Improving Vaginal Health for HIV-1 Prevention:

Comparison of Different Collection Methods for Vaginal Microbiota Profiling to Analyze Molecular Bacterial Vaginosis.

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The vaginal microbiota (VMB) is a key mediator of vaginal health and susceptibility to infection.

Women are at a higher risk of acquiring human immunodeficiency virus (HIV) and other sexually transmitted infections compared to men. In addition to socioeconomic factors, there are a number of biological features that contribute to this elevated risk, including the vaginal microbiota (VMB). A predominantly *Lactobacillus* VMB is associated with robust vaginal barrier integrity and reduced susceptibility to HIV-1; however, effects on vaginal health can vary between *Lactobacillus* strains. Conversely, a highly diverse VMB with low *Lactobacillus* abundance is associated with inflammation, diminished barrier function, the clinical condition bacterial vaginosis (BV), and elevated HIV susceptibility.

Examining the VMB profile is essential to elucidate mechanisms through which the VMB influences vaginal inflammation, barrier integrity, and clinical outcomes, such as risk of BV and HIV infection. **In order to establish VMB sampling methods that produce high quality, reliable profiles, VMB profiles ascertained from cervicovaginal lavages (CVLs), nurse- and self-collected vaginal swabs were compared.**

References

Wessels JM, et al. The relationship between sex hormones, the vaginal microbiome and immunity in HIV-1 susceptibility in women. Dis Model Mech. 2018 Aug 28;11(9):dmm035147.

Mtshali A, et al. HIV susceptibility in women: The roles of genital inflammation, sexually transmitted infections and the genital microbiome. J Reprod Immunol. 2021 Jun;145:103291.

Comparison of Sampling Methods for VMB Profile Analysis

23 Women 18-40 years old (from Toronto area)

Questionnaire

- Demographics
- Contraceptive use
- Sexual health & history
- Concomitant medications & supplements

VMB Samples Collected:

- Cervicovaginal lavage (CVL)
- Nurse-collected vaginal swab
- Self-collected vaginal swab

VMB Profiling using 16S rRNA Gene Sequencing

1. Genomic DNA extraction
2. Nested PCRs: 16S gene, V3-V4 region of 16S gene
3. Illumina MiSeq sequencing
4. Process reads (Cutadapt, Trimmomatic, DADA2)
5. Assign taxonomy using SILVA 138.1
6. Analysis in R & GraphPad

Protocols available at: https://www.surettelab.ca/protocols

23 16S rRNA gene VMB Profiles

- 14 matched CVL, nurse- & self-collected swabs
- 9 matched CLV & nurse-collected swabs

Sequencing depth was independent of sample type.

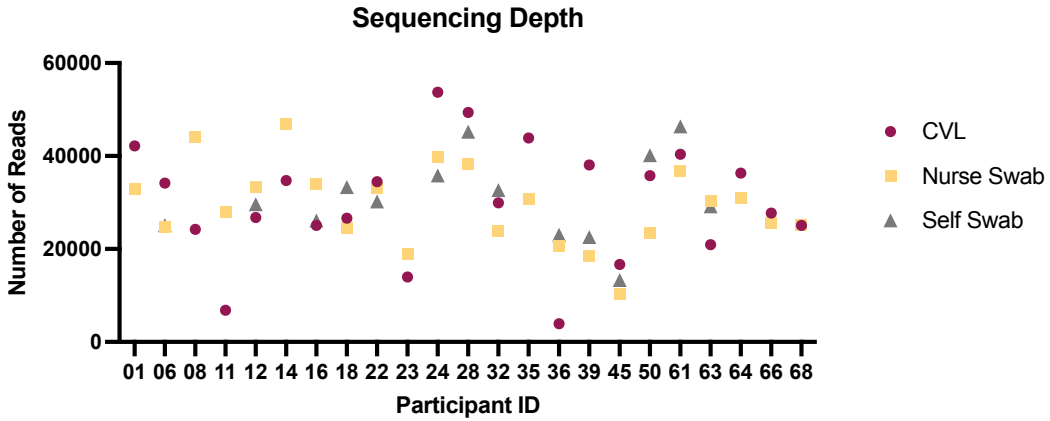

Figure 1. 16S rRNA gene sequencing depth of VMB samples. Statistical significance between sample types was evaluated by ANOVA with Tukey's post-hoc test (p=0.90).

Alpha diversity was not significantly different between sample types.

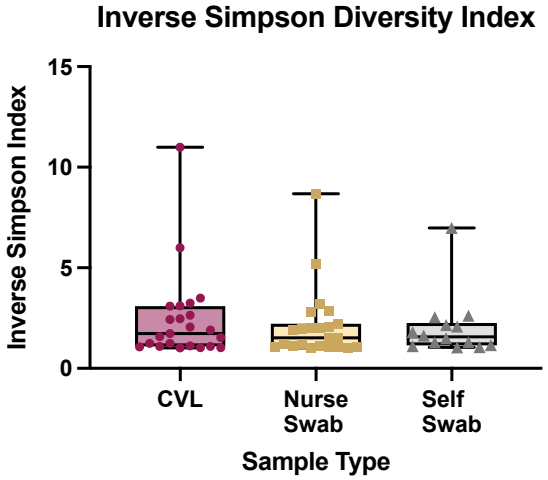

Figure 2. Assessment of VMB alpha diversity using the inverse Simpson diversity index. Statistical significance between sample types was evaluated by ANOVA with Tukey's post-hoc test (p=0.76).

Clustering by participant in principal component analysis (PCoA) indicated that the VMB profile was consistent between sample types.

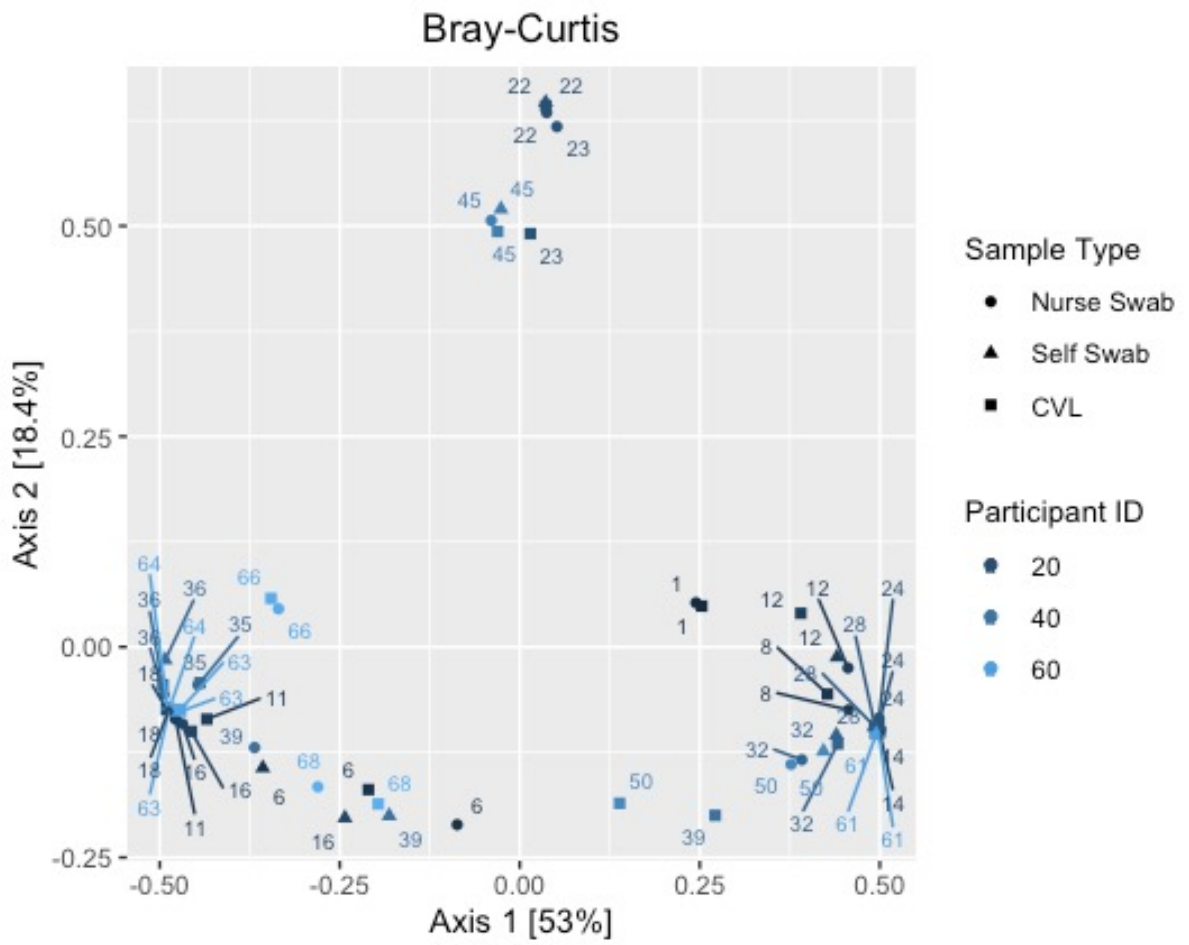

Figure 3. PCoA of Bray Curtis dissimilarity between samples. Beta diversity was evaluated by performing PCoA based on Bray Curtis dissimilarity metric.

16S rRNA profiles were consistent between VMB sampling types.

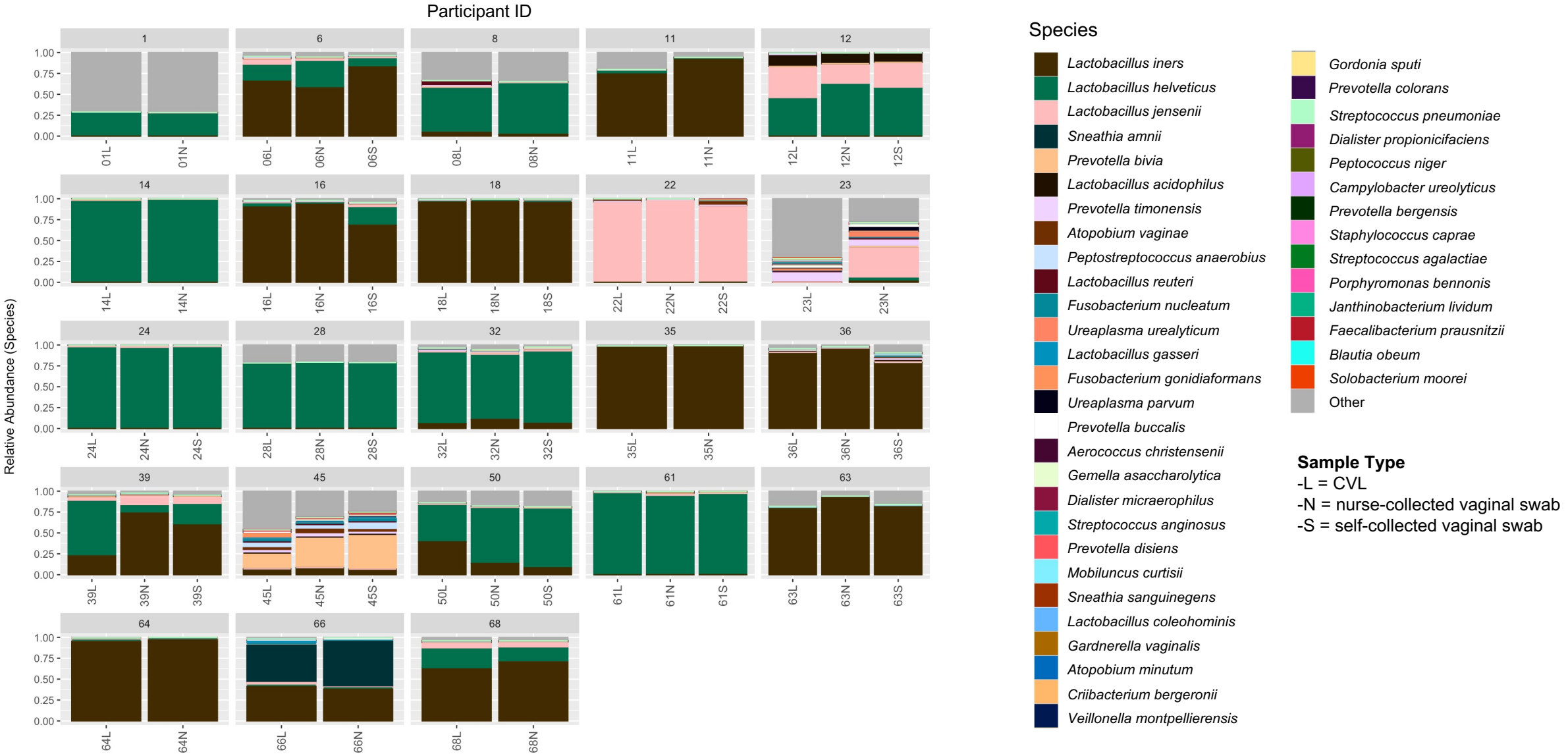

Figure 4. Relative abundance of species in 16S rRNA sequenced VMB samples. Samples are organized by participant, and each sample is labelled with the participant ID number and –L for CVL, -N for nurse-collected vaginal swab, of -S for self-collected vaginal swab.

Summary

- Sequencing depth varied by individual sample
- Some samples had low sequencing depths, which may reflect a low amount of bacteria collected when sampling
- No significant differences in alpha diversity were observed between sample types
- The 16S rRNA gene profiles were consistent between sample types, evidenced by relative abundance profiles and clustering observed with PCoA
- Issues with SILVA species-level taxa assignment were identified in our VMB samples:
  - *Lactobacillus* taxa identified were inconsistent with previous VMB profile reports; we believe *L. crispatus* was mis-identified as *L. helveticus.* Preliminary data using cpn60 gene sequencing with cpndb_nr taxa assignment as an alternative to 16S rRNA gene sequencing suggests this is the case (data not shown).
  - A number of amplicon sequence variants were not assigned taxa at the species level

Conclusion

CVLs, nurse- and self-collected vaginal swabs are all suitable sampling methods for profiling the VMB.

Therefore, CVLs and vaginal swabs, collected with or without a nurse, can be implemented in clinical studies.

Acknowledgements

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