The Role of the Vaginal Microbiome in Microbicide Research

Jeanne Marrazzo, MD, MPH
University of Washington, Seattle

First International Workshop on Microbiome in HIV Pathogenesis, April 2015
Acknowledgements:
UW-FHCRC Genital Health Study Team

- David Fredricks
- Kathy Ringwood
- Tina Fiedler
- Kathy Thomas
- Sujatha Srinavasan
- Congzhou Liu
- Kathy Agnew
- Nancy Dorn
- Dana Kubulis
- Dwyn Dithmer
- Laura Sycuro
DISCUSSION

• The “optimal” human vaginal microbiota
  – Definition
  – Relationship to health outcomes
  – Relationship to local immunity

• Disruptions of the microbiome and HIV acquisition / transmission
  – What do we know?
  – What don’t we know?
  – Is there a roadmap for optimizing understanding and consequently, outcomes (like HIV prevention)?
  – How can this be applied to microbicide research?
The Classical Spectrum of Vaginal Bacteria

• Traditional cultivation & determination of $\text{H}_2\text{O}_2$ production by *Lactobacilli*
  
  $\text{H}_2\text{O}_2$ - producing LB (*L. crispatus, L. jensenii*) predominate in ‘optimal’ environment (Nugent score = 0); lactic acid also critical
    
    – Underestimates presence of more fastidious LB (*L. iners*)

• Overgrowth of “commensal” anaerobes as community diversifies; may eventuate in bacterial vaginosis (BV)

<table>
<thead>
<tr>
<th>pH &lt; 4.7</th>
<th>pH &gt; 4.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nugent = 0</td>
<td>Nugent 7 -10</td>
</tr>
<tr>
<td>Amsel’s +</td>
<td></td>
</tr>
</tbody>
</table>
Benefits of an Optimal Vaginal Environment

- Optimal birth outcomes (short-term)
  - Normal birth weight
  - Normal timing of delivery
  - Fewer pregnancy-associated infections

- Optimal health outcomes (long-term)
  - Transfer of maternal microbiota to infant
    - Lower rates of autoimmune diseases (asthma), metabolic disorders (Dominguez-Bello 2010; Torrazza 2011; Neu 2011)
    - Mediated by rapid colonization of skin, gut, genital tract with maternal microbiota
  - Protection from pathogens & dysbiosis
    - BV, HIV, chlamydia, gonorrhea, trichomonas
    - Lower levels of genital HIV in HIV+ women (Sha 2005)
Bacterial Vaginosis

- Abnormal discharge and amine odor; ~1/2 symptomatic
- 15% to 20% fail initial antibiotic treatment
- Recurrence in majority who respond (75% annual incidence)

pH ≥ 4.7
Nugent 7 -10
Amsel’s +

Bradshaw 2008; Sobel 2008, Marrazzo 2010
Fig. 1. Forest plot of relative risk estimates of incident HIV infection by bacterial vaginosis status, stratified by HIV-risk group. Studies are identified by the references. The horizontal lines represent the 95% confidence intervals (CI). Overall heterogeneity $P = 0.7$. 

Atashili 2006
BV & Increased HIV Acquisition

• Loss of H$_2$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

Bacterial Vaginosis in HIV-Infected Women Induces Reversible Alterations in the Cervical Immune Environment

Anuradha Rebbapragada, PhD,* Kathryn Howe, PhD,* Charles Wachihi, MCChB,† Christopher Pettengell, BSc,* Sherzana Sunderji, BSc,* Sanja Huibner, BSc,* T. Blake Ball, PhD,‡ Francis A. Plummer, MD,‡ Walter Jaoko, PhD, MBChB,‡ and Rupert Kaul, MD, PhD*‡§
**Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Analysis among African Couples**

Craig R. Cohen\(^1,2\), Jairam R. Lingappa\(^3,4,5\), Jared M. Baeten\(^3,4,6\), Musa O. Ngayo\(^7\), Carol A. Spiegel\(^8\), Ting Hong\(^3\), Deborah Donnell\(^9\), Connie Celum\(^3,4,6\), Saidi Kapiga\(^10\), Sinead Delany\(^11\), Elizabeth A. Bukusi\(^1,3,5,7\)

<table>
<thead>
<tr>
<th>Model</th>
<th>HR</th>
<th>Adjusted HR*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-visit BV</td>
<td>3.62 (1.74-7.52)</td>
<td>3.06 (1.35-6.95)</td>
</tr>
<tr>
<td><strong>Sensitivity Analyses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current visit BV</td>
<td>5.30 (2.21-12.74)</td>
<td>3.97 (1.67-9.43)</td>
</tr>
<tr>
<td>More severe BV status</td>
<td>7.19 (2.59-19.94)</td>
<td>6.98 (2.12-23.0)</td>
</tr>
</tbody>
</table>

*Fixed covariates: age, geographic region, partner HSV-2 status, circumcision, randomization assignment and STD; Time-dependent covariates: pregnancy, hormonal contraception, plasma HIV-1 RNA, unprotected sex with study partner, CD4 count, outside partners, no. of sex acts with study partner, genital ulcer disease.*

Log$_{10}$ HIV RNA concentration in plasma and female genital secretions compared by vaginal flora category

<table>
<thead>
<tr>
<th>Vaginal Flora</th>
<th>Log$_{10}$ HIV Mean ± SD</th>
<th>P-Value vs. normal vaginal flora</th>
<th>P-Value* vs. normal vaginal flora</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genital HIV RNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal flora</td>
<td>3.04 ± 0.99</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intermediate vaginal flora</td>
<td>3.25 ± 1.01</td>
<td>0.0035</td>
<td>0.058</td>
</tr>
<tr>
<td>BV</td>
<td>3.23 ± 0.99</td>
<td>0.0023</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>Plasma HIV RNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal flora</td>
<td>3.81 ± 1.00</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intermediate vaginal flora</td>
<td>3.96 ± 1.07</td>
<td>0.037</td>
<td>N/A</td>
</tr>
<tr>
<td>BV</td>
<td>3.99 ± 1.07</td>
<td>0.0056</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Modest increase: 0.2 log$_{10}$

*After controlling for plasma HIV RNA

Cohen PloS Med 2012
- Modest increase in HIV shedding unlikely to account for increased HIV transmission risk observed

  - Increases in characteristic cytokines are due to BV, not HIV (Mitchell 2012)

- In this cohort, 35%, 15%, and 52% had BV at enrollment, throughout follow-up, and at least one time over 2 years

- Attributable risk of abnormal vaginal bacteria to HIV acquisition risk may be substantial, even if other STI not common

*After controlling for plasma HIV RNA
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)

Kyongo 2015
A spectrum of anaerobic bacteria has long been known to characterize BV

- **Gardnerella vaginalis**
  - Facultative, capnophilic, pleomorphic Gram-variable rod
  - Key component of Nugent scoring
  - Heavily represented in biofilms associated with BV
  - Universal in BV; present in 35%-40% of normal women

- **Mobiluncus mulieris & M. curtisii**
  - Anaerobic, curved, motile Gram variable rods
  - 85% of women with BV; 38% without

- **Mycoplasma hominis**
  - 60% of women with BV, 10% without

- Various anaerobes, variably present: *Porphyromonas, Prevotella, Peptostreptococcus, Veillonella*
Fluorescence Micrographs of Vaginal-Fluid Smears Analyzed by FISH with Labeled Oligonucleotide Probes Targeting Bacterial rRNA

BVAB-1 (green) + BVAB-2 (red) + DAPI (blue)
BVAB-1 (green), BVAB-2 (red), DAPI (blue)

- BVAB1: Family Lachnospiraceae
  - Butyrate producing bacteria in GI tract; see also Zozaya-Hinchcliffe *JCM* 2010

- BVAB2: No close family affinities
BVAB-3 (red)

- BVAB3: No close family affinities
- Associated with cervicitis (Gorgos *ISSTDR* 2009; Sycuro *ASM* 2012)
Hierarchical Clustering of Vaginal Bacterial Communities with 16S rDNA PCR & Pyrosequencing

A. Women with BV have diverse heterogeneous communities.

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

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

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

Srinivasan 2012
Categories of Vaginal Community State Types

I  L. crispatus
II  L. gasseri
III  L. iners
IVA modest LB spp; some anaerobes
IVB Diversity Group
   Atopobium, Prevotella, Parvimonas, Sneathia, Gardnerella, Mobiluncus, Peptoniphilus
V  L. jensenii

Vaginal Microbiome of Reproductive Age Women; Ravel PNAS 2010
Defining the Vaginal Immune Environment

• Measure soluble mediators related to traditional measures of vaginal bacteria (GS, culture)
• Increasing use of epithelial cell co-culture systems and histochemistry (Pyles 2014)
  • Higher levels of SLPI; low levels of proinflammatory cytokines (Fichirova 2011; Jiang 2012)
  • Few WBC, particularly PMN, in vaginal fluid
  • Intact cervicovaginal epithelium (glycogen fuel for LB), absence of major biofilm
• Very early use of functional assays & “omics”
  – CVL from healthy women had anti-E. coli activity & correlated with protein recovery; L. crispatus / L. jensenii proteins associated with inhibitory activity (Kalyoussef 2012)
• Production of sialidase (IgA destruction), glycosidase, volatile amines; IL-8 increase variable; ?SCFA (Mirmontsef 2012)

Yudin 2003; Caucci 2004; Valore 2006; Cherpes 2008; Rebbapragada 2008; Mitchell 2009
Local Immunology with BV

- SCFA are produced by anaerobic bacteria & regulate immune responses in gut (metabolomic perspective)

Fig. 2 SCFAs are present in vaginal fluid. Vaginal fluids were collected from women with or without BV by cervical-vaginal lavage. The levels of acetate, propionate, and butyrate were measured by gas chromatography. P-values are given comparing non-BV and BV for each SCFA.

Mirmontsef 2010
Some consistency in the “inflammatory” nature of the environment in BV, but not completely.

Need all components of metadata:

- Specific BV-associated bacteria: relationships to clinical features & innate immunity
- Contributions of behavior (sex, vaginal product use, antibiotics, diet) over TIME
- Hormonal environment: menses; exogenous hormones (contraception; ERT)
- Ultimately, link to endpoints of interest (PROM, PTD, PID, HIV transmission)
Microbicides: The Challenges

• In women at highest HIV risk, need to consider & measure key features of the vaginal microenvironment:
  • Vaginal bacterial communities & consequent local immune milieu
  • Biofilm with BV
  • Semen / sex
  • Hormones & menses

2013
Bacterial Taxa Associated with Amsel’s Criteria

- Leptotrichia amnionii & Eggerthella sp. associated with each criteria
- Stars denote bacteria present in >75% of women with BV
- Taxa in bold denote those associated with Amsel’s criteria as a composite unit

Srinivasan 2012
BVAB & The Host: Complex Interactions

• *Gardnerella vaginalis*
  – Biofilm associated with BV and treatment failure
  – Extracellular DNA critical for biofilm integrity; destroyed by DNAase (Hymes JID 2013)

• *Atopobium vaginae*
  – Biofilm; more BV-specific

Sialidase levels & other aspects of immune response may also be dictated in part by composition of anaerobic community (Marconi 2012; Anderson B 2011)

Epithelial cell lines respond differently to different LB or BVAB (Eades 2010)

M. indolicus induces IL-8 secretion from cervical & vaginal epithelial cells; proinflammatory potential (Sycuro, ASM 2012)
G. vaginalis biofilm - scanning electron microscopy (SEM)
Gardnerella vaginalis Outcompetes 29 Other Bacterial Species Isolated From Patients With Bacterial Vaginosis, Using in an In Vitro Biofilm Formation Model

Patrícia Alves, Joana Castro, Cármen Sousa, Tatiana B. Cereija, and Nuno Cerca

Institute for Biotechnology and Bioengineering, C University of Minho, Campus de Gualtar, Braga,

Original article
Evidence for Gardnerella vaginalis uptake and internalization by squamous vaginal epithelial cells: implications for the pathogenesis of bacterial vaginosis

Christy N. Marrs, Susan M. Knobel, Wen Qin Zhu, Stephanie D. Sweet, Ahsen R. Chaudhry, Donald J. Alcendor

RESEARCH ARTICLE
Genetic and biochemical diversity of Gardnerella vaginalis strains isolated from women with bacterial vaginosis

Milda Pleckaityte, Migle Janulaitiene, Rita Lasickiene & Aurelija Zvirbliene

1Institute of Biotechnology, Vilnius University, Vilnius, Lithuania; and 2National Public Health Surveillance Laboratory, Vilnius, Lithuania
• Planktonic vs. biofilm systems
• Persister cells & relative antibiotic resistance

Role of the BV biofilm in protecting BV-associated bacteria from the antimicrobial effects of current therapeutic approaches
Effect of Menses

Woman without (C) and another with *L. iners* (D)

Srinivasan 2009
**Biomedical HIV Prevention Efficacy Trials, 2014-2016**

*Trial end dates are estimates; due to the nature of clinical trials, the actual dates may change. For full trial details, see www.avac.org/pxrd.*

**This table only includes efficacy evaluations of biomedical strategies in HIV-negative people. There are ongoing pilot and demonstration projects of oral PrEP, an open-label evaluation of 1% tenofovir gel in the community where CAPRISA 008 took place and numerous Phase I and II trials of other options.**
ARV-based Prevention Pipeline

**Pre-Clinical**
- IPM
- Pop Council
- IPM
- IPM
- IPM
- IPPC NIAID
- IPM
- Pop Council
- ImQuest
- PBS
- Mintaka
- Pop Council
- IPM
- Pop Council

**Phase I**
- IPM
- GSK
- CONRAD
- IPM
- Jansen
- CONRAD
- Albert Einstein
- TailMed
- CONRAD
- IPM
- Pop Council

**Phase II**
- HPTN/ACTG
- CONRAD
- CONRAD
- IPM
- Gilead

**Phase III**
- CONRAD
- IPM

**Active Drug**
- TFV: Tenofovir
- TDF: Tenofovir disoproxil fumarate
- TNC: Tenofovir/emtricitabine
- TFV/FTC

**Delivery System**
- Oral pills
- Vaginal tablet
- Vaginal gel
- Rectal gel
- Vaginal ring
- Long-acting injectable
- Vaginal film
- Thin film polymer
- PBS: Phosphate buffered saline
- Nano-fiber

**AVAC**

November 2014
avac.org/infographics
Moving Forward

• Employ frequent sampling
  – Enhance understanding of dynamic nature of microbiota & its diversity

• Integrate host immunity, behavior, microbiology
  – Cross-disciplinary approach required: separating contributions of sexual behaviors & innate immunity…
  – BV as a dysbiosis that may often involve exchange then maintenance of microbial communities
  – Community cultivation: novel approaches
  – Incorporate “omics” approach: metabolomics, proteomics, genomics