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Insights into fungal diversity and dynamics of vaginal mycobiota

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Abstract
Although less studied than its bacterial counterpart, the fungal component of the vaginal microbiota plays a critical role in maintaining vaginal homeostasis. Most research on the composition of the vaginal mycobiota has focused on pathological conditions, with relatively few studies involving healthy women. To gain comprehensive insights into the vaginal mycobiota of Algerian women in two different age groups, we performed a targeted metagenomic analysis using ITS2 region sequencing data from 14 vaginal samples collected from healthy women in reproductive and postmenopausal stages. A single dominant fungal species per individual was observed in both young and postmenopausal women, with differences in fungal community composition between the two groups being related to hormone levels. Our results show that *Candida* and *Saccharomyces* were the dominant genera in both young and postmenopausal women. Notably, the postmenopausal group had twice as many species, along with the presence of uncommon taxa such as *Dipodascus* and *Fusarium*, indicating greater taxonomic diversity. These findings suggest that menopause is associated with increased microbial variability, likely due to hormonal changes that disrupt the vaginal environment. This study paves the way for more extensive analyses involving diverse age groups and ethnic backgrounds.

Keywords Vaginal mycobiota · Childbearing · Menopause · Algerian women

Introduction

Like many other organs, the vagina hosts billions of microbes that form a homeostatic and mutualistic relationship with the host [1]. Unlike other human body sites with native microbiota, healthy vaginal microbiota is distinct in that it is predominantly composed of a single genus, *Lactobacillus*, in most individuals [2]. Although the fungal component of the microbiota, known as the mycobiota, represents only about 0.1% of vaginal microorganisms [3], it plays a crucial role in interactions such as commensalism and pathogenesis. Vaginal fungi are often from the genus *Candida*, with *Candida albicans* being the most common species, frequently present in asymptomatic women [4]. Additionally, over 20 other fungal genera have been identified in the vaginal environment, including *Cladosporium*, *Pichia*, *Aspergillus*, *Rhodotorula*, *Eurotium*, and *Alternaria* [5–7]. Some of these, such as *Cryptococcus*, *Galactomyces*, and *Saccharomyces*, have confirmed commensal roles in the microbiota of the gut, mouth, skin, and vagina [8].

The vaginal mycobiota is dynamic and responsive to a variety of internal and external factors. Physiological changes, medical treatments, and systemic health conditions can all contribute to shifts in its composition, resulting in transient or persistent changes in fungal communities. Throughout a woman's life, major physiological transitions such as puberty, menstruation, pregnancy, and menopause can alter the vaginal microbiota, often driven by hormonal fluctuations that affect the vaginal environment. For example, during pregnancy, increased estrogen levels

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can promote the growth of certain microbial populations, including fungi [9]. The use of antibiotics, while targeting bacterial infections, may inadvertently disrupt the natural balance of the vaginal microbiota. This disruption may suppress beneficial bacteria and allow opportunistic fungi such as *Candida* species to proliferate, potentially leading to yeast infections. One study observed that antibiotic treatment for bacterial vaginosis resulted in a transient increase in vaginal fungal abundance in some women [4]. In addition, medications that modulate the immune system or alter hormone levels may affect the composition of the vaginal microbiota. For example, corticosteroids, which suppress immune function, may facilitate fungal overgrowth [10–11]. Similarly, hormonal contraceptives have been associated with changes in the vaginal microbial environment, although their specific effects on the mycobiota require further research [12]. Systemic health conditions may also affect the vaginal mycobiota. For example, individuals with diabetes are more susceptible to *Candida* infections due to elevated blood glucose levels that promote fungal growth. In addition, immunocompromised conditions, whether due to disease or medical treatment, can alter the vaginal microbiota and increase the risk of opportunistic fungal infections [13].

The influence of the mycobiota extends beyond the human body, interacting with environmental factors and other microbiota. Given the complex relationships between fungi, bacteria and bacteriophages, there is a growing interest in studying fungal components within the One Health framework [14].

Several studies have shown that the composition of the vaginal microbiota can vary among different ethnic groups. For example, research on North American women from four ethnic groups (White, Black, Hispanic, and Asian) revealed differences in their vaginal bacterial communities [2]. Similarly, studies of Algerian women identified distinct ethnic-specific characteristics in their vaginal bacterial composition [15]. While these studies focused primarily on bacterial differences, they suggest that ethnic background may influence the broader vaginal microbial environment, potentially affecting fungal populations as well. To address this gap, further studies are needed that specifically examine fungal populations in different ethnic groups.

Our work represents a first step in this direction, providing a prospective study of the composition and dynamics of the vaginal mycobiota in healthy Algerian women. Two age groups were considered: young women of childbearing age and postmenopausal women. A metagenomic approach based on ITS rRNA gene sequencing was used to register the relative abundance of fungi and changes over time. Results were compared and discussed in relation to hormone levels and body mass index.

Table 1 List of study subjects, age, body mass index, and estradiol dosages

Subject	Age	BMI	Estradiol pg/mL
1Y	29	32.2	239
2Y	23	24.5	295
3Y	35	29.68	231.8
4Y	30	26.52	16.36
5Y	28	22.22	147.9
6Y	29	32.86	206.4
7Y	26	30.11	52.62
1 M	75	26.66	15.84
2 M	63	42.44	31.71
3 M	59	40.44	5.47
4 M	48	37.89	20.22
5 M	60	30	<5
6 M	56	32.97	15.96
7 M	56	36.44	15.86

Y - young women, M - menopausal women, BMI - body mass index

Materials and methods

Study design and sample collection

A total of 14 healthy women attending the gynecological service of a private health unit in the city of Bejaia (northeastern Algeria) participated in this study. Seven women were of childbearing age (28.5 ± 3.7 years) and coded as Y, while seven women were postmenopausal (59.5 ± 8.2 years) and coded as M. Their body mass index (BMI) was calculated using weight (kg) divided by the square of height (m_2) (Table 1).

All participants provided written informed consent prior to participation in this study. The consent process included clearly explaining the purpose, procedures, risks, and benefits of the study and ensuring that participants understood their rights to voluntary participation. A detailed medical history was taken, focusing on previous pregnancies, gynecologic symptoms, infection status, and any previous antibiotic or hormone therapy. Women were excluded if they were pregnant, receiving antibiotic or corticosteroid treatment, using contraceptives, or had systemic diseases such as diabetes or immunocompromised states. Their health status was certified by the obstetrician-gynecologist in charge of sampling.

Sample collection was performed by the attending gynecologist using a sterile speculum without antiseptic cleansing of the exocervix. A brush was inserted into the endocervix and rotated to collect an adequate amount of vaginal secretions, which were then collected in sterile Falcon tubes. Samples were immediately frozen and stored at -80 °C until DNA extraction. In addition, a blood sample was taken from each participant to measure estradiol levels.

DNA extraction

Total DNA from the 14 frozen vaginal swabs was extracted using the QIAamp PowerFecal DNA Kit (Qiagen Hilden, Germany) according to the specifications provided by the manufacturer. DNA amounts were quantified by using BioSpectrometer (Eppendorf AG, Hamburg, Germany) and DNA quality was assessed using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA).

Estradiol dosage

Blood samples were analyzed for estradiol using conventional Elecsys 2010 and Cobas 6000 c601 (Roche, Basel, Switzerland) analyzers with electrochemiluminescence (ECL) technology as described by Mackens et al. [16] and Chen et al. [1]. The analyzers were calibrated according to the manufacturer's instructions using both adult and postmenopausal controls. Normal ranges for estrogen hormones in adult women were up to 400 pg/mL. In postmenopausal women, the normal range was up to 20 pg/mL. Samples were run in duplicate on each analyzer to avoid systemic bias. Estradiol dosages are listed in Table 1.

Amplicon profiling

Sequencing was performed on the GIGA platform of the Faculty of Veterinary Medicine of the University of Liège in Belgium. For fungal DNA sequencing, the Internal Transcribed Spacer region ITS2 was amplified, and libraries were prepared for each sample using universal primers with Illumina overhang adapters targeting the ITS2 region. The forward primer ITS3KYO2 (5'-GATGAAGAACGYAGYRAA-3'), and the reverse primer ITS4 (5'-TCCTCCGCT TATTGATATGC-3') were used for their broad coverage of fungal taxa [17–18]. Single-organism DNA (*Saccharomyces cerevisiae*) was used as a positive control, while a no-template control was used as a negative control to confirm the PCR amplification. Each PCR product was purified using the Agencourt AMPure XP Ball Kit (Beckman Coulter, Pasadena, USA) and subjected to a second round of PCR for indexing, using Nextera XT index 1 and 2 primers. After purification, the PCR products were quantified using the Quant -IT PicoGreen (Thermo-Fisher Scientific, Waltham, USA) and diluted to 10 ng.μL^{-1}. A final qPCR quantification of each library sample was performed using the KAPA SYBR FAST qPCR Kit (KapaBiosystems, Wilmington, USA) before standardization, pooling, and sequencing on a MiSeq sequencer using v3 reagents (ILLUMINA, USA). Data processing was performed using the MOTHUR v1.44 package and the VSearch algorithm [19] for alignment, clustering, and chimera detection, following methods previously described by Gérard et al. [20]. A rarefied table of 10,000 reads per sample was used for subsequent analyses. Alignment and taxonomic identification were performed using the MOTHUR software with the UNITE database v9.0 for 5.8 S rDNA gene sequences. All the biosample raw reads have been deposited at the National Center for Biotechnology Information (NCBI) and are available under BioProject accession number PRJNA630844. Data obtained from NGS analysis were analyzed for alpha diversity using the Shannon index (SI) that combines richness and evenness, emphasizing the proportional abundance of OTUs [21–22]. GraphPad Prism version 8.00 for Windows (GraphPad Software) was used to generate graphical representations of the SI and to assess statistical significance using one-way ANOVA followed by Tukey's post-hoc test ($p<0.05$). Beta diversity was assessed to evaluate the dissimilarity in fungal community composition between samples [23], using Principal Coordinates Analysis (PCoA) with the FactoMineR package in R version 3.5.2 (r-project.org).

Results and discussion

Targeted metagenomic analysis of the ITS2 region was performed to assess and compare the overall fungal composition of the vaginal mycobiota in seven women of childbearing age and seven postmenopausal women. After data cleaning, a total of 127,314 sequences were clustered into operational taxonomic units (OTUs) with 97% identity. Two phyla, *Ascomycota* and *Basidiomycota*, with 23 genera and 26 species, were detected (Table 2).

Mycobiota diversity

The Shannon Index (SI) was used as a synthetic numerical measure of alpha diversity. Although the values were not particularly high, an increase in the index was observed in postmenopausal women (M group) (0.18 ± 0.4) compared to women of childbearing age (Y group) (0.06 ± 0.1), with a non-significant p-value ($p>0.6$) (Fig. 1a). Principal component analysis (PCA) of beta diversity in vaginal mycobiota composition (Fig. 1b) also indicated greater taxonomic diversity in the M group, as evidenced by a higher graphical dispersion. Despite the lower number of sequences and taxa observed in the mycobiota compared to bacterial composition analyses [15], a similar trend was evident. Postmenopausal women exhibited greater diversity in both DNA sequences (alpha diversity) and fungal taxa (beta diversity).

Table 2 Sequence read counts of fungal OTUs in the two sampled groups

OTU	Identified taxa	Y $n=7$	M $n=7$	Phylum
OTU 1	*Cladosporium halotolerans*	0	11	a
OTU 2	*Candida albicans*	39,492	20,860	a
OTU 3	*Candida dubliniensis*	1	5,042	a
OTU 4	*Candida glabrata*	9,879	1,636	a
OTU 5	*Candida krusei*	9,878	9,725	a
OTU 6	*Candida orientalis*	6	0	a
OTU 7–13	*Candida* sp.	0	8	a
OTU 14	*Debaryomyces hansenii*	0	1	a
OTU 15	*Dipodascus australiensis*	0	9,994	a
OTU 16	*Geotrichum* sp.	0	1	a
OTU 17	*Wickerhamomyces* sp.	0	1	a
OTU 18	*Nakaseomyces* sp.	27	25	a
OTU 19	*Saccharomyces cerevisiae*	9,989	19,866	a
OTU 20	*Nakazawaea molendini*	0	1	a
OTU 21	*Pichia occidentalis*	0	1	a
OTU 22	*Fusarium incarnatum*	0	553	a
OTU 23	*Fusarium proliferatum*	0	76	a
OTU 24	*Sarocladium sp.*	0	2	a
OTU 25	*Ascomycota* NI	0	1	a
OTU 26	*Agaricales* ge.	243	0	b
OTU 27	*Phlebia rufa*	270	0	b
OTU 28	*Resupinatus* sp.	59	0	b
OTU 29	*Malassezia restricta*	56	54	b
OTU 30	*Rhodotorula mucilaginosa*	0	2	b
OTU 31	*Trichosporon asahii*	54	0	b
OTU 32	*Vishniacozyma victoriae*	0	244	b

a– *Ascomycota*; b– *Basidiomycota*; sp.– species; ge.– genus; NI– not identified; Y - young women; M - menopausal women

Mycobiota composition

The composition of the fungal community in each sample was analyzed (Fig. 2). 13 genera with a relative abundance greater than 0.01% were identified (Fig. 2a), most of which have been previously reported as common components of the vaginal mycobiota and confirmed by culture-based and/or molecular methods [24–26]. One of the most striking findings was the presence of a single dominant fungal species per individual in both young and postmenopausal women.

The predominant genus was *Candida* (85.71% in Y and 42.85% in M), followed by *Saccharomyces* (14.29% in Y and 28.57% in M). While the genus *Candida* is represented by several species (Fig. 2b), including *C. albicans* (dominant in three Y samples and three M samples but present in 75.57% of all samples), *C. krusei* (dominant in one Y sample and one M sample), *C. glabrata* (dominant in one Y sample but present in other Y and M samples), *C. dubliniensis* (dominant in one M sample but also detected in one Y sample), and *C. orientalis* (detected in one Y sample), the genus *Saccharomyces* was represented exclusively by *S. cerevisiae* (dominant in one Y sample and two M samples).

The genus *Dipodascus* was represented by *Dipodascus australiensis*, which was identified as dominant in one M sample but absent in all others. As it has not been reported in the literature as part of the vaginal mycobiota, it is more likely to be a contaminant or incidental finding rather than a consistent component of the vaginal microbiota.

Other fungal genera were detected in very low abundance (0.01–2.70%) in Y samples, including *Phlebia*, *Resupinatus*, and *Trichosporon*, and in M samples, including *Fusarium*, *Sarocladium*, *Rhodotorula*, and *Vishniacozyma*. *Malassezia* and *Nakaseomyces* were found in both Y and M samples, each with an abundance of less than 0.55% and 0.27% respectively.

Among all identified species, two species dominated the vaginal mycobiota: *C. albicans*, which was more prevalent in the Y samples, and *S. cerevisiae*, which was more prevalent in the M samples (Fig. 2b). *C. albicans* was either

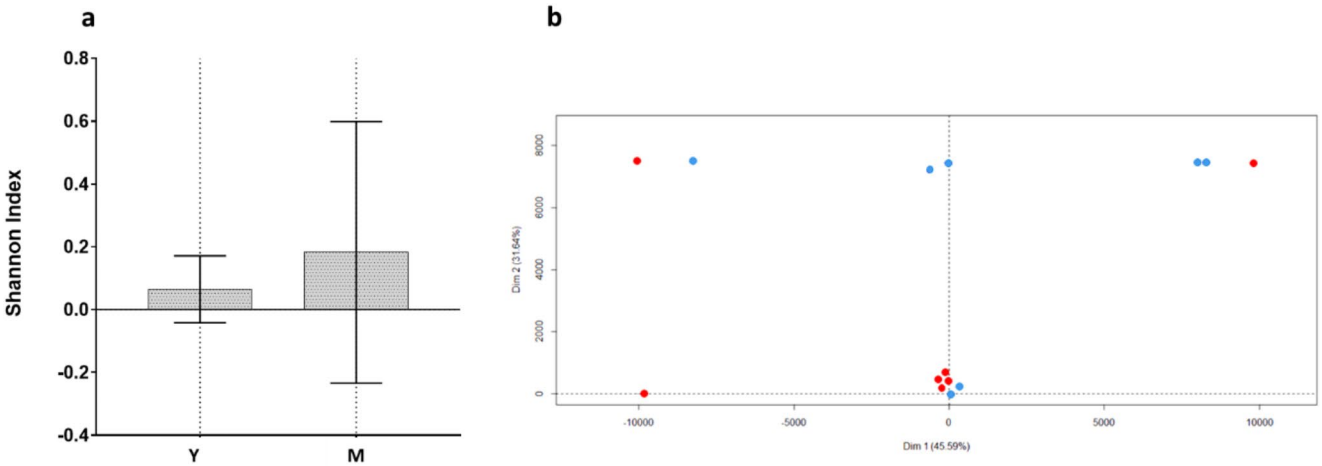

Fig. 1 Alpha- and beta-diversity of vaginal mycobiota. **a**: Shannon Index; **b**: Principal Component Analysis (PCA) of vaginal mycobiota Y (blue) and M (red) samples. Y: young women; M: menopausal women

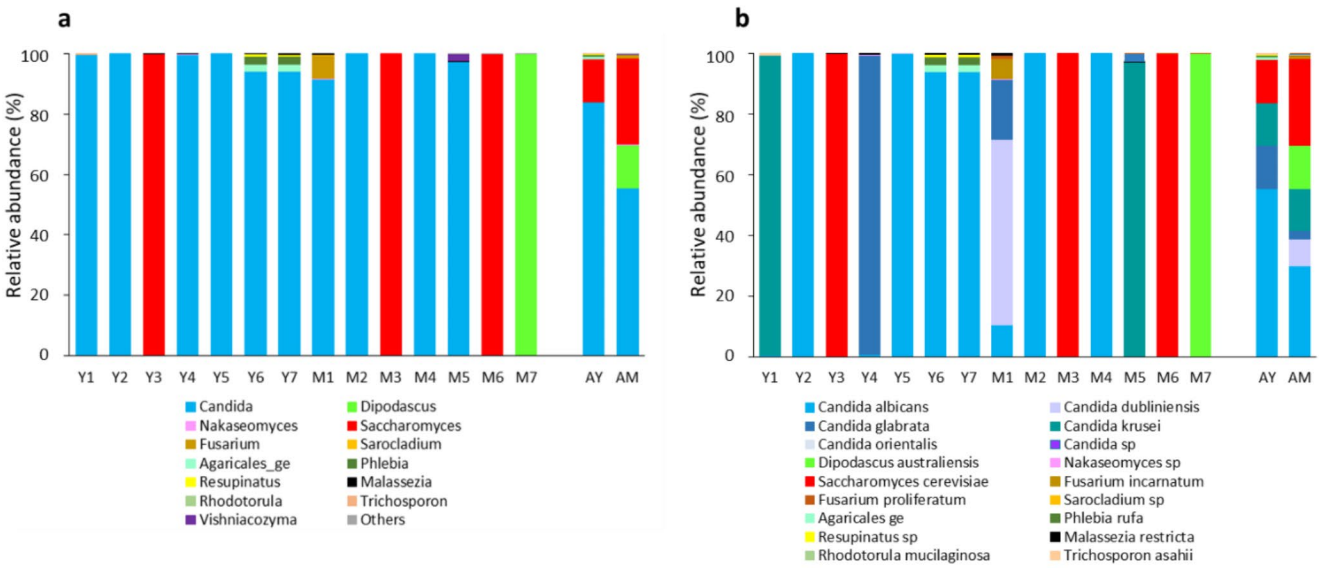

Fig. 2 Relative abundance of fungal genera (**a**) and species (**b**) in the vaginal mycobiota. Taxa with relative abundances below 0.01% were grouped as 'Others'. Y: young women; M: menopausal women; Y1-Y7, M1-M7: individual relative abundance; AY, MY: average relative abundance

dominant or present in all Y samples and in 50% of the M samples. *C. albicans* is commonly found as part of the vaginal microbiota in healthy women, where it coexists without causing infection, and functions as a commensal organism. The dominant presence of lactobacilli in a healthy vaginal environment plays a critical role in maintaining a low pH, which inhibits the growth of *C. albicans* and other opportunistic pathogens [27]. Furthermore, this species is known to regulate its virulence factors in response to hormonal levels and immune mechanisms [28]. In addition, its existence in a non-pathogenic state is largely maintained by a balanced interaction with the host immune system and the surrounding microbial communities. The second most important species was *S. cerevisiae*, which was either dominant or present in 16.66% of Y samples and 50% of M samples. *S. cerevisiae*, commonly known as baker's or brewer's yeast, has been studied in relation to the vaginal microbiota, although it is less common than other yeasts such as *C. albicans*. Some research suggests that *S. cerevisiae* may be present in the vaginal microbiota of healthy women, potentially contributing to the microbial ecosystem without causing infection [8].

In addition to these observations, it was evident that M samples exhibited greater taxonomic diversity, with a total of 21 species identified compared to only 12 species identified in the Y group across all samples (Table 2; Fig. 2). Furthermore, species such as *Dipodascus australiensis*, *Fusarium incarnatum*, and *Fusarium proliferatum*, found exclusively in M samples, are rare. *D. australiensis* has been reported as an environmental yeast [29], and macroconidia of *Fusarium* species have been noted as unusual findings in cervical smears [30].

Mycobiota composition and physiological indicators

All physiological and medical history parameters collected from the women in the study were analyzed to understand the changes occurring in the vaginal mycobiota. Our results showed a significant difference in estradiol levels between young women (ranging from 16.36 to 295 pg/mL) and postmenopausal women (ranging from 5 to 31.71 pg/mL). BMI also showed consistent variation, ranging from 22.22 to 32.86 in young women and from 26.66 to 42.44 in postmenopausal women (Table 1).

These differences may be rated to the higher alpha and beta diversity (Fig. 1) and the greater taxonomic complexity (Fig. 2) observed in the M group. Similar trends have been observed in studies investigating the variability of vaginal bacterial communities among Algerian women [15]. Furthermore, the increase in vaginal microbiota variability during menopause is well documented in the literature, often attributed to hormonal fluctuations, particularly the decrease in estrogen levels. These hormonal changes lead to changes in the vaginal environment, including an increase in pH, which can disrupt the balance of microbial communities [31].

The detection of uncommon taxa, such as *Dipodascus* and *Fusarium*, in the M group, in addition to the taxa already described in the vaginal mycobiota such as *Candida* and *Saccharomyces*, further indicates the more pronounced

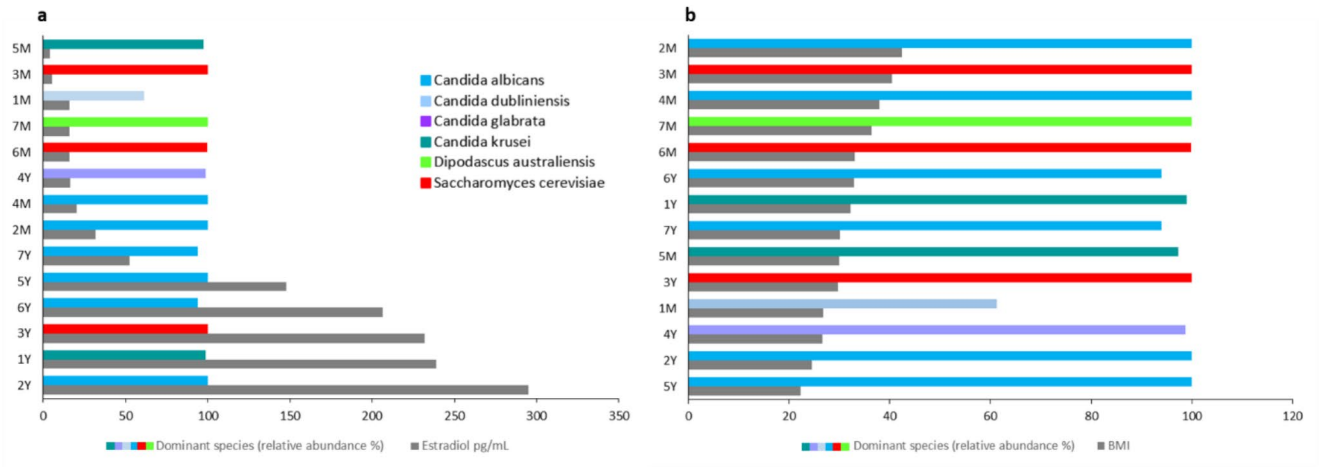

Fig. 3 Graphical representation of the dominant species following estradiol levels (**a**), and BMI values (**b**)

changes in the vaginal mycobiota at postmenopausal age. We created a graphical representation of the dominant species in each sample, along with estradiol levels (Fig. 3a) and BMI (Fig. 3b). Significantly greater taxonomic variability was observed in samples corresponding to lower estrogen levels (Fig. 3a). Specifically, samples with less than 16 pg/mL had 0% species identity, whereas samples with more than 16 pg/mL had 75% species identity. In contrast, no relationship was observed between BMI and the distribution of dominant species across samples (Fig. 3b).

Given that all other physiological parameters were healthy in both young and premenopausal women, the increased variability of the vaginal microbiota during menopause likely reflects a normal physiological response, representing a new microbial equilibrium adapted to hormone-related changes.

Conclusion

Analysis of the vaginal mycobiota revealed a single dominant fungal species per individual, with distinct differences between young and postmenopausal women, where *Candida* and *Saccharomyces* were the predominant genera. The presence of twice as many species in the postmenopausal group, along with uncommon taxa such as *Dipodascus* and *Fusarium*, highlights the pronounced taxonomic diversity observed in this group. These findings suggest that menopause is associated with greater microbial variability, likely due to hormonal changes that disrupt the vaginal environment. Despite the reduced number of samples, the results suggest a new microbial equilibrium in postmenopausal women, with taxonomic changes reflecting age-related physiological adaptations. Further research with larger sample sizes and consideration of additional physiological parameters is needed to elucidate the underlying mechanisms and broader implications of these findings, as well as to allow for comprehensive ethnic comparisons.

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Author contributions Conceptualization: Anca Lucau-Danila, Djamel Drider & Farida Bendali; Methodology: Anca Lucau-Danila, Djamel Drider; Software: George Daube, Bernard Taminiau, Nacim Barache; Validation: Liza Ouarabi, Anca Lucau-Danila, Djamel Drider, Farida Bendali; Formal analysis: Liza Ouarabi & Nacim Barache; Investigation: Liza Ouarabi, Anca Lucau-Danila; Resources: Liza Ouarabi, Anca Lucau-Danila, Djamel Drider, Farida Bendali, George Daube, Bernard Taminiau, Nacim Barache; Data Curation: Liza Ouarabi, Anca Lucau-Danila, Djamel Drider, Farida Bendali, George Daube, Bernard Taminiau & Nacim Barache Writing - Original Draft: Liza Ouarabi; Writing - Review & Editing: Liza Ouarabi, Anca Lucau-Danila, Djamel Drider Farida Bendali, Nacim Barache; Project administration & funding acquisition: Anca Lucau-Danila & Djamel Drider.

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Data availability The sequences gathered here and related to vaginal mycobiota are deposited at the National Center for Biotechnology Information (NCBI) (accession no. PRJNA630844).

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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