

Lactobacillus Iners Is The Predominant Species In The Vaginal Microbiome Of Women With High-Risk HPV-Infection: Experience From A Tertiary Referral Colposcopy Centre In Singapore

IM AGGARWAL¹, QJ NG¹, S KOO², MM TAN², JJ WANG¹, FH CHIN¹, M QI¹, WY HO¹, ZY NG¹, FH CHIN¹, EC TAN²



¹Department of Gynaecological Oncology, KK Women's and Children's Hospital, Singapore

²KK Research Centre, KK Women's and Children's Hospital, Singapore

INTRODUCTION

Persistent HPV infection is a necessary prerequisite for development of cervical intraepithelial neoplasia (CIN) and cervical cancer. Numerous studies have looked at the correlation of vaginal microbiome profile, HPV infection and CIN. We present a cross sectional analysis of vaginal microbiome profiles of women with high-risk HPV infection attending a tertiary colposcopy centre in Singapore.

METHOD

- This is a cross-sectional study of 48 women attending our colposcopy centre in KK Women's and Children's Hospital, Singapore between November 2022 and January 2023.
- After IRB approval and patient consent, vaginal swabs were collected using DNA Genotek OMNIgene.VAGINAL(OMR-130) kit.
- DNA was isolated using QIAamp PowerFecal Pro DNA kit. Sequencing library was prepared according to Illumina 16S metagenomic sequencing workflow for the V3 and V4 variable regions of 16S rRNA gene.
- Data was analysed using QIIME 2 (version 2023.5) using DADA2 as quality control method and SILVA 138 and Greengenes2 2022.10 as reference sequences for taxonomic classification. The analysed data were also verified against those generated by Illumina 16S Metagenomics BaseSpace app which utilized RefSeq RDP 16S v3 database. Statistical analysis was performed in QIIME 2 and R.
- The metrics computed for alpha diversity were Shannon's entropy, Faith's Phylogenetic Diversity, and Pielou's Evenness and the metrics computed for beta diversity were Jaccard distance, Bray-Curtis distance, as well as unweighted and weighted UniFrac distances.
- Patient's data, including demographics, high-risk HPV infection status, pap smear result, histology result and outcomes, were collected anonymised in a password-protected proforma.

RESULTS

Study Population

- A total of 48 women (including 14 postmenopausal) were included in the study. The median age was 37 years (range 23 -73). This multiethnic cohort included Chinese (77.1%), Malay (10.4%), Indian (4.2%) and Caucasian (8.3%).
- The various HPV subtypes isolated were HPV 16/18 (11.4%), HPV others (65.7%) and multiple genotypes (22.9%).

Vaginal Microbiome

- Firmicutes, Actinobacteriota, and Bacteroidota were the main phyla noted. *Lactobacillus iners* was the main species isolated with 16 of the 48 samples belonging to community state type III.
- Other species included *Gardnerella vaginalis*, *Atopobium vaginae*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Sneathia sanguinegens*.
- 20 samples were depleted of lactobacilli, representing community state IV. The dominant species isolated in HPV16/18 was different for each sample (n = 5) whereas the dominant species in HPV others was *Lactobacillus iners* (9/30).
- The dominant species in low grade histology were *Lactobacillus iners* (7/23) followed by *Gardnerella vaginalis* (5/23) while the dominant species in high grade histology were *Lactobacillus iners* (2/4) and *Sneathia spp.* (2/4).
- The dominant species in normal Pap smear (9/12) and low grade Pap smear (10/18) was *Lactobacillus spp.*

DISCUSSION

- Numerous studies have shown an association between changes in the composition of the vaginal microbiota and infection with human papillomavirus (HPV). However, there are not many studies in Asian women with HPV infection showing the distribution of the vaginal microbiome.
- Our study showed that *L. iners* was the predominant species in the vaginal microbiome of women with high-risk HPV infection. There was no *L. crispatus* seen in our study population.
- There was no difference in alpha and beta biodiversity between women with HPV 16/18 and women with other high-risk HPV subtypes.
- There was also no difference in alpha and beta biodiversity between women with single high-risk HPV subtype and women with multiple high-risk HPV subtypes.
- As this is a small study of only 48 women, our next step is expanding our study population.

CONCLUSIONS

Lactobacillus iners was noted to be the predominant species in the vaginal microbiome of women with high-risk HPV infection. More studies need to be done to compare the vaginal microbiome profile in women without high-risk HPV infection.

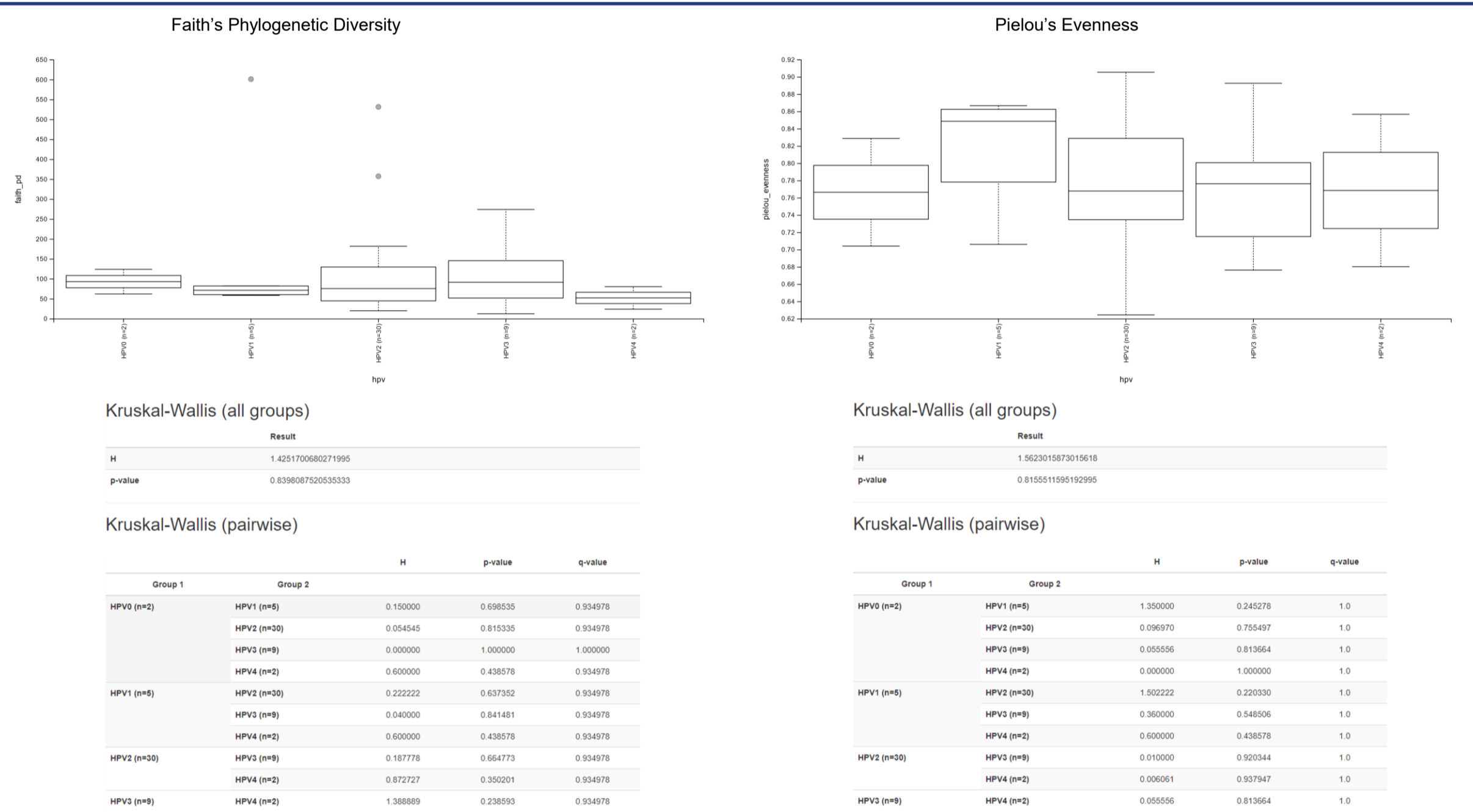


Figure 1 Alpha diversity metrics: Faith's Phylogenetic Diversity (a) and Pielou's Evenness (c) between the different HPV categories. Kruskal-Wallis test (all groups and pairwise) are performed for each metric (b and d, respectively). HPV 0= no HPV status, HPV 1= HPV 16/18, HPV 2= other hrHPV, HPV 3= multiple hrHPV subtypes, HPV 4= no hrHPV

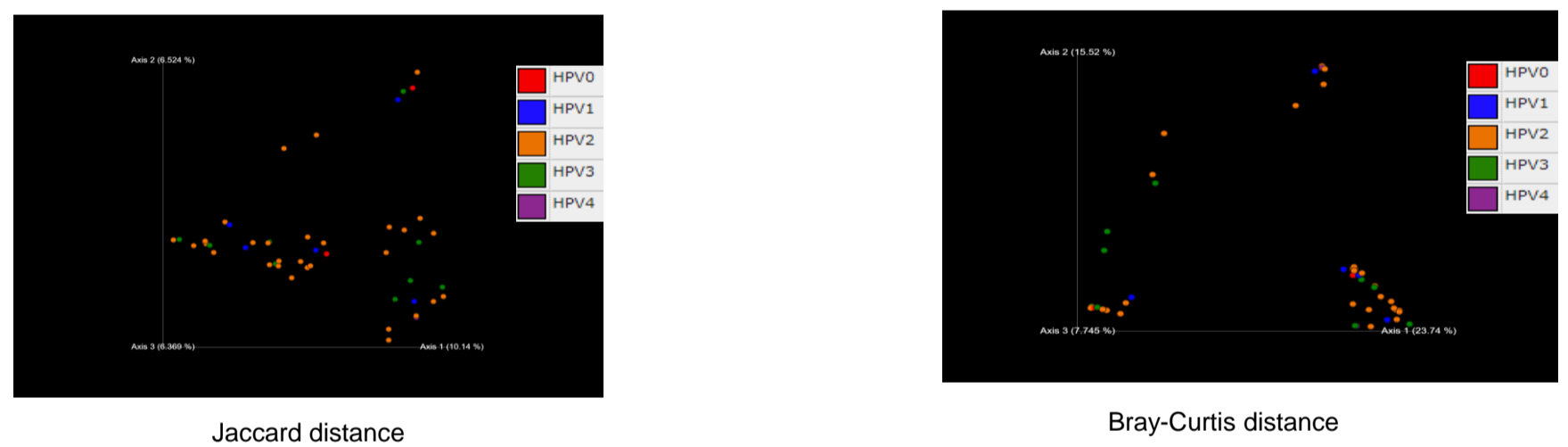


Figure 2 Beta diversity metrics: PCoA plots of Jaccard distance and Bray-Curtis distance between different categories of

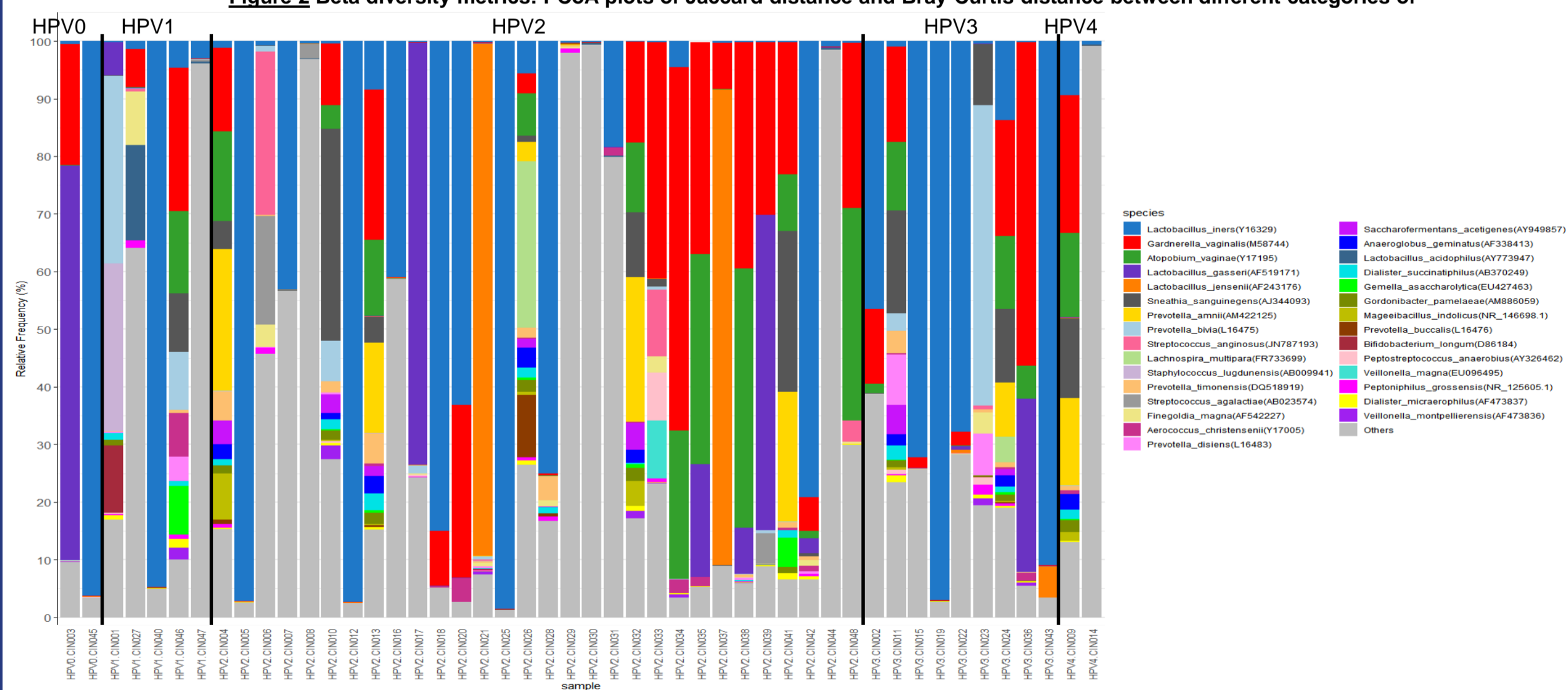


Figure 3 The relative abundance of 30 most abundant bacterial species across all samples grouped according to HPV categories based on RefSeq RDP 16s v3 database.

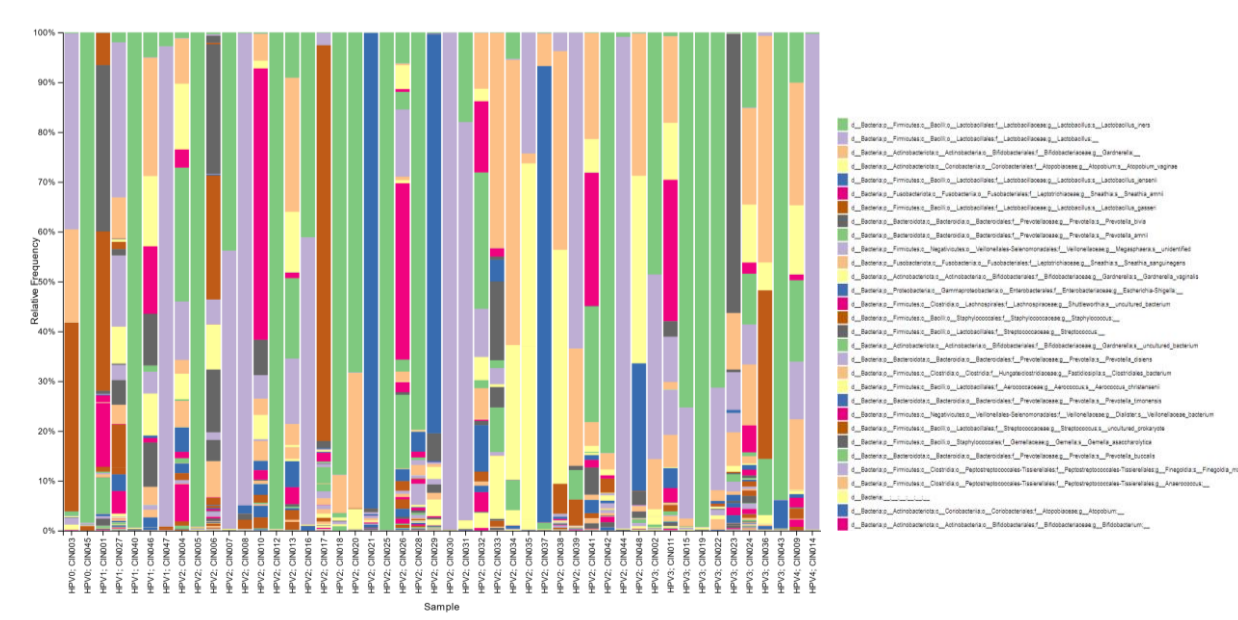


Figure 4 The taxonomic composition of bacterial species grouped according to HPV categories based on Silva 138 database. Only 30 most abundant species are shown in the legend.

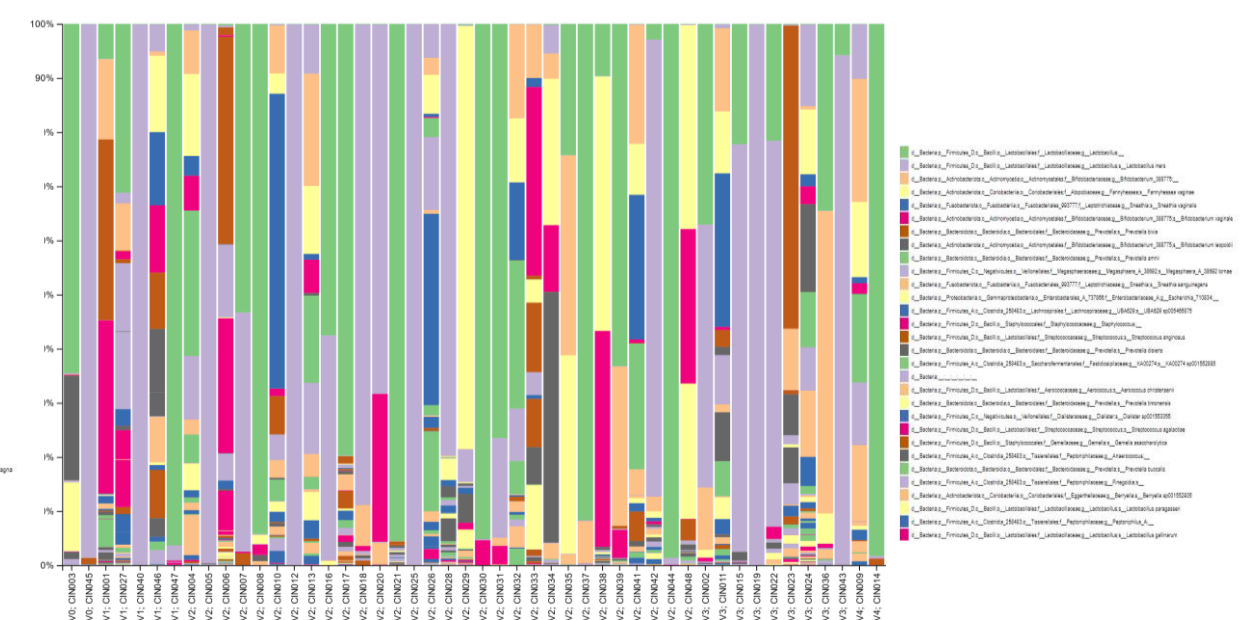


Figure 5 The taxonomic composition of bacterial species grouped according to HPV categories based on Greengenes2.2022.10 database. Only 30 most abundant species are shown in the legend.

REFERENCES

- Verstraeten H, Vieira-Baptista P, De Seta F, Ventolini G, Lonnee-Hoffmann R, Lev-Sagie A. The Vaginal Microbiome: I. Research Development, Lexicon, Defining "Normal" and the Dynamics Throughout Women's Lives. *J Low Genit Tract Dis.* 2022;26(1):73-78.
- De Seta F, Lonnee-Hoffmann R, Campisciano G, et al. The Vaginal Microbiome: III. The Vaginal Microbiome in Various Urogenital Disorders. *J Low Genit Tract Dis.* 2022;26(1):85-92.
- Ventolini G, Vieira-Baptista P, De Seta F, Verstraeten H, Lonnee-Hoffmann R, Lev-Sagie A. The Vaginal Microbiome: IV. The Role of Vaginal Microbiome in Reproduction and in Gynecologic Cancers. *J Low Genit Tract Dis.* 2022;26(1):93-98.
- Qiu Q, Lin Y, Ma Y, et al. Exploring the Emerging Role of the Gut Microbiota and Tumor Microenvironment in Cancer Immunotherapy. *Front Immunol.* 2021;11:612202.
- Norenstog J, Du J, Olvsson M, Verstraeten H, Engstrand L, Brusselaers N. The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG.* 2020;127(2):171-180.
- Martin DH, Marrazzo JM. The Vaginal Microbiome: Current Understanding and Future Directions. *J Infect Dis.* 2016;214 Suppl 1(Suppl 1):S36-S41.