

# Vaginal microbiota of asymptomatic reproductive-age women in Estonia

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## INTRODUCTION

Although well-studied with the classical microbiology approach, the knowledge of the healthy vaginal microbial communities remains still unclear. Though it is critical to determine "normal baselines" to compare them with the shift in microbial composition caused by diseases.

Recent studies using cultivation-independent methods have shown that the community composition of microbes in the vagina can vary greatly between healthy women and the common understanding of the healthy vaginal microbial community dominated by *Lactobacillus spp.* has revealed greater complexity (e.g. Ravel et al. 2010, Zhou et al. 2007). However, the broad-scale studies addressing this topic are rather scarce.

Hereby, we have applied metagenomic pyrosequencing and taxonomic identification to assess the individual microbial profile of Estonian women with asymptomatic urogenital tract.

## RESULTS

The sequences were in average 300 bases long and were assigned with a 97% identity threshold to operational taxonomic units (OTUs) (Fig. 2).

Individual vaginal bacterial communities acquired by the analysis of 16S rRNA gene sequences, were grouped into 7 clusters based on the similarities of OTU compositions within the community (Fig. 1). Only clusters  $\geq 15$  members were included. Most frequent taxons were *Lactobacillus* and *Gardnerella*, which were dominant, respectively, in 5 and 2 clusters (see Table 1. for the community compositions of these groups).

The remaining 136 vaginal communities were considered as a nonrelated heterogenous group. Most frequent OTUs of the non grouped samples are shown in Table 2.

## CONCLUSIONS

This is the first study using HTP pyrosequencing of bacterial 16S rRNA gene for characterizing vaginal microbiota in the population of annually monitored Estonian women declaring no medical complaints. Our results of separately clustered samples are supporting the recent view of the broad variation amongst the healthy vaginal microbial communities.

Results of the current pilot study using metagenomic approach enable us to investigate some further the conditions associated with the deviation from the "normal" microflora.

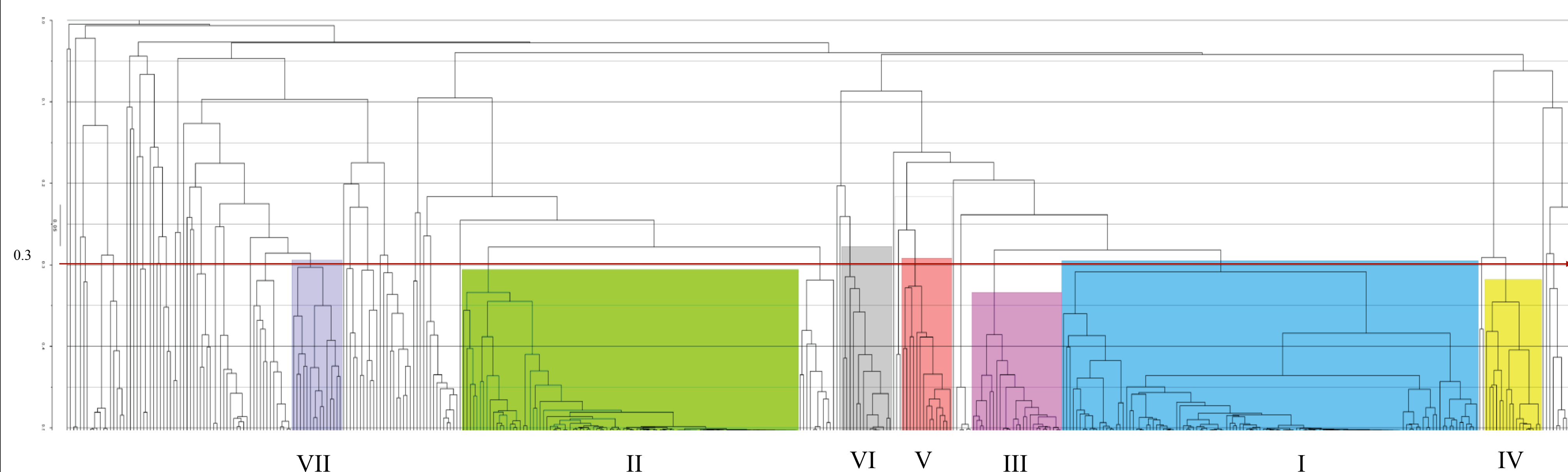
## MATERIAL AND METHODS

All the 450 human subjects in the study were healthy pre-menopausal non-pregnant ethnically homogenous women 15-44 (mean 31.1  $\pm$  6.4) years of age without complaints and symptoms in urogenital tract. The lack of infections was confirmed by gynecologists during the physical examination of patients while samples were taken from individual's vaginal fornix. Sampling period was June - October 2010. The individual samples were stored at -20° C until extracting total DNA with BioSprint 96 DNA Blood Kit (Qiagen). Pyrosequencing analysis of the 16S rRNA gene V2-V3 hypervariable regions for parallel tagged sequencing on the 454® Platform were performed by Roche/454 Life Sciences sequencer (GS junior). Used primers:

**5F** 5' TTGGCAGTCTCAGnnnnnnnn**AGTTTGATCCTGGCTCAG** 3' and **357R** 5' GTCTCCGACTCAGnnnnnnnn**CTGCTGCCTYCCGTA** 3' (McKenna et al. 2008). The 8-bp barcode sequence is marked as „n"s.

The sequences in the initial pretrimmed dataset (757282 sequences) were checked for chimeric artifacts, pyrosequencing noise and non-unique sequences were removed. The reference sequences of aligned 16S rDNA were obtained from SILVA ribosomal RNA database. Taxonomic assignments were done by Ribosomal Database Project (RDP) Naïve Bayesian classifier. OTUs with a 97% identity threshold were defined using the average neighbor clustering algorithm of mothur 1.17.2 software (Schloss et al. 2009). Statistical analyses for the regression curve and OTU frequencies were performed with the R 2.12.1.

The study protocol and informed consent document were reviewed and approved by the Ethics Committee of Medical Research in Tallinn.



**Fig. 1.** The linkage clustering due to the similarities between individual bacterial communities. Horizontal axis represents 450 samples of individual vaginal communities. Defined groups consisting  $\geq 15$  members. Similarity level = 0.3 (similarity is calculated by Yue & Clayton measure).

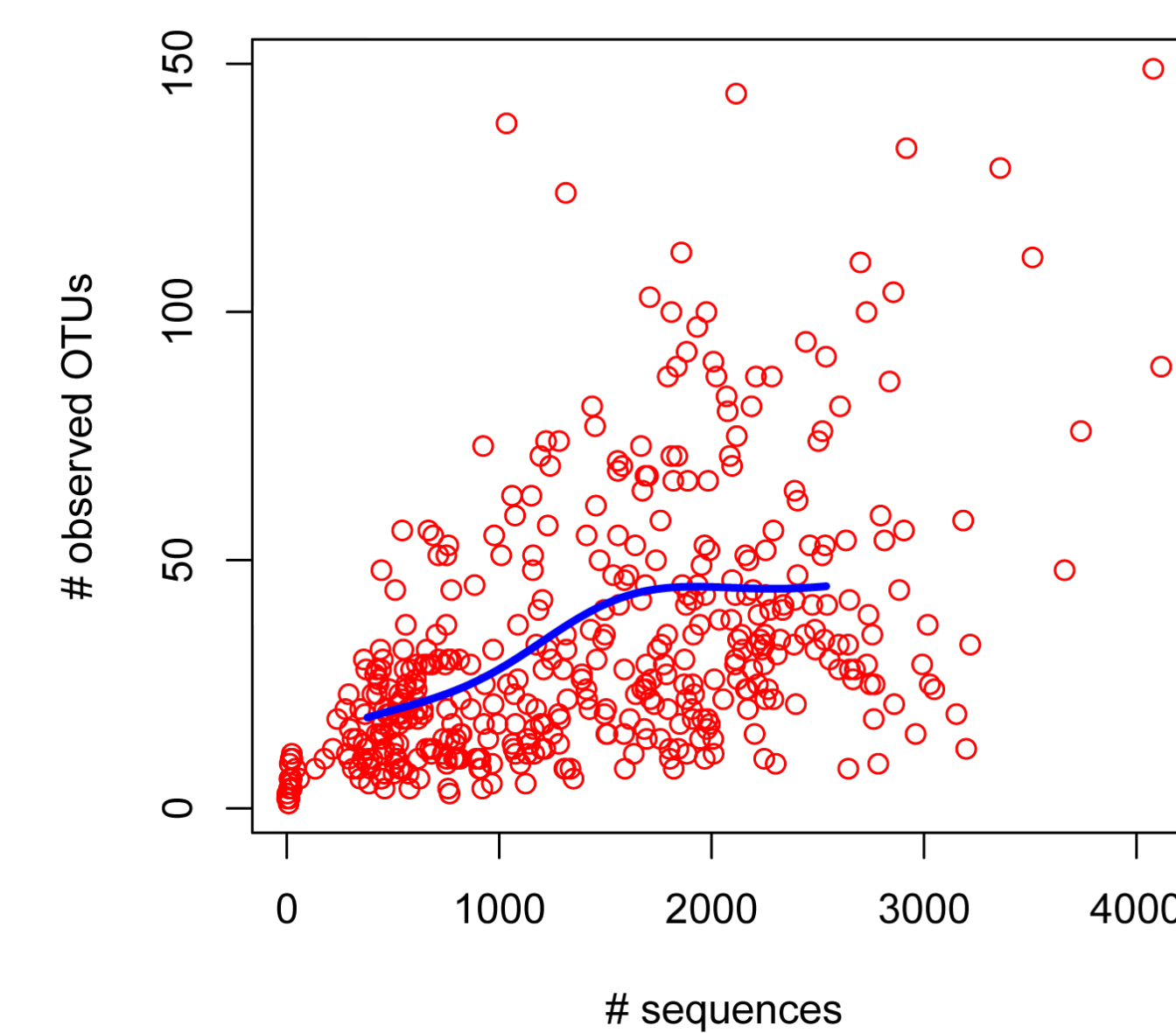
**Table 1.** Means of the frequencies of OTUs within each individual cluster. Cluster numbers are in accordance with Fig. 2. Consensus confidence specifies the percentage of the sequences within an OTU matching to the agreed taxonomy.

I (n=124 members)			II (n=101 members)			III (n=27 members)			VII (n=15 members)		
OTU (consensus confidence threshold %)	OTU ID	Mean $\pm$ SD	OTU (consensus confidence threshold %)	OTU ID	Mean $\pm$ SD	OTU (consensus confidence threshold %)	OTU ID	Mean $\pm$ SD	OTU (consensus confidence threshold %)	OTU ID	Mean $\pm$ SD
<i>Lactobacillus</i> (82)	1	0.77 $\pm$ 0.16	<i>Lactobacillus</i> (96)	2	0.83 $\pm$ 0.15	<i>Lactobacillus</i> (82)	1	0.48 $\pm$ 0.07	<i>Gardnerella</i> (100)	3	0.42 $\pm$ 0.08
<i>Gardnerella</i> (100)	3	0.03 $\pm$ 0.08	<i>Lactobacillus</i> (82)	1	0.04 $\pm$ 0.07	<i>Lactobacillus</i> (96)	2	0.38 $\pm$ 0.11	<i>Coriobacteriaceae</i> (97)	7	0.19 $\pm$ 0.13
<i>Lactobacillales</i> (92)	5	0.03 $\pm$ 0.06	<i>Lactobacillales</i> (92)	5	0.03 $\pm$ 0.05	<i>Lactobacillales</i> (92)	5	0.03 $\pm$ 0.05	<i>Veillonellaceae</i> (98)	29	0.04 $\pm$ 0.04
<i>Lactobacillus</i> (77)	14	0.03 $\pm$ 0.07	<i>Gardnerella</i> (100)	3	0.02 $\pm$ 0.04	<i>Gardnerella</i> (100)	3	0.02 $\pm$ 0.04	<i>Prevotella</i> (89)	10	0.03 $\pm$ 0.05
<i>Lactobacillus</i> (96)	2	0.02 $\pm$ 0.06			<i>Ureaplasma</i> (100)	9	0.01 $\pm$ 0.02	<i>Bacteria</i> (100)	26	0.03 $\pm$ 0.06	
<i>Gardnerella</i> (100)	33	0.01 $\pm$ 0.04						<i>Aerococcus</i> (100)	15	0.02 $\pm$ 0.05	
								<i>Lactobacillus</i> (82)	1	0.02 $\pm$ 0.04	
								<i>Prevotella</i> (87)	21	0.02 $\pm$ 0.04	
								<i>Sneathia</i> (100)	18	0.02 $\pm$ 0.04	
								<i>Lachnospiraceae</i> (89)	30	0.02 $\pm$ 0.06	
								<i>Prevotella</i> (78)	28	0.01 $\pm$ 0.04	
								<i>Sneathia</i> (100)	8	0.01 $\pm$ 0.02	
								<i>Clostridiales</i> (83)	36	0.01 $\pm$ 0.02	

**Table 2.** Means of the frequencies of OTUs from the comparison of **non-clustered** vaginal communities, n=136.

Consensus confidence specifies the percentage of the sequences within an OTU matching to the agreed taxonomy.

heterogenous group (n=136 members)		
OTU (consensus confidence threshold %)	OTU ID	Mean $\pm$ SD
<i>Gardnerella</i> (100)	3	0.19 $\pm$ 0.22
<i>Lactobacillus</i> (96)	2	0.10 $\pm$ 0.19
<i>Lactobacillus</i> (82)	1	0.07 $\pm$ 0.13
<i>Lactobacillales</i> (84)	11	0.05 $\pm$ 0.17
<i>Lactobacillus</i> (97)	6	0.04 $\pm$ 0.11
<i>Lactobacillales</i> (92)	5	0.04 $\pm$ 0.11
<i>Coriobacteriaceae</i> (97)	7	0.03 $\pm$ 0.07
<i>Prevotella</i> (89)	10	0.03 $\pm$ 0.08
<i>Sneathia</i> (100)	8	0.03 $\pm$ 0.08
<i>Prevotella</i> (100)	20	0.03 $\pm$ 0.06
<i>Streptococcus</i> (98)	12	0.02 $\pm$ 0.11
<i>Bifidobacterium</i> (100)	13	0.02 $\pm$ 0.10
<i>Lactobacillales</i> (87)	23	0.01 $\pm$ 0.09
<i>Enterobacteriaceae</i> (100)	19	0.01 $\pm$ 0.09
<i>Sneathia</i> (100)	18	0.01 $\pm$ 0.05
<i>Streptococcus</i> (87)	16	0.01 $\pm$ 0.05
<i>Gardnerella</i> (100)	33	0.01 $\pm$ 0.06
<i>Ureaplasma</i> (100)	9	0.01 $\pm$ 0.04



**Fig. 2.** The blue line depicts the conditional mean of number of observed OTUs (assigned by RDP Classifier) showing their relation to the number of sequences. Circles depict 450 samples of individual vaginal communities.

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