ANALYSIS OF THE CERVICAL MICROBIOME AND POTENTIAL BIOMARKERS FROM POSTPARTUM HIV-POSITIVE WOMEN DISPLAYING CERVICAL INTRAEPITHELIAL LESIONS

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The cervical microbiota is important to maintain local homeostasis and several studies have associated complications during pregnancy and the postpartum period with changes in the cervicovaginal bacterial communities. However, the cervical microbiota diversity and composition of HIV-positive women in the postpartum period remains unknown. Therefore, the aim of this study was to evaluate the postpartum cervical microbiota profiles of HIV-positive women displaying diverse cervical intraepithelial neoplasia. We analyzed cervical smear samples of 80 HIV+ women collected from 2010 to 2013. DNA was extracted and the bacterial 16S rRNA gene (V3-V6 region) was PCR-amplified and processed for next-generation sequencing in an Illumina HiSeq 2500 platform. After sequencing, reads were processed and compared against the Greengenes database. All bioinformatics analyses were carried out using QIIME and the graphs and statistics were performed in R. All participants were under antiretroviral treatment at the time of the collection. The median CD4+ T-cell count and HIV viral load values were 563 cells/ul and 376 copies/ml, respectively. In the 16S analysis, we identified four community state types (CSTs). CST III (L. iners-dominant) and CST IV (high-diversity) were found in 41% and 59% of samples, respectively. We did not find association of any CST to postpartum period (6 or 12 months), HPV status and cytology (normal or lesion). However, five bacterial genera were associated with cervical lesions (Gardnerella, Aerococcus, Schlegelella, Moryella and Bifidobacterium), with significant odds ratio (OR) of 40 (2.28-706) for the presence of Moryella and 3.5 (1.36-8.9) for Schlegelella. In the current study, we report the first data on the cervical microbiota of HIV-positive women in the postpartum period. We showed here that postpartum HIV-positive women present a stable cervical microbiota of high-diversity. Our results highlight that specific microbiota species may serve as sensors for changes in the cervical microenvironment associated with cervical lesions.

Table 2 CST distribution according to postpartum period, cervical cytology and HPV status

		CST III % (N/Total)	CST IV % (N/Total)	p-value[1]
Postpartum	06 months	27 (9/33)	36 (17/47)	0.403
	12 months	73 (24/33)	64 (30/47)	
Cervical Cytology	Normal	54 (18/33)	51 (24/47)	0.759
	Lesion	46 (15/33)	49 (23/47)	
HPV status	Positive	67 (22/33)	77 (36/47)	0.328
	Negative	33 (11/33)	23 (11/47)	

[1] Pearson's chi-square test.
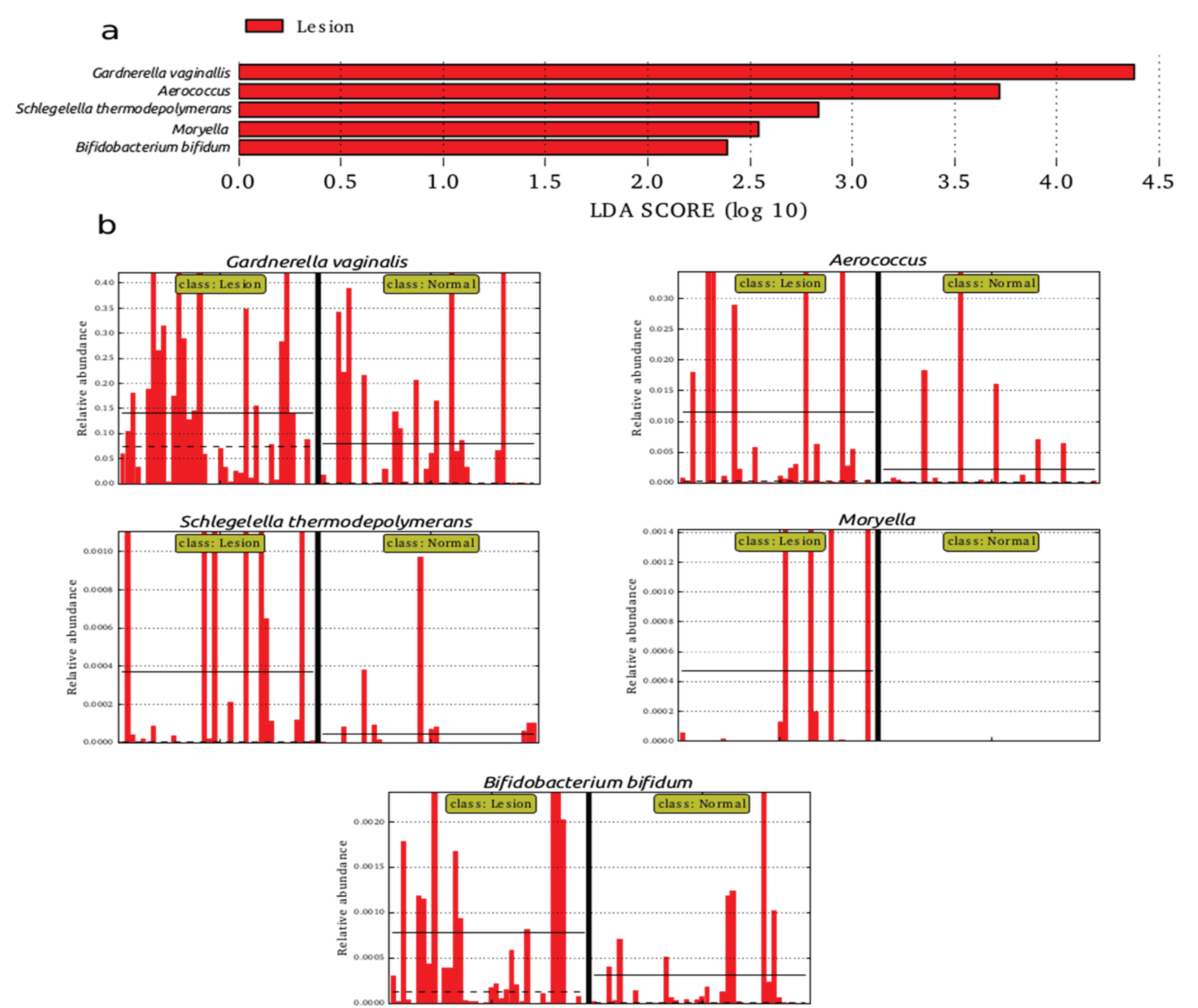
Figure 3 Analysis of cervical lesion putative biomerkers using LEfSe. (a) Histogram of the LDA scores (log 10) showing bacteria that presented higher relative abundance in cervical lesions (red) when compared to normal cytology. Only statistically significant differences are shown. (b) Histograms showing the relative abundance of the five specific taxa for each sample in the lesion and normal groups, separated by a black thick line. The solid and dotted black lines in the graphs indicate the mean and median relative abundance values for each group, respectively.

Table 1 Frequency of cervical cytology and HPV status at six and 12 months postpartum

		06 months % (n=26)	12 months % (n=54)	p-value[1]
Cervical Cytology	Normal	65 (17/26)	46 (25/54)	0.109
	Lesion	35 (9/26)	54 (29/54)	
HPV status	Positive	92 (24/26)	63 (34/54)	0.006
	Negative	8 (2/26)	37 (20/54)	
Cytology–HPV positive	Normal	58 (15/26)	15 (8/54)	0.003
	Lesion	38 (9/26)	48 (26/54)	
Cytology–HPV negative	Normal	8 (2/26)	31 (17/54)	0.556
	Lesion	0 (0/26)	6 (3/54)	

[1] Pearson's chi-square test.

Figure 1 Heatmap generated by unsupervised hierarchical clustering analysis of cervical microbiome of the studied participants. CSTs were determined using clustering based on Bray-Curtis dissimilarity and average linkage and are shown in color-coded groups at the bottom and also by the dendrogram at the top of the Figure. HPV status, cervical cytology and postpartum period are also color-coded according to the legend at the right of the Figure. CST III is L. iners-dominant; CST IV-A has a low proportion of Lactobacillus and a high-diversity; CST IV-B.1 is G. vaginalis-dominant and CST IV-B.2 is Prevotella-dominant.
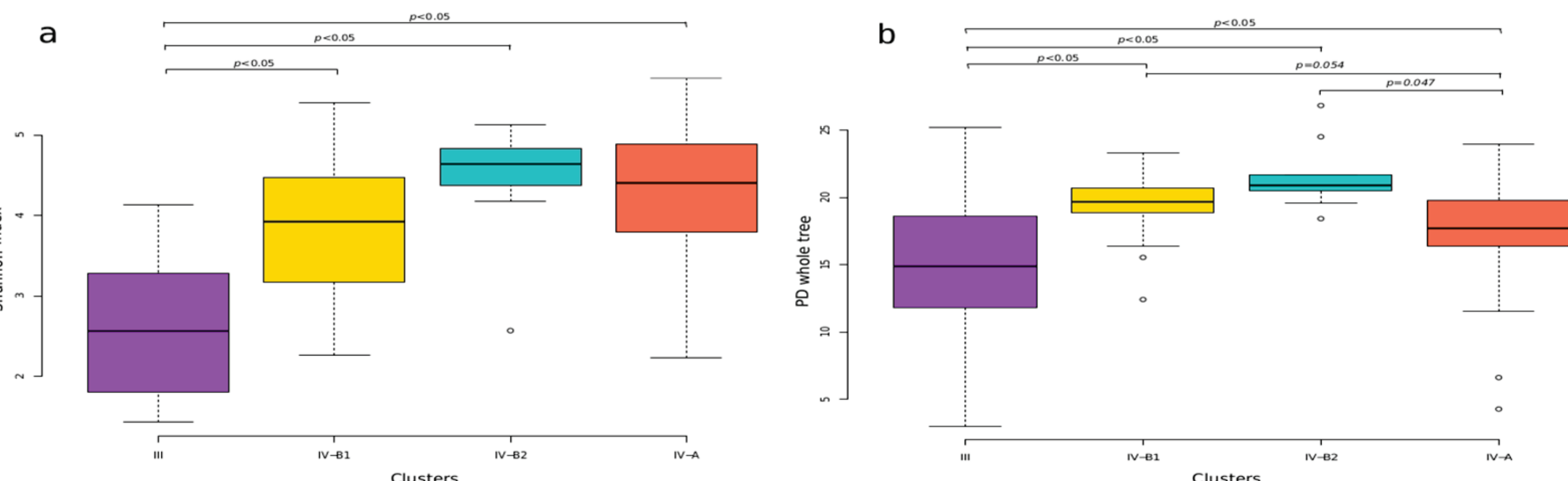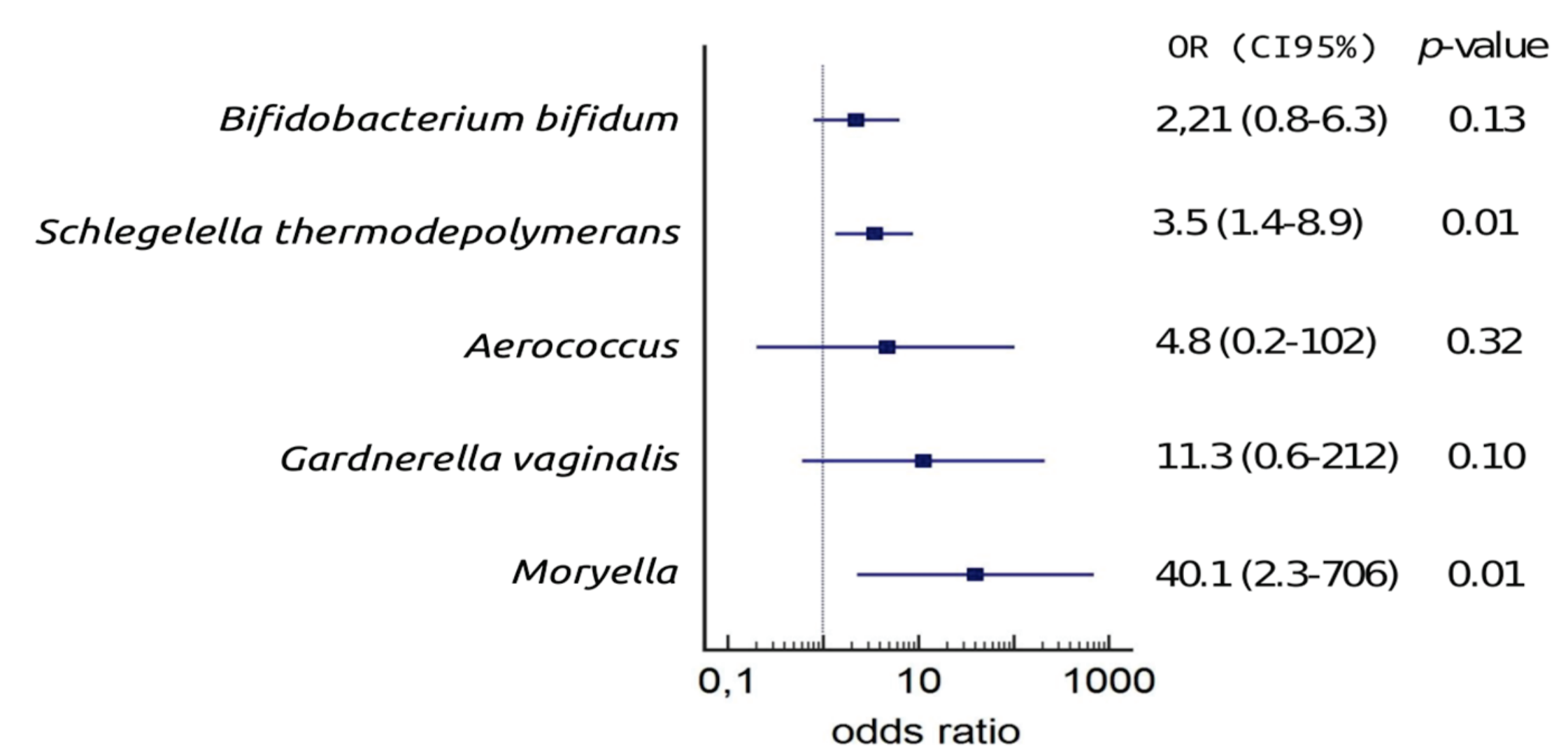

	OR (CI95%)	p-value
Bifidobacterium bifidum	2,21 (0.8-6.3)	0.13
Schlegelella thermodepolymerans	3.5 (1.4-8.9)	0.01
Aerococcus	4.8 (0.2-102)	0.32
Gardnerella vaginalis	11.3 (0.6-212)	0.10
Moryella	40.1 (2.3-706)	0.01

Figure 4 Forest plot showing the odds ratio of the occurrence of specific bacteria in cervical lesions. Odds ratio (OR) was calculated for the presence of the analyzed taxa using the normal cytology as reference (OR = 1). The OR values with their respective CI95% and associated p-values are depicted at the right of the Figure.
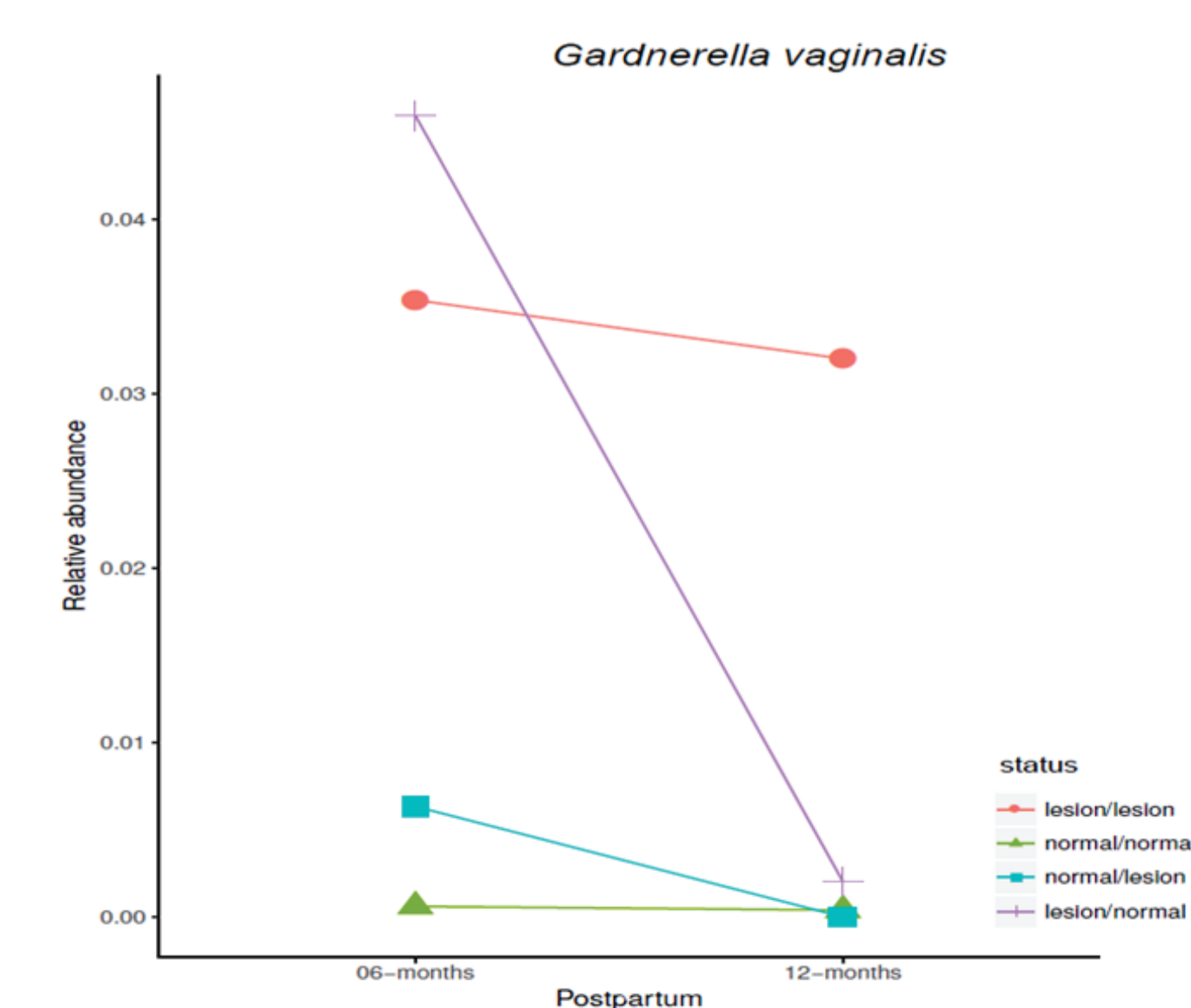

Figure 5 Longitudinal analysis of Gardnerella vaginalis abundance at six and twelve months postpartum in paired samples. The median relative abundance at six and twelve months postpartum of the paired samples that regressed (purple), progressed (blue), or maintained normal (green) or lesion (pink) cytology results is shown. The Wilcoxon test was performed with 95% confidence interval, and the p-value was only significant in the regression group.

Figure 2 Box plot analysis of alpha diversity using the (a) Shannon index and (b) phylogenetic diversity. Analyses in (a) and (b) were performed for each CST determined. The Student's t test was carried out to compare diversities between each CST, and the significant and borderline p-values at the 0.05 confidence level are represented at the lines above each graph.