Temporal Dynamics of the Vaginal Microbiota in Reproductive Age Women

Larry J. Forney
University of Idaho
Lactobacillus spp. are characteristic of vaginal microbiota in “normal” healthy reproductive age women.

Growth of non-indigenous organisms, including pathogens, is restricted.

The mechanisms are unknown:

- low pH (≤4.5)
- lactate
- other organic acids
- hydrogen peroxide (?)
- bacteriocins
- others?
Development of vaginal microbial communities

- **Birth**
  - Rapidly colonized

- **Puberty**
  - Increased estrogen; colonization and succession

- **Reproductive age**
  - Glycogen metabolized to produce lactic acid

- **Menopause**
  - Decreased estrogen, less glycogen, less lactic acid
Bacterial vaginosis (BV)

- BV is the most common cause of vaginal symptoms prompting medical evaluation.
- BV is characterized by decreased numbers of lactic acid producing bacteria, elevated vaginal pH, and a “disturbed” microbiota.
- Diagnosis based on clinical criteria or Gram stain (Nugent score).
- Numerous epidemiological studies have associated BV with major adverse outcomes, including increase risk for acquiring STIs, including HIV, and pre-term labor.
- Not all women are at equal risk to experience BV.

Hypothesis: Differential risk can be accounted for by differences in the composition, structure, function and dynamics of their vaginal communities.
Research Questions

- What kinds of bacterial communities are found in vaginas of asymptomatic, healthy women?
- Are these communities dynamic, such that the species composition and evenness varies over time?
- What factors determine vaginal community composition?
- Are changes in community composition associated with increased risk to disease?
The diversity of vaginal bacterial communities

A cross-sectional study
Study Design

- 410 asymptomatic healthy women
- Four ethnic groups equally represented:
  - Caucasian, Black, Hispanic, Asian
- Self-collected mid-vaginal swabs
- Metadata
  - Vaginal pH measurements (Inverness VpH glove)
  - Nugent scores (used for diagnosis of BV)
  - Questionnaire (health and sexual history, hygiene, etc.)
- Determine bacterial community composition by 454 pyrosequencing of barcoded V1-V2 region of 16S rRNA genes
  - Assign *Lactobacillus* sequences to species level

Species level classification using speciateIT (speciateIT.sourceforge.net)
Vaginal community composition

- Represent 95% of healthy women in these ethnic groups. - 5% are different

- These “community states” form five distinct clusters - multiple kinds of core microbiomes.

- All include significant numbers of lactic acid bacteria, though not necessarily species of *Lactobacillus*. Function may be conserved.
Community states dominated by *Lactobacillus* spp. are found on the four outer vertices (45%).

Community states with higher species evenness are found at an inner vertex within the tetrahedron (30%).

Intermediate forms and found along the edges of the tetrahedron (25%).

Community states dominated by *Lactobacillus* spp. are found on the four outer vertices (45%).

Community states with higher species evenness are found at an inner vertex within the tetrahedron (30%).

Intermediate forms and found along the edges of the tetrahedron (25%).
Hypotheses: Ecological models

Dynamic equilibrium hypothesis - The composition of a community is somewhat invariant over time and exists in a single dynamic equilibrium.

Community space hypothesis - Communities can occupy any position in community space over time and throughout a woman’s lifetime.

Community resilience hypothesis - Communities may vary in composition but are resilient and return to a single community state.

Alternative equilibrium states hypothesis - Communities composition can change over time, but the number of alternative states is limited.

Longitudinal studies of the vaginal microbiota
The dynamics of vaginal bacterial communities

A longitudinal study
39 women were enrolled in (2004) as part of another study done at Johns Hopkins, Baltimore, MD

- Self-collected swabs were obtained twice-weekly for 16 weeks (total of 1,107 samples)

- Vaginal smears and daily diaries mailed to the lab weekly

- Nugent Gram stain score (0-10 scale)

- 56% African-American and 36% white women

- Analyze community composition by 454 pyrosequencing of barcoded V1-V2 region of 16S rRNA genes (>3.5 million reads)
Longitudinal profiles
Longitudinal profiles
Longitudinal profiles

Subject 3

Patient 3

Microbiome Data

Phylotype

Corynebacterium
L. crispatus
L. iners
Anaerococcus
Prevotella
L. gasseri
Finegoldia
Campylobacter
Facklamia
Streptococcus
Aurantimonadaceae.1

Peptontiphilus
Parvimonas
Eggerthella
Mobiluncus
Megasphaera
Prevotella
Aerococcus
Gardnerella
L. inera
Atopobium

Menses
Tampon
Douching

Nugent [low, intermediate, high]
Vaginal Intercourse
Anal Sex
Oral Sex
Digital Penetration
Sex Toy
Lubricant

Weeks
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Subject 17

Patient 17

Microbiome Data

Phylotype

Atopobium
L. iners
Gardnerella
Aerococcus
Prevotella
Megasphaera
Mobiluncus
Eggerthella
Parvimonas
Peptontiphilus

Menses
Tampon
Douching

Nugent [low, intermediate, high]
Vaginal Intercourse
Anal Sex
Oral Sex
Digital Penetration
Sex Toy
Lubricant

Weeks
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Longitudinal profiles
Vaginal community dynamics

Age 45, Monogamous: Yes, Contraceptive: tubal ligation, Male sex partner: Yes

- Atopobium
- Lactobacillales
- Porphyromonas
- L. unclassified
- Faecalibacterium
- Dialister
- Actinomyces
- Bacteroides
- Veillonella
- Peptoniphilus
- Streptococcus
- Corynebacterium
- Prevotella
- Incertae_Sedis_XI.1
- Actinobaculum
- Campylobacter
- Parvimonas
- Prevotella
- Eubacterium
- Streptococcus
- Peptostreptococcus
- Veillonella
- Anaerococcus
- L. iners
- L. crispatus

0.0e+00 0.0e+00
Stability versus Constancy