The Vaginal Microbiome & HIV-1 Acquisition

Jeanne Marrazzo, MD, MPH
UAB Division of Infectious Diseases
MTN Regional Meeting
September 2016
Discussion

- How might a healthy vaginal environment dominated by *L. crispatus* help protect against HIV infection?
- What is the molecular approach to defining vaginal microbiology?
- What are the implications for understanding relationship to HIV acquisition risk?
- What are the most important next steps?
Background

- A healthy vaginal environment dominated by *L. crispatus* helps protect against HIV infection

- *L. crispatus* can be grown and studied in the laboratory, as can some of the bacteria commonly found in bacterial vaginosis. However...

- To define the entire spectrum of bacteria in the vagina, especially anaerobes, molecular methods are needed: 16S rRNA approach
The 16S rRNA gene

- **Present in all bacteria** (codes for small subunit of ribosomal RNA complex, necessary for protein synthesis)
- **Has properties of a molecular clock**
  - rDNA sequence similarities between species correlate with evolutionary relatedness (time to common ancestor)
  - Little evidence of horizontal gene transfer or recombination
- **Conserved regions**: useful for broad range PCR
- **Variable regions**: useful for identifying species
Hierarchical Clustering of Vaginal Bacterial Communities with 16S rDNA PCR & Pyrosequencing

A. Hierarchical clustering of vaginal bacterial communities

Scale bar = KR distance; colored bars = most abundant taxa in each sample

B. Taxonomic composition

Women with BV have diverse heterogeneous communities.

Women who don’t have BV are dominated with either L. iners or L. crispatus.

A = Amsel criteria
N = Nugent score
Red = BV+; Green = BV-

Srinivasan 2012
Contribution of Various Infections (PAR%) to HIV Acquisition Over Time

Masese AIDS 2015
Role of vaginal microbiota in genital inflammation and enhancing HIV transmission

Jo-Ann Passmore, PhD
University of Cape Town
CAPRISA
National Health Laboratory Service

Brent Williams, PhD
Center for Infection & Immunity,
Mailman School of Public Health,
Columbia University
# Association between genital inflammation and HIV acquisition

<table>
<thead>
<tr>
<th></th>
<th>HIV+</th>
<th>HIV-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital inflammation present*</td>
<td>19</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Genital inflammation absent</td>
<td>39</td>
<td>52</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>58</td>
<td>116</td>
</tr>
</tbody>
</table>

**Odds Ratio** 3.2 (95% CI: 1.3 – 7.9)  
**p-value** 0.014

*Women with 5 or more pro-inflammatory cytokines or chemokines (MIP-1a, MIP-1b, IL-8, IP-10, TNF-a, MCP-1, IL-6, IL-1a, IL-1b) above the 75th percentile  
Significant after adjusting for age, urban/rural, condom use, hormonal contraceptives, number of sex acts, number of returned used applicators, HSV-2 status*
Vaginal microbiome cluster CST4 is linked with genital inflammation and HIV
**Prevotella bivia** is strongly associated with genital inflammation and HIV acquisition

<table>
<thead>
<tr>
<th></th>
<th>P. bivia+ OR*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>19.2</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(95% CI: 4.0-92.4)</td>
<td></td>
</tr>
<tr>
<td>HIV+</td>
<td>12.7</td>
<td>p=0.006</td>
</tr>
<tr>
<td></td>
<td>(95% CI: 2.1-77.8)</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted odds ratio

22 women were HIV positive & had inflammation – 9/22 (41%) had *P. bivia*

Women with *P. bivia* were **19 times** more likely to have genital inflammation and **13 times** more likely to acquire HIV
Vaginal bacteria associated with increased risk of HIV acquisition in African women


Disclosure: RSM has a research grant from Hologic Corp, paid
Vaginal bacteria associated with increased risk of HIV acquisition in African women

**Background:** Disruption of the vaginal microbiota associated with risk of HIV.

**Methods**
- Nested case-control
- Microbiota at pre-SC (N=72) or acute infection (N=15) sample vs. negative controls
- Characterized microbiota by deep sequencing and qPCR

**Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=262)</th>
<th>Cases (N=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>29 (23-36)</td>
<td>26 (22-30)</td>
</tr>
<tr>
<td><strong>Married</strong></td>
<td>199 (76%)</td>
<td>66 (76%)</td>
</tr>
<tr>
<td><strong>DMPA</strong></td>
<td>37 (14%)</td>
<td>18 (21%)</td>
</tr>
<tr>
<td><strong>Pregnant</strong></td>
<td>57 (22%)</td>
<td>20 (23%)</td>
</tr>
<tr>
<td><strong>BV</strong></td>
<td>67 (29%)</td>
<td>32 (42%)</td>
</tr>
</tbody>
</table>
Overall vaginal bacterial community diversity in 57 cases versus their 57 matched controls

Shannon Diversity Index higher in cases (median 0.9, IQR 0.4-2.3) vs. controls (median 0.7, IQR 0.1-1.4), p=0.03
Adj. ORs for association between bacterial quantity & HIV acquisition: 5 species associated with HIV

**Eggerthella sp. type 1**
- Undetectable
- T1
- T2
- T3

**Gemella asaccharolytica**
- Undetectable
- T1
- T2
- T3

**Leptotrichia / Sneathia**
- Undetectable
- T1
- T2
- T3

**Megasphaera**
- Undetectable
- T1
- T2
- T3

**Mycoplasma hominis**
- Undetectable
- T1
- T2
- T3

N=87 cases + 262 controls; undetectable compared to 1st, 2nd, 3rd tertile
Why the Difference?

• Different populations of women
  – Region; unmeasured variables
  • Exposure to male partners’ microbiome

• Different techniques to define microbiome
  – McClelland characterized microbiota by deep sequencing, then used data to select bacterial taxa (some genus and some species level) to investigate using highly sensitive qPCR probes
  – CAPRISA used proteomic approach to search for bacterial peptides that were then associated with a database derived from earlier 16S rRNA work; no specific qPCR, but estimated bacterial abundance by summing protein spectral counts
What Next?

• Use VOICE repository to study relationship between vaginal microenvironment & HIV-1 acquisition
  – HIV incidence 5.7/100,000 p-y; BV 42.5 cases/100 p-y

• Nested case-control study using qPCR to target specific BVAB and lactobacilli with proteomic profiling to associations of their presence and concentrations with HIV-1 acquisition risk

• Define relationship between vaginal microbiome and efficacy of TFV gel in participants by assessing associations between BVAB-specific qPCR and proteomic profiling, serum and vaginal TFV levels, and HIV-1 acquisition risk
Conclusions

• A *L. crispatus*-dominant vaginal microbiome is associated with lower prevalence and incidence of HIV
• More research is needed on the role of BV-associated anaerobes in increasing HIV risk
• Maintenance of this environment might reduce the risk of acquiring these infections and should be further studied
  – Balkus *JID* 2016
Acknowledgements

- Slim Abdool-Karim
- Scott McClelland
BV & Increased HIV Acquisition

- Loss of $\text{H}_2\text{O}_2$ (directly virucidal)
- Activation of CD4 by alkaline pH
- Upregulation of cytokines that promote local HIV replication (TNF-alpha, IL-1 beta) & increased shedding
  - HIV shedding increased with intermediate flora or BV (Rebbapragada 2008; Coleman 2007; Sha 2005; Tanton 2011)
    - Not in all prospective studies (Wang 2001; Moreira 2009)
  - Successful BV treatment: decreases in IL-1 beta, IL-8, RANTES & activated CD4 T-cells at endocervix, including those expressing CCR5 and CD69 (Rebbapragada 2008)
- Kyongo 2015; Cone 2015
BV & Increased HIV Transmission

- Bacteria may activate Langerhans cells and CD4+ T-cells (Donoval, 2006; deJong 2009)
  - May involve direct stimulation by BVAB of relevant immune targets in male genitalia
  - BVAB / LB shared in male & female partners (Bukusi 2011; Gray 2009; Marrazzo 2009)
  - Male circumcision changes microbiota of penis, and reduces women’s risk of subsequent BV (Price 2010; Gray 2008; Liu 2013)
## The Microbiome & Other STI-Related Syndromes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bacteria</th>
<th>References</th>
</tr>
</thead>
</table>
| Cervicitis     | *M. indolicus*  
*L. crispatus* | Gorgos 2015     |
| Urethritis     | *Sneathia spp*              | Manhart 2013   |
| Endometritis   | *Sneathia spp*  
BVAB-1  
*Atopobium vaginae* | Haggerty 2016 |