



Vaginal bacterial microbiome composition in women living with HIV in Meru, Kenya

Thomas Atenya^{1*}, Sophia Mogere⁶, Celestine Kemunto^{2,3}, Joan Simam^{1,5}, Cynthia N. Mugo Mwenda¹, Frank G. Onyambu^{2,1}

¹Meru University of Science and Technology, Meru, Kenya, ²Centre for Molecular Biosciences and Genomics, ³Kenyatta University,

⁵National Cancer Institute of Kenya, ⁶University of Nairobi

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ABSTRACT

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The immune system status and the vaginal microbiome represent risk factors for hrHPV and women living with HIV are susceptible to persistent HPV infections. This study characterized the vaginal bacterial communities in women living with HIV in Meru. A cross-sectional study was conducted involving 38 women living with HIV at the Meru Teaching and Referral Hospital. Genomic DNA extraction and 16S rRNA amplification was carried out. Bioinformatic analysis was performed using R and Cutadapt was used to perform sequence quality control.

We identified 6,481 bacterial taxa. Lactobacillus and Gardnerella were the most abundant genera in the study population. In conclusion, this study shows the status of vaginal microbiome in Kenyan women living with HIV. Follow up studies are recommended to include control group of HIV-negative women.

Introduction

Kenya has a total of 1,377,784 people living with HIV as per the National Syndemic Diseases Control Council (NSDCC) statistics, with a 5.31% prevalence among women (NSDCC 2023). Women living with HIV are six times more likely to develop cervical cancer in comparison to those without HIV, due to their immunocompromised status (WHO, 2024). In Sub-Saharan Africa, there is a substantial correlation between HIV and HPV-associated dysplasia, with HIV-infected women having a higher prevalence of hrHPV genotypes compared to HIV-negative women (Castle et al., 2020; Klein et al., 2019, 2020; Taku et al., 2020; Tchouaket et al., 2023).

The human microbiome constitutes a community made up of symbiotic microbes which function in the maintenance of human physiology and general body fitness (Ma et al., 2024; Nieves Delgado & Baedke, 2021). A healthy vaginal microbiome is dominated by Lactobacilli which modulate genital health through the production of lactic acid and hydrogen peroxide (Sharifian et al., 2023). A Lactobacillus-dominant vaginal microbiome protects women against invading pathogens whereas hrHPV thrives in a diverse microbiome with an abundance of Gardnerella vaginalis and Atopobium vaginae (Santella et al., 2022; Sharifian et al., 2023).

*Corresponding author: Thomas Atenya

Email: thomasatenya43@gmail.com

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Few studies describing the vaginal microbiome of women living with HIV have been conducted in Kenya, most of which are cross-sectional. This study sought to characterize the vaginal microbiome of a representative sample group of women living with HIV at the Meru Teaching and Referral Hospital's (MeTRH) HIV clinic in Meru County, Kenya. We identified bacterial communities in the vagina of HIV patients with or without HPV.

Materials and Methods

Study site

This study was carried out at the Meru Teaching and Referral Hospital's (MeTRH) HIV clinic in Meru County, located in the Eastern region of Kenya.

Ethical considerations

Ethical approval for the study was obtained from the Meru University of Science and Technology Institutional Research Ethics Review Committee (MIR-ERC); approval number MIRERC004/2024

Study design and population

From a cohort of 303, we randomly selected 38 participants to explore the relationship between HPV status and the vaginal bacterial microbiome.

Sample and data collection

Semi-structured questionnaires were used to collect data from the participants including socio-demographic and behavioural characteristics. Vaginal swabs were collected using the Evalyn brush (Rovers® Medical, Netherlands) through a self-sampling procedure and the samples were transported to the Centre for Molecular Biosciences and Genomics (CMB) Nairobi, for laboratory analysis.

DNA extraction and quantification

DNA extraction was done using the Wizard Genomic DNA Purification Kit (Promega Corporation, Wisconsin, USA). The Qubit™ dsDNA HS assay kit was used to quantify DNA yield after extraction (Thermo Fisher Scientific, Massachusetts, USA). Samples with a concentration of ≥ 1.5 ng/ μ l were selected for sequencing.

High-risk HPV detection and 16S rRNA sequencing

The HPV Screening PCR Kit was used to detect hr-HPV genotypes (GeneProof, Brno, Czech Repub-

lic). Real-time PCR was performed on the Bio-Rad CFX-96 platform (Bio-Rad, California, USA). Deep sequencing of the V3-V4 region was performed using MGIEasy Fast Library Prep kit (MGI Tech, Shenzhen, China). Libraries were circularised to form a DNA Nanoball through rolling circle amplification. The DNB was sequenced on the combinatorial probe-anchor synthesis (cPAS)-based DNBSEQ-G99 (MGI Tech, Shenzhen, China) platform.

Data analysis

Data pre-processing and taxonomic classification

Cutadapt was used to perform sequence quality control by trimming gene-specific 16S rRNA V3-V4 primers from the sequence. DADA2 filtered out poor quality reads, denoised sequence data and assigned taxonomy. Phyloseq was used to perform diversity analysis and generate data visualizations (v1.52.0).

Results

The median age of women was 35 years (29-40). Most women reported occasional condom use during sexual intercourse (21/38). Contraceptive use was common (22/38), with the majority indicating use for more than 5 years. Most women in the study had a minimum level of education as secondary school (20/38). Finally, most participants reported being single (21/38). It was observed that none of the socio-demographic characteristic of the participants had a statistically significant relationship with vaginal bacterial microbiome status.

Vaginal microbiota composition and abundance

We identified 6,481 bacteria from 32 samples belonging to the following phyla: Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria, Tenericutes, Proteobacteria, Saccharibacteria and Spirochaete. Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria were the most abundant phyla in the study population, in a decreasing order.

Firmicutes was the most abundant vaginal microbiome phyla followed by Actinobacteria, Bacteroidetes, Fusobacteria. Spirochaetes and Proteobacteria were the least abundant phyla as shown in **Table 1 below**.

Phylum	Absolute abundance	Relative abundance
Firmicutes	3508	48.07%
Actinobacteria	1928	26.42%
Bacteroidetes	854	11.70%
Fusobacteria	562	7.70%
Proteobacteria	173	2.37%
Tenericutes	137	1.88%
Patescibacteria	70	0.96%
Euglenozoa	34	0.47%
Spirochaetes	28	0.38%
Protalveolata	3	0.04%

Table 1: Abundance of phyla in the vaginal microbiome in women living with HIV I Meru

Discussion

In this study, we described the vaginal microbiome in Kenyan women living with HIV. We observed that *Lactobacillus* was the most abundant genus in our study population, which is typically the most dominant genus in the healthy vaginal microbiome (Ntuli et al., 2022; Reimers et al., 2016). This finding shows that despite the diversity and presence of pathogenic bacterial genera; the vaginal microbiome strives to maintain an abundant *Lactobacillus*. However, the high abundance of dysbiosis-related bacteria genera such as *Gardnerella*, *Sneathia* and *Streptococcus* could be attributed to the participants' HIV status. HIV infection has been associated with the proliferation of dysbiotic bacteria including *Lactobacillus iners*, *Prevotella bivia*, *Atopobium*, and *Gardnerella*, thereby increasing susceptibility to hrHPV (Badial et al., 2018; Klein et al., 2019, 2020; Shvartsman et al., 2023). HIV is associated with increased progression risk from sub-clinical to clinical HPV disease. A decrease in the abundance of *Lactobacilli* promotes the growth of pathogenic bacteria resulting in increased susceptibility to viral infections such as HPV (Zeber-Lubecka et al., 2022, 2024).

One of the main limitations of this study was the small sample size. Another limitation was the inability to assign taxonomy up to the species which is also attributable to the small sample size.

Conclusion

Lactobacillus and *Gardnerella* are the most abundant bacterial genera in the vaginal microbiome of women living with HIV. However, a control group of HIV-negative women should be carried out to confirm this.

Recommendation

Subsequent studies should recruit more participants to minimize the bias that might arise with small sample sizes

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