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# RECENT INSIGHTS INTO THE VAGINAL MICROBIOTA

S. Condori, S. Ahannach, L. Vander Donck, E. Oerlemans, J. Dillen, C. Dricot, T. Gehrmann, I. Spacova, S. Lebeer

Department of Bioscience Engineering, Research Group Environmental Ecology and Applied Microbiology (ENdEMIC), University of Antwerp, Antwerp, Belgium

*Corresponding Author:* Sarah Lebeer, DO; email: sarah.lebeer@uantwerpen.be

**Abstract –** Research on the vaginal microbiome and its relation to women's health has steadily gained momentum in recent years. Traditionally, vaginal microbiome studies have focused on the pathogenesis of various diseases. Recent publications focus more on health and support a key role for *Lactobacillus* species and their dominance in maintaining a healthy state in the human vagina. In this narrative review, we synthesize promising novel insights related to vaginal microbiota published from April 2021 to March 2022. Hereby, we focus specifically on advances in understanding the vaginal microbiota composition by using innovative sequencing and detection tools. In addition, microbiota composition-influencing factors, potential microbe-based diagnostics and therapies are discussed.

**Keywords:** Vaginal microbiota, Microbiome, Sequencing, Probiotics, Prebiotics, Vaginal conditions.

## VAGINAL MICROBIOTA COMPOSITION: FROM BACTERIA AND BEYOND

### Bacterial Members and Interacting Microbes

The vaginal microbiota is the collection of microorganisms inhabiting the human vagina. As all microbiota in general[1], it includes bacteria, fungi, viruses, archaea and protists, but the relative abundance of microorganisms other than bacteria is not yet well mapped. Since more than a decade, advanced tools for next-generation sequencing of specific gene regions and entire genomes have allowed us to move from characterization by microscopy and culture-dependent methods towards a more comprehensive understanding of microbiota residing in the vagina. These approaches have revealed that most vaginal bacterial communities are dominated by a limited group of bacteria, which is different from many other human body sites, such as the gut. In one of the first larger studies on the vaginal microbiome, Ravel et al[2] have proposed to group the vaginal microbiota into five community state types (CST): one type characterized by a dominance of *Lactobacillus crispatus* (CST-I), one by *L. gasseri* (CST-II), one by *L. iners* (CST-III), one by *L. jensenii* (CST-V), and a diverse community (CST-IV) composed of different facultative and anaerobic bacteria[2]. The same research team has later expanded this CST framework into thirteen CSTs, where CST-I and CST-III were each split into an A and B subcategory, with respectively a higher and lower relative abundance of *Lactobacillus* spp., and a further subdivision of CST-IV into seven groups[3]. In addition, different updates have been suggested by other teams for these CSTs over the years[4,5]. For example, a sixth CST (CST-G) dominated by members of the *Gardnerella* genus has been suggested by Mancabelli et al[4].

We have recently highlighted that the CSTs are not arbitrary separated groups and should be seen as a continuum with different *Lactobacillus* species often co-dominating[5]. Figure 1 (inner circle) summarizes the most dominant and prevalent bacterial microbiota identified in the largest Western European study[5].

When describing the different dominant and prevalent vaginal microbiota members, it is important to realize that they occur in a community undergoing continuous dynamic interactions that influence the microbial composition. Based on our recent compositional correlation network analysis, we observed different co-occurrence patterns for vaginal bacteria[5]. We have described them as six main modules with the following key taxa (i) *Lactobacillus crispatus*/*Lactobacillus jensenii*/*Limosilactobacillus*, (ii) *Lactobacillus iners*/*Ureoplasma*, (iii) *Gardnerella* and many others such as *Megasphaera, Gemella, Atopobium* and *Sneathia*, (iv) *Prevotella* and *Dialister*, (v) *Anaerococcus* and many others such as *Finegoldia, Staphylococcus* and *Peptoniphilus*, and (vi) a module containing different gut-derived taxa. Interestingly, when eigentaxa were made from the members of each module, the *L. crispatus*-module was negatively associated with the number of vaginal complaints. In contrast, the *Gardnerella*-module was associated with changes in discharge and increasing age. These results align with other recent studies[4,6-8] where *L. crispatus* and *Gardnerella* have been associated with positive and adverse vaginal health outcomes, respectively. *Gardnerella vaginalis* (proposed reclassification as *Bifidobacterium vaginale* according to the Genome Taxonomy Database) is of particular interest, as this species was historically considered as the causal agent of bacterial vaginosis (BV)[9]. BV is a common cause of vaginal complaints and has traditionally been and remains the main focus in vaginal microbiome research, with its high prevalence, associated adverse health effects, unclear etiology and difficulties in diagnosis and treatment, as also recently reviewed[10-15]. BV is characterized by immune disruption[16-19] and clinical symptoms such as an abnormal and malodorous discharge and itching. It is associated with an increased risk of acquiring sexually transmitted infections, preterm birth, pelvic inflammatory disease and endometriosis[11]. Besides *Gardnerella,* many other taxa are typically found in the diverse BV microbiota profiles, including members of the same module such as *Atopobium* and *Sneathia,* but also *Prevotella* and *Dialister*[20]*. Prevotella* has also been associated with another vaginal condition, aerobic vaginitis, which is characterized by

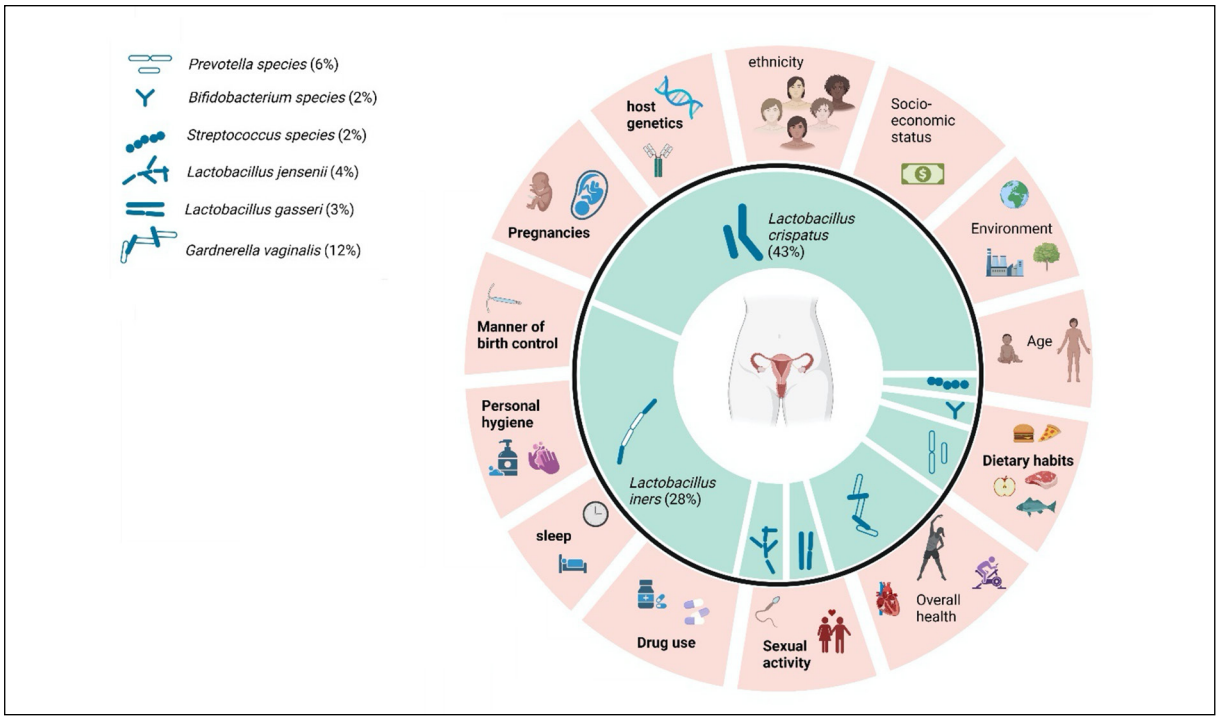


**Figure 1.** Dominant members of the vaginal microbiota (*inner circle*) and the prevalence of its dominance in a Belgian cohort[5]. Key determinants (*outer circle*) influencing the vaginal microbiome. Figure created with BioRender.com.

(strong) inflammation and/or vaginal wall thinning and was previously mostly associated with aerobic bacteria such as *Streptococcus* and *Staphylococcus*[21,22]. Despite that *Prevotella* itself has been associated with adverse health outcomes such as preterm birth[23]. Recent therapies for BV have still been mainly aimed at inhibition of *Gardnerella* and its biofilm[24-27]. However, *Gardnerella s*pecies are also commonly present in the vagina and lower urinary tract of healthy women[28]. Possibly, the pathogenic potential of specific vaginal microbiota members such as *Gardnerella* is strain-specific, because the jury is still out on the pathogenic role of *Gardnerella* species and types according to Koch's postulates[9,29]. The specific role for other main members of the vaginal bacterial community such as *L. iners* is also still controversial[30,31]. For example, increasing evidence shows that this species co-occurs with *L. crispatus*[5], while some studies[32,33] also suggest a more pathobiont role such as in preterm birth in specific geographic regions such as India[32], UK[33], but not China[34]. The other *Lactobacillus* species *L. jensenii* and *L. gasseri*, which are dominant in 3-4% of healthy women in many geographical regions and prevalent in 47% and 27% respectively[5], are less explored. They are generally associated with vaginal health[35], but their role should be further substantiated.

## FUNGI

Besides bacteria, fungi are also present in the human vagina, where they form the vaginal mycobiota. Their prevalence and abundance are not yet well mapped, but they seem to occur much less and in lower microbial load than bacteria[36]. Yet, they can play an important role in vaginal health, both in a negative (causing disease)[37] and positive way (promoting health)[38]. *Candida albicans* is to date the main fungus studied as causative agent in fungal vaginal infections. However, other fungal species were identified in the vagina, including *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, *C. pseudotropicalis, C. stellatoidea, C. spencermartinsiae, C. lusitaniae*, *C. sake* and *C. dupliniensis*[39-41]. Ongoing challenges for identification of vaginal fungi based on sequencing tools are the fact that DNA extraction methods are often optimized for bacterial DNA[39,42], while fungi are often more difficult to lyse. This introduces bias towards genera and species (such as *Candida*) which are easier to lyse than other taxa. Overall, more research on the "average" fungal community and its role in vaginal health is necessary. Additionally, further research on polymicrobial interactions as well as a consensus on mycobiome analysis in the field will allow comparisons between these studies.

## Host-Associated Viruses and Bacteriophages

In addition to bacteria and fungi, recent advanced sequencing approaches highlight that the vaginal niche also harbors a previously undetected range of viruses. Up to now, studies describing vaginal host-associated viruses have mainly focused on viruses in the context of diseases such as cervical cancer (linked to human papilloma viruses, HPV) or conditions such as AIDS (with human immunodeficiency virus or HIV as the causative agents). Earlier this year, Madere & Monaco[43] have listed the main host-associated DNA eukaryotic viral members, namely *Papillomaviridae* (predominant), followed by other double stranded (ds) DNA viruses including *Polyomaviridae*, *Herpesviridae*, *Genomoviridae*, *Adenoviridae*, and *Poxviridae* and single-stranded DNA viruses such as those in the *Anelloviridae* family. In addition to these eukaryotic viruses, bacteriophages infecting bacterial cells (collectively named the phageome) are also relevant, but their role in vaginal health is only superficially studied. Nonetheless, there has been recent interest in the involvement of phages in the pathogenesis of BV[43,44]. *Caudovirales* bacteriophages, especially members of the *Myoviridae, Siphoviridae* and *Podoviridae* families, appear to dominate the vaginal phageome. Another recent study suggests that vaginal bacteriophages form two community groups, named viral state types one (VSTs). Here, 44.7% (n=17) displayed VST1, characterized by a high relative abundance of *Rhodococcus* viruses, *Spounavirinae* and *phi*[29] whereas VST2 was observed in 55.3% (n=21) which showed a high diversity[45]. Interestingly, when performing transkingdom association analysis (bacteriophages and bacteria) VST1 was associated with *Lactobacillus* dominated

profiles whereas VST2 was associated with high diversity bacterial profiles. These associations indicate a direct or indirect interaction between bacteriophage communities and host bacterial population. Phages may modulate the vaginal microbiota, because under certain stress conditions (e.g., vaginal pH, temperature, etc.) prophages can induce large-scale infection *via* the lytic phase, resulting in shifts of bacterial communities leading to establishment and/or maintenance of adverse vaginal outcomes[45]. In this sense, of special interest is the identification of prophages in *Lactobacillus* spp. used as probiotics, because if active (lytic cycle) they could impact lactobacilli probiotic effect and efficacy[46].

## ARCHAEA

Archaea also form part of the microbiota of the mouth and intestine[47] but *Methanobrevibacter smithii* is so far the only methanogen identified and isolated from vaginal samples[48]. In one landmark study[49], this archaeon was detected only in vaginal specimens from 33 women with BV and not in 92 women without BV (control group). This observation made the authors postulate that detection of *M. smithii* could be used as a biomarker for the laboratory diagnosis of BV, but this remains to be validated. While Archaea have long thought to not cause human disease, increasing knowledge on their host interactions reveals a more nuanced view[50].

When describing or analyzing the different vaginal microbiome members, it is important to consider that low abundant microorganisms are usually discounted from sequencing analysis as potential technical artifacts or contamination[51]. For example, *Chlamydia thrachomatis* is a major cause of sexually transmitted diseases worldwide, but it seems to be underdetected through 16S rRNA amplicon sequencing, while metagenomic shotgun sequencing has recently allowed for a clearer picture of its occurrence in endocervical, vaginal and rectal samples in Fijian women[52]. Recently, the candidate phyla radiation (CPR) (i.e., a large group of uncultured bacteria defined primarily *via* sequencing)[53] has also been proposed to be part of the vaginal microbiota[54]. It is thus important to further develop sequencing and culturing approaches to better understand this "dark matter" within the vaginal microbial communities. Indeed, some major advances have already been made in this area. For example, long read sequencing techniques such as Oxford Nanopore Sequencing allow more rapid analysis even in laboratory limited settings (e.g., in the gynecologist office) compared to state-of-the-art next-generation sequencing techniques such as metagenomic and amplicon sequencing. Moreover, full-length 16S rRNA sequencing (V1-V9) with the MinION Nanopore device for BV diagnosis permitted higher discriminatory power compared to short read V3-V4 Illumina reads, while also revealing the presence of common BV-associated and opportunistic pathogenic species with a quick turnaround time (less than a day) from sample collection to results[55,56]. Besides next-generation sequencing, another emerging diagnostic tool is direct on-swab metabolic profiling with desorption electrospray ionization mass spectrometry (DESI-MS)[57]. This method has the advantage of being a rapid, on-swab metabolic profiling that gives a more functional picture of key metabolites of the microbiota and host (e.g., pathobiont and inflammation status combined). It is thus very promising to see whether this approach can be further developed into rapid, point-of-care vaginal diagnostics to facilitate faster clinical decision making, more judicious use of antibiotics and targeted treatment strategies.

## DETERMINANTS OF *LACTOBACILLUS* DOMINANCE

The *Lactobacillus* dominance is typical for humans and has not been observed in other mammals[57]. It also remains consistent among women from different origins. For example, in women of European origin *L. crispatus*-dominated profiles are prevalent followed by *L. iners* (43.2% and 27.7%, respectively)[5]. Likewise, women from Sub-Saharan Africa display a vagina dominated by *Lactobacillus* spp., with *L. iners* as the dominant species[59]. Although other studies have shown the opposite, for instance a recent study in South Africa showed that 72.7% of women had high diversity profiles[45]. One popular hypothesis proposed to explain *Lactobacillus* dominance is centered around the availability of glycogen in the vaginal epithelium, which is postulated to be the main carbon source that accumulates inside vaginal

epithelial cells[60]. Glycogen is metabolized by lactobacilli[6] to either L-lactic acid and/or D-lactic acid depending on the species, lowering vaginal pH. The cooperation of lactic acid, the low environmental pH and potentially other lactobacilli-produced specialized metabolites[61] (e.g., antimicrobial peptides such as bacteriocins) are likely factors that help establish and stabilize lactobacilli-dominated communities. The inclusion of starch in the human diet since the advent of agriculture is proposed as one explanation for glycogen accumulation in vaginal cells but this is still very speculative[58]. Indeed, the uniqueness of the human vaginal microbiota composition (*Lactobacillus* dominance) is probably the result of an interplay of several factors during evolution, involving our ovarian or typical menstrual cycle, the contribution of vaginal cells to low pH favoring the growth of (lactic) acid-tolerant lactobacilli[62], active sexual life and the risk of microbial complications associated with pregnancy and childbirth[63]. Currently, most evidence seems to be available for the role of estrogen, with its role in driving maturation and proliferation of the vaginal epithelium and the accumulation of glycogen, thereby linking estrogen concentrations with lactobacilli abundances[60]. Estrogen concentrations fluctuate throughout the menstrual cycle, which is mirrored in *Lactobacillus* abundances[5,64-64]. Similarly, when exogenous estrogens are applied, such as in hormonal contraceptives, this is also associated with increased levels of *Lactobacillus*[5,67]. Associations between progestins in hormonal contraceptives and the vaginal microbiome are less clear[67]. Other hormones such as DMPA (depot medroxyprogesterone acetate) have been shown to impact cervical cells at the transcriptional level, by inducing the expression of epithelial chemotactic chemokine CCL3L3 (a chemotactic cytokine for CCR5+ CD4+ T cells)[68].

Recently, a role for semen is proposed within inflammation and immune responses[69]. Compared to other mammals that mostly mate during ovulation, humans engage much more frequently in sexual activities throughout the entire menstrual cycle as well as during gestation. Consequently, women are regularly exposed to sperm components, such as spermatozoa, penal microorganisms and sperm specific proteins, which can all be considered non-self, therefore increasing the risk for the development of antisperm immunity which complicates conception[70]. Indeed, the development of antisperm antibodies has been indicated as a causative factor for infertility and recurrent pregnancy loss[71] and is recently being used as a contraceptive strategy[72]. Nevertheless, there still exists a lot of discussion about the role of lactobacilli in (in/sub) fertility. For example, in a recent study, *L. crispatus* has been shown to inhibit sperm activity and mobility *via* agglutination[73], indicating the need for dedicated studies on the impact of probiotics on fertility.

Indeed, the role of hormones, semen[69] and other factors affecting the vaginal microbiome cannot be uncoupled with a role for the immune system. In fact, a recently proposed key factor for *Lactobacillus* dominance is specific selection for lactobacilli by the human immune system. In an exploratory study of Breedveld et al[74], (n=25), immunoglobin A (IgA) and G (IgG) levels (bound and unbound to bacteria) were measured, *via* flow cytometry and ELISA respectively, in three vaginal swabs across one menstrual cycle. They were analyzed according to their microbiota data and metadata, previously determined by van der Veer et al[75]. No significant inter- or intra- participant differences were observed for IgG levels, the most abundant immunoglobin in the vaginal mucosa. However, total IgA levels were consistently upregulated in *L. crispatus*-dominated profiles during the entire cycle. These data suggest that IgA might maintain a *L. crispatus*-dominated microbiota in the vagina, possibly *via* immune inclusion[76] as analogously observed for healthy microbiome composition in the intestinal tract[77]. Moreover, IgA is reported to stimulate bacterial metabolism, including short-chain fatty acid production, which again promotes immunoglobin production[74,78]. However, other elements of our innate and adaptive immune system are also postulated to play a role in the enrichment for lactobacilli, including the specific way of how lactobacilli interact with epithelial cells and toll-like receptors such as TLR2/6[21,79], and produce specific metabolites such as lactic acid, vitamins, indole-derivatives and histamines that all induce specific signaling pathways. The puzzle of these molecular interactions and bi-directional communication between host cells and lactobacilli remain to be largely uncovered. Therefore, it is also important to pay critical attention to the role of specific therapies such as antibiotics on the vaginal microbiome and its interplay with the immune system. For example, metronidazole treatment, the first-line therapy for BV, has been shown to affect chemokines expression, including inflammatory chemokines CXCL9 and CXCL10[80].

In addition to the effects of the host immune system, the vaginal microbiome composition can be affected by other host-associated factors. For instance, obese women appear to have a lower prevalence of *Lactobacillus*-dominated vaginal microbiome, a higher prevalence of a more diverse vaginal microbiota and increased levels of local inflammation compared to non-obese women[80,81]. Interestingly, in women who underwent bariatric surgery, only the ones with the greatest weight loss at 6 months post-surgery were more likely to have a *Lactobacillus*-dominant vaginal microbiota, whereas in women with light weight loss the vaginal microbiota did not significantly change[80]. It would therefore be relevant to also study dietary habits in more detail such as done in the Isala study[5]. However, it is important to note that to correctly uncover the dietary effect in vaginal microbiome studies, more observational and dedicated intervention studies are needed. For example, validated web-based, semi-quantitative simplified food frequency questionnaire (FFQ) based on three 24h dietary recalls could be applied for vaginal microbiota research as now done for the gut[82]. This dietary assessment tool should still be validated in large microbiome population studies.

Other personal lifestyle factors that could impact the vaginal microbiota composition include personal hygiene such as pubic hair grooming[84]. In addition, wearing cloths is typical for humans and not for other mammals, but its role on the vaginal microbiome is currently under-explored, but with a great application potential[85]. Poor quality of menstrual hygiene management has also been reported to impact the vaginal microbiome, with cloth use associated with a more diverse vaginal microbiome composition[86]. In our own Isala cohort, a menstrual cup appeared more beneficial for the *L. crispatus*-module, while pads were associated with an increased alpha diversity. The menstrual pads also significantly reduced the *L. crispatus*-module and increased the *Anaerococcus*-module, especially when used in the last 48h. Wiping the vulva from front to back after a bathroom visit was associated with lower levels of the gut taxa module in the vagina[5]. In addition, smoking can impact the vaginal microbiome[5,87]. This effect seems to be mainly observed as an overgrowth of *Gardnerella* spp. and *Mobiluncus* spp.[87] or the *Gardnerell*a module as defined in our Isala cohort[5].

## TOWARDS INTEGRATED VAGINAL MICROBIOME THERAPIES

An increasing number of diseases are linked to a disturbance of the vaginal microbiome. In addition to vaginal conditions that can be directly linked to an overgrowth of pathogens or pathobionts (see above), an increasing number of other, more general adverse health outcomes are being associated with the constellation of the vaginal microbiome. An important focus of current vaginal microbiome research is put on reproductive health problems such as failure to conceive, miscarriage, preterm rupture of membranes, preterm birth and peripartum infections, as recently reviewed[89-90]. Studies on endometriosis[91-94], gynecological cancers[94-97], and PCOS are also emerging[99-101].

The ultimate dream for every scientist working on the vaginal microbiome is contributing to the development of better therapies. Based on the available evidence on the vaginal microbiome, it seems evident to focus on the supplementation of *Lactobacillus crispatus* strains to women that lack this species. Yet, this strategy is much less obvious than it sounds. First of all, at present, we do not understand enough about the conditions and determinants that promote foreign *L. crispatus* dominance and persistence in a new female host. This is exemplified with a phase 2b trial with 228 women with BV who had completed a course of vaginal metronidazole gel[102]. 152 women were randomized to a treatment with *L. crispatus* CTV-05 (Lactin-V) and 76 to placebo. Recurrence of BV by week 12 was less (30%) in the Lactin-V group compared to placebo (45%), with *L. crispatus* CTV-05 still detected in 48% of participants 13 weeks after the last administration. The protective effect of the treatment was modest, similar to twice-weekly metronidazole and some comments have been raised that the effects are a bit less than hoped for. However, we must bear in mind that the vaginal microbiota is part of a complex female body and physiology, and it will be difficult for single strains to solve everything. Therefore, an important trend is the research on combination therapies that promote the colonization of (endogenous and exogenous) lactobacilli, but also other underlying host factors, such as inflammation. For example, lactoferrin has been

evaluated as a prebiotic (and immunomodulatory agent), recently reviewed by Arthym and Zimecki[103]. Another example to highlight that a single factor will be insufficient is the study with an intravaginal lactic acid gel: it was shown to have a poor BV treatment success[104].

Vaginal microbiota transplants could form the ideal combination therapy taking multiple factors into account, because these mixtures contain a complex vaginal microbial community, including lower abundant taxa that have keystone functions such as some phages and viruses with beneficial roles, immune factors such as cytokines and vitamins. In a recent pilot study, five patients with intractable BV, received a vaginal microbiota transplantation from healthy donors. Four (out of five) patients showed full long-term remission (based on improvement of symptoms, Amsel criteria, microscopic vaginal fluid appearance and reconstitution of a *Lactobacillus*-dominated vaginal microbiome) until the end of follow-up at 5-21 months[105]. Besides this promising result, remission in three patients necessitated repeated VMT, including a donor change in one patient, to elicit a long-standing clinical response. These are encouraging results that need to be validated in larger randomized, placebo-controlled clinical trials. Moreover, it will also be important to consider the partners in these treatments. For example, recent studies[106] have shown that standard BV treatment (metronidazole and imidazole) should be extended to partners of women at high risk of BV, as this can reduce BV recurrence from 50% to 17% within 12 weeks.

Finally, since the first microbes that colonize a newborn originate often from the vaginal microbiota, therapies for infants based on the vaginal microbiota are also explored. Maternal seeding involves transfer of vaginal fluid microbiome from mother to newborn – generally – a cotton gauze, with the potential to reduce the difference in microbiota composition of vaginal versus C-section born babies[107]. Yet, larger randomized controlled trials still need to prove if maternal seeding reduces the risk of C-section birth-associated diseases such as obesity and immune disorders.

## FUTURE PERSPECTIVES AND CONCLUDING REMARKS

The recent insights discussed in this review highlight that the field of vaginal microbiota research is quickly developing by gaining a better ecological understanding of the role of *Lactobacillus* taxa and their interaction with the host and other main vaginal microbiota members in health and disease. However, more mechanistic studies are needed to uncover these microbe-microbe and microbe-host interactions. For instance, it is currently not well understood how and *via* which molecules different strains and species of *Lactobacillus* spp. interact with each other, other residing bacteria and the host. The different molecules involved in such quorum sensing mechanisms (intra-, inter-, and trans-kingdom communication) remain to be largely uncovered. Certainly, advances in experimental approaches such as organ-on-a-chip (Organ Chip) microfluidic culture models of the human vaginal mucosa and multi-omics approaches will help researchers to dissect these complex interactions.

These more mechanistic modelling studies and clinical studies will also have to pay attention to standardizing protocols for analyzing, and including women from different ethnicities and age, to help us better understand the spectrum of vaginal microbiota determinants. For this, investigating the vaginal microbiota in a citizen science project context offers a unique opportunity to reach a broad community. The knowledge on all the determinates will improve the development of microbiome-modulation therapies that range from live biotherapeutic products with single strains or mixtures towards complex vaginal microbiota transfers, including microbiome seeding and combination therapies of probiotics, prebiotics, antibiotics, hormones, and immune-modulating agents. Most of these strategies are under development and still, with most of the studies in their pilot phase: only few of them have reached the clinical RCT phase 2. Yet, clinical solutions are not the only objective of vaginal microbiome research. With our citizen science project, Isala (https://isala.be/en/), we have experienced that it is also important to raise the awareness of the importance of a healthy vaginal microbiome and to empower women with knowledge of their own vaginal health. Disseminating this knowledge and providing women with better insights on which factors have which impact will allow women to take their vaginal health into their own hands.

## Conflict of Interest

SL is a voluntary academic board member of ISAPP (the International Scientific Association on Probiotics and Prebiotics, www.isappscience.org), and chairperson of the scientific advisory board of YUN (yun.be). She has also received research funding from different probiotic companies, but they were not involved in this manuscript. She is an inventor on several patent applications, some related to applications to the vaginal microbiome. Patent application EP20210606.8 (owned by the University of Antwerp and with SA, IS, and SL as inventors). The remaining authors have no conflict of interest to declare.

## Authors' Contribution

Manuscript preparation: SC, SA, EO, JD, CD, LVD, TH, IS, SL. Critical revision: SL.

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## ORCID ID

Sandra Condori: https://orcid.org/0000-0002-9494-6336; Sarah Ahannach: https://orcid.org/0000-0002-8274-5342; Jelle Dillen: https://orcid.org/0000-0003-4160-7645; Caroline Dricot: https://orcid.org/0000-0002-2465-9761; Gehrmann: https://orcid.org/0000-0003-4666-5406; Irina Spacova: https://orcid.org/0000-0003-0562-7489; Sarah Lebeer: https://orcid.org/0000-0002-9400-6918.

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