The Rich Diversity in the Vaginal Microbiome Impacts on Health and Disease

The Vaginal Human Microbiome Project at VCU (VaHMP-VCU) Les Pensières Fondation Mérieux, June 10-12, 2013

Human Microbiome Project NIH/NIAID 1UH2AI083263/4UH3AI083263
Project Specific Aims:

To address the following questions:

1. Do the *genes of the host* contribute to the composition of the vaginal microbiome and impact disease?

2. Are variations in the vaginal microbiome associated with *relevant infectious diseases* and conditions?

3. Are variations in the vaginal microbiome associated with *altered physiological or pathological states* of the host?
A broad-based community sampling project:

Goal to sample: 250 MZ/DZ twin pairs
4,000 clinical participants

Women’s clinics sampled:

Body Sites Sampled:
- Mid-vaginal, Cervical, Introital
- Buccal, Perianal
- Blood

Population:
Women’s clinics: VCU
- ~40,000 visits/year
- hot spot for STIs
- Mid Atlantic Twin Reg (170,000 twins)

Diverse demographics:
- ~50% African American
- ~ 25% Caucasian
- ~ 25% Latino
Now over 6,000 enrolled from clinics/ > 450 MZ/DZ twins to date
- >40,000 samples (7-11 from each participant)
- Very broad coverage of conditions of women’s health issues
- >1000 pregnant (~50 delivered preterm)
Vaginal Microbiome: the evolving paradigm...

‘Healthy’ pH: < 5.0; acidic
Microbiome monotonous
Dominated by *Lactobacillus*
Complex microbiome unhealthy
The ‘VCU Protocol’: three main approaches

1. Define the vaginal microbiome by metagenomic 16S rDNA sequencing

2. Define the metabolic potential of the vaginal microbiome by whole metagenomic shotgun sequencing

3. Assess the pathogenic potential of selected bacterial clones by de novo whole genome sequence analysis
First Step:

Metagenomic 16S rDNA Sequence Analysis (V1-V3)

Principal Component Analysis (L1-PCA*):
Microbiome Profiles from Different Body Sites
Vaginal Microbiome in Healthy Women is Diverse

HMP Consortium Healthy Cohort (143 samples)  VaHMP Clinically Healthy (366 samples)

So: even genus level classifications:

Vaginal microbiome exhibits rich diversity
Vaginal Microbiome in Healthy Women is Diverse

HMP Consortium Healthy Cohort (143 samples)  VaHMP Clinically Healthy (366 samples)

At species level: much greater diversity

Lactobacillus sp.

Gardnerella sp.
STIRRUPS

Species-level Taxon Identification of rDNA Reads using a USEARCH Pipeline Strategy

**Sneathia amnii**
(previously *Leptotrichia amnionii*)

Previously associated with:
- bacterial vaginosis
- spontaneous abortion
- preterm rupture of membranes
- Preeclampsia
- inflammation, histological chorioamnionitis, and/or amnionitis
- preterm birth
- Infant /maternal postpartum bacteremia
- Uncultured and poorly characterized

In our data
- *Sneathia amnii* is a ‘minor’ vagitype
- associated with BV
- excluded in pregnant women?

Does *S. amnii* have pathogenic potential?

Cultured and cloned:
- long, gram-negative, non-motile rods

Sequenced and analyzed the genome…
Characterization of *Sneathia amnii*

- Emerging pathogen??
- Identified in amniotic fluid
- Isolated from woman who delivered preterm
- Cytotoxic
- Implications for pregnancy??

**Harwich MD, Serrano MS, Fettweis JM, Alves JMP, Reimers MA, Vaginal Microbiome Consortium, Buck GA, and Jefferson KK. (2012) Genomic sequence analysis and characterization of *Sneathia amnii* sp. nov. BMC Genomics. 13, S4.**
Sequencing and analysis of bacterial strains

Approximately 40-50% of bacterial sequences from whole metagenome shotgun analysis

- are not derived from known bacterial species!
- the microbial ‘dark side of the moon’.

To date, over **250 bacteria** of interest cultured, colony cloned, and sequenced (HiSeq 2000/2500 and Roche 454).

• known species of vaginal relevance (e.g., *Lactobacillus* sp.; *Streptococcus* sp.; etc.)

• known species that have previously not been sequenced (*Sneathia amnii*, *Gardnerella vaginalis*; *Megasphaera* sp., etc.)

• previously unknown (emerging pathogens) taxa (e.g., "Ca. Mycoplasma girerdella"; OTUH1; new *Sneathia* sp.; etc.)
In Conclusion:

1. Species-level metagenomic rDNA analysis of thousands of community-based vaginal samples reveals extremely diverse vaginal microbiome profiles for both ‘healthy’ and ‘unhealthy’ women.

2. Broad microbiome analysis of these community-based samples using ‘deep coverage’ reveals up to 20 or more discrete ‘vagitypes’.

3. ‘Deep coverage’ suggests that different vaginal sites display similar ‘vagitypes’ (e.g., dictated by the abundant taxa), but, not surprisingly, differ in less abundant components of their microbiomes.

4. The vaginal microbiome probably exhibits decreased entropy during pregnancy

5. New taxa (e.g., *Sneathia amnii*, OTU-M1, etc.), potential pathogens of the female urogenital tract, are being identified and characterized.

6. OTU-M1 represents an likely completely new pathogen that is nearly absolutely correlated with disease (whether diagnosed or undiagnosed).
# VaHMP Team Membership

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