The vaginal microbiome, preterm birth and HIV

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Outline

- Overview of the vaginal microbiome
  - in general
  - in HIV positive women
- Vaginal microbiome and infectious etiology of preterm birth
- Preterm birth in HIV positive women
- The role of the vaginal microbiome in contributing to preterm birth in HIV positive women?
What is the importance of normal vaginal microbiota?

- Vaginal microbiota is the fine balance of organisms that exist in the vagina
- Normal vaginal microbiota is associated with ability to defend against vaginal/cervical pathogens
- Abnormal vaginal flora is associated with susceptibility to many infections, risk of post surgical complications and adverse perinatal outcomes
Culture-based Investigations of the Vaginal Microbiota

- *Lactobacillus* spp. - predominant
  - Aid in immune/host defense against pathogens (*Sobel 1999*)
  - Influence fertility and reproductive success (*Eschenbach 1989, Hillier 1999*)
- Shift to increased diversity of mixed anaerobic bacteria (*Gardnerella vaginalis, Bacteroides* spp.)
  - Increased risk of STI acquisition + transmission (*Hillier 1998, Wiesenfeld 2003*)
  - Increased risk of preterm birth (*Hillier 1995*).
Culture Independent Investigations

- Clusters of 4-7 defined as community state types (CST) distinguished by dominant bacterial taxa
- Most prevalent and dominant—*Lactobacillus (L)* *inners*, followed by *L crispatus*, *L gasseri*, *L jensenii*
- Suggestion that non-lactobacillus dominant communities may be “healthy” in some women
## Defined Vaginal Community State Types

<table>
<thead>
<tr>
<th>Ravel et al 2011</th>
<th>Gajer et al 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Lactobacillus (L) crispatus</td>
</tr>
<tr>
<td>II</td>
<td>L. gasseri</td>
</tr>
<tr>
<td>III</td>
<td>L. iners</td>
</tr>
<tr>
<td>IV</td>
<td>Heterogenous mix</td>
</tr>
<tr>
<td>V</td>
<td>L. jensenii</td>
</tr>
</tbody>
</table>

| I | Lactobacillus (L) crispatus |
| II | L. gasseri |
| III | L. iners |
| IVA | Bifidobacterium, Dialister, Streptococcus, and Bacteroides |
| IV | Gardnerella, Prevotella, Megasphaera, bacterial vaginosis-associated bacteria (BVAB), and Mobiluncus |
| V | L. jensenii |
Factors + Behaviors associated with Vaginal Microbial Diversity and CST

- **Ethnicity**: Black and Hispanic women present with more non-Lactobacillus dominant CST IV vs. Asian and White women (Zhou 2007, Zhou 2009, Ravel 2011)

- **Menstrual cycle**: Menses associated with shift to CST III and CST IV and/or increased bacterial diversity in some but not all women (Gajer 2012, Chaban 2014)

- **Sexual activity**: Recent sexual activity negatively impacts community stability, shift to CST IV in some women (Gajer 2012)

- **Symptoms**: Self-reported abnormal discharge associated with increased bacterial diversity (Drell 2013)
Microbiome profiling approach (Money, Hill et al.)

Total genomic DNA extraction → \textit{cpn60 UT} PCR → \textit{cpn60} PCR amplicon libraries

Assembly

Pyrosequencing
GS Junior
454 Sequencing System
## Community State Types

<table>
<thead>
<tr>
<th>CST</th>
<th>N (%)</th>
<th>Median Nugent Score</th>
<th>Shannon Diversity Index</th>
<th>Dominant OTU (Prevalence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>153 (49%)</td>
<td>0</td>
<td>0.96 ± 0.07</td>
<td><em>L. crispatus</em> (100%)</td>
</tr>
<tr>
<td>II</td>
<td>13 (4%)</td>
<td>0</td>
<td>1.09 ± 0.18</td>
<td><em>L. gasseri</em> (100%)</td>
</tr>
<tr>
<td>III</td>
<td>49 (16%)</td>
<td>0</td>
<td>1.17 ± 0.1</td>
<td><em>L. iners</em> (100%)</td>
</tr>
<tr>
<td>IVA</td>
<td>19 (6%)</td>
<td>2</td>
<td>2.48 ± 0.35</td>
<td><em>Bifidobacterium</em> spp (45%), <em>Prevotella</em> spp (79%), <em>Atopobium vaginae</em> (53%), <em>Proteobacteria</em> (89%)</td>
</tr>
<tr>
<td>IVB</td>
<td>31 (10%)</td>
<td>8</td>
<td>2.26 ± 0.12</td>
<td><em>Gardnerella vaginalis A</em> (100%), <em>Megasphaera genomsp.</em> (94%), <em>Prevotella timonensis</em> (87%), BVAB3 (74%)</td>
</tr>
<tr>
<td>IVC</td>
<td>20 (6%)</td>
<td>5</td>
<td>1.71 ± 0.15</td>
<td><em>Gardnerella vaginalis B</em> (100%)</td>
</tr>
<tr>
<td>V</td>
<td>25 (8%)</td>
<td>0</td>
<td>1.55 ± 0.15</td>
<td><em>L. jensenii</em> (100%)</td>
</tr>
</tbody>
</table>
Characteristics of vaginal microbiome of HIV positive women

- HIV-positive women have a higher prevalence (> 30%) of bacterial vaginosis (BV) compared to HIV-negative women (Atashili et al. 2008)
- HIV-positive women with BV have higher genital tract HIV load (Cu-Uvin et al. 2001)
- Significant association between decreased vaginal lactobacilli and increased genital HIV RNA levels (Sha et al. 2005) and low CD4 count (Mane et al. 2012)
CPN60 based comparisons – HIV-/+

Healthy women, n= 310
Average age 30.1

- Caucasian: 64.5%
- Black: 19.4%
- Asian: 19.4%
- South Asian: 3.9%
- Aboriginal: 1.9%
- Other/Multiple Ethnicities: 2.9%

HIV-positive women, n= 54, average age 36.6

- Caucasian: 33.3%
- Black: 24.1%
- Asian: 16.7%
- South Asian: 7.4%
- Aboriginal: 7.4%
- Other/Multiple Ethnicities: 11.1%
Community state type
I – L. crispatus
II – L. iners
III – L. crispatus/iners
IVA – Gardnerella Group A
V – Mixed 1
VI – Mixed 2
IVB – Gardnerella Group B
IVC – Gardnerella Group C

Nugent score
Low (0-3)
Intermediate (4-6)
High (7-10)
No cells
Missing

Healthy women
n=310 women
OTU are at least 10% of at least 1 woman's profile

Low (0-3)
Intermediate (4-6)
High (7-10)
No cells
Missing
Lactobacillus crispatus
Community Groups

HIV+ women

Megasphaera sp.  
Prevotella timonseasia
Atopobium vaginae
Clostridium genomosp

Bifidobacterium breve

Gardnerella vaginalis

Lactobacillus iners
Lactobacillus crispatus
Bifidobacterium breve
Lactobacillus gasseri
HIV viral load suppressed vs unsuppressed

**LDA Scores of Significant Bacteria by Viral Load Groupings (p<0.02 for all species)**
Preterm delivery

- Preterm birth rates vary from country to country with 8% in Canada and 13% in US

- Accounts for 75% of neonatal morbidity and mortality including long term neurologic morbidity

- Cost to the health care system: $10-13 billion per year in US
Infant mortality associated with preterm birth globally
Causes of preterm birth- overall

- 30% indicated births – maternal or infant disease
- 40-50% due to idiopathic preterm birth – related to infection/inflammation
- 15-30% secondary to PPROM

- i.e. up to 55-80% due to dysbiosis
Intrauterine Infection

Figure 1. Potential Sites of Bacterial Infection within the Uterus.

BV and adverse pregnancy outcomes

- Leitch H & Kiss H – meta analysis 2007
- 32 studies, 30,518 patients
- BV associated with
  - 2.16 OR [1.56-3.0] for birth <37 wks
  - 1.29 OR [0.92-1.82] for <34 wks
  - 1.34 OR [0.59-3.06] for < 32 wks
- BV assoc with late miscarriage
  - 6.32 OR [3.65-10.94]
Consequences of Chorioamnionitis

- More common w/ maternal BV
- ~15-30% of women with PPROM
- Neonatal complications more common in preterm infants
  - Neonatal sepsis, pneumonia, respiratory distress
- Severe neonatal morbidity with PPROM
- Neurodevelopmental delay and cerebral palsy

PPROM: Evidence for role of infection

- Infection is most common identifiable risk factor
- Bacterial vaginosis is associated with preterm delivery and PPROM (Denney and Culhane. 2009. Semin Fetal Neonatal Med 14: 200-203)
- Ct, Trich, Gc infection associated with PPROM and PTD (Locksmith and Duff. 2001. Semin Perinatol 25: 295-309)
- Group B streptococcal urinary infection is associated with preterm delivery (Nomura et al. 2005. IJGO 91:69-70)
Preterm Rupture of the Membranes

Membrane stretch (IL-8)

Dec. tensile strength

Localized defects

Dec. collagen content in amnion

Amniotic Extracellular matrix degradation

Apoptosis

Prod’n of Prostaglandins E2 & F2α

Uterine irritability

Prod’n of Prostaglandins E2 & F2α

Genital tract infection (IL-1, TNFα)

Relaxin

Glucocorticoid Prod’n

Parry and Strauss. NEJM. 1998; 338: 663-70.
PPROM study in BC population (March of Dimes)

- Prospective enrollment of women at 24+0-33+6 with idiopathic PPROM
- Enrollment of 52 women completed
- Women are consented, have vaginal swabs taken at admission/time of PPROM diagnosis, weekly then at delivery and the neonate is followed for outcomes
- We hypothesized that specific microbiome clusters would correspond with latency and infectious/inflammatory morbidity in infants
- Batched metagenomic samples in progress – preliminary results only available.
Most PPROM profiles are not dominated by *Lactobacillus*
Uncultivated bacteria cause preterm birth

- Han et al, J Clin Micro 2009
- Intramniotic infection detected by 16s RNA based methods detected bacteria in ‘sterile’ amniotic fluid – Fusobacterium, Letotrichia, Bergeyella, Peptostreptococci
- PCR pos women more often had chorio, funisitis and preterm infant with early neonatal sepsis
18 cases of preterm delivery/72 controls (term)

Three intervals: 6.9 – 22.1 weeks;
   22.2 – 29.8 weeks;
   29.9 – 41 weeks

- L. crispatus, L. jensenii, L. gasseri, L. vaginalis increases.
- A. vaginae, G. vaginalis, Ureplasma parvum, Eggerthella, Parvimonas micra, Dialister spp type 2, Gernella, BVAB1/2 decreases.
Compare microbial abundance over time using the differences of Logs i.e. $\log_{10} (a) - \log_{10} (b)$.

- Zero-inflated negative binomial mixed-effects models (ZINBLME)
- Negative binomial linear mixed effects (NBLME)
- Poisson linear mixed effects (PLME) models

Found no difference in the microibota of women who delivery preterm compared to term by relative abundance or bacterial load
Diversity of microbiome correlates to PTB

- Hyman et al – Repro sciences 2014;21(1):32
- 46 women at high risk for PTB, 42 low risk
- Although low numbers of preterm births – diversity correlated with PTB compared to term birth

- 277 women (170 term, 107 preterm)
- Oral, vaginal, stool, placental swabs & tissue
- No causal differences in the vaginal microbiome of preterm women (p=0.254), but significant variation in the placental microbiome (p=0.012), attributable to case of spontaneous PTB.
- Taxa associated were Mycoplasma and Fusobacterium
Vaginal microbial profiles – overview CPN60 based data
HIV and pregnancy

- Advancement of antiretroviral therapy for the prevention of mother to child transmission – success story of 25% transmission to less than 1% due to extensive use of ARV’s

- Temporal progression from monotherapy (1994) to dual nucleosides to NNRTI’s to PI’s to PI’s with boosting (ritonavir)
Association of preterm birth with ARV’s

- Watts et al. (US) - (J Infect Dis. 2013 Feb 15;207(4):612)
  - 1869 births – overall 18.6% preterm, with adj OR 1.55 for PTB if exposed to PI’s during 1st trimester, not assoc with later start of ARV

- Sibiude et al. (France) – (Clin Inf Dis 2012;54:1348)
  - 9.2% PTB increased to 14.3% by 2009 with use of combination ARV’s. Associated with boosted more than unboosted regimens

- Short et al. (UK) – HIV Medicine 2014;15:233
  - 13% PTB – assoc with short term cART compared to long term?
BC HIV surveillance data

MTC transmission rate
- 0/230

Preterm birth rate
- 18% (38/230) <37 weeks ga
- 4% (9/230) <34 weeks ga

**Background preterm birthrate in BC is 9.3%**

Mean Birthweight (g)
- Term Infants = 3204.6 ± 30.4
- Preterm Infants = 2361.9 ± 85.9
BC surveillance data – 1996-2013

- HAART Type and Preterm Birth

<table>
<thead>
<tr>
<th>Variable</th>
<th>HAART Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All N = 230</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>37 (16%)</td>
</tr>
</tbody>
</table>

No significant difference in proportion of preterm birth between HAART types
### BC Surveillance data – Infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Preterm</th>
<th>Term</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>STI in pregnancy</td>
<td>73</td>
<td>15 (41)</td>
<td>58 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Abnormal Nugent score (4-10)</td>
<td>54</td>
<td>14 (38)</td>
<td>40 (21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HCV-Ab positive</td>
<td>88</td>
<td>25 (68)</td>
<td>63 (33)</td>
<td>&lt;0.00015</td>
</tr>
</tbody>
</table>

Increased risk of PTB among women with Abnormal Nugent scores and HCV-Ab+ women
CONCLUSIONS of BC perinatal HIV surveillance

- HIV positive pregnant women on HAART are at increased risk of preterm birth versus background population
- We did not detect any differences in preterm birth rate between HAART regimen types
- In our population, substance use and sub-optimal HAART exposure are significantly associated with preterm birth
Summary

- Preterm birth rates are increasing with no viable approaches to prevention.
- Vaginal microbiome can both protect against adverse pregnancy outcome and can result in adverse pregnancy outcome.
- Metagenomics presents a novel new approach to understand the microbiome and the potential for new screening and prevention approaches.
New Opportunities

- PTB clearly associated with vaginal dysbiosis in most studies
- HIV associated with high rates of preterm birth
- Could understanding the role of the vaginal microbiome in HIV associated preterm birth permit new diagnostics and new prevention options?
The Vogue/Perinatal HIV research teams

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- Evelyn Maan

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- Greg Gloor

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**BCCDC**
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