Community ecology and the human vaginal microbiome

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common wisdom about the vaginal microbiome
things we thought we knew
differences among women
community composition
community dynamics
community stability
resistance and resilience
bacterial vaginosis: the curse of Koch’s postulates
community genetics
resource competition
genome amelioration
vaginal microbiome [common wisdom]

Albert Döderlein, (German obstetrician and gynecologist, 1860–1941), first described the ‘Döderlein bacillus’ in 1894.
vaginal microbiome [common wisdom]

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vaginal microbiome [common wisdom]

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

- Postulated mechanisms of protection:
  - low pH ($\leq 4.5$)
  - lactic acid
  - antimicrobial compounds
  - others

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vaginal microbiome [changes through life]
(or so the story goes)

Birth
Sterile in utero but colonized by *Lactobacillus*

Pre-puberty
Loss of *Lactobacillus*; abundance of strict anaerobes

Puberty
Increased estrogen; community shift

Reproductive age
Glycogen metabolized to produce lactic acid

Menopause
Decreased estrogen, less glycogen, less lactic acid
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It isn't what we don't know that gives us trouble, it's what we know that ain't so.

~Mark Twain
vaginal microbiome [common wisdom]

Women are more or less the same, and their vaginal microbiota are more or less stable.
vaginal microbiome [common wisdom]

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Ravel et al. (2011) PNAS 108 S1:4680-7
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vaginal microbiome [differences among women]

How much variation is there in the vaginal microbiota of healthy women?

Cross-sectional study

- 396 healthy, asymptomatic women of reproductive age equally representing Asian, black, Caucasian and Hispanic ethnic groups

- Determine bacterial community composition by pyrosequencing of 16S rRNA genes (V1-V2 region)
  - Assign *Lactobacillus* sequences to species

vaginal microbiome [variation in composition]

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

- Function may be conserved.
  - All include significant numbers of lactic acid bacteria, though not necessarily species of *Lactobacillus*.

Prevalence of community states varies among racial groups

vaginal pH

<table>
<thead>
<tr>
<th>Race</th>
<th>Mean pH ± SD</th>
<th>Median pH ± MAD&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Caucasian</td>
<td>4.5 ± 0.66</td>
<td>4.4 ± 0.59</td>
</tr>
<tr>
<td>Black</td>
<td>4.7 ± 0.66</td>
<td>4.7 ± 1.00</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4.8 ± 0.64</td>
<td>5.0 ± 0.74</td>
</tr>
<tr>
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<td>Overall</td>
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<sup>a</sup>Median absolute deviation (MAD)

For a significant proportion of women (>50%) the vaginal pH is >4.5
## vaginal pH

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A call for **personalized medicine**

[Today the message is: you are not normal, you're not healthy, and the fact that you are Hispanic or Black is not so important.]
Language matters

Normal
Language matters

Normal

Not Average
Language matters

Atypical

Normal

Not Average
Language matters

Atypical  Strange

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Not Average
Language matters

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Odd

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Where did we get the idea that a vaginal pH >4.5 is not “healthy”?

- Countless review articles
- Centers for Disease Control
  - “… an elevated pH (i.e., >4.5) is common with BV or trichomoniasis … ”
- Commercial OTC kits to measure vaginal pH
  - “A disturbance in the micro ecological balance in the vagina (e.g. dysbiosis) is often connected with an increase in the pH level. Vaginal pH levels that are higher than 4.5 can lead to many gynaecological problems.”
- Human Microbiome Project
  - Exclusion criterion: vaginal pH greater than 4.5 at screening visit

http://www.cli-online.com/index.php?id=1019&tx_ttproducts_pi1%5Bproduct%5D=2948
http://hmpdacc.org/doc/HMP_Clinical_PROTOCOL.pdf
Effect of pH on *Enterococcus faecalis* growth

![Graph showing the effect of pH on Enterococcus faecalis growth](image-url)
If we assume vaginal pH data are normally distributed, then ...

According to the 2000 census there were 143.4 million women in the United States. Therefore, 14.3 million women (1 in 10) are in the tails of the distribution.
Normal and healthy
Normal and healthy

World Health Organization

Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Citation: Preamble to the Constitution of the World Health Organization as adopted by the International Health Conference, New York, 19-22 June, 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, no. 2, p. 100) and entered into force on 7 April 1948.
vaginal microbiome [community dynamics]

Do vaginal bacterial communities vary over time and can temporal changes be linked to behaviors or other factors?

Can we develop ways to identify women with an increased risk to bacterial vaginosis, sexually transmitted infections, and yeast infections?
Gajer et al. (2012) Science Translational Medicine, 4:132ra52
Profile of Vaginal Community State Types

Alternative hypotheses

(1) Dynamic equilibrium hypothesis
A community is comparatively invariant over time and exists in a single dynamic equilibrium.

(2) Community space hypothesis
A community can and does occupy any position in community space over time and throughout a woman’s lifetime. These changes are postulated to occur in response to hormonal cycles, an individual’s habits and practices, changes in diet, or some other ecological force.
Alternative hypotheses

(3) Alternative equilibrium states hypothesis
A community can change over time.
The number of alternative states are limited in number and governed by unknown factors.

(4) Community resilience hypothesis
A community resides in a single region of space.
The community can change to a transitional state in response to disturbance, but the resistance and resilience of a community determine the extent and duration of a change, whereas homeostatic mechanisms drive communities back to their “ground state”.

Tuesday, April 9, 13
Several kinds of bacterial communities occur in the human vagina, but the community function of lactic acid production is apparently conserved.

These communities differ among women in different ethnic groups and can change markedly over short periods of time.

These differences may have important consequences that should accounted for in risk assessment and disease diagnosis.

We need to think critically and reevaluate ‘healthy and normal’, and in doing so account for differences among ethnic groups and individuals.
stability of community structure and function

- communities that differ in species composition likely differ in terms of stability

- the disturbed state itself could elicit signs and symptoms of disease (e.g. BV)

- communities in a disturbed state are more susceptible to invasion, which may increase risk to infection or disease by ‘weedy’ species (e.g. STIs)
bacterial vaginosis: a system out of kilter?

“Bacterial vaginosis (BV) is a condition in women where the normal balance of bacteria in the vagina is disrupted and replaced by an overgrowth of certain bacteria. It is sometimes accompanied by discharge, odor, pain, itching, or burning.”

“Although BV will sometimes clear up without treatment, all women with symptoms of BV should be treated to avoid complications.”

vaginal microbiome [bacterial vaginosis]

**BV is common**

Bacterial vaginosis (BV) is the most common vaginal infection in women of childbearing age.

Complaints of vaginal discharge, vaginal odor, and/or vulvar itching account for more than 10 million physician office visits in the United States annually. The overall prevalence of BV in the United States is 29.2% but differs among racial groups.

**BV is associated with adverse consequences**

Linked to preterm delivery, low birth weight infants, pelvic inflammatory disease, and increased risk to acquiring sexually transmitted infections and HIV.
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The curse of Koch’s postulates
Diagnosis of BV
(by Amsel criteria)

1. Homogeneous, thin, grayish-white vaginal discharge

2. The presence (>20%) of “clue” cells (squamous epithelial cells with adherent bacteria)

3. Vaginal pH > 4.5

4. Amine (‘fishy’) odor after addition of KOH to vaginal secretions
community genetics

an approach to understanding community ecology that integrates across genetics, evolutionary biology, population and community ecology

Understanding ecological networks of bacterial populations and the host: key to developing strategies to maintain health

**Resource competition**

- All resources are derived from the host
- Complex resource pool, diverse community
- System operates as a plug flow reactor
metabolome

Figure 9. Lactate levels in vaginal samples with high (>5.5) or low (<4.5) pH.

Figure 10. Schematics of the urea cycle. Red is higher at high pH, green means higher at low pH. Putrescine accumulate in high pH samples, and is associated with vaginal odors.
Comparative genomics

Lactobacillus spp.
- Ameliorated genomes
- Well-known nutritional requirements
- Only found in the human vagina
- Essential nutrients must be obtained from the host or other members of the microbiota

L. iners
- Most prevalent among women
- Smallest genome of common vaginal lactobacilli
- Associated with unstable communities
Where now ...

What other functions are performed by vaginal microbial communities?

What are the ecological interactions that occur?

How is health maintained when vaginal communities are not dominated by lactobacilli?
HOST

ENVIRONMENT

MICROBIOTA

MUTUALISM
Coevolution of an ecological network
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Boticelli Nascita di Venere (transl. The Birth of Venus), ca. 1486
ecosystem management
Current Treatments Often Fail
Personalized Medicine

Disease

Tx

Tx

Tx

Tx
These trees will not grow here
now what?