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The Vaginal Microbiome Composition of Premenopausal and Postmenopausal Women: A Systematic Review

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Abstract

Introduction: The vaginal microbiome is a critical modulator of vaginal immunology and undergoes constant changes throughout life. While the vaginal microbiota of premenopausal women is extensively studied, its composition and implications on vaginal health in peri-menopausal and postmenopausal women remains unclear. Notably, differences in vaginal microbiome composition may influence susceptibility to mucosal infections, including bacterial vaginosis (BV), sexually transmitted infections (STIs), and human immunodeficiency virus (HIV).

Methods: With no time restrictions, a systematic search of Ovid EMBASE and Ovid Medline was conducted from inception to May 2024. Three independent reviewers screened titles, abstracts, and full texts and extracted data on population demographics, sequencing methods, BV diagnostic criteria, and vaginal microbiome composition. The inclusion criteria were studies comparing premenopausal and postmenopausal women with outcomes reporting vaginal bacterial composition. Secondary outcomes include hormone or estrogen levels and glycogen content.

Results: Five studies met the inclusion criteria. These studies span two countries, with participants aged 18 to 61 years. Four studies used 16S rRNA gene sequencing; one used bacteria-specific qPCR. Premenopausal women predominantly exhibited *Lactobacillus*-dominated community state types (CSTs) I and III, with higher glycogen levels and lower vaginal pH compared to postmenopausal women. The vaginal microbiomes of postmenopausal women are largely presented as CST-IV, characterized by reduced *Lactobacillus* spp. and increased diversity of other anaerobes. Estrogen therapy partially restored *Lactobacillus* dominance, improving microbial stability. Transition rates between CSTs were more frequent in premenopausal women, typically between *Lactobacillus crispatus*-dominant (CST I) and high-diversity anaerobic-dominant (CST IV).

Discussion: Age-related changes in the vaginal microbiome underscore the impact of hormonal decline and glycogen availability on microbial dynamics. The differences in vaginal microbiome composition access life stages may reflect age-related susceptibility to urogenital conditions. Estrogen therapy emerges as a potential intervention to restore microbial balance and improve vaginal health outcomes in postmenopausal women.

Conclusion: Further research is needed to explore targeted interventions supporting microbial resilience across life stages. Such interventions could inform strategies to prevent urogenital conditions and enhance overall vaginal health for women as they age.

Keywords: vaginal microbiome; *Lactobacillus*; bacterial vaginosis; menopause; estrogen therapy; community state types; glycogen; hormonal changes; vaginal pH; urogenital health

Introduction

The female genital tract is a dynamic ecosystem crucial to reproductive and sexual health, with the vaginal microbiome playing an important role in maintaining the vaginal immune milieu. An optimal vaginal microbiome is characterized by a predominance of *Lactobacillus crispatus*, which is associated with decreased pro-inflammatory cytokines and chemokines and a more acidic vaginal pH via greater lactic acid production, offering enhanced immune protection against sexually transmitted infections [10]. In contrast, bacterial vaginosis [BV] is a vaginal clinical condition characterized by reduced *Lactobacillus* spp. and increased diversity and density of other facultative anaerobes like *P. bivia* and *G. vaginalis*. BV is associated with subclinical vaginal inflammation, characterized by increased levels of pro-inflammatory cytokines, as well as epithelial barrier disruption, which likely contributes to the 60% increased risk of acquiring human immunodeficiency virus [HIV] and increased risk for other sexually transmitted infections [STIs].

Menopause involves dramatic changes in systemic hormonal levels and alterations of the vaginal environment. Premenopausal women generally have estradiol levels ranging from 30–400 pg/mL, which support glycogen

accumulation, foster *Lactobacillus* proliferation, and maintain a vaginal pH of ≤4.5 [1, 2, 7]. Estradiol levels, however, fluctuate significantly across a woman's lifespan, peaking during the reproductive years, beginning to decline in perimenopause [typically around age 45–55], and falling below 40 pg/mL postmenopause. This decline results in reduced glycogen availability, decreased *Lactobacillus* abundance, and a more basic vaginal pH of 6–7.5 [8, 9]. While it is well-established that the vaginal microbiome differs significantly between reproductive-age and postmenopausal women, the extent and mechanisms of these differences require further exploration.

This systematic review aims to explore age-related changes in the vaginal microbiome and their implications for vaginal health. Specifically, it examines the differences in microbiome composition between premenopausal and postmenopausal women, focusing on the roles of hormonal fluctuations and glycogen availability in shaping bacterial community state types [CSTs]. Enhanced understanding of these dynamics could inform targeted interventions to maintain microbial balance across life stages.

Methods

A systematic electronic search on Ovid EMBASE and Ovid Medline was performed from inception to May 2024 without time restrictions. Only English studies were considered. The search strategy is documented in Appendix A. A research librarian was consulted during the entire process. Eligible studies were managed using Covidence [Veritas Health Innovation, Australia]. Three independent reviewers [AA, SR, SS] performed title, abstract, full-text screening, and data extraction. Conflicts were resolved by a fourth reviewer [JT]. Inclusion criteria were 1] cohorts consisting of both premenopausal/reproductive age and postmenopausal biological females aged 18 years or older and 2] the analysis of the vaginal microbiome composition using sequencing techniques. Exclusion criteria included biological males, biological females with STIs [HIV, Chlamydia, HPV, Syphilis, etc.], prepubescent biological females, and/or biological females with primary amenorrhea. Additional literature searches were performed on PubMed between October 2024 and December 2024. Using pre-piloted forms, three authors [AA, SR, SS] independently extracted data, including study title, country, year, journal, and study type. Population details included total participants and breakdown by menopausal status [premenopausal, postmenopausal, perimenopausal] with corresponding numbers and percentages. We recorded median and mean ages, sequencing methods, and BV diagnostic criteria [Amsel, Nugent, molecular]. Data on Community State Types [CSTs], pH values, Nugent scores, and BV prevalence were extracted by the menopausal group. Other non-microbiome results were also noted. Discrepancies were resolved with JT, and no assumptions were made regarding missing data. Studies were excluded using PRISMA guidelines.

Results

The systematic search resulted in 947 articles [Figure 1]. After removing 44 duplicates, we performed title and abstract screening of 901 studies. 76 studies underwent a full-text screen for eligibility. We excluded 32 studies from this, including two with the wrong outcome and 8 with an incorrect study population. The final systematic review consists of five articles and 303 female participants.

Study Characteristics

The characteristics of the included articles are listed in Table 1. Among the included studies, two were longitudinal observational studies [2, 9], while the remaining 3 were prospective cohort studies [5, 6, 11]. They were from two different countries: four studies from the United States of America [n=263 patients], and one from Sweden [n=40]. The participant age varied widely, ranging from 18 to 98 years. Four studies utilized 16s rRNA gene sequencing to assess cervicovaginal microbiome composition, and one study used quantitative PCR [qPCR]. One study focused on the impact of menopause-related hormonal changes on the vaginal microbiota, particularly the decline in estrogen levels and its effect on *Lactobacillus* abundance and microbial diversity [2]. Estrogen therapy use was reported in one study [2].

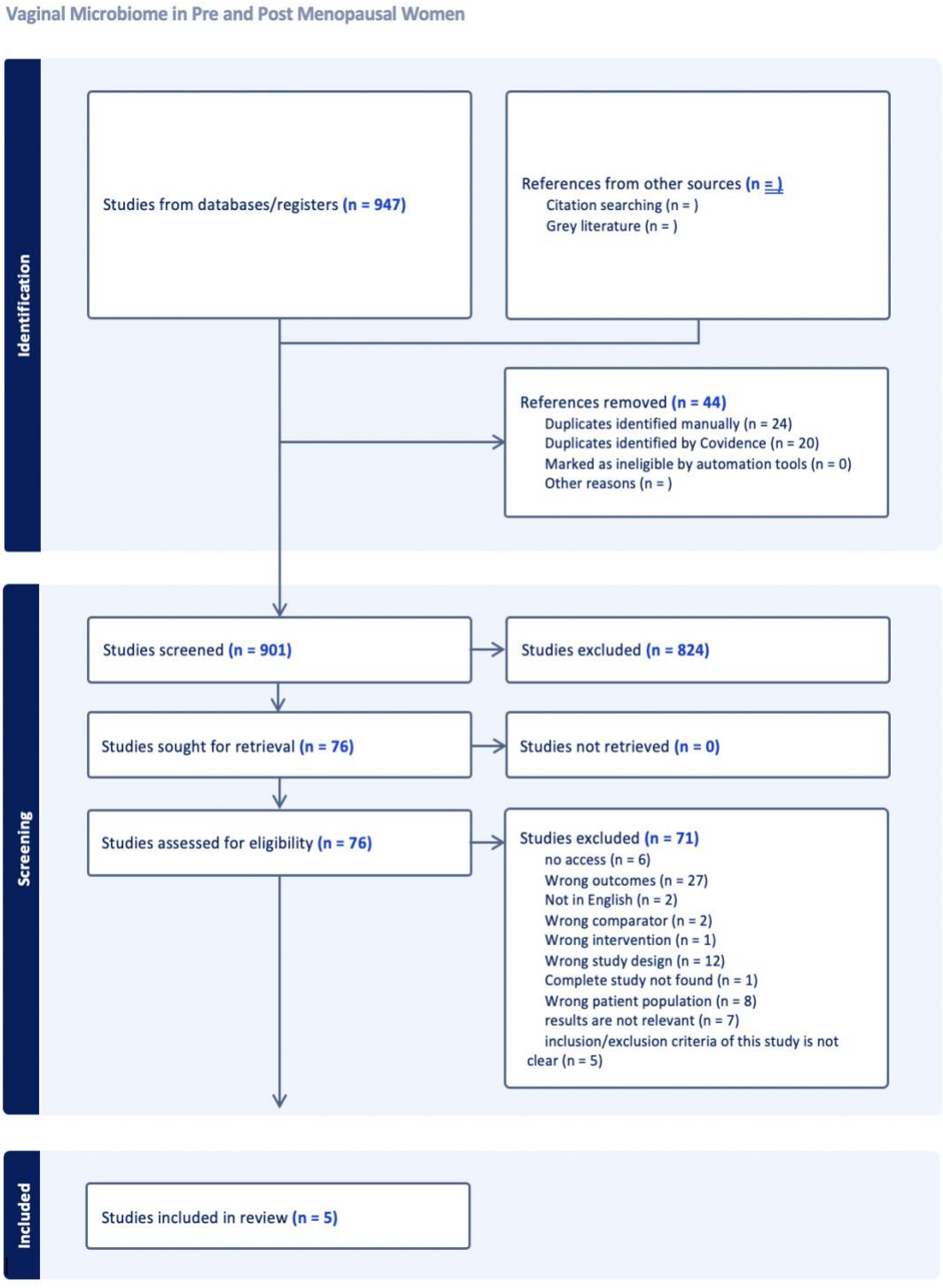

Figure 1. PRISMA

Table 1. Among the included studies, two were longitudinal observational studies

Author, year	Study Design	Country of Study	N	Age	Sequencing method	CSTs (Pre menopausal)	CSTs (Post menopausal)
Brotman 2014	Longitudinal observational study	USA	87	NR	16S rRNA gene amplification and pyrosequencing	Premenopausal women predominantly had CST I (*L. crispatus*), with Lactobacillus spp. dominating in 83% of participants, but L. iners was less protective and did not correlate with vaginal atrophy symptoms.	In postmenopausal women, CST IV-A with low Lactobacillus and higher bacterial diversity is linked to vaginal atrophy, while reduced dominance of Lactobacillus spp., especially L. crispatus and L. iners, may contribute to vaginal dryness and dysbiosis.
Gustafsson 2011	Cross Sectional Study	Sweden	40	Premenopausal: 40 years Postmenopausal: 60 year	Partial 16S rDNA sequencing	CST I (*L. crispatus*)was the most common vaginal species, with oestradiol levels positively influencing its presence, although no clear link was found between hormone levels and bacterial presence in the rectal flora.	Lactobacillus crispatus is less prevalent, with L. gasseri and L. plantarum more commonly found, and while lower estrogen levels correlate with reduced L. crispatus in the vagina, no clear link exists between hormone levels and lactobacilli in the rectal flora.
Mirmonsef 2015	Longitudinal observational study	USA	23	Pre-menopausal: 40 years Post-menopausal: 55.5 years	Bacteria-specific quantitative PCR (qPCR)	Higher glycogen levels in premenopausal women were strongly correlated with *L. iners* and *L. jensenii*, supporting a Lactobacillus-rich, acidic vaginal environment conducive to vaginal health.	Postmenopausal women have lower Lactobacillus levels due to reduced glycogen, a higher vaginal pH, and varying Lactobacillus correlations with glycogen despite low estrogen.
Stennett 2024	Longitudinal observational study	USA	102	Pre-menopausal: 32 years Post-menopausal: 59 years	16S rRNA gene amplicon sequencing	Frequent shifts between CST III (*L. iners*) and CST IV among premenopausal women indicated less microbiota stability, driven by menstrual cycle variations.	Postmenopausal women in CST IV show stable, low-Lactobacillus microbiota with reduced estrogen, affecting glycogen and resilience, though some maintain stable levels of L. iners.
Fields 2020	Longitudinal observational study	USA	51	NR	16S rRNA gene sequencing	70% of premenopausal women showed Lactobacillus presence in both urinary and vaginal environments, supporting a protective, low-diversity microbiome profile.	Lack of estrogen in postmenopausal women reduces Lactobacillus, increases diversity, and raises infection risk.

Table 2. Other findings

Author, year	pH	Glycogen levels	Hormone/Estrogen	Behavioral practices
Brotman 2014	NR	NR	NR	NR
Gustafsson 2011	NR	NR	Hormone fluctuations were significant in pre-menopausal women, but no correlation was found between hormone levels and microbiota in either pre- or post-menopausal women.	NR
Mirmonsef 2015	Premenopausal: 4 Postmenopausal: 4.6	Glycogen levels strongly correlated with Lactobacillus abundance in both pre- and post-menopausal women, unaffected by HIV status.	NR	NR
Stennett2024	NR	NR	NR	Vaginal microbiota stability was consistent across race, BMI, and hormonal contraceptive use, with low hormonal therapy and minimal antibiotic impact reported.
Fields 2020	NR	NR	Estrogen supplementation restored Lactobacillus levels in post-menopausal women, aligning their microbiota profiles closer to pre-menopausal women.	NR

Community State Types

Community State Types [CSTs] are classifications of the vaginal microbiome based on the dominant bacterial species and overall microbial composition. Two studies [n=189] classified the vaginal microbiome into five CSTs: *L. crispatus* dominant [CST I]; *L. gasseri* dominated [CST II]; *L. iners* dominated [CST III]; low *Lactobacillus* and a higher abundance of *Anaerococcus, Peptoniphilus,* and *Prevotella* [CST IV-A]; a mixture of *Gardnerella*, *Atopobium* [recently reclassified as *Fannyhessea vaginae]*, *Prevotella,* and *Finegoldia* [CST IV]; *L. jenesenii* dominated [CST V]. Three studies [n=114] listed the bacteria found in the vaginal microbiome and did not refer to CSTs.

Premenopausal Women

There was a total of [n=154] premenopausal/ reproductive-age women in five included studies. The median vaginal pH in premenopausal women was 4.0.

One study reported that CST I was predominant in 46% of premenopausal women [n=14], while *Lactobacillus* spp dominated the vaginal microbiota [n=25, 83%] in these participants as well [Brotman et al., 2014]. In contrast, one study reported approximately 39% [n = 32] of premenopausal women presented with vaginal microbiomes consistent with CST-IV, or a high diversity of facultative anaerobic bacteria [11].

Amongst the three studies that did not identify vaginal microbiome composition by CSTs, one study reported that *L. crispatus* was the most commonly detected bacterial species in the vaginal flora of premenopausal women. Other species found in the premenopausal vaginal microbiome were *L. acidophilus, L. gasseri, L. jensenii,* and *L. vaginalis,* albeit at lower densities. Another study reported *L. iners* to be the most common species in the vaginal microbiome.

Another study detected *Lactobacillus* species in the urinary tract of premenopausal women [n= 7, 70%] as a proxy measure of the vaginal and urinary microbiota. Across the included studies, the vaginal microbiota in premenopausal women was predominantly composed of *Lactobacillus* species.

Postmenopausal Women

The vaginal microbiome composition was classified in [n=149] postmenopausal women across five studies. The studies highlighted significant shifts in microbial profiles associated with reduced glycogen availability in cervicovaginal secretions, lower *Lactobacillus* abundance, and increased microbial diversity. The median vaginal pH in postmenopausal women was 4.6.

One study reported that 29% of postmenopausal women exhibited a vaginal microbiome profile consistent with CST IV-A, characterized by high diversity and density of bacteria such as *Anaerococcus*, *Peptoniphilus*, *Prevotella*, *Streptococcus* species, and *G. vaginalis*. [2] Similarly, CST IV-B, also prevalent among postmenopausal females, featured a higher abundance of Atopobium and *G. vaginalis*, both associated with elevated vaginal pH levels around 5.0 or higher. The remaining 53.1% of postmenopausal women were classified into other CSTs, including CST I [*Lactobacillus crispatus*-dominated, 25%], CST III [*Lactobacillus iners*-dominated, 18%], and CST II and V [*Lactobacillus gasseri* and *Lactobacillus jensenii*-dominated, 10.6%], associated with lower pH values [closer to 4.0]. Additionally, a longitudinal cohort study sampled postmenopausal women twice weekly over 8 weeks to evaluate CST stability and transitions. Compared to premenopausal women, postmenopausal participants exhibited longer persistence in CST IV [median 42.3 days] and lower CST transition rates [14.6% per sampling interval]. Transitions from CST IV-A or IV-B to CST III or CST I were most common, indicating limited shifts toward *Lactobacillus*-dominated profiles.

One study investigated the impact of estrogen on the microbial diversity and presence of *Lactobacillus* in the vaginal and urinary microbiota. Catheterized urine and vaginal swabs were collected. Significant differences in vaginal *Lactobacillus* abundance were found among groups: postmenopausal not using estrogen [$p = 0.049$], postmenopausal on estrogen [$p = 0.06$], and premenopausal. Alpha diversity was significantly different among these groups [$p < 0.001$]. In the urinary microbiota, *Lactobacillus* was present in 70% of premenopausal women, 78% of those on estrogen, and 48% of those not on estrogen.

One study identified CST I [*L. crispatus* dominant], in 25% of postmenopausal women, while CST III [*L. iners* dominant], was present in 18%. In total, *Lactobacillus* species were the dominant bacteria in 54% of postmenopausal women. Similarly, another study observed that *L. crispatus* was less frequently present in the vaginal microbiota of postmenopausal women [n=2, 10%], with *L. gasseri* being more common [n=5, 25%]. Low estradiol levels in this group were associated with the reduced presence of *L. crispatus*.

Glycogen

Glycogen content in the vagina plays a critical role in maintaining a healthy microbial environment by supporting the growth of *Lactobacillus* species and sustaining vaginal pH. A study found a positive correlation between *L. iners* and free glycogen. [$r = 0.6$, $p < 0.0001$], *L. jensenii* was also positively correlated with glycogen levels [$r = 0.4$, $p < 0.0006$]. [9] In contrast, no significant correlation was observed between *L. crispatus* and glycogen.

In postmenopausal women, *Lactobacillus* density was lower, which was associated with reduced glycogen levels, with a median glycogen level of 0.002 µg/µl, compared to 0.065 µg/µl in premenopausal women [$p = 0.03$]. L. *iners* and L. *jensenii* were positively correlated with glycogen levels [$r = 0.7$, $p < 0.0001$ and $r = 0.65$, $p < 0.0006$, respectively]. [9]

Other Findings

5 studies reported secondary findings about the vaginal microbiome composition of premenopausal versus post-menopausal females.

While most pre-menopausal females had a *Lactobacillus* spp, dominant vaginal microbiome, one study also reported transitions between CST III and CST IV in females who had either an *L.* iners or high-diversity vaginal microbiome at previous visits [11]. Hormonal changes strongly influenced microbiota composition. Estrogen therapy in postmenopausal women restored *Lactobacillus* levels, aligning their microbiota with pre-menopausal profiles [2, 5]. Hormonal fluctuations, specifically changes in estrogen and progesterone levels, during the menstrual cycle influenced CST transitions in pre-menopausal women, although *Lactobacillus* remained dominant [6].

Discussion

This systematic review highlights significant differences in the vaginal microbiome between premenopausal and postmenopausal women. Premenopausal women typically exhibit a *Lactobacillus*-dominated microbiota, particularly *L. crispatus*, while those without this dominance often show *L. iners* or high-diversity microbiomes, which offer less immune protection. *L. iners*, though part of the *Lactobacillus* genus, is associated with higher levels of proinflammatory cytokines like IL-6 and IL-8, which can disrupt the epithelial barrier and increase susceptibility to infections. Menopause leads to a decline in *Lactobacillus* levels, increased microbial diversity, and a higher prevalence of BV-associated bacteria such as *Atopobium*, *G. vaginalis*, and *Prevotella*. Estrogen decline during menopause also causes vaginal epithelial atrophy, reducing glycogen availability and resilience, contributing to a non-optimal microbiome and increased infection risk [2]. However, postmenopausal women have lower HIV risk than younger women, likely due to behavioural and immune differences. These findings underscore the intricate relationship between hormones, microbiome composition, and infection risk, highlighting the need for a holistic approach to vaginal health across life stages.

The vaginal microbiome composition is associated with the vaginal immune profile and subsequent risk of vaginally-acquired mucosal infections. *L. crispatus* is commonly regarded as an "optimal" facultative anaerobe, given its association with decreased levels of proinflammatory cytokines like IL-1α, IL-6, and IL-8, all of which are associated with female HIV acquisition risk at elevated levels. This cytokine suppression reflects *L. crispatus'* role in maintaining epithelial integrity and immune protection. In contrast, postmenopausal women are more likely to have a vaginal microbiome consisting of an array of facultative anaerobes like *G. vaginalis*, *Atopobium*, and *Prevotella*, all of which are associated with increased HIV biomarkers in cervicovaginal secretions and therefore increased infection risk. These differences in microbial composition may partially explain age-related vaginal conditions, such as atrophic vaginitis and bacterial vaginosis, which are more prevalent during postmenopause. The decline in *L. crispatus* abundance and associated immune protection likely contributes to increased susceptibility not only to HIV but also to other sexually transmitted infections (STIs) in postmenopausal women. Addressing these shifts through targeted interventions, such as probiotics or estrogen therapy, may be crucial for mitigating mucosal infection risks in aging populations.

Brotman et al. found that postmenopausal women had lower levels of estrogen and thus a lower presence of *Lactobacillus*. As estrogen levels decline during menopause, glycogen production decreases, reducing the nutrient supply for *Lactobacillus*. Over time, this decline contributes to a shift in the vaginal microbiome, with lower *Lactobacillus* abundance and an increase in anaerobic bacteria, such as *G. vaginalis*, associated with higher pH levels. The reciprocal relationship between vaginal pH and *Lactobacillus* levels further exacerbates this shift, as a higher pH environment fosters the growth of BV-associated bacteria. This highlights the critical role of estrogen in maintaining glycogen levels, which directly influence the composition and function of the vaginal microbiome. Estrogen supplementation has the potential to restore glycogen production and *Lactobacillus* dominance, improving vaginal health. However, some studies suggest that estrogen therapy may not always be the optimal treatment despite its effects on microbiome composition, as it may not fully restore the vaginal environment or may carry risks for certain individuals [4].

This systematic review has limitations, including small sample sizes, methodological variability in sequencing techniques and BV diagnostic criteria, and a lack of longitudinal data to capture long-term microbial changes. Ethnicity also plays a crucial role, with ACB women and Hispanic women more likely to exhibit BV-associated or *L. iners*-dominant microbiomes. These populations are disproportionately affected by BV but were underrepresented in the reviewed studies, introducing demographic bias. Future research should prioritize longitudinal designs and more diverse sample populations to better understand how hormonal changes and ethnicity interact to shape microbiome composition and related health risks across life stages.

Conclusions

This systematic review highlights significant differences in the vaginal microbiome between premenopausal and postmenopausal women, driven by hormonal changes, particularly declines in estrogen, which in turn reduce glycogen availability. Premenopausal women typically exhibit a *Lactobacillus*-dominated microbiome, while postmenopausal women show reduced *Lactobacillus* levels, increased microbial diversity, and a higher prevalence of anaerobes. These findings underscore the complex interplay between hormonal shifts, microbial composition, and immune responses, which may influence inflammation and

infection risk. Future research should focus on standardized methodologies, longitudinal designs, and the role of systematic hormone levels in shaping microbial dynamics, addressing current gaps to inform therapies integrating hormonal and microbiome-based interventions.

List of Abbreviations
ACB: African, Caribbean, and Black
BV: bacterial vaginosis
CST: community state types
HIV: human immunodeficiency virus
STIs: sexually transmitted infections

Conflicts of Interest
The authors declare that they have no conflict of interest.

Ethics Approval and/or Participant Consent
This study is a systematic review and did not involve the collection of primary data or direct interaction with human participants. As such, ethics approval and participant consent were not required, as the study solely utilized previously published and publicly available data in accordance with standard ethical guidelines for reviews.

Authors' Contributions
AA: made contributions to the design of the study, collected and analyzed data, drafted the manuscript, and gave final approval of the version to be published.
SS: Contributed to study design and planning, assisted with the collection and analysis of data, and gave final approval for the published version.
SR: made substantial contributions to the design of the study, the collection of data and interpretation and analysis of the data, revised the manuscript critically, and gave final approval of the version to be published.

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