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Introduction

A healthy vaginal microbiota is strongly correlated with adequate physiological pH. Moreover, the vaginal microbial population is mandatory in order to prevent pathogens and limit sexually transmitted viral infections. Among them, HPV represents one of the leading infections that cause minor and severe problems such as cancer. The introduction of next generation sequencing (NGS) technology in the microbial population evaluation highlights the possible relationship among vaginal microbiota and HPV. Finally, these studies aim to prevent an increase in viral clearance with the goal of healthy vaginal microbiota balance. Our study goes in the same direction. Indeed, here we reported that an unbalanced vaginal microbial population is correlated with HPV-positive patients. With a deeper analysis of our sequenced microorganisms, we define that the lactobacilli percentage is reduced in HPV patients compared to healthy; at the same time we notice an increased value for the biodiversity of the bacterial flora in favour of opportunistic pathogenic species such as *Gardnerella vaginalis* and anaerobic bacteria. Furthermore, a higher proportion of unclassified bacteria are present in positive HPV patients.

Materials and methods

The study included 23 healthy women (13 HPV-positive and 10 HPV-negative) aged 20-50 at enrolment, coming from the Department of Obstetrics and Gynecology of the University of Campania "Luigi Vanvitelli" in Naples (Italy). The presence of HPV DNA has been examined with a linear array HPV genotyping test. DNA isolation from the swabs. For isolation of bacterial microbiome DNA from clinical vaginal swab samples, a DNA isolation kit (QIAamp DNA Microbiome kit; cat. no. 51704) according to the manufacturer's protocols was used. Isolated DNA concentrations were measured using a Qubit® 2.0 Fluorometer and normalized to 1 ng/μL. The V3-V6 regions of the 16S rRNA gene were amplified with PCR Target using Kit Microbiota solution B (ARROWforNGS, cod. AD-002.024). The amplicon pools were prepared for sequencing with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and the gene was amplified by PCR "index" using Illumina adapter overhang nucleotide sequences following Illumina protocols. The amplified products of the "PCR index" were quantified and normalized to 10nM. The pool of amplicons obtained was processed using the MiSeq Reagent Nano Kit v2 (500-cycles) Kit (cat. n. MS-103-1003, Illumina). Bioinformatics and statistical analysis: libraries were sequenced using a 900 bp paired-end run on a MiSeq-Illumina platform. Sequencing data was available within approximately 48 hours. The data obtained were processed with the MicrobAT system (Microbiota Analysis Tool) of the company SmartSeq S.r.l.

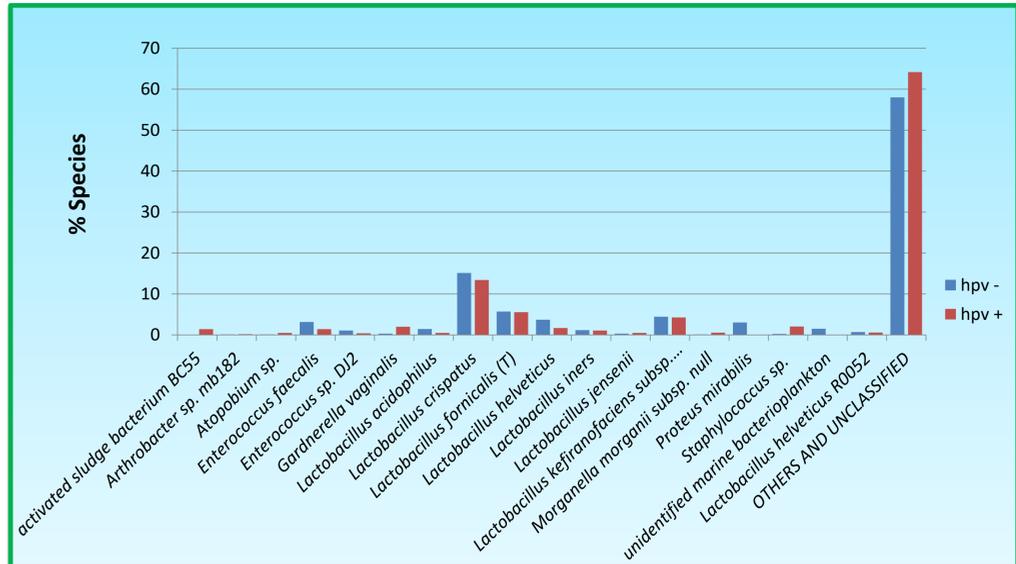
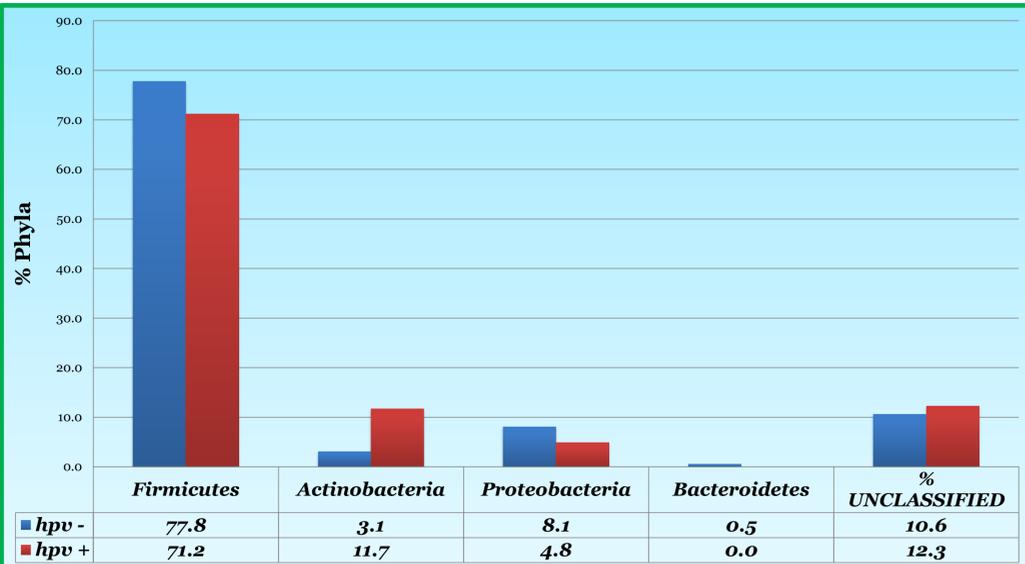


Figure 1. Representation of the vaginal microbiota at Phylum level. The relative abundance of Firmicutes is lower in positive HPV (red) samples compared with negative (blue) HPV samples.

Figure 2. Representation of the vaginal microbiota at species level. The relative abundance of *Gardnerella vaginalis* and *Staphylococcus sp.* species is higher in positive HPV samples (red) than in negative (blue) HPV samples.

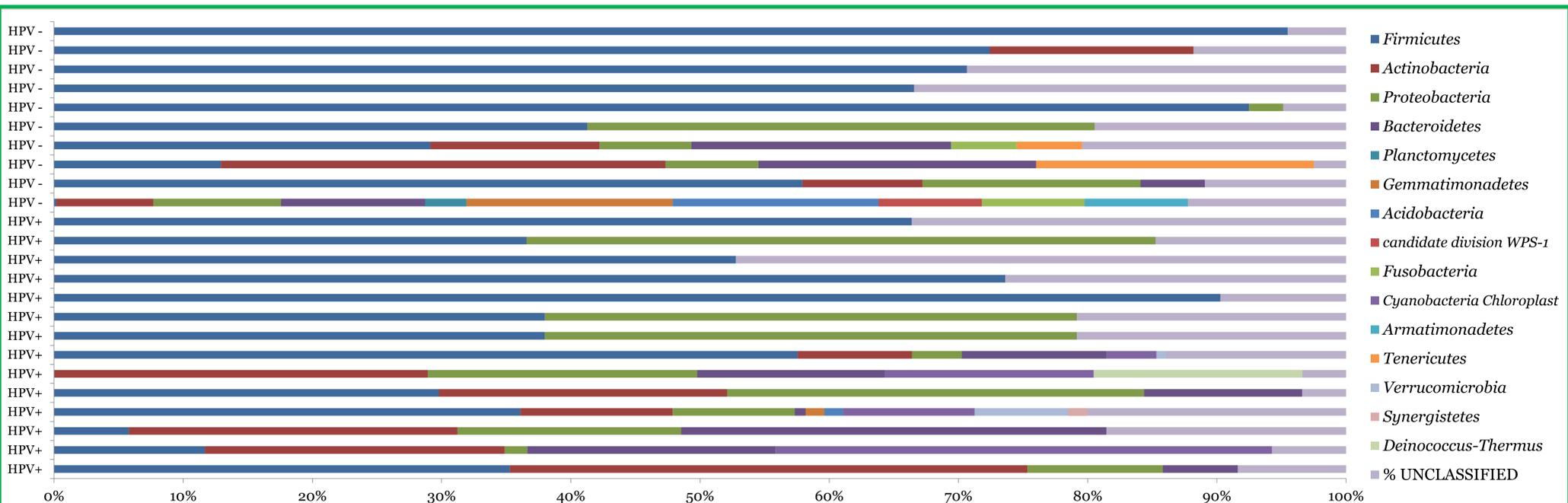


Figure 3. Representation of the individual vaginal microbiota at phylum level. The biodiversity of the vaginal microbiota in HPV positive cases is greater than that of the vaginal microbiota of healthy subjects.

Results and conclusions

The study included 23 healthy women (13 HPV-positive and 10 HPV-negative) aged 23-50 at enrolment. The vaginal microbiota composition was determined by sequencing 16S rRNA gene fragments (V3-V6) on Illumina HiSeq. The majority belonged to the Phylum Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria, to 90.8% of all sequencing reads. The Firmicutes occupy most of the normal vaginal flora, with a lower percentage in positive HPV women. The Actinobacteria are increased in subjects infected with HPV; Proteobacteria, on the other hand, occupy a reduced percentage in the group infected with HPV. The *Lactobacillus* represent the dominant genus of the vaginal flora, with the species *L. iners*, *L. jensenii*, and *L. crispatus*, but there was no significant difference between the two groups. *Gardnerella vaginalis* and *Staphylococcus sp.* species are more representative in HPV positive patients. Noteworthy, the increase of vaginal microbiota biodiversity is correlated with HPV positive cases.

The altered population of the vaginal microbiota plays a functional role in HPV infection, progression and clearance. This relationship leads to the possibility of probiotic therapies development. Furthermore, further studies are mandatory in order to understand if the vaginal microbiota imbalance can promote or be a consequence of HPV infection.