Diversity & Dynamics of the Human Vaginal Microbiota

Johanna B. Holm, PhD
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Host - Microbe Interactions, The Metaorganism

Bosch & McFall-Ngai, Zoology 2013
Human Vagina - Microbe Interactions
What is the vaginal microbiota?

Patient with Normal Clinical Exam

L: *Lactobacillus* type

Spiegel, Amsel, Holmes, 1983
What is the vaginal microbiota?

L: *Lactobacillus* type
g: *Gardnerella* type

Patient with Normal Clinical Exam

Spiegel, Amsel, Holmes, 1983
What is the vaginal microbiota?

L: *Lactobacillus* type

G: *Gardnerella* type

P: gram-positive cocci

B: gram-negative rods

Patient diagnosed with Bacterial Vaginosis

Patient with Normal Clinical Exam
Bacterial Vaginosis

- Condition of unknown etiology
- Disturbance of vaginal microbial ecology
- Replacement of *Lactobacillus* predominant flora with an overgrowth of *Gardnerella vaginalis* and mixed anaerobic organisms
- The most common cause of symptomatic vaginal discharge in reproductive age women
- Associated with a variety of conditions including post-surgical infection, pelvic inflammatory disease, preterm birth and HIV acquisition

Definition modified from Klebanoff et al. 2010
What is the vaginal microbiota?

L: *Lactobacillus* type

g: *Gardnerella* type

p: gram-positive cocci

b: gram-negative rods

Patient diagnosed with Bacterial Vaginosis

Patient with Normal Clinical Exam
Vaginal - Microbe Interactions

Mechanisms of protection by microbiota:

• Fermentation of sugars to lactic acid => low pH
  *Lactobacillus* spp., but also: *Atopobium, Streptococcus, Staphylococcus, Megasphaera, and Leptotrichia*

• Produce antimicrobials (i.e. bacteriocin peptides)

• *Lactobacillus* competitively exclude other bacteria and yeast from adhering to epithelial cells *(Boris et al. 1998)*

<table>
<thead>
<tr>
<th># Adhering to vaginal epithelial cells</th>
<th>Candida albicans</th>
<th>Gardnerella vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus absent</td>
<td>22.5 ± 5</td>
<td>34.5 ± 10</td>
</tr>
<tr>
<td>Lactobacillus present</td>
<td>5.5 ± 3</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

Simplified for clarity
Metataxonomic Profiling, 16S rRNA gene

A dot is one sequenced amplicon.

Taxonomic Assignment:
- Lactobacillus crispatus
- Lactobacillus iners
- BVAB1
- Bifidobacterium breve
- Mycoplasma genitalium
- Atopobium
- Megasphaera

PECAN
Per sEquenCe tAxonomic assigNer
Within a woman, microbial composition can be **dynamic**. How about across many women?

Species come and go over time

Lactobacillus iners
Lactobacillus crispatus
Prevotella
Anaerococcus
Aerococcus
Sneathia
Lactobacillus jensenii
Gardnerella
Gemella
Actinomyces

Compare one time point across many women

Lactobacillus iners
Lactobacillus crispatus
Gardnerella vaginalis
Lactobacillus jensenii
Lactobacillus gasseri
g_Streptococcus
Megasphaera sp type 1
Atopobium vaginae
Leptotrichia amnionii
Prevotella genogroup 2
Sneathia sanguinegens
Prevotella genogroup 1
BVAB1
Bifidobacterium breve
Dialister sp type 2
Prevotella genogroup 3
Anaerococcus tetradius
Prevotella bivia
Finegoldia magna
g_Staphylococcus
BVAB2
Ureaplasma parvum
Microbial compositions can be **diverse**.

396 Women:
- 97 Hispanic
- 97 Asian
- 104 Black
- 98 White

**CST I:** *Lactobacillus crispatus*
**CST II:** *Lactobacillus gasseri*
**CST III:** *Lactobacillus iners*
**CST IV:** non-*Lactobacillus*
**CST V:** *Lactobacillus jensenii*

*Ravel et al. 2011*
Microbial compositions can be **diverse**.

Many taxa  
=  
High Diversity  
=  
High Diversity Index
Microbial compositions can be diverse.

CST distribution differs among ethnic groups
($\chi^2 = 36.8$ on 10 df $P < .0001$)
What affects dynamics and diversity of vaginal microbiota?
What affects dynamics and diversity of vaginal microbiota?

Host Physiology
- Age
- Menstrual Cycle
- Pregnancy

Host Behavior
- Smoking
- Exposure to HIV
- Exposure to *Chlamydia*
Age

Prepuberal girls:
- High pH
- Diverse microbiota
- Low levels of estrogen
- Mucus
- Squamous epithelium
- Low levels of glycogen
- Thin vaginal mucosa

Adult women:
- Low pH
- H₂O₂
- L. iners
- L. crispatus
- Mucus
- Squamous epithelium
- Thick vaginal mucosa
- Glycogen
- Deposition of glycogen
- Degradation to glucose

Reviewed by Petrova, et al. 2013
Age - Menopause

Vulvovaginal Atrophy (VVA)

Sturdee et al. 2010
Age - Menopause

Vulvovaginal Atrophy (VVA)

- Lack of lubrication
- Dryness
- Soreness
- Spotting
Age - Menopause

Genitourinary Syndrome of Menopause
Vulvovaginal Atrophy (VVA)

- Lack of lubrication
- Dryness
- Soreness
- Spotting
What happens to the microbiota in menopausal women?

Age - Menopause

Genitourinary Syndrome of Menopause
Vulvovaginal Atrophy (VVA)
Age - Menopause

87 Women
30 pre-menopausal
29 peri-menopause
28 post-menopause

Association between the vaginal microbiota, menopause status and signs of vulvovaginal atrophy

Rebecca M. Brotman, PhD, MPH\textsuperscript{1,2}, Michelle D. Sh Ardell, PhD\textsuperscript{2}, Pawel Gajer, PhD\textsuperscript{1}, Doug Fadrosh, MS\textsuperscript{1}, Kathryn Chang, RN\textsuperscript{3}, Michelle Silver, ScM\textsuperscript{3}, Raphael P. Viscidi, MD\textsuperscript{4}, Anne E. Burke, MD, MPH\textsuperscript{5}, Jacques Ravel, PhD\textsuperscript{1,6}, and Patti E. Gravitt, PhD, MS\textsuperscript{3}

Brotman, et al. 2014
Postmenopausal women are more likely to be classified as CST IV versus CST I.
There is an association between non-\textit{Lactobacillus} vaginal microbiota and menopause stage
Age - Menopause

There is an association between non-\textit{Lactobacillus} vaginal microbiota and menopause stage.

There is an association between non-\textit{Lactobacillus} vaginal microbiota Vulvovaginal Atrophy.

Reviewed by Petrova, \textit{et al.} 2013
Age - Menopause

There is an association between non-\textit{Lactobacillus} vaginal microbiota and menopause stage

Does lower \textit{Lactobacilli} spp and VVA increase susceptibility to infection?

There is an association between non-\textit{Lactobacillus} vaginal microbiota Vulvovaginal Atrophy
How are the vaginal microbiota affected by menstruation?
Temporal Dynamics of the Human Vaginal Microbiota

Paweł Gajer,¹,²* Rebecca M. Brotman,¹,³* Guoyun Bai,¹,² Joyce Sakamoto,¹,²†
Ursel M. E. Schütte,⁴,⁵‡ Xue Zhong,⁵,⁶,⁷‡ Sara S. K. Koenig,¹,² Li Fu,¹,² Zhanshan (Sam) Ma,⁴,⁵‖
Xia Zhou,⁴,⁵ Zaid Abdo,⁵,⁶,⁷ Larry J. Forney,⁴,⁵‡ Jacques Ravel¹,²¶

32 women
Self Collected 2x/week
16 weeks
Menstruation

Menses

Menstruatio

Instability

Stability

Vaginal microbiota most dynamic during menses
Menstruation

Vaginal microbiota most dynamic during menses

Does instability mean susceptibility?
How dynamic is the vaginal microbiota throughout pregnancy?
Pregnancy

32 Non-pregnant
22 Pregnant

More *Lactobacillus* in pregnant women
Pregnancy

More stability as pregnancy progresses

Romero et al. 2014
What affects dynamics and diversity of vaginal microbiota?

**Host Physiology**

- **Age** – Microbial diversity increases with menopause (reduced estrogen)
- **Menstrual Cycle** – Microbiota more dynamic with menses (unstable)
- **Pregnancy** – Microbiota less diverse and dynamic with pregnancy
What affects dynamics and diversity of vaginal microbiota?

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What affects dynamics and diversity of vaginal microbiota?

Host Physiology

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Host Behavior

- Smoking
- Exposure to HIV
- Exposure to *Chlamydia*
What affects dynamics and diversity of vaginal microbiota?

**Host Physiology**
- Age
- Menstrual Cycle
- Pregnancy

**Host Behavior**
- Smoking
- Exposure to HIV
- Exposure to *Chlamydia*
Smoking

Artist Kevin Berlin, East Meets West #2
<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Non-Smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Non-Smokers</td>
<td></td>
<td></td>
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<tr>
<td>&amp;</td>
<td></td>
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</tr>
<tr>
<td>20 Smokers</td>
<td></td>
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</tbody>
</table>
Smoking

20 Non-Smokers & 20 Smokers

Smokers were 25-fold more likely to have low proportions of vaginal *Lactobacillus* sp. (CST IV)

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Brotman *et al.*, 2014
Biogenic Amines (BAs) can allow various bacteria to survive in low pH environments, and enhance growth rates of pathogens.
Biogenic Amines (BAs) can allow various bacteria to survive in low pH environments, and enhance growth rates of pathogens.
Smoking increases the diversity of vaginal microbiota
Smoking increases the diversity of vaginal microbiota.

Do women who smoke have increased susceptibility?

Brotman et al., 2014
Sexually Transmitted Infections

Vaginal microbiota & HIV
In 2020, 0.5-2 million new HIV infections

WHO, 2016
CVM is critical to reproductive health as a lubricant and possibly as a line of defense against STIs.

**HIV must penetrate CVM** to reach target cells in the vaginal epithelium.

CVM that immobilizes HIV virions can directly reduce or even prevent initial infection.

Some women appear better protected than others.

What role does CVM play in protection against HIV infection?
Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1

CVM collected from 31 women

HIV-like Particles Mobile

HIV-like Particles Trapped

n=14

n=17
What was the difference between CVM samples?
STIs - Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1

Nunn et al. 2015
STIs - Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1

Nunn et al. 2015
STIs - Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1

L. crispatus
L. iners
G. vaginalis
L. jensenii
L. gasseri
Others

Group 1
Group 2
Group 3

pH

Total LA (% w/v)

Nunn et al. 2015
STIs - Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1
STIs - Cervicovaginal mucus (CVM), the vaginal microbiota and HIV-1

$L. crispatus$ dominated communities enhance virus trapping relative to $G. vaginalis$ and $L. iners$ dominated communities

What’s the deal with $Lactobacillus iners$ communities?
Vaginal microbiota alter the composition of CVM, enhancing protection from HIV infection
Vaginal microbiota alter the composition of CVM, enhancing protection from HIV infection.

All *Lactobacillus* species were not created equal.
Sexually Transmitted Infections

Vaginal microbiota & *Chlamydia*
A “healthy” vaginal microbiota is thought to be the first line of defense against STI.

Estimated rate of transmission after contact with a partner infected by *C. trachomatis* is estimated to be 25-40%, even lower for HIV (Katz et al. 1992).
STIs – *Chlamydia trachomatis* (CT) infections

CHARM Project

324 ♀ & ♂ Enrolled

222 ♀ CT + Visit 1

Questionnaire
Vaginal/Cervical Samples
Treatment (Az)

Visit 2 (3 months):
129 ♀ CT +
93 ♀ CT -
How did the microbiota differ between Visit 1 and Visit 2 for women that cleared CT?
STIs — *Chlamydia trachomatis* (CT) infections
Following CT infection and azithromycin treatment, women switch from CST IV to CST III.
STIs – *Chlamydia trachomatis* (CT) infections

CT+ women (Visit 1)

CT- (cleared) women (Visit 2)
Why are women with *L. iners* dominated microbiota susceptible to infection by CT?
Chlamydia infection is associated with a low Lactobacillus state.

Following clearance of Chlamydia infection by antibiotics, Lactobacillus iners dominated states are common.
Conclusions

Regarding factors of host physiology:

\[ \uparrow \text{Estrogen} = \uparrow \text{Lactobacillus} \]
\[ \& \]
\[ \uparrow \text{Microbial Stability} = \downarrow \text{Microbial Diversity} \]
Conclusions

CST IV: a normal state that carries risks?

Smoking, reduced trapping of HIV by CVM, and *Chlamydia* infection associated

But at any given time, 25% of women are in this state
**Conclusions**

*Lactobacillus iners* dominated communities show less protective properties (higher pH, lower lactic acid)

Strain-level differences

All *Lactobacilli* are not created equal
Acknowledgments

Dr. Jacques Ravel

Dr. Rebecca Brotman
Hormonal Contraceptives are associated with decreased prevalence of Bacterial Vaginosis

Rifkin, et al., 2009; Riggs et al., 2007; Baeten et al. 2001; Klebanoff
Hormonal Contraceptives
Hormonal Contraceptives are associated with decreased prevalence of Bacterial Vaginosis

How do the microbiota change?

Rifkin, et al., 2009; Riggs et al., 2007; Baeten et al. 2001; Klebanoff
Hormonal Contraceptives

125 women
Sampled prior to and after onset of oral HC use

Preliminary Data:
11 women
Hormonal Contraceptives

CST I: *Lactobacillus crispatus*
CST II: *Lactobacillus gasseri*
CST III: *Lactobacillus iners*
CST IV: non-*Lactobacillus*
CST V: *Lactobacillus jensenii*
Hormonal Contraceptives

Trends of increasing *Lactobacillus* spp. and decreasing CST IV with oral HC’s

CST I: *Lactobacillus crispatus*
CST II: *Lactobacillus gasseri*
CST III: *Lactobacillus iners*
CST IV: non-*Lactobacillus*
CST V: *Lactobacillus jensenii*
Temporal Dynamics of Vaginal Microbiota

32 women self-collected, 2x/week 16 weeks

Temporal Dynamics of Vaginal Microbiota

JSD = Jensen-Shannon Divergence Index = Vaginal Community Stability over time = Stability (for this talk)
Temporal Dynamics of Vaginal Microbiota

High JSD = Low Stability

Regardless of community state type!
Longitudinal trajectories of 4 women who completed the 16-week smoking cessation intervention

Brotman, BMC ID, 2014
Biosynthesis of BAs can allow various bacteria to survive in low pH environments. BAs have also been shown to enhance growth rates of various pathogenic bacteria, and shield them from host innate immunological defenses. Women who smoke, and particularly smokers with BV, may have increased susceptibilities to STIs.
The feminine product aisle
<table>
<thead>
<tr>
<th></th>
<th>Number (%) of intervals with the factor</th>
<th>OR</th>
<th>p Value</th>
<th>95% CI</th>
<th>aOR</th>
<th>p Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Day before</td>
<td>82 (19.90)</td>
<td>1.45</td>
<td>0.34</td>
<td>0.88</td>
<td>3.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days before</td>
<td>120 (29.13)</td>
<td>1.34</td>
<td>0.39</td>
<td>0.69</td>
<td>2.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Days before</td>
<td>215 (52.18)</td>
<td>1.94</td>
<td>0.08</td>
<td>0.92</td>
<td>4.11</td>
<td>2.28</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Menstrual hygiene</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Not menstruating</td>
<td>358 (87.10)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Tampon</td>
<td>13 (3.16)</td>
<td>2.75</td>
<td>0.20</td>
<td>0.58</td>
<td>13.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pad</td>
<td>36 (8.76)</td>
<td>0.91</td>
<td>0.84</td>
<td>0.37</td>
<td>2.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tampon and pad</td>
<td>4 (0.97)</td>
<td>3.60</td>
<td>0.13</td>
<td>0.68</td>
<td>18.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rectal sex</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 Day before</td>
<td>2 (0.49)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2 Days before</td>
<td>3 (0.73)</td>
<td>4.14</td>
<td>0.00</td>
<td>2.15</td>
<td>7.96</td>
<td>4.48</td>
<td>0.00</td>
</tr>
<tr>
<td>3 Days before</td>
<td>5 (1.22)</td>
<td>4.06</td>
<td>0.00</td>
<td>1.60</td>
<td>10.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vaginal douching</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 Day before</td>
<td>15 (3.85)</td>
<td>3.91</td>
<td>0.10</td>
<td>0.78</td>
<td>19.52</td>
<td>3.71</td>
<td>0.10</td>
</tr>
<tr>
<td>2 Days before</td>
<td>23 (5.60)</td>
<td>1.57</td>
<td>0.51</td>
<td>0.41</td>
<td>5.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days before</td>
<td>31 (7.56)</td>
<td>1.25</td>
<td>0.74</td>
<td>0.33</td>
<td>4.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vaginal lubricant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Day before</td>
<td>12 (2.92)</td>
<td>7.51</td>
<td>0.13</td>
<td>0.56</td>
<td>100.09</td>
<td>11.75</td>
<td>0.01</td>
</tr>
<tr>
<td>2 Days before</td>
<td>23 (5.60)</td>
<td>2.83</td>
<td>0.16</td>
<td>0.65</td>
<td>12.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days before</td>
<td>31 (7.56)</td>
<td>1.41</td>
<td>0.42</td>
<td>0.61</td>
<td>3.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Brotman et al, STI, 2010
Many lubricants and their ingredients have overall component concentrations (osmolalities) higher than those of the human body.
Vaginal lubricants are widely used and vastly understudied

› Most lubricants are formulated with high concentrations of humectants (glycerol, propylene glycol) that make them markedly hypertonic to mucosal epithelia.

› These concentrated humectants make the lubricants feel comfortably warm when applied.

› Hypertonic lubricants have molecular concentrations higher than in the body’s cells, causing cells to shrink and die, and increasing mucosal susceptibility to infection.

› There is little *in vivo* evidence available on how the vaginal or rectal ecosystems respond to lubricant use.

› Epidemiologic studies suggest vaginal lubricants are associated with development of BV.
Gynecology and Lubricant Effects Study (GALE)

- Recruiting women presenting for TVUS
- Standardized product and confirmation of dose and date
- Observational study of women enrolled in HMP
- Evaluating vaginal microbiota, lactic acid isomers, immunology and epithelial shell shedding
Hormonal Contraception
Longitudinal (HCL) Study
Table 1. Association between hormonal contraception and community state type (CST)† compared to women on no HC in a longitudinal study

<table>
<thead>
<tr>
<th>CST</th>
<th>Dominant bacteria</th>
<th>OCP §</th>
<th>Ring</th>
<th>Implant</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>L. crispatus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>L. gasseri</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>L. iners</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IV-A*</td>
<td>Low-Lactobacillus</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>IV-B*</td>
<td>Low-Lactobacillus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>L. jensenii</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

† CSTs reflect the clustering of samples based on bacterial composition and abundance.

* CST IV-A is characterized by various species of anaerobic bacteria including Anaerococcus, Peptoniphilus and Prevotella spp., whereas CST IV-B had higher proportions of bacteria from the genera Gardnerella, Atopobium and Megasphaera among others.

§ The "+" is increased, "-" is decreased and 0 is not statistically significant.

N=125 women and >4500 samples
Longitudinal profile of all women on the HCL study over 2 years. (n=108 women)
HCL study: Change in CST over time with OCP initiation, \((n=11 \text{ women and 385 observations})\).

There are drops in CST IV-B and increases in CST III.