

Impact of Antiretroviral Treatment on Vaginal Microbiome Composition in People Living with HIV

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Abstract

Background

The vaginal microbiome is a critical determinant of reproductive health and HIV transmission, yet its composition in West African populations remains poorly characterised. Antiretroviral therapy (ART) has dramatically reduced HIV-related morbidity, but its impact on the vaginal ecosystem is incompletely understood. We used PacBio long-read 16S rRNA sequencing to characterise the vaginal microbiome of five women in Lagos, Nigeria: three HIV-positive (one with only a baseline sample, two with both a baseline and a one-month follow-up after ART initiation) and two HIV-negative controls, yielding a total of seven samples. We compared microbiome composition by age, *Lactobacillus* diversity, pathogen prevalence, and strain consistency; assessed the effect of ART; and screened for potentially novel bacterial taxa.

Results

The vaginal microbiome was dominated by *Lactobacillus* (mean relative abundance 68.2%). Species identified included *L. iners*, *L. crispatus*, *L. mulieris*, and *L. jensenii*. HIV-positive women exhibited higher abundances of BV-associated genera (*Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, *Mobiluncus*) and lower *Lactobacillus* compared to HIV-negative controls. DESeq2 analysis identified 163 differentially abundant ASVs, with 124 enriched in HIV-positive samples and 39 (all *Lactobacillus*) enriched in HIV-negative controls. HIV-positive individuals showed greater strain-level variability (mean Jaccard distance 0.51) compared to HIV-negative controls (0.23). Two participants with paired samples showed divergent ART responses: one maintained high *Lactobacillus* abundance (~ 99%), while the other increased from 0% to 99% after one month. We detected 39 candidate novel taxa with < 97% sequence identity to known species.

Conclusions

HIV-positive women in Lagos harbour a vaginal microbiome with lower *Lactobacillus*, higher BV-associated pathogens, and greater inter-individual strain variability compared to HIV-negative controls. ART initiation may affect *Lactobacillus* abundance differently across individuals. These findings provide a foundation for larger longitudinal studies in West African populations. Larger cohorts with comprehensive clinical metadata are urgently needed to validate these exploratory observations and inform interventions to restore vaginal eubiosis.

Background

The vaginal microbiome is a critical determinant of reproductive health and a key factor in HIV transmission. Yet, its composition in West African populations, particularly among women living with

HIV, remains poorly characterised [1–4]. In Nigeria, an estimated 1.9 million people live with HIV, with women accounting for nearly 60% of cases [5, 6]. Lagos State, the country's most populous urban centre, has an HIV prevalence of approximately 3.2% among pregnant women [7]. Although antiretroviral therapy (ART) has dramatically reduced HIV-related morbidity and mortality, its impact on the vaginal microbiome, a key determinant of reproductive health and HIV transmission, remains incompletely understood [8].

The vaginal microbiome is typically dominated by *Lactobacillus* species, which produce lactic acid and antimicrobial compounds that protect against sexually transmitted infections, including HIV [9, 10]. A shift towards a non-*Lactobacillus*-dominant state, characteristic of bacterial vaginosis (BV), is associated with increased HIV acquisition and transmission [10–12]. HIV-positive women often exhibit lower *Lactobacillus* abundance and higher levels of BV-associated anaerobes, such as *Gardnerella* and *Prevotella* [12, 13]. However, how ART initiation modulates these communities is unclear. Some studies suggest ART restores eubiosis, while others report persistent dysbiosis [14]. Moreover, most vaginal microbiome research has been conducted in Western or South African cohorts. Given the geographic and ethnic variation in microbiome composition observed globally, these findings may not be directly transferable to Nigerian women.

The impact of ART on the vaginal microbiome is particularly complex and remains poorly resolved [15]. Although ART effectively suppresses systemic viral replication, its local effects on the vaginal ecosystem are highly variable and may depend on baseline community structure, host immune reconstitution, and the specific drug regimen used [16–18]. As our pilot data and other recent reports suggest, women can exhibit divergent trajectories following ART initiation [8, 15]. Some maintain a stable, *Lactobacillus*-dominant environment, while others experience a dramatic recovery from a near-complete absence of *Lactobacillus* to nearly 100% dominance within just one month of treatment. Conversely, a subset of individuals may fail to restore eubiosis, maintaining a persistent dysbiotic state characterised by high abundances of anaerobes such as *Gardnerella* and *Prevotella* [8, 15]. This heterogeneity carries important clinical implications. Restoration of a *Lactobacillus*-rich environment could reduce local inflammation, decrease HIV shedding, and lower the risk of other sexually transmitted infections [9, 10, 12]. Conversely, persistent dysbiosis may perpetuate residual inflammation and maintain an elevated risk of HIV transmission to partners, despite systemic viral suppression [1, 3, 11, 12]. The mixed and often contradictory findings reported in the literature, combined with the complete absence of longitudinal data from West Africa, highlight a critical knowledge gap regarding the individualised nature of the vaginal microbiome response to ART [4, 8, 15].

To address this gap, we conducted a pilot study using PacBio long-read 16S rRNA sequencing to characterise the vaginal microbiome of five women in Lagos: three HIV-positive (one with only a baseline sample, two with both a baseline and a one-month follow-up after ART initiation) and two HIV-negative controls, yielding a total of seven samples. Our objectives were to compare microbiome composition by age, *Lactobacillus* diversity, pathogen prevalence, and strain consistency; to assess the effect of ART;

and to identify potentially novel bacterial taxa. This exploratory study provides a foundation for larger longitudinal investigations in West African populations.

Methods

Study design and participants

Vaginal swabs were collected from five participants: three HIV-positive individuals (two with a follow-up visit after one month of antiretroviral therapy (Dolutegravir 50mg, lamivudine 300mg and Tenofovir 300mg, all combined in one) initiation, one with only a baseline visit) and two HIV-negative controls, resulting in a total of seven samples. All participants provided written informed consent. The study was approved by the College of Medicine, University of Lagos Health Research Ethics Committee on 6th of January 2022 (Approval number CMUL/HREC/11/21/966). Participant metadata are summarised in Supplementary Table S1.

Sample collection and DNA extraction

Vaginal swabs were collected in a DNA/RNA ShieldTM (Cat. No R1100-50 Zymo Research, USA) stored at -80 °C until processing. Total genomic DNA was extracted using the Miniprep Kit (Zymo Research, Catalogue No. D4300) following the manufacturer's instructions. Seven metagenomic DNA samples were obtained.

16S rRNA gene amplification and PacBio sequencing

The V1-V9 hypervariable region of the bacterial 16S rRNA gene was amplified using primers M13 tailed forward primer /5AmMC6/GTAAAACGACGGCCAGT(N)n and M13 tailed reverse primer /5AmMC6/CAGGAAACAGCTATGAC(N)n. PCR products were barcoded, Library preparation was done with SMRTbell@prep kit 3.0 PacBio 102-182-700 and sequenced on the PacBio Sequel platform (one SMRT cell). Raw reads were demultiplexed and provided as .fastq.gz files.

Quality control and preprocessing

Raw reads were inspected with FastQC v0.11.9 and aggregated with MultiQC v1.14. Based on per-base quality profiles, reads were truncated at 1450 bp to remove the low-quality tail. Filtering was performed with DADA2 v1.26.0 using filterAndTrim (maxEE = 2, truncQ = 2, rm.phix = TRUE). Remaining reads were denoised with the DADA2 algorithm (dada function), and amplicon sequence variants (ASVs) were inferred using the makeSequenceTable function. Chimeras were removed with removeBimeraDenovo (consensus method).

Taxonomic assignment

Taxonomy was assigned to each ASV using the DADA2 assignTaxonomy function against the SILVA v138.1 reference database (nr99). Species-level assignment was attempted with addSpecies against the

corresponding SILVA species file. For ASVs that remained unclassified at the species level, we performed additional BLASTn searches against the NCBI nt database (accessed May 2026) to identify *Lactobacillus* species and to screen for potentially novel taxa (cut-off <97% identity to any named species).

Diversity and statistical analyses

Data handling and phyloseq

All downstream analyses were performed in R v4.3.3 using the phyloseq v1.42.0 package. ASVs with zero counts were pruned. Relative abundance profiles were generated by transforming counts to percentages.

Alpha diversity

Observed ASV richness and Shannon diversity index were calculated. Differences between HIV status groups and age categories (<30 years vs ≥30 years) were visualised with boxplots.

Beta diversity

Bray-Curtis dissimilarity was computed on relative abundance data. Principal coordinate analysis (PCoA) was used for ordination. Permutational multivariate analysis of variance (PERMANOVA, *adonis2* with 999 permutations) was performed to test the effects of HIV status and age group on community composition.

Genus composition and pathogen prevalence

Relative abundances of all genera were visualised as stacked bar plots. A subset of predefined pathogenic genera (*Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, *Mobiluncus*, *Mycoplasma*, *Ureaplasma*, *Megasphaera*) was extracted, and their relative abundances were summarised in a heatmap and a CSV table.

***Lactobacillus* analysis**

ASVs assigned to the genus *Lactobacillus* were extracted. Their relative abundances were visualised as a heatmap, and a table of ASV-level abundances was exported. BLASTn against NCBI nt was used to assign species names to each *Lactobacillus* ASV (top hit with ≥99% identity).

Differential abundance (HIV+ vs HIV-)

Differential abundance testing was performed using DESeq2 v1.38.3 on raw counts, with HIV status as the explanatory variable. Size factors were estimated using the geometric mean of positive counts. Log₂ fold changes and Wald test p-values were calculated. ASVs with an adjusted p-value <0.1 were considered differentially abundant. Results were visualised with a volcano plot.

Strain consistency (Jaccard similarity)

To assess strain-level consistency across groups, ASV tables were converted to presence/absence (1 if count >0). Jaccard distances were calculated, and mean within-group distances were computed for each combination of HIV status and age group.

Novel microbe detection

ASV sequences were exported as a FASTA file and searched against the NCBI nt database using BLASTn. Any ASV with <97% identity to the top hit and lacking a reliable genus assignment in the SILVA taxonomy was flagged as a candidate novel species.

Results

Study participants and sequencing output

Vaginal swabs were collected from five participants: three HIV-positive individuals (one with only a baseline sample, two with both a baseline and a one-month follow-up after ART initiation) and two HIV-negative controls, yielding a total of seven samples. After quality filtering and chimera removal, a total of 342 amplicon sequence variants (ASVs) were retained across all samples, with a mean of 37,368 reads per sample (range 30,397–41,185).

Overall microbial community composition

The vaginal microbiome was dominated by the genus *Lactobacillus* (mean relative abundance 68.2%, range 12.5–96.8%). Other abundant genera included *Gardnerella* (mean 12.3%), *Prevotella* (7.1%), *Bifidobacterium* (3.4%), and *Shuttleworthia* (2.1%). A complete genus-level composition for each sample is shown in Figure 1A, with rare genera (<1% per sample) grouped as “Other” in Figure 1B.

Lactobacillus diversity and species identification

We detected 104 ASVs belonging to the genus *Lactobacillus*. Their species assignments, based on BLASTn against the NCBI nt database ($\geq 99\%$ identity threshold), are listed in Supplementary Table S1. The identified species were *Lactobacillus iners*, *L. crispatus*, *L. mulieris*, and *L. jensenii*. The dominant species varied between individuals: *L. iners* was prevalent in most samples, while *L. crispatus* was also detected in several participants.

Age-stratified alpha diversity

Shannon diversity and observed ASV richness were compared between age groups (<30 years, n=4 vs ≥ 30 years, n=3) and HIV status. Overall, the mean Shannon index was 3.01 (SD 1.19) for HIV-positive women under 30 years, 2.72 (SD NA) for HIV-positive women ≥ 30 years, and 2.51 (SD 0.562) for HIV-negative women ≥ 30 years (Figure 2A). No statistically significant differences were observed ($p > 0.05$, Wilcoxon). The raw diversity values are provided in Supplementary Table S2.

Boxplots of Shannon diversity index (left) and observed ASV richness (right) stratified by age group (<30 years vs ≥30 years) and HIV status. No significant differences were observed (Wilcoxon, $p > 0.05$).

Beta diversity and community structure

Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity revealed that samples from HIV-positive individuals (both ART-naïve and after one month of ART) clustered separately from the two HIV-negative controls (Figure 2B). However, PERMANOVA testing the effects of HIV status and age group on community composition was not significant (pseudo-F = 1.157, $R^2 = 0.3665$, $p = 0.476$). The separation observed in the PCoA was primarily driven by higher relative abundances of *Lactobacillus* in HIV-negative controls and increased *Gardnerella* and *Prevotella* in HIV-positive samples.

Principal coordinate analysis (PCoA) of Bray-Curtis dissimilarities. Each point represents a sample, coloured by HIV status and shaped by age group. Ellipses indicate 95% confidence intervals. PERMANOVA testing the effects of HIV status and age group was not significant ($p = 0.476$).

Pathogen prevalence

We screened for eight genera commonly associated with bacterial vaginosis or vaginal dysbiosis. A total of 129 ASVs were assigned to these genera. *Gardnerella* was detected in all HIV-positive samples (mean relative abundance 15.6%) but only in one HIV-negative control (0.8%). *Prevotella* and *Atopobium* were present in five of seven samples, while *Sneathia* and *Mobiluncus* were found only in HIV-positive women (Table 1, Figure 3). No *Mycoplasma* or *Ureaplasma* were detected.

Table 1. Number of samples positive for each pathogenic genus by HIV status.

Presence defined as relative abundance > 0% (any detection). HIV+ n = 5, HIV- n = 2.

Pathogen	HIV+ (n=5)	HIV- (n=2)
<i>Gardnerella</i>	5	2
<i>Prevotella</i>	5	2
<i>Atopobium</i>	5	2
<i>Sneathia</i>	5	2
<i>Mobiluncus</i>	5	2
<i>Megasphaera</i>	5	2
<i>Mycoplasma</i>	0	0
<i>Ureaplasma</i>	0	0

Very low relative abundances (often <0.1%) were detected for some genera in HIV-negative samples; this may represent background noise or low-grade colonisation. These genera are typically considered

BV-associated, and their presence in healthy controls is not unprecedented (see Discussion).

Stacked bar plot showing the abundance of eight genera associated with bacterial vaginosis or vaginal dysbiosis in each sample. Samples are grouped by HIV status. *Gardnerella* was detected in all HIV-positive samples (mean 15.6%) but in only one HIV-negative control. *Sneathia* and *Mobiluncus* were found exclusively in HIV-positive women.

Differential abundance between HIV-positive and HIV-negative individuals

DESeq2 analysis identified 163 ASVs that were differentially abundant between HIV-positive and HIV-negative women (adjusted $p < 0.1$, $|\log_2 \text{fold change}| > 1$). Among these, 124 were enriched in HIV-positive samples, including several ASVs assigned to *Gardnerella*, *Prevotella*, and *Shuttleworthia*. Conversely, 39 ASVs were enriched in HIV-negative controls, all belonging to *Lactobacillus* (e.g., *L. iners*, *L. crispatus*). The full list is provided in Supplementary Table S4, and a volcano plot is shown in Figure 4.

DESeq2 analysis was performed on raw ASV counts. Each point represents an ASV. The x-axis shows the $\log_2$ fold change (HIV+ vs HIV-), and the y-axis shows the $-\log_{10}$ of the unadjusted p-value. ASVs with an adjusted p-value < 0.1 and $|\log_2 \text{FC}| > 1$ are coloured red (enriched in HIV+) or blue (enriched in HIV-). Grey points are not significantly different.

Strain consistency across populations

Jaccard similarity analysis of ASV presence/absence showed that HIV-negative controls had the highest within-group similarity, indicating a more consistent strain-level composition (mean Jaccard distance 0.23). HIV-positive individuals, regardless of ART exposure, exhibited greater within-group dissimilarity (mean Jaccard distance 0.51). Pairwise distance matrices are provided in Supplementary Table S5.

Novel microbe detection

We searched all 342 ASVs against the NCBI nt database. A total of 39 ASVs showed less than 97% sequence identity to any named species in the top BLAST hit. Many of these also lacked a reliable genus assignment from SILVA. These ASVs are listed in Supplementary Table S6 as candidate novel bacterial taxa. Their sequences have been deposited in GenBank under accession numbers PRJEB124225.

Association with antiretroviral therapy (exploratory analysis)

Among the five HIV-positive individuals, two provided a follow-up sample after one month of ART (participants PND02 and PND03). In participant PND02, *Lactobacillus* abundance remained high (~99%) at both time points. In participant PND03, *Lactobacillus* abundance increased from 0% at baseline to 99% after one month of ART. Conversely, *Gardnerella* (not shown) showed the opposite pattern (data not shown). No single ASV reached statistical significance due to the small sample size. These divergent individual responses highlight the need for larger longitudinal studies to understand how ART affects the vaginal microbiome.

Relative abundance of *Lactobacillus* before (First) and after one month of ART (Second) in the two HIV-positive participants who provided paired samples (PND02 and PND03). Participant PND02 maintained high *Lactobacillus* abundance (~99%) at both time points, while participant PND03 showed an increase from 0% to 99% after ART. No ASV reached statistical significance (exploratory analysis).

Discussion

This pilot study used PacBio long-read 16S rRNA sequencing to characterise the vaginal microbiome of five women (three HIV-positive, two HIV-negative). Despite the small sample size, several patterns emerged that are consistent with the literature and provide new insights into HIV-associated vaginal dysbiosis and the effect of antiretroviral therapy. Together, these findings address our primary objectives: age-associated differences in alpha diversity were not statistically significant; *Lactobacillus* diversity comprised four species with high inter-individual variation; BV-associated pathogens were more prevalent and abundant in HIV-positive women; HIV-positive individuals exhibited greater strain-level variability; ART initiation showed divergent individual responses; and 39 candidate novel taxa were identified. Each of these findings is discussed in turn below.

Lactobacillus dominance and species composition

As expected, the vaginal microbiome was dominated by *Lactobacillus* (mean relative abundance 68.2%). BLASTn-based species assignment ($\geq 99\%$ identity) identified *L. iners*, *L. crispatus*, *L. mulieris*, and *L. jensenii*. *L. iners* was the most prevalent species, a finding consistent with observations from many populations, particularly in sub-Saharan Africa [2, 4,19]. The presence of *L. crispatus* in several participants is notable because *L. crispatus*-dominated communities (community state type I) are associated with the lowest risk of adverse reproductive outcomes, including HIV acquisition [12, 13]. The high inter-individual variability in *Lactobacillus* species observed here may influence susceptibility to HIV and other sexually transmitted infections, as different species exhibit varying capacities for hydrogen peroxide production, lactic acid generation, and epithelial barrier protection [9, 10]. This variability underscores the importance of species-level resolution, which PacBio full-length 16S rRNA sequencing enabled, rather than genus-level characterisation alone.

HIV-associated shifts: increased pathogens, reduced *Lactobacillus*

HIV-positive women had higher relative abundances of *Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, and *Mobiluncus*, and lower *Lactobacillus* compared to HIV-negative controls. These genera are hallmarks of bacterial vaginosis and have been linked to increased HIV acquisition and transmission [1, 3, 11, 12]. The mechanisms linking these anaerobes to HIV risk are multifaceted. *Gardnerella* produces vaginolysin, a cytolyisin that disrupts the epithelial barrier and may facilitate HIV shedding [1,20]. *Prevotella* and *Sneathia* are potent inducers of pro-inflammatory cytokines, which recruit CD4+ T cells to the genital mucosa, providing target cells for HIV infection [3,21]. The exclusive detection of *Sneathia* and *Mobiluncus* in HIV-positive individuals further supports the concept that HIV infection promotes a more diverse and anaerobe-rich vaginal ecosystem, even in the absence of clinical BV symptoms [4,22]. This

finding aligns with studies demonstrating that immune dysregulation associated with HIV, including reduced mucosal CD4⁺ T cell responses and altered cytokine profiles, may permit overgrowth of anaerobes that are normally kept in check by a robust *Lactobacillus*-dominated community [12, 13].

Effect of antiretroviral therapy

The two participants with paired samples showed divergent responses to one month of ART. One participant maintained high *Lactobacillus* abundance (~99%) at both time points, while the other experienced an increase from 0% to 99% *Lactobacillus* after ART. This suggests that ART initiation may have individualised effects on the vaginal microbiome, possibly mediated by baseline composition, immune reconstitution, or direct drug effects [8, 15, 16]. Larger studies have reported mixed results: some show restoration of *Lactobacillus* dominance following ART, while others describe persistent dysbiosis despite systemic viral suppression [8, 15]. The divergence observed in our pilot data highlights the need for personalised monitoring and larger longitudinal cohorts to determine whether specific baseline characteristics predict favourable microbiome responses to ART. It is also plausible that different ART regimens exert distinct effects on the vaginal ecosystem, an area that remains poorly explored [18]. Our observations are hypothesis-generating and require validation in cohorts with adequate statistical power and comprehensive clinical metadata.

Pathogen prevalence and clinical relevance

The universal detection of *Gardnerella* in HIV-positive samples and its presence in both HIV-negative controls at very low abundances (<0.1%) is consistent with reports that *Gardnerella* is a key BV-associated bacterium that produces vaginolysin, a cytolysin that disrupts the epithelial barrier and may facilitate HIV shedding [1, 11]. The absence of *Mycoplasma* and *Ureaplasma* in our samples may reflect the small sample size or geographic variation, as these genera are more prevalent in some sub-Saharan African populations [2, 4]. It is also possible that the detection thresholds of our sequencing approach, combined with the relatively high stringency of our quality filtering, limited the identification of very low-abundance taxa. The clinical relevance of these findings extends beyond HIV transmission: BV-associated anaerobes have been linked to adverse pregnancy outcomes, pelvic inflammatory disease, and increased risk of other sexually transmitted infections [1, 3]. Longitudinal studies integrating microbiological data with clinical diagnoses such as Nugent score or Amsel criteria are needed to determine whether the microbial shifts observed here correspond to symptomatic BV or represent subclinical dysbiosis.

Strain consistency and transmission implications

HIV-negative controls had substantially higher within-group Jaccard similarity (0.23) than HIV-positive individuals (0.51), indicating more consistent strain-level composition. This finding is consistent with the concept that HIV infection promotes vaginal dysbiosis and increased inter-individual variability [1, 4]. Greater strain-level variability in HIV-positive women may reflect the cumulative effects of chronic immune activation, recurrent infections, and antibiotic exposure, all of which are more common in this

population [3]. Whether specific *Lactobacillus* strains are protective against HIV acquisition remains an active area of research. Strain-level differences within the same species can influence functional properties such as adhesion to epithelial cells, production of antimicrobial peptides, and modulation of local immune responses [9, 10]. The Jaccard similarity metric used here provides a conservative estimate of strain sharing; future studies employing metagenomic shotgun sequencing or cultured isolates would enable more precise strain tracking and functional characterisation.

Candidate novel microbes

The detection of 39 ASVs with less than 97% sequence identity to known species suggests the presence of potentially novel bacterial taxa in the vaginal niche. This is not unexpected, as many vaginal bacteria remain uncultured and underrepresented in reference databases [4]. The use of PacBio long-read sequencing, which captures nearly full-length 16S rRNA genes, provides greater phylogenetic resolution than short-read platforms and increases the likelihood of identifying divergent taxa [2]. However, confirmation of novelty requires phylogenetic analysis, including construction of neighbour-joining or maximum-likelihood trees to establish placement within known genera or families. Ideally, isolation in culture would permit comprehensive characterisation, including genome sequencing, metabolic profiling, and assessment of interactions with host cells. The sequences of these candidate taxa have been deposited in GenBank to facilitate future identification by other researchers working on vaginal microbiomes in African populations.

Limitations and future directions

The primary limitation of this study is the small sample size ($n=5$ participants, 7 samples), which precludes robust statistical inference and generalisation. The PERMANOVA for HIV status and age was not significant, likely due to low power rather than absence of biological effect. Additionally, the lack of singletons in our dataset, resulting from strict chimera removal and filtering, may underestimate true richness. The one-month ART follow-up window is short, and we had no paired samples from HIV-negative controls over time. Furthermore, we lacked clinical data such as CD4 count, viral load, ART regimen details, and Nugent score for BV diagnosis. These variables could influence both baseline microbiome composition and the response to ART, and their absence limits our ability to attribute observed differences solely to HIV status or ART. The cross-sectional comparison between HIV-positive and HIV-negative women also means that causality cannot be inferred. Future work should include a larger cohort with comprehensive clinical metadata, longer follow-up periods, and integration of host immune and metabolic measurements. Such studies would enable identification of predictors of favourable microbiome responses to ART and inform the development of interventions such as probiotic supplementation or targeted antimicrobial therapy to restore eubiosis and reduce HIV-associated comorbidities and transmission risk.

Conclusions

This pilot study demonstrates that HIV-positive women harbour a vaginal microbiome with lower *Lactobacillus*, higher *Gardnerella* and *Prevotella*, and greater inter-individual strain variability compared to HIV-negative controls. ART initiation may have individualised effects on *Lactobacillus* abundance, ranging from stable high levels to dramatic increases. The identification of specific *Lactobacillus* species and candidate novel taxa provides a foundation for future mechanistic and translational research. Ultimately, understanding how HIV and ART shape the vaginal ecosystem could inform interventions to restore eubiosis and reduce HIV-associated comorbidities. Larger longitudinal cohorts are urgently needed to validate these exploratory findings.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the College of Medicine, University of Lagos Health Research Ethics Committee on 6th of January 2022 (Approval No. CMUL/HREC/11/21/966). All participants provided written informed consent before enrolment.

Availability of Data and Materials

Raw sequence reads have been deposited in the NCBI Sequence Read Archive under accession number PRJEB124225. The ASV table, taxonomy, metadata, and all analysis scripts are available at <https://github.com/bbeckley-hub/Vaginal-Microbiome-Study-Nigeria>.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

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Authors' contributions

Adesola Olalekan: Conceptualisation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Brown Beckley: Formal analysis, Data curation, Software, Visualization, Writing – original draft, Writing – review & editing.

Ogunsanya Oluwabunmi: Investigation, Methodology, Writing – review & editing.

Felix Agomma: Investigation, Data curation, Writing – review & editing.

Olarenwaju Ademuyiwa: Clinical data handling, patient follow-up, Writing – review & editing.

All authors read and approved the final manuscript.

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Figures

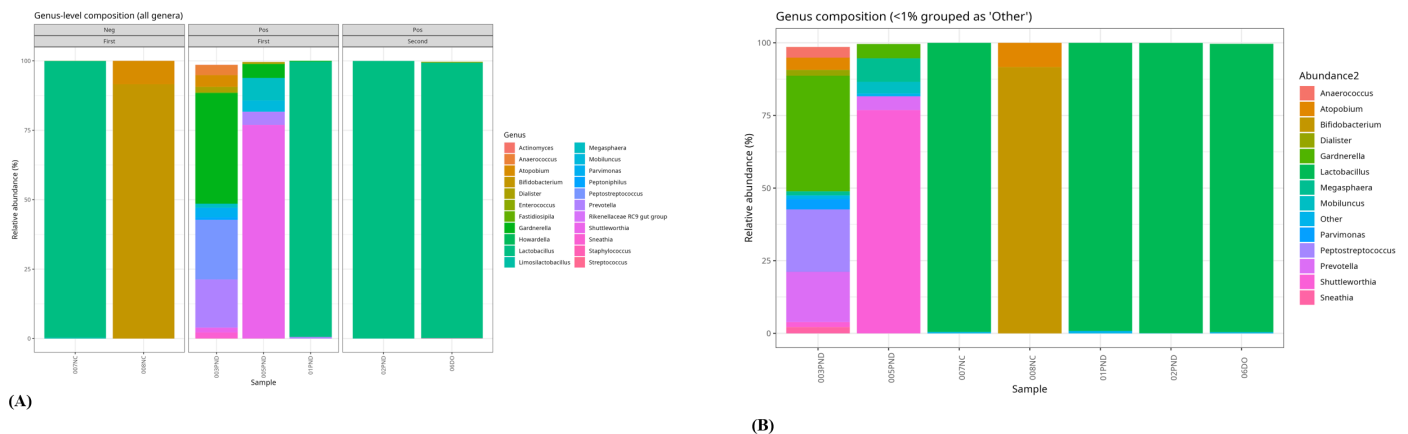


Figure 1

Genus-level composition of the vaginal microbiome.

(A) Stacked bar plot showing the relative abundance of all bacterial genera detected in each sample. Samples are grouped by HIV status (Pos = HIV-positive, Neg = HIV-negative) and time point (First, Second, or No follow-up). **(B)** Same as (A) but with genera representing less than 1% of the total in a given sample aggregated into the category “Other”.

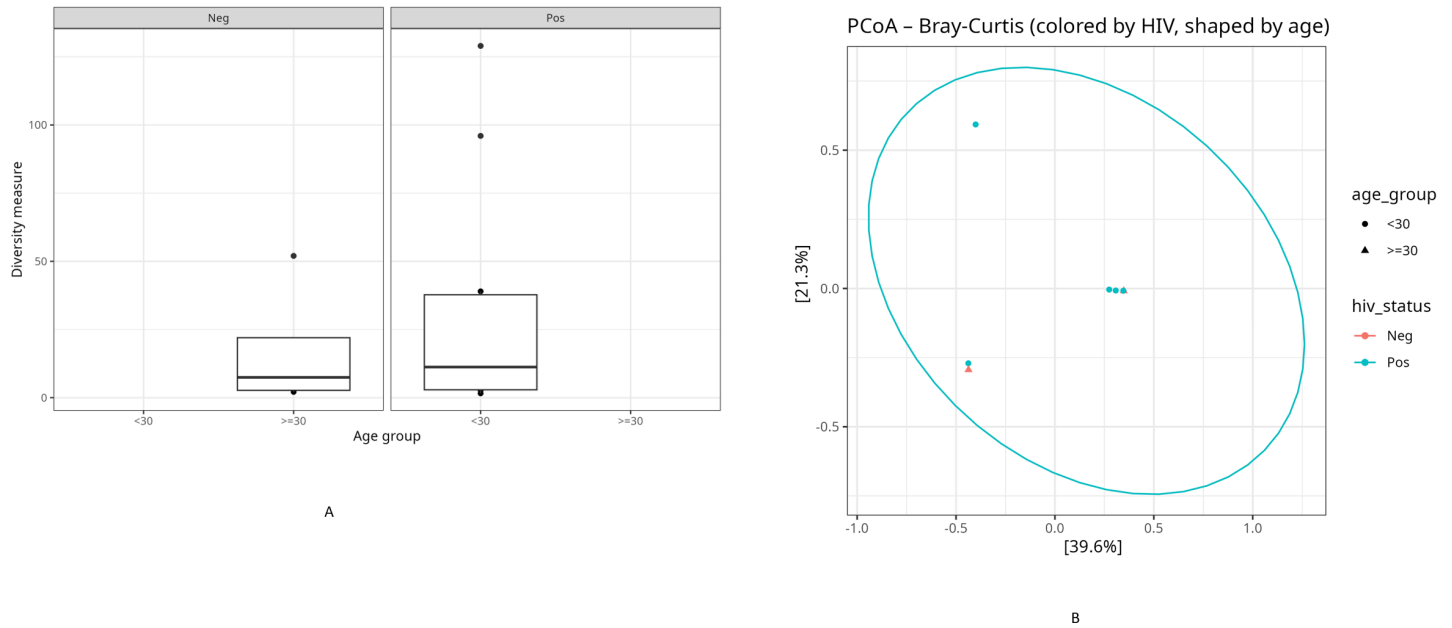


Figure 2

A. Alpha and beta diversity of the vaginal microbiome.

Boxplots of Shannon diversity index (left) and observed ASV richness (right) stratified by age group (<30 years vs ≥30 years) and HIV status. No significant differences were observed (Wilcoxon, $p > 0.05$).

B. Alpha and beta diversity of the vaginal microbiome.

Principal coordinate analysis (PCoA) of Bray-Curtis dissimilarities. Each point represents a sample, coloured by HIV status and shaped by age group. Ellipses indicate 95% confidence intervals. PERMANOVA testing the effects of HIV status and age group was not significant ($p = 0.476$).

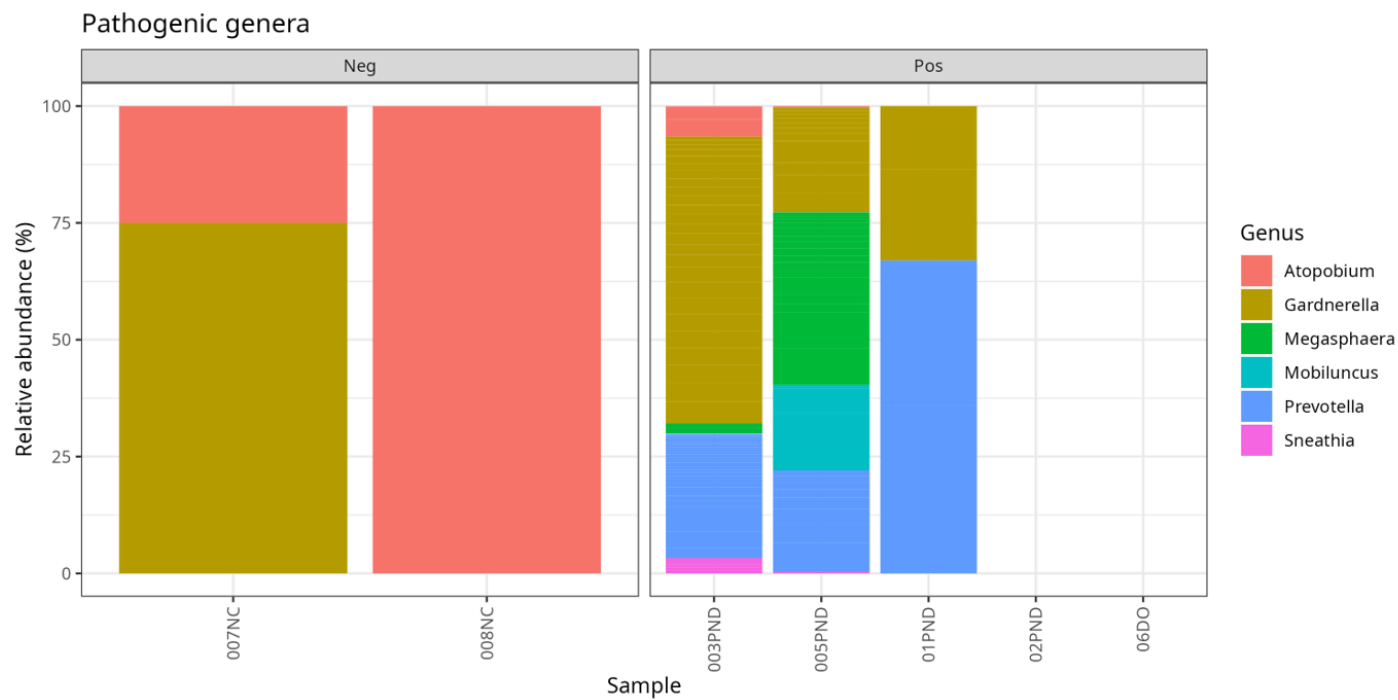


Figure 3

Relative abundance of pathogenic genera.

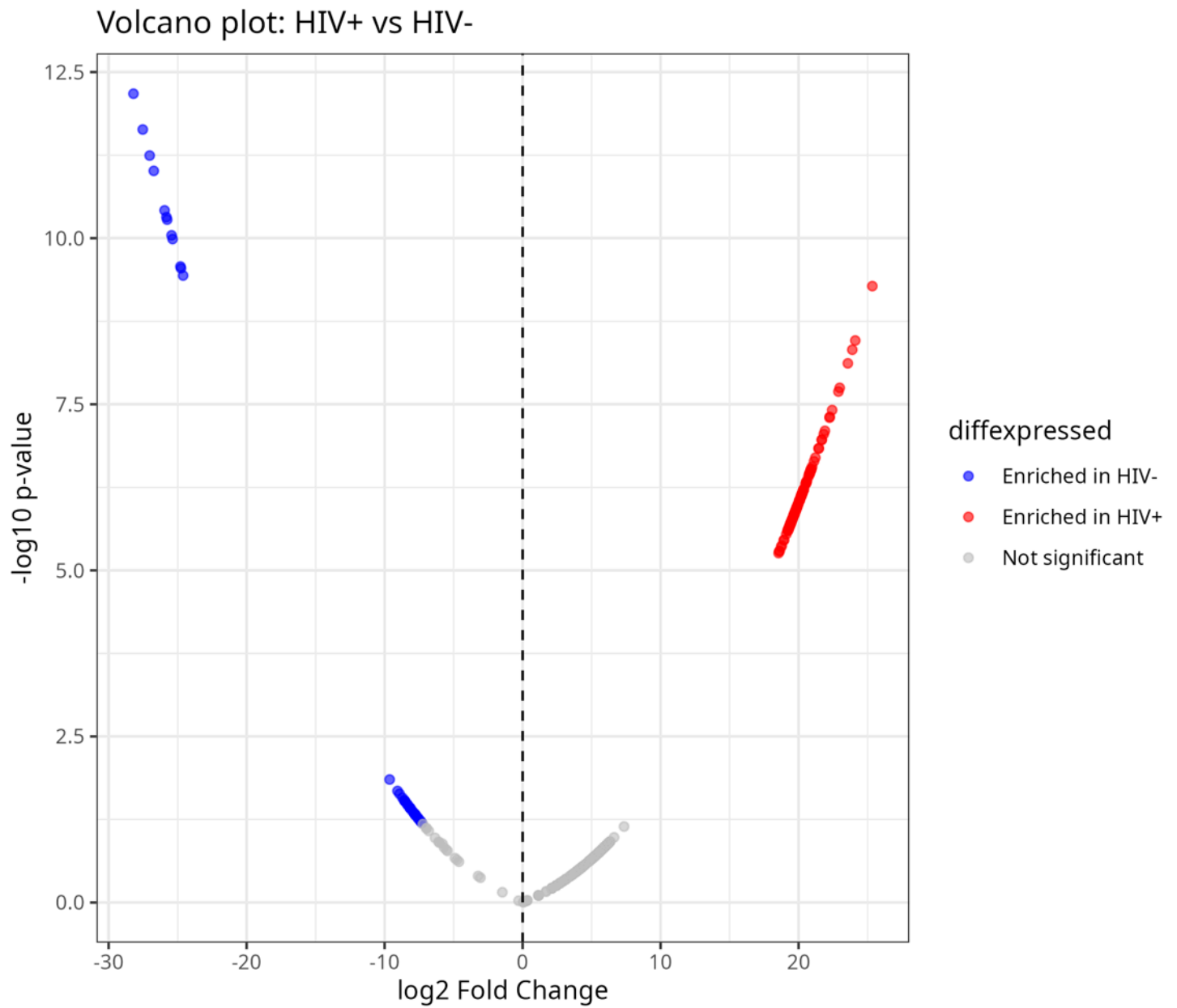


Figure 4

Volcano plot of differentially abundant ASVs between HIV-positive and HIV-negative women.

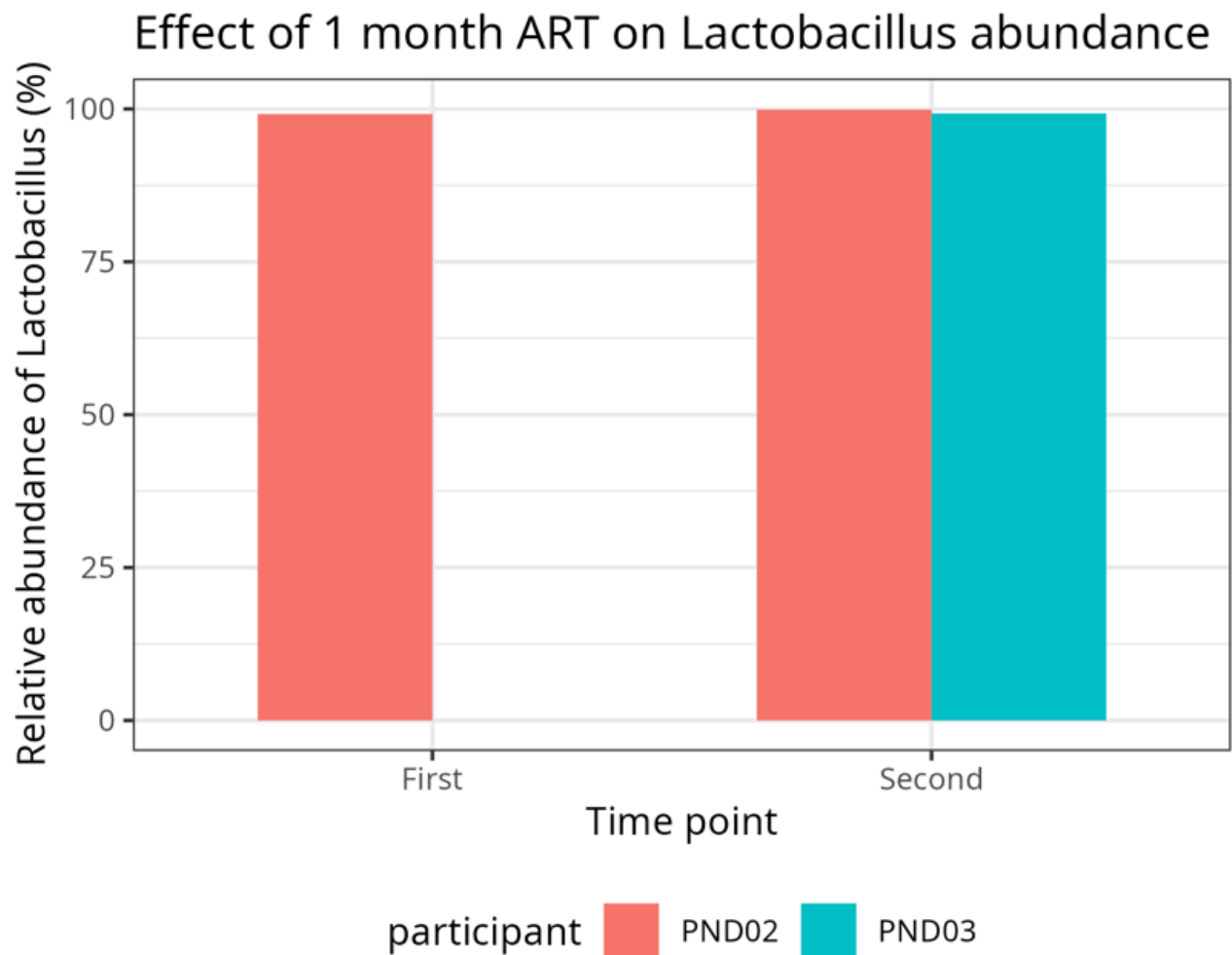


Figure 5

Effect of one month of antiretroviral therapy (ART) on *Lactobacillus* abundance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1S6.csv](#)
- [SupplementaryFigureS1.png](#)