Medical Mycology, 2026, **64**(4), myag026
https://doi.org/10.1093/mmy/myag026
Published: 25 March 2026
Original Article

Characterization of the vaginal microbiome and its metabolic potential in Colombian patients with recurrent vulvovaginal candidiasis

Jeiser Marcelo Consuegra-Asprilla, Yesid Cuesta-Astroz, and Ángel González*

Basic and Applied Microbiology Research Group (MICROBA), School of Microbiology, Universidad de Antioquia, Medellín 050010, Colombia
*To whom correspondence should be addressed. Angel Gonzalez, MSc, PhD. School of Microbiology, Universidad de Antioquia; Calle 67 No. 53-108; Of: 5-103, Medellín, Colombia. Tel: +57-604 219-5489; Fax: +57-604 219-8494; E-mail: angel.gonzalez@udea.edu.co

Abstract

Recurrent vulvovaginal candidiasis (RVVC) is a multifactorial condition in which vaginal microbiota dysbiosis plays a key role. This study aimed to characterize the vaginal microbiome of patients with RVVC using metagenomic sequencing. Vaginal scraping samples were collected from 34 women aged 20–47 years and classified into three groups: (1) 14 women with RVVC who had experienced 3–7 episodes of VVC in the previous year; (2) 9 women with severe RVVC, defined as ≥8 episodes in the last year; and (3) 11 healthy women as controls. The results revealed an increased relative abundance of bacteria associated with bacterial vaginosis—including *Gardnerella vaginalis*, *G. swidsinskii*, and *Prevotella bivia*—as well as higher levels of *Lactobacillus iners* in both RVVC groups. In contrast, healthy women showed a greater abundance of *L. crispatus* and *L. gasseri*. Diversity analyses indicated lower α-diversity in the healthy group compared to RVVC patients. Metabolic potential profiling showed a differential increase in sequences related to the phosphotransferase system, fructose/mannose metabolism, pentose phosphate pathway, and cysteine/methionine and purine metabolism in RVVC groups relative to controls; no significant differences were observed between RVVC groups, indicating that microbial profiles alone do not correlate with the degree of disease severity. These findings provide relevant insights into the taxonomic and functional characteristics of the vaginal microbiome in women with RVVC and may support the development of targeted therapeutic strategies.

Lay summary

Women with recurrent candidiasis show changes in their vaginal microbiome, with fewer protective bacteria and an increase in other microorganisms associated with imbalance. These findings show an association between microbiome alterations and recurrent infections and may help identify factors linked to recurrence.

Keywords vulvovaginal candidiasis, RVVC, *Candida albicans*, microbiome, metagenomic, shotgun sequencing

Introduction

Vulvovaginal candidiasis (VVC) is the second most common cause of vaginitis worldwide, affecting approximately 75% of the female population at least once in their lifetime.[1] It is estimated that approximately 10% of women experience a phenomenon known as recurrent vulvovaginal candidiasis (RVVC), which is defined as three or more episodes of VVC in 1 year.[2] Both VVC and RVVC are considered multifactorial conditions, and their presentation has been related to factors such as pregnancy, alterations in the innate or adaptive immune response; use of oral contraceptives; use of broad-spectrum antibiotics or antifungals; corticosteroid therapy; individual behavioral factors; immunosuppression due to human immunodeficiency virus (HIV), diabetes; as well as dysbiosis of the vaginal microbiota.[3,4] In this sense, observational studies suggest that variations in the vaginal microbiota are linked to VVC/RVVC since some species of the genus *Lactobacillus* spp. have a protective role at the vaginal mucosa, due to the acidification of the medium by the production of lactic acid, hydrogen peroxide (H_2O_2) and biosurfactants, which inhibit the proliferation of *Candida* spp. and therefore, prevent the development of VVC.[5] Similarly, it has been described that a decrease in the number of *Lactobacillus* spp. at the vaginal environment is associated with the proliferation of pathogenic bacteria such as *Gardnerella vaginalis* and *Prevotella* spp. as well as with an increase in the *in situ* production of proinflammatory cytokines such as IL-1β and IL-12.[6]

Received: 26 January 2026. **Revised:** 19 March 2026. **Accepted:** 20 March 2026

On the other hand, previous studies examining the vaginal microbiota in patients with VVC have reported that, compared with healthy women, VVC is often associated with lower relative abundances of *L. crispatus* and higher relative abundances of *L. iners*,[7] although these patterns are not observed in all cases. However, most of these studies have been conducted using 16S rRNA-based sequencing techniques,[8–11] which have limited accuracy in species-level annotation compared to metagenomic sequencing, since the latter allows a more robust species-level characterization in each microbiome.[12–14] Nevertheless, recently, full-length 16S rRNA gene amplicon sequencing using nanopore-based methods has been shown to achieve species–level classification in gut microbiota context and capture broader taxonomic diversity than short partial amplicons alone. However, even with these improvements, partial 16S amplicon approaches targeting single or paired hypervariable regions remain limited in reliably distinguishing closely related species due to the constrained sequence information provided by short reads; whereas shotgun metagenomic sequencing directly samples genomic sequences from across the entire microbial genomes, enabling more accurate and comprehensive species–level taxonomic profiles as well as functional potential analyses.[15] Therefore, in the context of RVVC, more evidence is needed to better characterize the association between vaginal microbiome composition and RVVC. Thus, the objective of this study was to characterize the vaginal microbiome in healthy women and patients diagnosed with RVVC using metagenomic sequencing techniques.

Materials and methods

Ethical aspects

This study was approved by the Bioethics Committee for Human Research (Record No. 21-28-935) of the University Research Center (SIU) of the Universidad de Antioquia.

Population and type of study

A descriptive cross-sectional study was conducted from September 2022 to October 2023, which included samples from 34 Colombian women between 20 and 47 years of age, these samples were obtained in a previous study conducted by our research group.[16] The samples were divided into three groups: Group (1) 11 healthy women (control group), corresponded to women without diagnosis, symptoms or clinical history of vulvovaginal candidiasis or bacterial vaginosis at the time of sampling; Group (2) 14 women diagnosed with RVVC who at the time of sample collection had presented between 3 and 7 episodes of VVC in the last year; and Group (3) 9 women diagnosed with RVVC who presented $\geq$8 episodes of VVC in the last year (severe RVVC). The following exclusion criteria were considered: being pregnant; being under treatment with antibiotics, antifungals, or corticosteroids in the 7 days prior to sample collection; being under hormone replacement therapy or having autoimmune diseases or other underlying diseases such as HIV infection, diabetes, cancer, among others. Additionally, those patients who met the study's inclusion criteria signed an informed consent form for subsequent sample collection.

Sampling, DNA extraction, and genomic sequencing

Vaginal scraping samples were obtained from all women participating in the study by scraping them with a cytobrush using a sterile speculum. Subsequently, the samples underwent metagenomic DNA extraction using the ZymoBIOMICS™ DNA Miniprep kit and following the manufacturer's instructions. Additionally, the concentration, quality, and integrity of the DNA samples were evaluated using Nanodrop and agarose gel electrophoresis, respectively. DNA libraries were prepared by mechanical DNA fragmentation using the Covaris system. Genomic libraries were constructed from this material, a process that included DNA end repair, the addition of specific adapters, and fragment size purification, followed by PCR amplification using KAPA HiFi HotStart DNA Polymerase. Prior to sequencing, the fragment lengths of each library were verified using the Agilent 2100 system to ensure quality. Finally, DNA nanoball (DNB) sequencing was performed using the DNBSEQ-G400 platform (BGI, Shenzhen, China). These DNBs were sequenced using a 150-base-paired read configuration (PE150). The metagenomics sequence data were deposited in the SRA database (PRJNA1356845). Link: https://www.ncbi.nlm.nih.gov/sra/PRJNA1356845.

Quality control of readings and metagenomic analysis

The raw data obtained in FASTQ format were passed through a bioinformatics quality filter. The SOAPnuke tool was used.[17] SOAPnuke software filter parameters: '-n 0.001 -l 20 -q 0.5 –adaMis 3 –minReadLen 150'; the filtering process included the following steps: (i) filter adapter: if the sequencing read matched 50.0% or more of the adapter sequence (maximum mismatches of three bases are allowed), the entire read was removed; (ii) filter read length: if the sequencing read length was less than 150 bp, the entire read was discarded; (iii) remove *N*: if the *N* content in the sequencing read represented 0.1% or more of the entire read, the entire read was removed; (iv) low-quality data filter: if bases with a quality score lower than 20 in the sequencing read accounted for 50.0% or more of the entire read, the entire read was discarded; and (v) obtaining clean reads: the output read quality score system was set to Phred+33.

In order to remove reads from the human host, the Bowtie2 version 2.5.4 tool was used to map the reads against the human reference genome GRCh38. This process of removing reads from the host was complemented using the Kraken2 version 2.1.3 program through the script (https://github.com/jenniferlu717/KrakenTools?tab=readmeovfile#extract_kraken_readspy). The percentages of microbial readings before and after filtration are summarized in Supplementary Table 1.

To determine the taxonomic composition of each sample, the Kraken2 tool version 2.1.3 was used, which compares the reads obtained by sequencing against the PLUSPF database. Subsequently, to obtain a more accurate estimate of the abundance of each species and correct for potential biases in the initial assignment, the Kraken2 results were refined using the Bracken tool (version 2.9).[18] Additionally, to quantify alpha diversity within each sample, the abundance data were normalized using the cumulative sum scaling methodology with the metagenomeSeq v1.48

package.[19] With the normalized data, alpha diversity indices, such as community structure indices and the Shannon index, were calculated using the vegan v2.6.10 package of R version 4.5.0. Additionally, to evaluate the dissimilarity in the taxonomic composition between samples (beta diversity), distance matrices were calculated using Bray–Curtis. The statistical significance of differences in microbial composition among samples was assessed using permutational multivariate analysis of variance (PERMANOVA) using the adonis function from the vegan package v2.6.10 in R version 4.5.0. Principal component analysis (PCoA) was performed based on Bray–Curtis distances.

Additionally, co-occurrence analyses were performed using Spearman's correlation coefficient, taking into account ($|r_s| > 0.6$, P-value $< .05$) and using the following packages of the R version 4.5.0 program: tidyverse, for the manipulation and visualization of the databases used; igraph, to build and analyze the correlation networks between microbial taxa; Hmisc, which was used to calculate Spearman's correlation coefficients and their respective statistical significance values using the rcorr function; reshape2, which was used to organize data matrices that could be subsequently graphed using the ggraph package.

To perform a functional analysis of the vaginal microbiome in each group, contigs were reconstructed from high-quality reads of each sample using the MEGAHIT tool.[20] The quality of each assembly was assessed using the QUAST tool.[21] From these contigs, protein-coding genes were predicted using the Prodigal tool in its metagenomic mode.[22] To assign a metabolic function to each gene, protein sequences were annotated with eggNOG-mapper v2 and compared with the eggNOG v5.0 database by alignment with DIAMOND.[23] Finally, all statistical analyses were performed using R version 4.5.0 and GraphPad Prism version 9.0, considering P-values $\leq .05$.

Results

The abundance of microbial genera that shape the vaginal microbiome differs between healthy women and women with RVVC

From metagenomic analyses of the vaginal microbiome, differences in the abundance of bacterial genera were observed among the three groups evaluated. In this regard, the *Lactobacillus* genus was the most abundant in all three groups, with an average abundance percentage of 70.33% (96.66% in the healthy group, 53.39% in the RVVC group, and 62.50% in the severe RVVC group). In addition to *Lactobacillus*, the 10 most abundant genera included *Gardnerella* (8.97%), *Prevotella* (4.58%), *Aerococcus* (1.55%), *Fannyhessea* (1.14%), *Candida* (1.13%), *Ureaplasma* (0.37%), *Sneathia* (0.28%), *Metamycoplasma* (0.27%), and *Bifidobacterium* (0.085%) (Fig. 1).

At the species level, the analyses revealed that the most abundant species in the group of healthy women was *L. crispatus* (80.17%), unlike the groups of women with RVVC, where the most abundant species was *L. iners*, with an average relative abundance of 52.16%. Furthermore, compared to the group of healthy women, a significant increase in the abundance of bacterial species such as *G. vaginalis* (10.15%), *G. swidsinskii* (4.5%), and *P. bivia* (11.61%) was observed in the groups of patients with RVVC

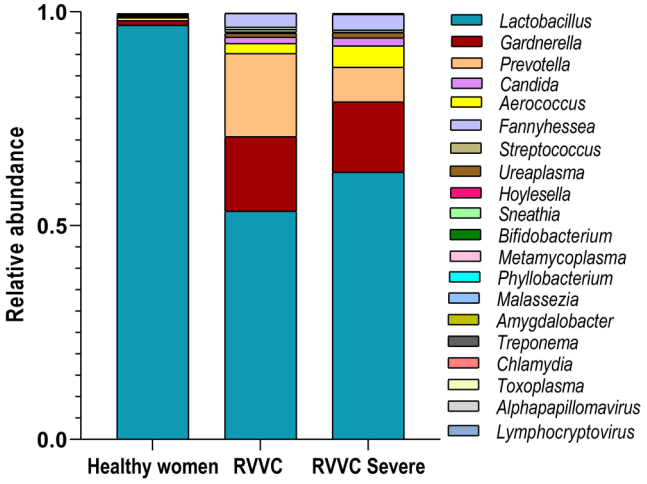

Figure 1 Composition of the vaginal microbiome of healthy women and women with RVVC, at the genus level. Thirty-four samples were evaluated, divided into three groups: Group 1: healthy women ($n = 11$, control group); Group 2: women diagnosed with RVVC ($n = 14$) who had experienced 3–7 VVC episodes in the past year; Group 3: women diagnosed with RVVC ($n = 9$) who had experienced ≥8 VVC episodes in the past year (severe RVVC).

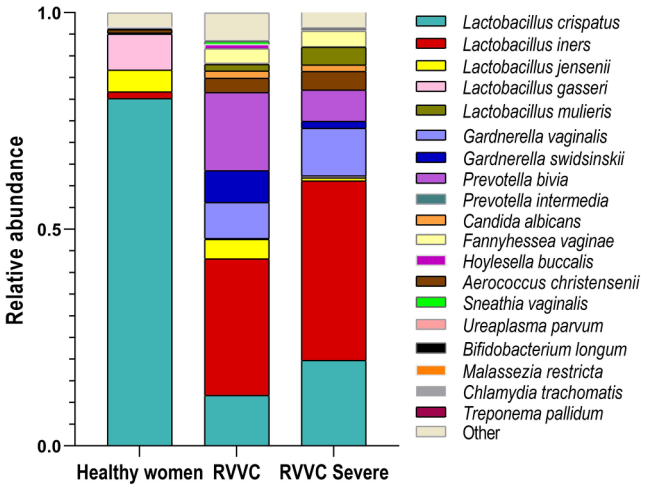

Figure 2 Composition of the vaginal microbiome of healthy women and women with RVVC, at the species level. Thirty-four samples were evaluated, divided into three groups: Group 1: healthy women ($n = 11$, control group); Group 2: women diagnosed with RVVC ($n = 14$) who had experienced 3–7 VVC episodes in the past year; Group 3: women diagnosed with RVVC ($n = 9$) who had experienced ≥8 VVC episodes in the past year (severe RVVC).

(Figs 2 and 3). At the species level, no significant differences in the bacterial composition were observed between the two groups of patients with RVVC.

Considering the importance of *Lactobacillus* in the vaginal microbiota, species-specific analyses of this genus were performed. These analyses showed that, in addition to differences in the relative abundances of *L. crispatus* and *L. iners*between healthy women and patient groups, the groups of women with RVVC presented a significant decrease in the abundance of *L. gasseri* in comparison with healthy group (Fig. 3). No differences in abundance were observed among the three groups of

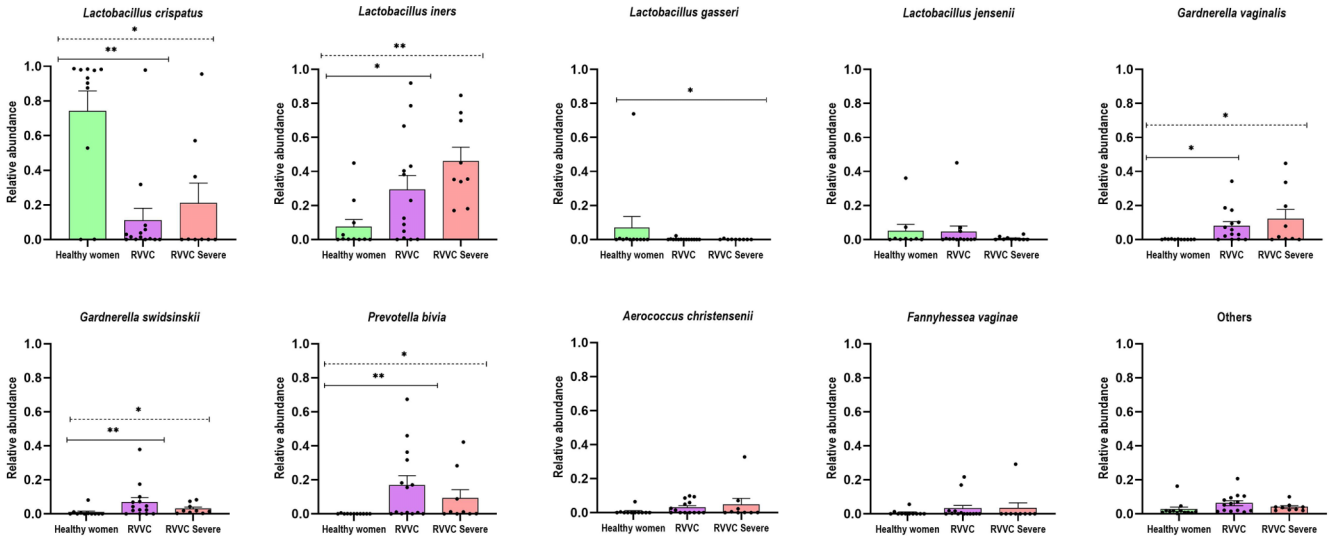

Figure 3 Comparison of the relative abundance of the most representative bacterial species in the groups evaluated. The results indicate significant differences in the relative abundance of some bacteria between the different groups tested. Results are expressed as mean ± standard error (SEM). $*P$-value $< .05$; $**P$-value $< .001$.

women studied for the other *Lactobacillus* species evaluated (Supplementary Fig. 1).

Patients with RVVC have greater α-diversity compared to healthy women

α-Diversity analyses were performed by calculating the Shannon–Wiener index. These analyses demonstrated that the α-diversity of the vaginal microbiome in the groups of women with RVVC and severe RVVC was significantly higher than that of the healthy women (P-value = .0052 and P-value = .016, respectively). However, no significant differences were observed between the RVVC and severe RVVC groups (P-value = .254) (Supplementary Fig.S2). In addition to the above, the effects size was calculated using the rank-biserial correlation coefficient (r(rb)); these analyses revealed large effect sizes for comparisons between healthy women and RVVC (r(rb) = −0.87) and between healthy women and severe RVVC (r(rb) = 0.73), while the comparison between RVVC and severe RVVC showed a small effect size (r(rb) = −0.04). Additionally, a PCoA based on Bray–Curtis distances by Adonis/PERMANOVA was performed to evaluate β-diversity at the level of the vaginal microbiome. In this analysis, the first principal component explained 44.4% of the variation, and the second principal component explained 25.8% of it. From the PCoA, it was determined that no differential clustering patterns were observed between the groups (Supplementary Fig. 3).

Analysis of the interactions between *Candida albicans* and the main microorganisms that shape the vaginal microbiota in patients with RVVC

Based on the clustering patterns obtained, three co-occurrence network analyses were performed, considering significant correlations between species ($|r_s| > 0.6$, P-value $< .05$). The characteristics of the interactions varied in each of the groups evaluated. Regarding the group of patients with RVVC and severe RVVC, a negative correlation was observed between *C. albicans* and *L. crispatus*. Additionally, in the three groups evaluated, a positive correlation pattern was observed between *C. albicans* and *L. iners*. Furthermore, the correlation patterns between *C. albicans* and bacteria associated with vaginal infections varied depending on the group studied, as observed in the co-occurrence graphs (Fig. 4).

Metabolic pathway analyses of the vaginal microbiome show significant differences between groups of healthy women and patients with RVVC

Predictive analyses of metabolic potential were performed based on metagenomic data between the groups of healthy women and women with RVVC. The results allowed us to identify the main metabolic pathways in the context of the vaginal microbiome, taking into account the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Supplementary Fig. 4). Significant differences were observed between healthy women and women with RVVC in the proportion of gene sequences related to purine metabolism (Fig. 5), as well as in gene sequences involved in the carbohydrate phosphotransferase system (PTS); fructose and mannose metabolism; the pentose phosphate pathway; and cysteine and methionine metabolism (Fig. 6A–D). Noteworthy, only a significant difference in pentose phosphate pathway was observed between the groups of women with RVVC.

Discussion

Candida spp. are part of the vaginal microbiota of healthy women, with *C. albicans* being the most common species.[24] However, in the presence of risk factors such as the use of broad-spectrum antibiotics, pregnancy, corticosteroids, diabetes, and immune sys-

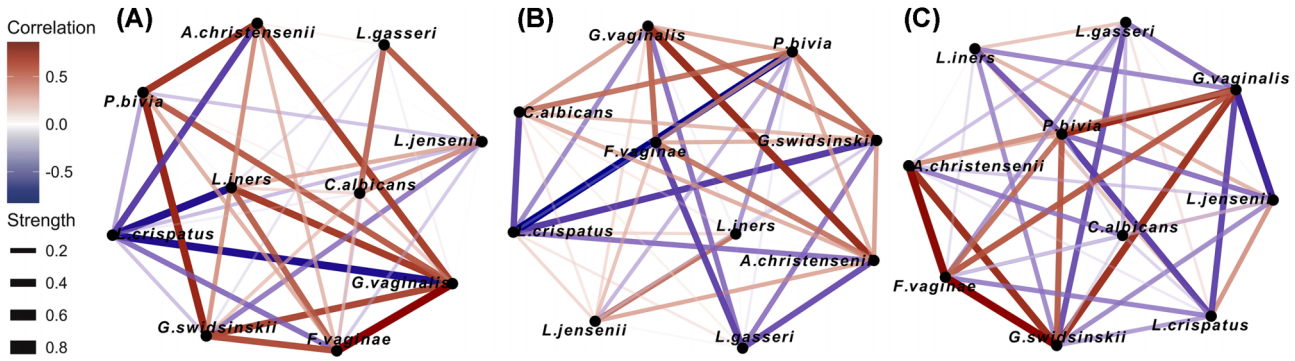

Figure 4 Analysis of the interrelationship between the main microorganisms that are part of the vaginal microbiome. Co-occurrence network diagram in the group of healthy women (A), in women with RVVC (B), and in women with severe RVVC (C). Edges represent significant correlations between pairs of microorganisms. The thickness and opacity of the edges represent the absolute strength of the correlation. Red edges represent a positive correlation, blue represents a negative correlation, and white or light tones represent neutral correlations. Only microorganisms that were significantly correlated are included. ($|r_s| > 0.6$, P-value $< .05$.)

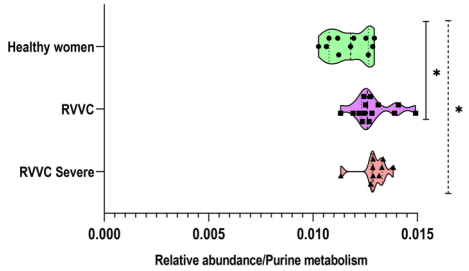

Figure 5 Proportion of sequences related to purine metabolism. The microbiome analysis was performed on healthy women and women with VVC. Results are expressed as mean ± standard error (SEM). ∗P-value $< .05$.

tem disorders, among others,[25] *C. albicans* can transition from a commensal to a pathogenic state.

During this transition, the yeasts initiate the formation of hyphae, which are characterized by their ability to adhere, penetrate, and invade the vaginal epithelium,[26] which can lead to the development of VVC and, in some cases, RVVC.[27] The vaginal microbiome has been reported to play a key role in the pathogenesis of vaginal infections, which is why vaginal dysbiosis has been associated with the risk of acquiring infections such as VVC/RVVC.[28] It has been described that in healthy women of reproductive age, the microbiota is characterized by the presence of a species of *Lactobacillus*, whose relative abundance can exceed 90%.[29] This is confirmed by the results of relative abundance observed in the present study, where in the group of healthy women, the most abundant bacterial genus was *Lactobacillus*, with a percentage greater than 95%, unlike the groups of women with RVVC or severe RVVC where said abundance did not exceed 65% with respect to the total identified genera. Additionally, it has been reported that in patients with VVC/RVVC generally there is an increase in the abundance of bacterial genera associated with bacterial vaginosis such as *Gardnerella*, *Prevotella*, and *Atopobium*,[30] which agrees with the results of the present study, where in the groups of patients with RVVC and severe RVVC presented an increase in the relative abundances of the genera *Gardnerella*, *Prevotella*, *Aerococcus*, and *Fannyhessea*.

Five types or groups of vaginal bacterial communities (CST) with a predominance of *Lactobacillus* spp. have been reported: *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III), and *L. jensenii* (CST V), while the CST IV group presents a predominance of facultative anaerobic bacteria, mainly *G. vaginalis*.[31] Regarding the above, in this study, it was observed that the group of healthy women was associated with the CST I type, while the groups of patients with RVVC and severe RVVC were associated with the CST III type, this is interesting since it has been reported that the CST III type is more common in women of Asian and Caucasian ethnicity, while in Latin women the CST IV group is the most common.[32] This apparent contradiction may reflect regional and cohort–specific differences in vaginal microbiome composition, which can be influenced by factors such as lifestyle, diet, and sexual practices that vary even within broadly defined ethnic groups. Although at the genus level, the vaginal microbiome is relatively stable, with *Lactobacillus* spp. the most abundant genus in both the group of healthy women and in the groups of patients with RVVC and severe RVVC, in the latter two, a notable change in the composition of *Lactobacillus* species is observed.

In the group of healthy women, the most abundant species were *L. crispatus* followed by *L. gasseri*, while in the groups of women with RVVC, the most abundant species was *L. iners*. Considering the above, it has been reported that the metabolic capacity of *Lactobacillus* species plays a key role in pathogenesis of RVVC. Similar to the findings of this study, other authors have reported that in patients with RVVC there is a decrease in the relative abundance of D-lactic acid-producing *Lactobacillus* species such as *L. crispatus* and an increase in L-lactic acid-producing species such as *L. iners*, the latter isoform being associated with less protection against pathogens at the vaginal level.[7,33] The production of D-lactic acid promotes a vaginal pH < 4.5, which inhibits the morphological change of *C. albicans* from yeast to hypha.[34]

Additionally, unlike *L. crispatus*, *L. iners* does not produce hydrogen peroxide (H_2O_2), suggesting that this species exerts a lower protective capacity compared to *L. crispatus*.[34] On the other hand, it has been reported that *L. iners* has the ability to produce inerolysin, which is a cholesterol-dependent cytolytic toxin that can cause damage to the vaginal epithelium, thus promoting *Candida* spp. invasion as well inflammation.[29]

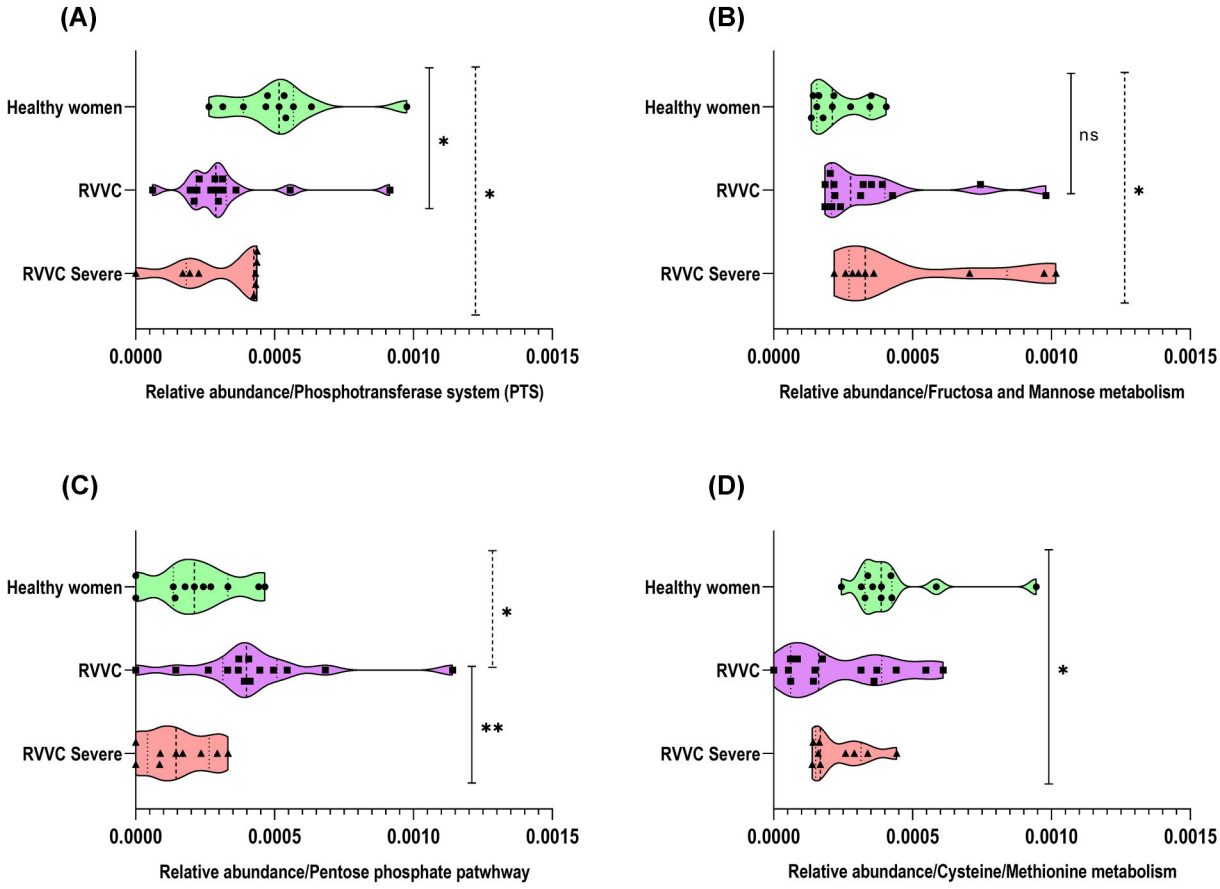

Figure 6 Proportion of sequences related to different metabolic pathways of the vaginal microbiome. (A) Phosphotransferase system (PTS), (B) fructose and mannose metabolism, (C) pentose phosphate pathway, and (D) cysteine and methionine metabolism. Results are expressed as mean ± standard error (SEM). ∗*P*-value < .05; ∗∗ *P*-value < .001.

As in the present study, Liang et al. reported a high α-diversity in patients with RVVC compared to healthy women.[30] This could be related to the fact that in patients with vaginal infections, an increase in the richness of species belonging to genera such as *Gardnerella*, *Prevotella*, *Faecalibacterium*, *Atopobium*, or opportunistic aerobic bacteria such as *Streptococcus* spp., *Staphylococcus aureus*, coagulase-negative staphylococci, and *Escherichia coli* has been observed.[10,28]

From co-occurrence analysis, inferences can be made about the possible ecological interactions that occur between microorganisms in the vaginal microenvironment; these analyses showed that in the group of patients with RVVC and severe RVVC, the interaction between *L. iners* and *C. albicans* is positive and neutral, respectively; thus, it has been described that *L. iners* can promote the growth and proliferation of *C. albicans* due to the upregulation of the expression of genes associated with biofilm formation such as *HWP1*, *ALS3*, and *ECE1*.[35] However, it is important to note that these observations reflect associations rather than direct causal effects. On the other hand, in the group of healthy women, positive or neutral interaction patterns were observed between *C. albicans* and *Lactobacillus* spp., which could be related to the fact that in healthy women, *Candida* spp. have been reported to be found mainly in its commensal state with a low to moderate fungal load, which is an indicator of eubiosis of the vaginal microbiota.[36,37]

Additionally, the analysis of the metabolic potential led to the observation that in the groups of patients with RVVC, there is an enrichment in genes related to purine metabolism. This finding is significant since it has been reported that in fungal pathogens such as *C. albicans*, *de novo* purine biosynthesis is essential during infection, since it may confer a selective advantage against the host immune response. In line with this, it has been reported that the elimination or interruption of enzymes of the *de novo* purine pathway in *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *C. albicans* is associated with a decrease in their virulence.[38] However, in the absence of direct metabolomic measurements, the functional interpretation of enriched KEGG pathways associated with purine metabolism pathways should be considered cautiously; for that reason, the enrichment in genes related to purine metabolism in RVVC groups could also reflect a higher bacterial load or a relative increase in housekeeping genes, rather than an actual metabolic change; moreover, some authors have also reported that communities dominated by anaerobes may appear functionally enriched simply due to broader genomic repertoires in comparison with *L. crispatus*.[39–41] Additionally, in the present study it was observed that in the groups of patients with RVVC, there is a lower proportion of sequences associated with the carbohydrate PTS; a system responsible for carbohydrate metabolism, especially in the catabolic and anabolic processes of glucose.[42] *Lactobacillus* spp. can acidify the vaginal microenvironment by producing lactic acid from substrates such as glycogen or other carbohydrates; thus, the decreased proportion of PTS-associated sequences in the microbiome of women with RVVC

could be due to the decreased relative abundance of *Lactobacillus* spp. and a possible decrease in glycogen levels in these patient groups.[43] Additionally, the reduction in the proportion of PTS-associated sequences observed in the RVVC patient groups could also suggest that the microorganisms present, in addition to glycogen metabolism, could be using alternative routes to obtain energy.[44] It has been reported that fructose is one of the least abundant carbohydrate sources in the vagina. Compared to the group of healthy women, patients with RVVC exhibited a predominance in the relative abundance of *Gardnerella* spp. This is interesting, considering that bacteria such as *G. vaginalis* have the ability to metabolize both fructose and starch, unlike other bacteria such as *L. iners*, which do not have this ability.[45]

A higher proportion of sequences associated with the pentose phosphate pathway was identified in the group of patients with RVVC compared to the group of healthy women. This finding is related to other studies in which it was identified that women with asymptomatic bacterial vaginosis and low relative abundance of *L. crispatus* presented a higher proportion of sequences related to the pentose phosphate pathway.[46] With respect to the proportion of sequences related to cysteine and methionine metabolism, it was observed that in the group of patients with RVVC, this proportion is lower compared to the group of healthy women. This result is in line with the findings of a study conducted in South Africa, in which higher levels of cysteine were observed in vaginal discharge from healthy women, with a dominance of *Lactobacillus* spp., compared to women with vaginal dysbiosis.[47] Taking the above into account, differential results were observed in the metabolic potential of some biological processes depending on the severity of RVVC, which clearly indicates that the study of metabolic interactions in these microorganisms that make up the vaginal microbiota may be key to elucidating biological markers or mechanisms by which RVVC can occur in a severe and recurrent manner.

Unlike amplicon sequencing, shotgun sequencing allows for the detection of gene function, enabling inferences about the metabolic potential of a given microbiome. Accordingly, this study analyzed the metabolic potential in samples from healthy women and patients with RVVC. However, it should be emphasized that further *in vitro* and *in vivo* experiments are required to validate these findings and identify potential therapeutic targets for RVVC. Moreover, research with larger sample sizes in Latin American women is needed to confirm the findings described in this study, and thereby better understand the dynamics between the vaginal microbiota and the risk of acquiring RVVC.

This study has several limitations that should be acknowledged. First, the relatively small sample size, particularly within the subgroup of women with severe RVVC, may limit the generalizability of the findings and reduce statistical power to detect subtle microbiome differences; in that order, the absence of significant differences between RVVC and severe RVVC groups should be interpreted with caution, as the limited sample size may reduce statistical power. Therefore, the possibility of observing subtle biological differences cannot be ignored. Second, the cross-sectional study design precludes any inference of causality or temporal relationships between vaginal microbiome alterations and RVVC, and it remains unclear whether the observed microbial patterns precede or result from recurrent infections. In addition, functional pathway predictions were inferred from metagenomic data and were not validated by transcriptomic or metabolomic analyses. Finally, samples were collected at a single time point, and longitudinal studies are needed to assess microbiome dynamics over time and their association with recurrence patterns.

Conclusions

In conclusion, our study provides relevant information on the characteristics of the vaginal microbiome in a cohort of Colombian women with RVVC. The results of this study confirm that susceptibility to experiencing recurrent episodes of RVVC could be associated with an increased abundance of bacteria associated with vaginal infections, such as *G. vaginalis* and *P. bivia*. Interestingly, no significant differences were found between the groups of women with RVVC and severe RVVC that would allow correlation with the degree of severity, clearly suggesting that factors other than the composition of the vaginal microbiota could be associated with the degree of severity or recurrence. These findings highlight the complexity and importance of studying the vaginal microbiome in the context of RVVC, to search for potential therapeutic strategies that allow preventing and treating these types of infections from a personalized and differential approach.

Acknowledgments

We acknowledge funding from the Programmatic Health Sciences Call, 2019–2020, Committee for Research Development (CODI), Universidad de Antioquia, Medellín, Colombia.

Author contributions

Jeiser Marcelo Consuegra-Asprilla (Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing), Yesid Cuesta-Astroz (Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing), Ángel González (Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing)

Supplementary data

Supplementary data are available at *Medical Mycology* online.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Metagenomic sequence data supporting the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1356845 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1356845). There are no restrictions on data access.

References

1. Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007; 369(9577): 1961–1971. https://doi.org/10.1016/S0140-6736(07)60917-9.
2. Galán JSJ, Poliquin V, Gerstein AC. Insights and advances in recurrent vulvovaginal candidiasis. *PLoS Pathog*. 2023; 19(11): e1011684.
3. Rosati D, Bruno M, Jaeger M, Ten Oever J, Netea MG. Recurrent vulvovaginal candidiasis: an immunological perspective. *Microorganisms*. 2020;8(2): 144. https://doi.org/10.3390/microorganisms8020144.
4. Ge G, Yang Z, Li D, Zhang N, Chen B, Shi D. Distinct host immune responses in recurrent vulvovaginal candidiasis and vulvovaginal candidiasis. *Front Immunol*. 2022; 13: 959740. https://doi.org/10.3389/fimmu.2022.959740.
5. Jang SJ, Lee K, Kwon B, You HJ, Ko G. Vaginal lactobacilli inhibit growth and hyphae formation of *Candida albicans*. *Sci Rep*. 2019; 9(1): 8121. https://doi.org/10.1038/s41598-019-44579-4.
6. Macklaim JM, Clemente JC, Knight R, Gloor GB, Reid G. Changes in vaginal microbiota following antimicrobial and probiotic therapy. *Microb Ecol Health Dis*. 2015; 26: 27799.
7. McKloud E, Delaney C, Sherry L, *et al.* Recurrent vulvovaginal candidiasis: a dynamic interkingdom biofilm disease of *Candida* and *Lactobacillus*. *mSystems*. 2021; 6(4): e00622–21. https://doi.org/10.1128/msystems.00622-21.
8. Qing W, Shi Y, Chen R, *et al.* Species-level resolution for the vaginal microbiota with short amplicons. *mSystems*. 2024; 9(2): e01039–23. https://doi.org/10.1128/msystems.01039-23.
9. Coolen MJL, Post E, Davis CC, Forney LJ. Characterization of microbial communities found in the human vagina by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Appl Environ Microb*. 2005; 71(12): 8729–8737. https://doi.org/10.1128/AEM.71.12.8729-8737.2005.
10. Sirichoat A, Buppasiri P, Faksri K, Lulitanond V. Dynamics and diversity of vaginal microbiota in bacterial vaginosis among Thai patients treated with metronidazole. *J Infect Public Health*. 2025; 18(2): 102646. https://doi.org/10.1016/j.jiph.2024.102646.
11. Virtanen S, Kalliala I, Nieminen P, Salonen A. Comparative analysis of vaginal microbiota sampling using 16S rRNA gene analysis. *PLoS One*. 2017; 12(7): e0181477. https://doi.org/10.1371/journal.pone.0181477.
12. Laudadio I, Fulci V, Palone F, Stronati L, Cucchiara S, Carissimi C. Quantitative assessment of shotgun metagenomics and 16S rDNA amplicon sequencing in the study of human gut microbiome. *OMICS*. 2018; 22(4): 248–254. https://doi.org/10.1089/omi.2018.0013.
13. Chen Y, LC F, Chai YH, Xu JF. Advantages and challenges of metagenomic sequencing for the diagnosis of pulmonary infectious diseases. *Clin Respir J*. 2022; 16(10): 646–656. https://doi.org/10.1111/crj.13538.
14. Odogwu NM, Olayemi OO, Omigbodun AO. The vaginal microbiome of Sub-Saharan African women: revealing important gaps in the era of next-generation sequencing. *PeerJ*. 2020; 8: e9684. https://doi.org/10.7717/peerj.9684.
15. Aja-Macaya P, Conde-Pérez K, Trigo-Tasende N, *et al.* Nanopore full length 16S rRNA gene sequencing increases species resolution in bacterial biomarker discovery. *Sci Rep*. 2025; 15(1): 26486. https://doi.org/10.1038/s41598-025-10999-8.
16. Consuegra-Asprilla JM, Rodríguez-Echeverri C, Posada DH, Gómez BL, González Á. Patients with recurrent vulvovaginal candidiasis exhibit a decrease in the fungicidal activity of neutrophils and the proliferation of peripheral blood mononuclear cells. *Mycoses*. 2024; 67(4): e13720. https://doi.org/10.1111/myc.13720.
17. Chen Y, Chen Y, Shi C, *et al.* SOAPnuke: a MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. *GigaScience*. 2018; 7(1): gix120. https://doi.org/10.1093/gigascience/gix120.
18. Lu J, Breitwieser FP, Thielen P, Salzberg SL. Bracken: estimating species abundance in metagenomics data. *PeerJ Comput Sci*. 2017; 3: e104. https://doi.org/10.7717/peerj-cs.104.
19. Paulson JN, Stine OC, Bravo HC, Pop M. Differential abundance analysis for microbial marker-gene surveys. *Nat Methods*. 2013; 10(12): 1200–1202. https://doi.org/10.1038/nmeth.2658.
20. Li D, Liu CM, Luo R, Sadakane K, Lam TW. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*. 2015; 31(10): 1674–1676. https://doi.org/10.1093/bioinformatics/btv033.
21. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013; 29(8): 1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
22. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinf*. 2010; 11: 119. https://doi.org/10.1186/1471-2105-11-119.
23. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol*. 2021; 38(12): 5825–5829. https://doi.org/10.1093/molbev/msab293.
24. d' Enfert C, Kaune AK, Alaban LR, *et al.* The impact of the fungus-host-microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives. *FEMS Microbiol Rev*. 2021; 45(3): fuaa060. https://doi.org/10.1093/femsre/fuaa060.
25. Gonçalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. *Crit Rev Microbiol*. 2016; 42(6): 905–927. https://doi.org/10.3109/1040841X.2015.1091805.
26. Min K, Neiman AM, Konopka JB. Fungal pathogens: shape-shifting invaders. *Trends Microbiol*. 2020; 28(11): 922–933. https://doi.org/10.1016/j.tim.2020.05.001.
27. Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: a systematic review. *Lancet Infect Dis*. 2018; 18(11): e339–e347. https://doi.org/10.1016/S1473-3099(18)30103-8.
28. Kalia N, Singh J, Kaur M. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. *Ann Clin Microbiol Antimicrob*. 2020; 19(1): 5. https://doi.org/10.1186/s12941-020-0347-4.
29. Taraskina AE, Frolova EV, Uchevatkina AE, *et al.* Mechanisms of interaction between yeast, bacterial biota, and local im-

mune response in recurrent vulvovaginal candidiasis. *Cell Tissue Biol*. 2025; 19(1): S25–S43. https://doi.org/10.1134/S1990519X25600322.
30. Liang Y, Huang Z, Fan S, *et al.* Highlight signatures of vaginal microbiota and metabolome contributed to the occurrence and recurrence of vulvovaginal candidiasis. *Microbiol Spectr*. 2024; 12(12): e01521–24. https://doi.org/10.1128/spectrum.01521-24.
31. Brooks JP, Buck GA, Chen G, *et al.* Changes in vaginal community state types reflect major shifts in the microbiome. *Microb Ecol Health Dis*. 2017; 28(1): 1303265.
32. Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S. *Lactobacillus* species as biomarkers and agents that can promote various aspects of vaginal health. *Front Physiol*. 2015; 6: 81. https://doi.org/10.3389/fphys.2015.00081.
33. Ceccarani C, Foschi C, Parolin C, *et al.* Diversity of vaginal microbiome and metabolome during genital infections. *Sci Rep*. 2019; 9: 14095. https://doi.org/10.1038/s41598-019-50410-x.
34. Vitali B, Pugliese C, Biagi E, *et al.* Dynamics of vaginal bacterial communities in women developing bacterial vaginosis, candidiasis, or no infection. *Appl Environ Microb*. 2007; 73(18): 5731–5741. https://doi.org/10.1128/AEM.01251-07.
35. Sabbatini S, Visconti S, Gentili M, *et al. Lactobacillus iners* cell-free supernatant enhances biofilm formation and hyphal/pseudohyphal growth by *Candida albicans* vaginal isolates. *Microorganisms*. 2021; 9(12): 2577. https://doi.org/10.3390/microorganisms9122577.
36. Yano J, Peters BM, Noverr MC, Fidel PL, Jr. Novel mechanism behind the immunopathogenesis of vulvovaginal candidiasis: neutrophil anergy. *Infect Immun*. 2018; 86(3): e01128–17. https://doi.org/10.1128/IAI.00684-17.
37. Jacobsen ID. The role of host and fungal factors in the commensal-to-pathogen transition of *Candida albicans*. *Curr Clin Microbiol Rep*. 2023; 10(2): 55–65. https://doi.org/10.1007/s40588-023-00190-w.
38. Chitty JL, Fraser JA. Purine acquisition and synthesis by human fungal pathogens. *Microorganisms*. 2017; 5(2): 33. https://doi.org/10.3390/microorganisms5020033.
39. Langille MGI, Zaneveld J, Caporaso JG, *et al.* Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*. 2013; 31(9): 814–821. https://doi.org/10.1038/nbt.2676.
40. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012; 489(7415): 242–249. https://doi.org/10.1038/nature11552.
41. McHardy IH, Goudarzi M, Tong M, *et al.* Integrative analysis of the microbiome and metabolome of the human intestinal mucosal surface reveals exquisite inter-relationships. *Microbiome*. 2013; 1: 17.
42. Roth P, Jeckelmann JM, Fender I, *et al.* Structure and mechanism of a phosphotransferase system glucose transporter. *Nat Commun*. 2024; 15(1): 7992. https://doi.org/10.1038/s41467-024-52100-3.
43. Mirmonsef P, Hotton AL, Gilbert D, *et al.* Free glycogen in vaginal fluids is associated with *Lactobacillus* colonization and low vaginal pH. *PLoS One*. 2014; 9(7): e102467. https://doi.org/10.1371/journal.pone.0102467.
44. McMillan A, Rulisa S, Sumarah M, *et al.* A multi-platform metabolomics approach identifies novel biomarkers associated with bacterial diversity in the human vagina. *Sci Rep*. 2015; 5: 14174. https://doi.org/10.1038/srep14174.
45. France MT, Mendes-Soares H, Forney LJ. Genomic comparisons of *Lactobacillus crispatus* and *Lactobacillus iners* reveal potential ecological drivers of community composition in the vagina. *Appl Environ Microb*. 2016; 82(24): 7063–7073. https://doi.org/10.1128/AEM.02385-16.
46. Pramanick R, Nathani N, Warke H, Mayadeo N, Aranha C. Vaginal dysbiotic microbiome in women with no symptoms of genital infections. *Front Cell Infect Microbiol*. 2021; 11: 760459. https://doi.org/10.3389/fcimb.2021.760459.
47. Bloom SM, Mafunda NA, Woolston BM, *et al.* Cysteine dependence of *Lactobacillus iners* is a potential therapeutic target for vaginal microbiota modulation. *Nat Microbiol*. 2022; 7(3): 434–450. https://doi.org/10.1038/s41564-022-01070-7.

Received: 26 January 2026. **Revised:** 19 March 2026. **Accepted:** 20 March 2026