vaginal microbiota and health, we still lack descriptions of the causal mechanisms and pathways. It is particularly difficult to determine whether the associations are driven by the microbiota influencing host physiology or by changes in host physiology affecting the composition of the microbiota. Parsing these tripartite associations will require the development of animal and cell culture model systems that incorporate the vaginal microbiota (Box 1).

Efforts to modulate the vaginal microbiota

Efforts to impart lasting change in the composition of the vaginal microbiota have largely proven unsuccessful. Standard-of-care antibiotic treatment for BV often yields only temporary resolution of the condition[208–210]. Other methods to repress the growth of BV-associated anaerobes and/or support the growth of lactobacilli include oestrogen therapy[171] and treatment with lactic acid[211] or boric acid[212] (Fig. 4). Many have also suggested probiotics to modulate the vaginal microbiota, either following antibiotic treatment

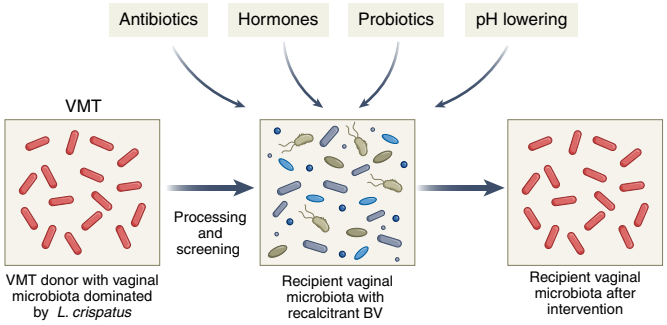

Fig. 4 | Vaginal microbiota interventions to treat bacterial vaginosis. Existing treatments include antibiotics (for example, metronidazole), oestrogen therapy (hormones), vaginal lactobacilli probiotics, and lactic acid and boric acid (to lower the pH). However, these interventions vary in their success and do not effectively prevent recurrent/recalcitrant BV. VMT is a promising intervention for BV. A suitable donor with a *Lactobacillus*-dominant vaginal microbiota is identified. Vaginal secretions are collected from the donor, screened for various STIs and processed. The processed vaginal secretions are then introduced into the vagina of a recipient who is typically experiencing recurrent/recalcitrant BV. The recipient may or may not be treated with antibiotics before the transplant. Success is defined as a long-lasting resolution of the BV of the recipient and a shift in their vaginal microbiota to the *Lactobacillus*-dominant configuration.

or primary treatment. Several vaginal probiotics containing *Lactobacillus* spp. have been designed and tested, and have largely yielded mixed results[213–220]. There are a number of reasons why the efficacies of these probiotics fell short of expectations. In some cases, the probiotic formulations did not utilize species that are common to the human vagina, opting instead to use those that were already in gut probiotics[215,220]. Other probiotics were given to women in the form of oral tablets with the expectation that such a probiotic might influence host physiology, creating a vaginal environment favourable for *Lactobacillus*[214,220]. A randomized, double-blind, placebo-controlled clinical trial[221] was conducted to test the efficacy of a vaginally delivered *L. crispatus* probiotic called Lactin-V. The probiotic was provided to women with BV, following metronidazole treatment, and resulted in a difference of 15% in the rate of BV recurrence between the treatment and placebo groups (30% versus 45% recurrence)[221]. This result is encouraging; however, more than one-quarter of the treated women experienced BV recurrence within 12 weeks. Identifying the factors that drive treatment failure will prove crucial for the development of more effective vaginal probiotics.

Promising results from studies reporting the efficacy of fecal microbiota transplants to treat recurrent *Clostridioides difficile* infections[222] have motivated the investigation of vaginal microbiota transplants (VMTs) as a potential approach to treat recurrent BV (Fig. 4). The concept involves sampling vaginal secretions from an individual with a *Lactobacillus*-dominant vaginal microbiota and introducing the sample into the vagina with recurrent and/or recalcitrant BV[223]. An exploratory study of women with recurrent BV indicated the potential efficacy for this approach, as long-term remission was achieved for four of the five recipients of the VMT[224]. It is not clear how VMT could be implemented at scale safely, as each donation requires extensive testing for vaginal pathogens and viruses (for example, HSV or HPV) and contains a relatively small bacterial load[225]. However, studies of the mechanisms of VMT are likely to yield new insights into the factors that influence the successful modulation of the vaginal microbiota. These insights could then be translated to traditional *Lactobacillus* probiotic formulations with increased safety and can be produced at scale.

Outlook

Over the past decade, we have learnt a great deal about the vaginal microbiome and how it relates to host health. Unfortunately, our reliance on observational studies and amplicon-based compositional survey data has stymied the progress towards a mechanistic understanding of these communities and their impact on host physiology. These observational studies have generated innumerable hypotheses that must be tested in the laboratory. Recent in vitro work has characterized aspects of the biology of individual bacteria (for example, on glycogen-debranching enzymes of the vaginal bacteria[96,98]), but these studies often do not include the microbiota and/or the host. A major barrier towards the development of a mechanistic understanding is the dearth of suitable model systems for in vitro experimentation. Although it is true that no model is perfect, some models are certainly better than others, and a cervicovaginal model that incorporates the vaginal microbiota is acutely needed. Progress must be made in the field of multi-omics as an integration of metagenomic, metatranscriptomic, metabolomic and immunology datasets could afford a detailed look into the biology of the microbiota–host relationship as it exists in vivo. Results from such in vitro and in vivo studies, along with interventional clinical trials, will help drive the development of advanced and innovative treatment options and preventative measures for the myriad of adverse health outcomes that affect individuals with a vagina and remain unaddressed.

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Competing interests

J.R. is co-founder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutics drugs for women's health. All other authors declare that they have no competing interests.

Additional information

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