

Opinion

Diversity in women and their vaginal microbiota

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Women's health is essential to global societal and economic wellbeing, yet health disparities remain prevalent. The vaginal microbiota plays a critical role in health, with research indicating that reduced levels of core bacteria, such as lactobacilli, are associated with conditions like bacterial vaginosis (BV) and increased infection susceptibility. Lower levels of vaginal lactobacilli are reported more frequently in women of African and Latin American descent compared with women of European and Asian descent. However, geographical and other study inclusion and analysis biases influence current research. This opinion highlights the need for a more comprehensive understanding of a 'healthy' vaginal microbiome. It underscores efforts to broaden global research on microbiome diversity in socially relevant contexts, avoiding inappropriate applications of terms such as race and ethnicity.

Women and their vaginal microbes

Western medicine, to this day, assumes an androcentric perspective at the expense of women. Women's bodies, and knowledge concerning their health have been neglected, controlled, and persecuted for centuries [1], resulting in a health disparity that persists today. Despite achieving a higher average age, older women are more frail than age-matched men [2]. This is associated with not only a severe social cost in the quality of life for 49.6% of the world's population, but also with an economic cost [3]. An often-asked question is how to narrow the **gender health gap** (see [Glossary](#)) to achieve these goals. The answer might lie – at least partially – in women's microorganisms. Conditions related to the female reproductive tract are among the most pressing health issues that women face and include noncommunicable gynecological and reproductive conditions and infections [4]. Many of these conditions have been associated with the vaginal microbial community or **microbiota** inhabiting the reproductive tract [5,6], consisting of bacteria, archaea, fungi, and viruses [7]. Some of the best-known early reports from the Western world on the microbes residing in the vagina date from 1892, when Albert and Gustav Döderlein described rod-shaped Gram-positive bacteria in the vagina to be associated with health by using a light microscope. These vaginal bacteria were named the Döderlein vaginal bacilli and were shown to be associated with lactic acid production and antagonistic action against staphylococcal growth [8,9]. With the development of molecular biology, knowledge about the vaginal microbiota composition has extended thanks to techniques such as qPCR, 16S rRNA amplicon sequencing, and metagenomics [10]. For example, a landmark paper by Ravel *et al.* [11] categorized the composition of the vaginal bacterial microbiota in **healthy women** into five vaginal community state types (CSTs) by using 16S amplicon sequencing. CST I was dominated by *Lactobacillus crispatus*, CST II by *Lactobacillus gasseri*, CST III by *Lactobacillus iners*, and CST V by *Lactobacillus jensenii*. A more diverse type, named CST IV, was not dominated by *Lactobacillus*

Highlights

The vaginal microbiome is essential for women's health. Current research in high-income countries points to *Lactobacillus crispatus* as the bacterial member with a reported beneficial role.

A healthy vaginal microbiome is known to generally have lactobacilli, but studies in women of non-European descent point to other bacterial taxa as dominant.

The role of other microbiota members – such as fungi and other eukarya, archaea, and viruses – is largely underexplored in diversity research on the vaginal microbiome.

Uncovering the composition of the vaginal microbiota across a variety of genetic, cultural, environmental, and health conditions is essential to understand the role of the vaginal microbiome in health and disease.

Citizen science has proven to advance women's health research by not only advancing scientific research but also by fostering collaborations and capacity building.

Inclusive microbiome research is still an attention point for the field, but an increasing number of initiatives are being launched.

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spp., but rather by a mix of more facultative or strict anaerobes such as *Gardnerella*, *Atopobium*, *Prevotella*, and *Finegoldia*. This classification has now been widely accepted and applied by the scientific community [12]. Initial drawbacks faced by CST classification, such as small sample size and simplification of the bacterial communities to five CSTs, have been overcome by expanding to seven CSTs and 13 sub-CSTs [13]. Yet, although this CST simplification is highly useful for statistical analyses, even applying this extended CST classification does not capture the full biology and functionality of the **vaginal microbiome**. For instance, CST-determining bacteria can co-exist in similar abundances, as has been reported in the Belgian Isala study where 10.4% of participants displayed a co-dominance of *L. crispatus* and *L. iners*, complicating the classification of these samples as CST I or CST III or a combination of both [14]. In the Isala study, it has been proposed to consider the whole complexity and continuity of the vaginal microbiota composition and functionality as much as possible, also for statistical analyses of lifestyle and life-course associations.

BV is associated with disease, but is not a disease per se

Irrespective of the approach used to categorize the vaginal microbiota (CSTs, microscopy, or other approaches, see further), epidemiological cohort studies and meta-analyses increasingly report on associations between the vaginal microbiota and several clinical conditions. For example, a reduction in lactobacilli has been associated with pregnancy complications, including preterm birth, as reported in a meta-analysis including research articles from 2014 to 2021 [15], urinary tract infections [16], endometritis [17], human papilloma virus (HPV) [18], HIV [19], and other sexually transmitted infections [20] in different cohorts across the world.

A reduction in lactobacilli and overgrowth of anaerobic bacteria is generally considered to be the non-optimal vaginal microbiota condition termed BV. The term BV has been used long before the advent of molecular diagnostic approaches and is currently under reconsideration since not all women with BV suffer from symptoms. Therefore, it is crucial to highlight that BV is not a disease and needs to be carefully evaluated in the correct context, taking aspects such as biogeographical bias into account, as we discuss further later. A BV diagnosis is generally based on the Nugent score [21] or Amsel criteria [22]. Both methods are widely used in high-income countries and in low- and middle-income countries (LMICs) highlighted in a previous meta-analysis [23]. The Nugent score is based on Gram staining, the identification of Gram-positive large bacillus-shaped bacteria (*Lactobacillus* spp. morphotypes), Gram-variable rods (known as *Gardnerella* spp. morphotypes), and curved Gram-variable rods (known as *Mobiluncus* spp. morphotypes). Based on the proportions of these morphotypes, a score that ranges from 0 to 10 is calculated. Since no symptoms are included in the Nugent score, it is possible to be positive for BV (score = 7–10), even though typical BV symptoms like abnormal discharge or odor are absent. This is, in fact, frequent and known as asymptomatic BV, which is also associated with an increased risk of adverse health outcomes [24,25]. The Amsel criteria might provide a more clinical diagnosis of BV because they are based on the following four signs: vaginal fluid pH above 4.5, positive whiff test (foul odor after adding 10% potassium hydroxide – KOH), presence of **clue cells**, and abnormal vaginal discharge. At least three of these signs must be present before BV is diagnosed [22]. Both approaches have their pitfalls; for instance, although lactobacilli are Gram-positive bacteria, *L. iners* has often been the cause of ambiguity in Gram-staining due to its thinner cell wall [26]. This can result in BV over-diagnosis by the Nugent method. Currently, there is no gold standard across the world for BV diagnosis, and molecular approaches are also increasingly applied. If women go to their general practitioner or gynecologist with symptoms, the standard treatment for BV is metronidazole or clindamycin vaginally or orally [27]. These treatments often lack sustained efficacy, with up to 60% of BV recurrence rate after treatment [28]. In addition, care should be taken with preventive strategies using BV-targeting antibiotics. For example, in a large meta-analysis of 13 studies

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(6915 participants) providing individual patient data, antibiotic treatment of BV to prevent preterm birth failed to show any benefit for metronidazole or clindamycin [29].

A new strategy to prevent and treat BV-associated symptoms and diseases could originate from directly modulating the vaginal microbiome, by, for example, vaginal microbiota transfers [30] and vaginal **live biotherapeutic products** [31]. These microbiota-based strategies are currently focused on *L. crispatus*. Indeed, a clear picture that has emerged from the variety of epidemiological studies on vaginal microbes and diseases – for instance, on HPV [32] and HIV [33], as well as functional and mechanistic studies in the laboratory – is that *L. crispatus* plays a key protective role in the vagina [34–38]. However, not all *L. crispatus* strains or clones appear to be protective since some women dominated with *L. crispatus* can still experience conditions such as vaginal cytotoxicity [39] or *Candida* infections [40,41]. In addition, the fact that *L. crispatus* is generally protective does not imply that women without *L. crispatus* are always more prone to health risks. In addition, an important consideration is that most epidemiological and cohort studies done so far have included mostly women of European ancestry, with less participation of women from other parts of the world, as we now discuss in more detail.

Vaginal microbiome research disparity across the globe

Efforts have been made to include women of reproductive age from different ethnic backgrounds by including cohorts of women with various ancestries living in high-income countries [42–45]. In those studies, a lack of lactobacilli and an increase in BV are reported to occur more often in self-identified Black and Latin American women compared with women of Asian or European descent living in the USA. Importantly, besides sociodemographics, these studies generally do not include the geographical history, birth country, cultural and dietary lifestyle, and socioeconomic status of the participants, which could all confound these associations [46]. For example, Anukam *et al.* [47] reported that healthy Black African Nigerian women without BV ($n = 13$) had *Lactobacillus* genera as the predominant microbiota. Another study with 62 women in South Africa reported that non-*Lactobacillus* taxa were most prevalent, but HPV and high-risk HPV infections were detected in 37.1% and 29.0% of the women respectively [48]. This is an important consideration, as a lot of vaginal microbiome research has so far focused on populations with increased risks for sexually transmitted infections (STIs), for example, in Africa [49–52]. It thus remains to be substantiated that women with different geographical backgrounds and ethnicity have indeed genuinely a different microbiota composition in the vagina in healthy conditions. This also includes a need for more studies in other countries across the globe, accounting for the plethora of lifestyle and life-course factors as well as genetics.

It is a general trend for all microbiome studies that LMICs are under-represented [53]. This is also clear from our recent search of the latest studies (2019–2024) investigating the vaginal microbiota using 16S rRNA amplicon sequencing (Figure 1), a widely used, cost-effective technique compared with shotgun sequencing. As in many other fields, increasing diversity in vaginal microbiome research is a real challenge since research infrastructure, technical and financial capacities are skewed towards high-income countries [50,53]; for instance, the costs of consumables for DNA extraction and sequencing are generally higher in LMICs. Metadata acquisition (detailed surveys), sample collection, and detailed microbiome analyses also come with the need for special recruitment, trained personnel, data processing, and security challenges [54,55]. To overcome these material challenges, LMIC researchers could participate in international collaborations, even though they possess the technical capacity to conduct the research independently. To prevent an imbalanced relationship – in which Western researchers extract material, data, and publication coauthorships from LMICs – policymakers can require the adherence to CARE (Collective Benefit, Authority to Control, Responsibility, and Ethics) principles [56],

Glossary

Citizen science: research conducted with the participation of citizens (general public) who can provide data (lower involvement) or participate in problem formulation, research priorities, data collection, data analysis, interpretation and result dissemination (high involvement).

Clue cells: vaginal epithelial cells covered in a large number of bacteria. When observed under the microscope, these cells display a stippled appearance.

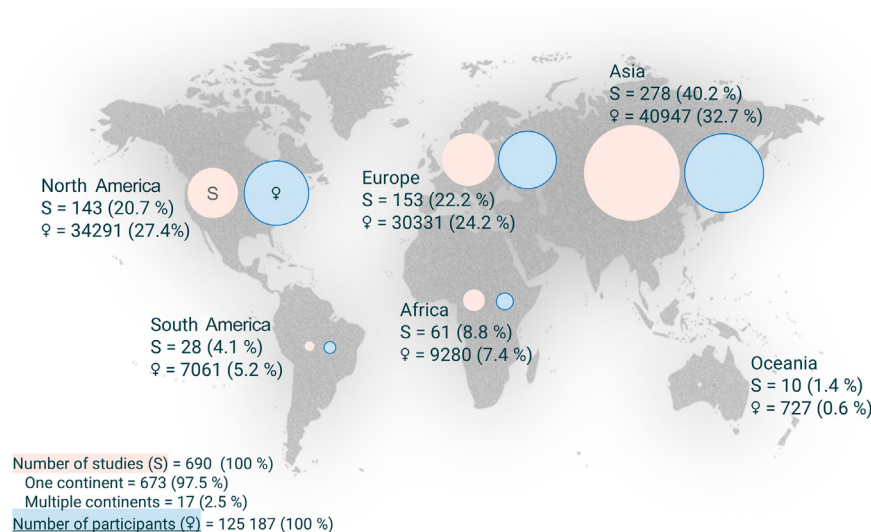
Gender health gap: the differences in health and wellbeing outcomes between men and women throughout life. In particular, the disparities that arise from the institutional and societal sexism that exists in private healthcare organizations, public healthcare institutions, regional, national, supranational and international legislation, academic research, research funding organizations, and society at large.

Healthy women: defining health is notoriously complex, also considering the WHO definition of health. In this opinion, we refer to women without vaginal complaints at the moment of donating vaginal swabs.

Live biotherapeutic products: therapeutic agents that use live microorganisms to treat or prevent disease. The FDA has adopted this category for a biological product that: (i) contains live organisms, such as bacteria; (ii) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and (iii) is not a vaccine.

Microbiota: an assembly of microorganisms belonging to different kingdoms [Prokaryotes (Bacteria, Archaea), Eukaryotes (e.g., Protozoa, Fungi, and Algae)].

Vaginal dysbiosis: literally, an imbalance in vaginal ecology. It refers specifically to a loss of *Lactobacillus* dominance in the vaginal microbiome, and an increase in bacterial diversity. However, the term dysbiosis is reductive, and presumes a specific set of microbiome compositions as acceptable or healthy, while compositions deemed to be ‘dysbiotic’ may, in other contexts, be viable for health.



Vaginal microbiome: in this opinion we adopt the microbiome definition of Berg *et al.* [83] which refers to the comprehensive set of material that can be collected from the vagina, including from the microorganisms themselves, the host, and the environment. This includes genomic, epigenetic, transcriptomic, proteomic, metabolomic, and conditional data that characterize the environment. It may be summarized as the total 'theater of activity' of the environment in which microbiota are found.

Trends in Microbiology

Figure 1. World distribution of the vaginal microbiome data studies using 16S rRNA amplicon sequencing (2019–2024). The total number of studies (light pink circles, S) and participants (light blue closed circles, ♀) are shown per continent; studies spanning multiple continents ($n = 17$, 2.5%) are not part of the counts per continent. The size of the circles is proportional to the percentage of studies (690 in total) or participants (125 187) in each continent, from January 2019 until September 2024. An initial extraction from PubMed using advanced search criteria^a retrieved 1177 studies, and after manual curation, only 690 studies were considered (systematic reviews, meta-analysis, studies based solely in re-analysis of data already published, or studies in animal subjects were excluded). When counting the total number of participants, we took care not to double count samples/participants (when used in more than one study). To the best of our knowledge, none are present. When defining the continent of transcontinental countries, the location of the participant's recruitment site was considered. ^aAdvanced PubMed search: ((([all[sb] NOT(animals [mh] NOT humans [mh])) AND (microbiota [mh] OR microbiota[Title/Abstract] OR microbiome[Title/Abstract])) AND (female genitalia [MeSH Terms] OR vagina[Title/Abstract] OR women[Title/Abstract] OR pregnancy[Title/Abstract] OR fertility[Title/Abstract] OR menstruation[Title/Abstract] OR menopause[Title/Abstract])) AND (('2019/01/01'[Date – Publication]: '2024/09/21'[Date – Publication])) NOT (Review[Publication Type])).

and promote capacity building in omics science (both wet-lab and *in silico*), especially in under-represented populations, to empower local researchers and foster indigenous data and research sovereignty [57].

Initiatives to increase global representation in the vaginal microbiome field

An increasing number of initiatives aim to close the (vaginal) microbiome data gap across the world – starting with initiatives such as the Vaginal Human Microbiome Project (VaHMP) datasets which map data on the vaginal microbiota (16S sequencing data) of women from different ethnic backgrounds living in the USA [58]. The VIRGO database [59] also originates from the USA and combines metagenomic, transcriptomic, and actual bacterial genome isolates. It now has data from six countries across different continents (America, Africa, Europe, Asia, and Oceania) in its most recent version (Ravel *et al.* in preparation). Another recent initiative is the Vaginal Microbial Genome Collection (VMGC), which contains metagenomic data from 14 countries (USA, China, South Africa, France, Fiji, Canada, Ireland, Italy, Denmark, Israel, Kenya, Sweden, Luxembourg, and New Zealand) [60]. They found that the mapping rate of vaginal metagenomes against the vaginal microbial gene and genome catalogs was higher in samples from Europe or North America than in those from China, Fiji, or South Africa, suggesting that these latter regions were less well represented. Another important initiative that has helped the field to move forward to include more diverse vaginal microbiota profiles is the Vaginal Microbiome Research

Consortiumⁱ. It has a specific dedicated part for Africa (VMRC4Africa Consortium)ⁱⁱ and Bangladesh (Asia), and it is partially sponsored by the Gates Foundation. The VMRC4Africa focuses on identifying and correlating the stability and resilience in the vaginal microbiota to further leverage these features to set up a framework for maintaining or restoring vaginal health. Initiatives such as the African Microbiome in Health and Disease Symposium organized by VMRC4Africa in October 2024 will help to promote more inclusive research across this continent. Hereby, it is important to highlight that other LMICs worldwide in Asia, Central and South America, and even Europe, should also not be left behind.

An interesting approach to involve more research on the vaginal microbiome across the globe in a bottom-up, locally-led approach is via **citizen science**. For example, our Isala project on the vaginal microbiome in Belgium managed to motivate a large number of participants ($n = 3345$), contributing to establishing a Western European reference map of the vaginal microbiome (Box 1) [14]. Since its launch, the citizen science framework and communication strategy of Isala has been extended to a global partnering network with researchers from various continents (America, Africa, Asia, and Europe), giving life to an Isala sisterhood, which aims to inspire research on women's health and microbiomes worldwide [61].

The Isala Sisterhood's mission includes advising other research groups to start their own citizen-science studies on women's health and build capacities (if needed) at the individual and institutional level. The sisterhood acknowledges that researchers and citizens across the world have invaluable diverse knowledge on various aspects of the vaginal microbiome and lifestyle, which benefit the whole Isala Sisterhood to better understand the underlying biology of the vaginal microbiome. Isala actively creates an ecosystem where research teams in various countries can conduct research independently and collaboratively through sharing their best practices (wet lab and *in silico* research, communication approaches, logistics, lifestyle insights, etc.), facilitating international mobility of young researchers, and ultimately supporting each other in closing the health equity gap. For example, in February 2023, researchers from the Uganda Isala sister project traveled to the Peruvian Isala sister project in rural communities in Lima and the remote Amazon Forest to exchange citizen-science practices concerning local cultures^v. Another example is the international collaboration between Isala in Belgium and the research teams from the sister projects in Cameroon, Peru, and Switzerland, to study the impact of menstrual products on the vaginal microbial ecosystem^{vi}. The Isala Sisterhood forms the umbrella for all these sustainable collaborations by cordially practicing Open Science, CARE and FAIR (Findable, Accessible, Interoperable, Reusable) data best practices [62], the Nagoya protocol [63], and the SAGER guidelines on Sex and Gender Equity in Research [64]. For science to fulfill the many societal needs of women's health, which are both complex and interconnected, the sisterhood actively practices transdisciplinary involvements and develops new technologies to understand women's health better. The Isala sisterhood aims

Box 1. The Isala Sisterhood project

Isala is a global citizen-science study on the female microbiomeⁱⁱⁱ. It was named after Isala Van Diest (1842–1916), the first female physician in Belgium, where the project originated. The project has now expanded to different countries and geographical areas across the world, referred to as 'The Isala Sisterhood'. Isala was an activist, feminist, and role model for many in Belgium (and beyond). Every sisterhood project has a uniquely chosen name honoring another role model^{iv}. Isala sisterhood projects are driven by multidisciplinary teams including microbiologists, gynecologists, primary care physicians, and other healthcare workers, molecular biologists, bioinformaticians, biostatisticians, communication experts, governmental organizations related to women's reproductive and sexual health. Since its launch, Isala has developed into an international sisterhood, with citizen-science studies already rolling in Peru (Laura), Switzerland (Marie), Cameroon (Leke), Nigeria (Dora), Spain (Manuela), USA (Bess), France (Madeleine), Hawaii (Aiona), Argentina (Cecilia), Anna (Poland), and more being planned (South-Africa, Morocco, Venezuela, Puerto Rico, Singapore, Ghana, Canada, India, Kenya, Uganda, UK, Ethiopia, Chile, Uruguay).

to bring its high-quality and robust research results together to help us to ultimately tackle – at least partly – the Sustainable Development Goals for 2030.

The challenges that remain: ethnicity, race, and the vaginal microbiome

The envisaged globalization and diversification of vaginal microbiome research, however, is not without its own set of challenges. A critical question remains on which approaches can be employed to examine the discrepancies between and within populations. It is common to see articles using the term ‘race’ or ‘ethnicity’ in studies of the vaginal microbiome to explain observed microbiological differences. However, scholars have repeatedly warned against the naive use of race as a category when discussing human health [65–67], particularly concerning the microbiome [68–70]. The history of ‘race’ or ‘ethnicities’ is intrinsically linked with social and political struggles for domination, which have shaped social and health inequalities for centuries around the world [71]. Racial categories are always blurred and more socially constructed than based on biological differences [72]. For example, many individuals in the USA would be categorized (either by themselves, or by others) as African American even if half or more of their ancestors were white European [67]. Additionally, even within a single African lineage, there appears more genetic variation than among European lineages [73]. Furthermore, the study of epigenetic markers illustrates how inequities are embodied as a consequence of racialized and economic injustices [74]. In the case of the postulated racial differences in the vaginal microbiome between women with African ancestry and white women in the USA introduced earlier, racialized hygiene behavior has, for example, been linked to the social category of ‘race’, including vaginal douching, which is a major risk factor for **vaginal dysbiosis** [75]. Therefore, disparities in BV and the vaginal microbiome, more generally, cannot be completely reduced to biology. Nevertheless, we cannot exclude that both potential biological as well as socially constructed differences can lead to health disparities. We thus invite the microbiome research community to critically reflect on the practice of delineating ‘race’ and ‘ethnicity’ in their studies. From a public health perspective, it is important to investigate if a particular population is at higher risk to elaborate tailored preventive measures. This investigation should focus on the actual environmental, socioeconomic, and healthcare disparities that place specific populations at higher risk rather than relying solely on race as a genetic or biological proxy. Therefore, while accounting for population structures, through methods such as those applied in genome-wide association studies [76–78] is a step forward, it is insufficient for a diversity analysis as it fails to account for the societal, cultural, and environmental effects, which can be variable, even within closely related individuals. As a way forward, we must consider a multidisciplinary approach, together with social scientists and anthropologists, as key for this type of work. Gathering data from diverse populations is thus important, but this diversity should be adequately considered. Only recently, *The Lancet* launched a guideline for reporting race and ethnicity to avoid continuing to increase and perpetuate disparity when studying one population group and claiming that the results represent the diversity of the global human population [79].

Concluding remarks

To promote better preventive, diagnostic, and therapeutic strategies for women affected by conditions associated with the vaginal microbiota, more research on the functions and diversity of the vaginal microbiota is urgently needed in different parts of the world, using metagenomics and multi-omics analysis. We are still understanding which bacteria can be considered pathogens and which are more protective. For instance, some researchers consider *Gardnerella vaginalis* (newly classified as *Bifidobacterium vaginale*) and *Prevotella* species as vaginal pathogens [80]. However, according to sequencing data, some *Gardnerella* species are highly prevalent in women with Amsel-negative diagnosis [81], and a considerable portion of healthy women do not display large abundances of *Lactobacillus* species. The continuous portrayal of lactobacilli as a gold standard for optimal vaginal health needs to be carefully (re)evaluated with unbiased

Outstanding questions

What is a healthy vaginal microbiome?

How many variations of a healthy vaginal microbiome exist?

Why are lactobacilli dominant?

Is *L. crispatus* relevant for all women or only for women from Western countries?

Which bacterial species are associated with health in women in different locations?

What are the dynamics of the vaginal microbiome related to the host and various environmental and lifestyle factors?

global diversity perspectives (see [Outstanding questions](#)). However, microbiome analysis costs are still high. Therefore, fair and equitable collaborations and capacity building are also key to increase the diversity in vaginal microbiome research. This way, we can better understand what a healthy vaginal microbiome looks like in each geographical location [82]. Finally, addressing biases and enhancing representation in vaginal microbiome research is crucial for developing robust, inclusive health interventions. Future studies should integrate diverse geographical and socioeconomic groups, consider sociocultural factors, and employ comprehensive and transdisciplinary methodologies to understand the vaginal microbiome's complexity and its implications for women's health globally.

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Name to be defined: South Africa.

Declaration of interests

S.C.C. has received travel support from Copan to participate in two scientific conferences to present the Isala Sisterhood. S.A. is a voluntary academic board member of the International Scientific Association on Probiotics and Prebiotics, Students, and Fellows Association (<https://www.isapp-sfa.com>). S.L. and G.V. are academic board members of the International Scientific Association on Probiotics and Prebiotics (www.isappscience.org), but this organization was not involved in this opinion article. M.C.C., S.L. and G.V. have received travel grants from ISAPP to participate in meetings. As a principal investigator (PI), S.L. has received research funding from several companies not involved in this opinion. She has been a scientific advisor for YUN and is now advising for Freya Biosciences. G.D. is the chairperson of Femicare vzw (<https://www.facebook.com/profile.php?id=100063440664962>) and has worked as a medical consultant for various industries. None of these organizations or companies was involved in the present opinion article. M.T., J.K., K.C.A., V.P.C., M.G.D.B., C.M., S.M., and T.G. declare no competing interests.

Resources

ⁱ<https://vmrc4health.org>

ⁱⁱ<https://vmrc4africa.org>

ⁱⁱⁱ<https://isala.be/en>

^{iv}<https://isala.be/en/studies>

^v<https://isala.be/en/isala-and-her-sisters-two-british-midwives-in-peru>

^{vi}<https://snis.ch/projects/the-impact-of-menstrual-health-management-on-the-vaginal-microbiome-linking-transdisciplinary-health-science-and-policy-to-improve-safety-and-hygiene-for-the-benefit-of-womens-health>

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