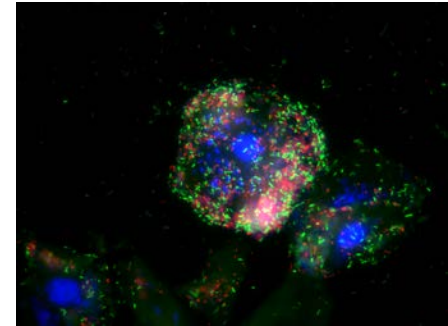
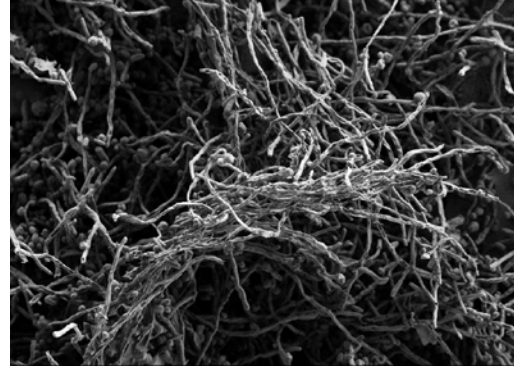


# The Vaginal Microbiome & HIV-1 Acquisition



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

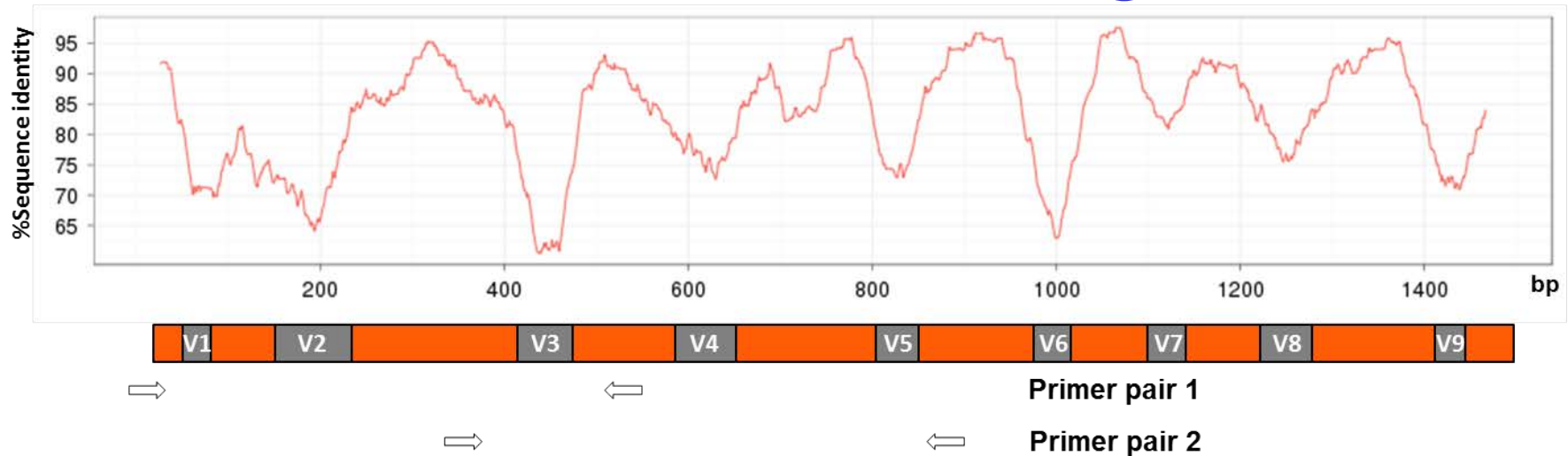
# Discussion

- 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

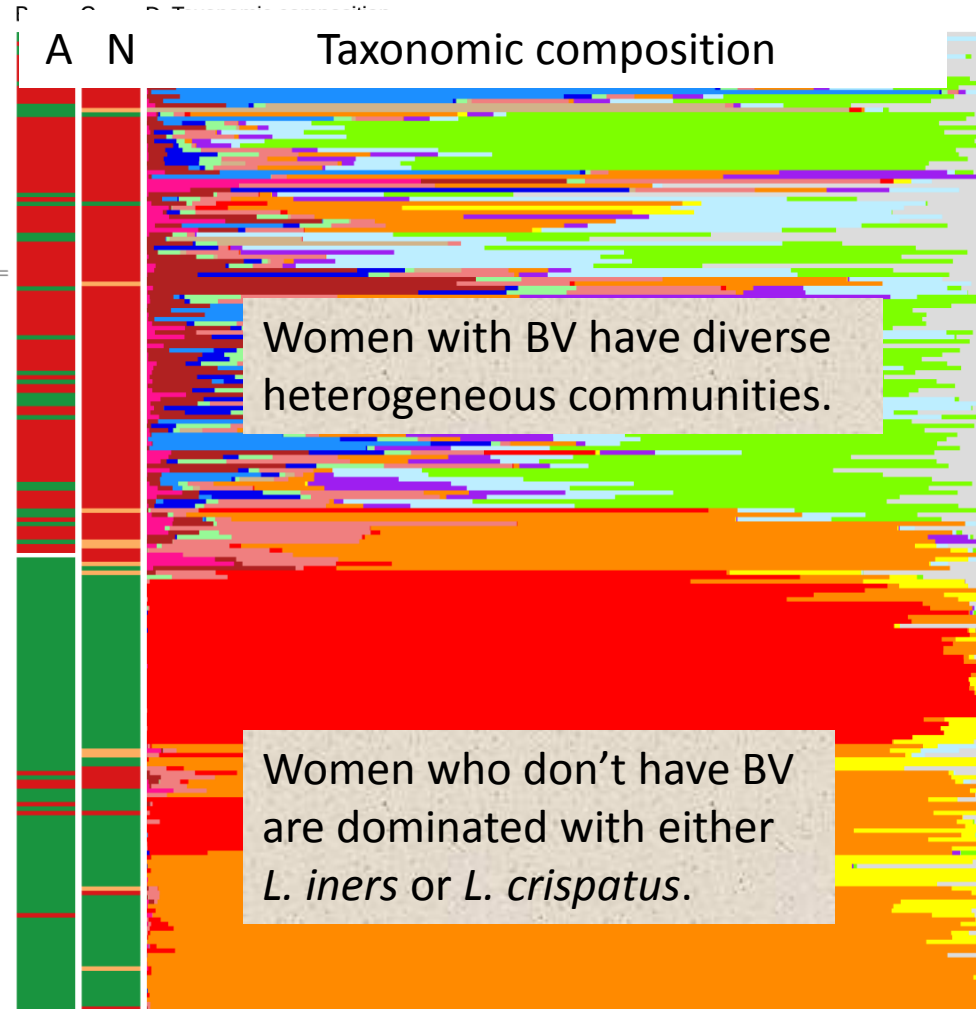
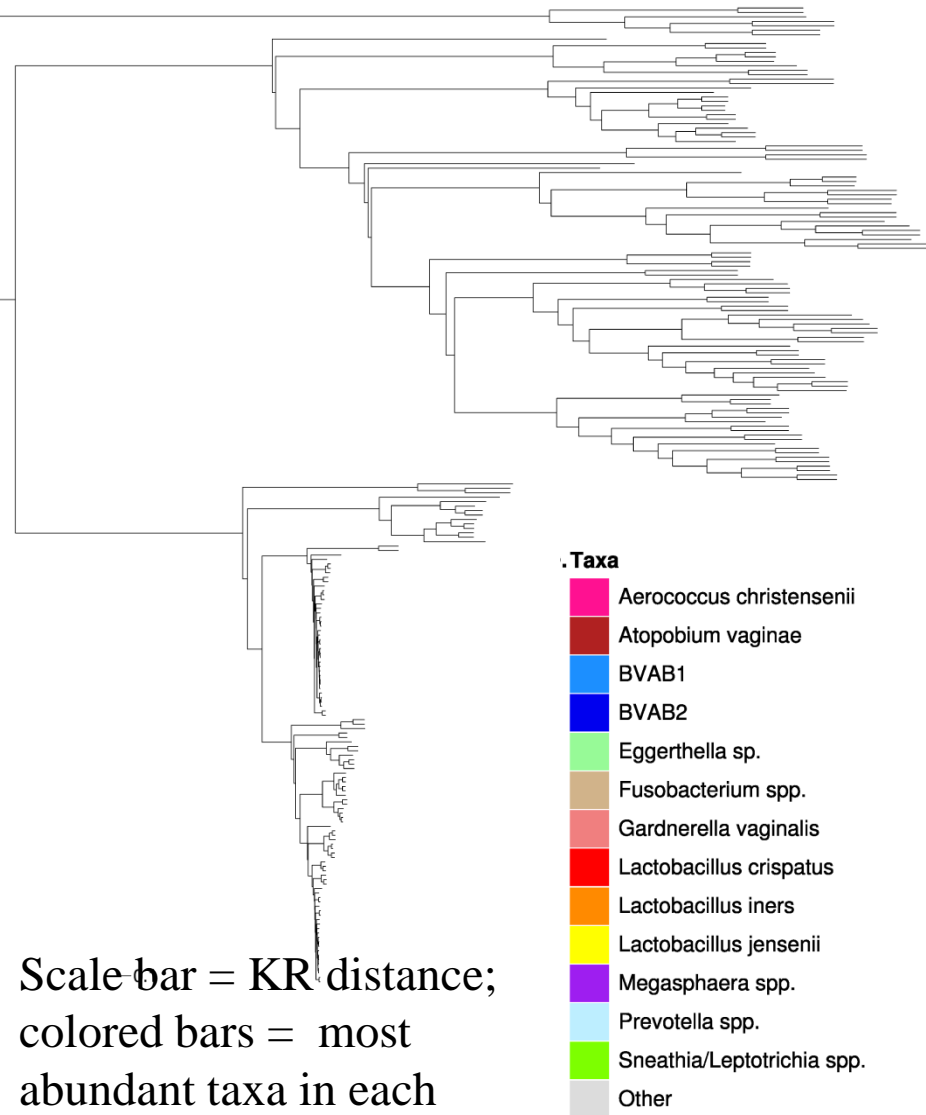
# The 16S rRNA gene



- **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**

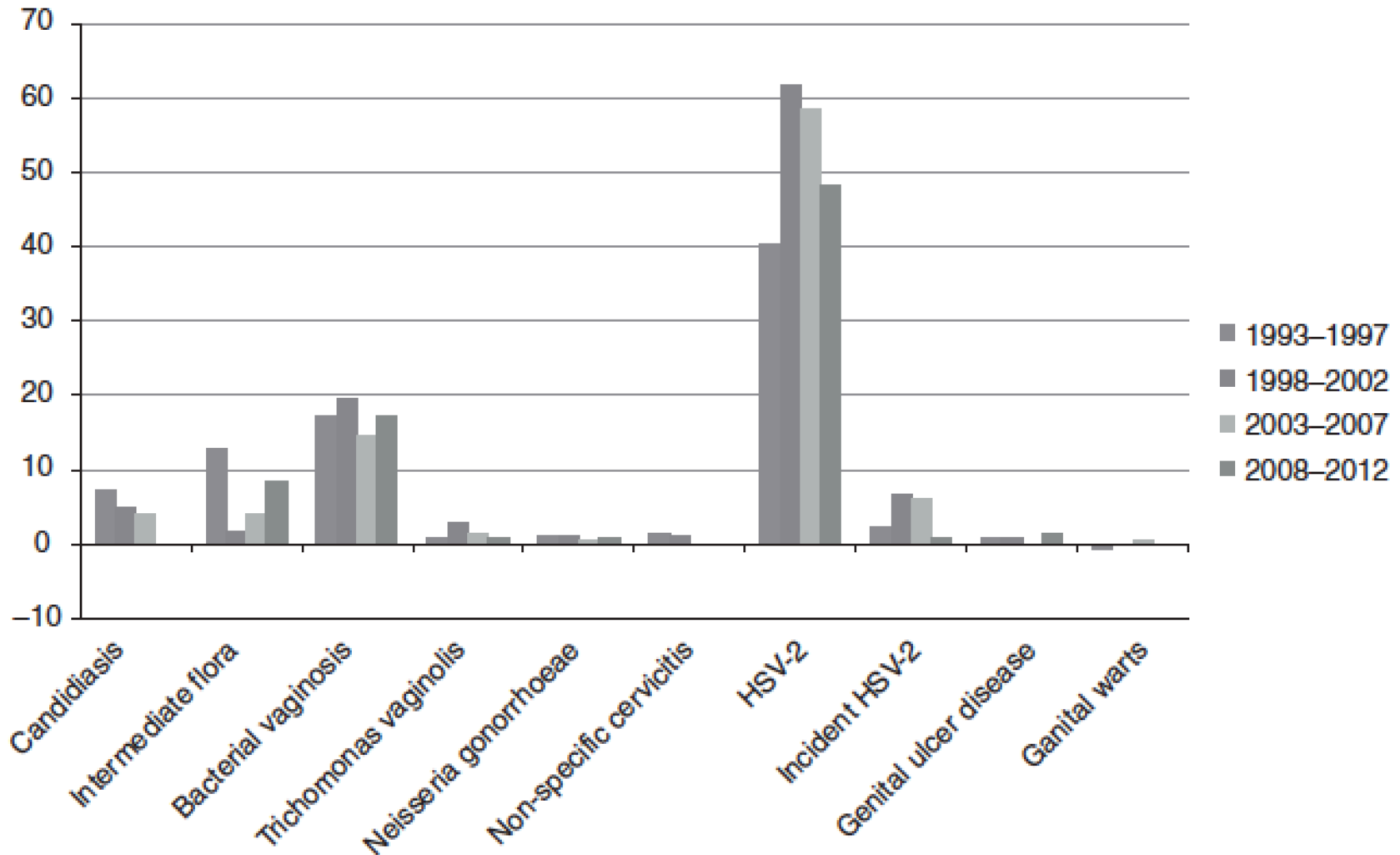
# Hierarchical Clustering of Vaginal Bacterial Communities with 16S rDNA PCR & Pyrosequencing

A. Hierarchical clustering of vaginal bacterial communities



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

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





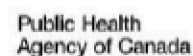
# Role of vaginal microbiota in genital inflammation and enhancing HIV transmission

**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

	HIV+	HIV-	Total
Genital inflammation present*	19	6	25
Genital inflammation absent	39	52	91
Total	58	58	116

**Odds Ratio**

**3.2 (95% CI: 1.3 – 7.9)**

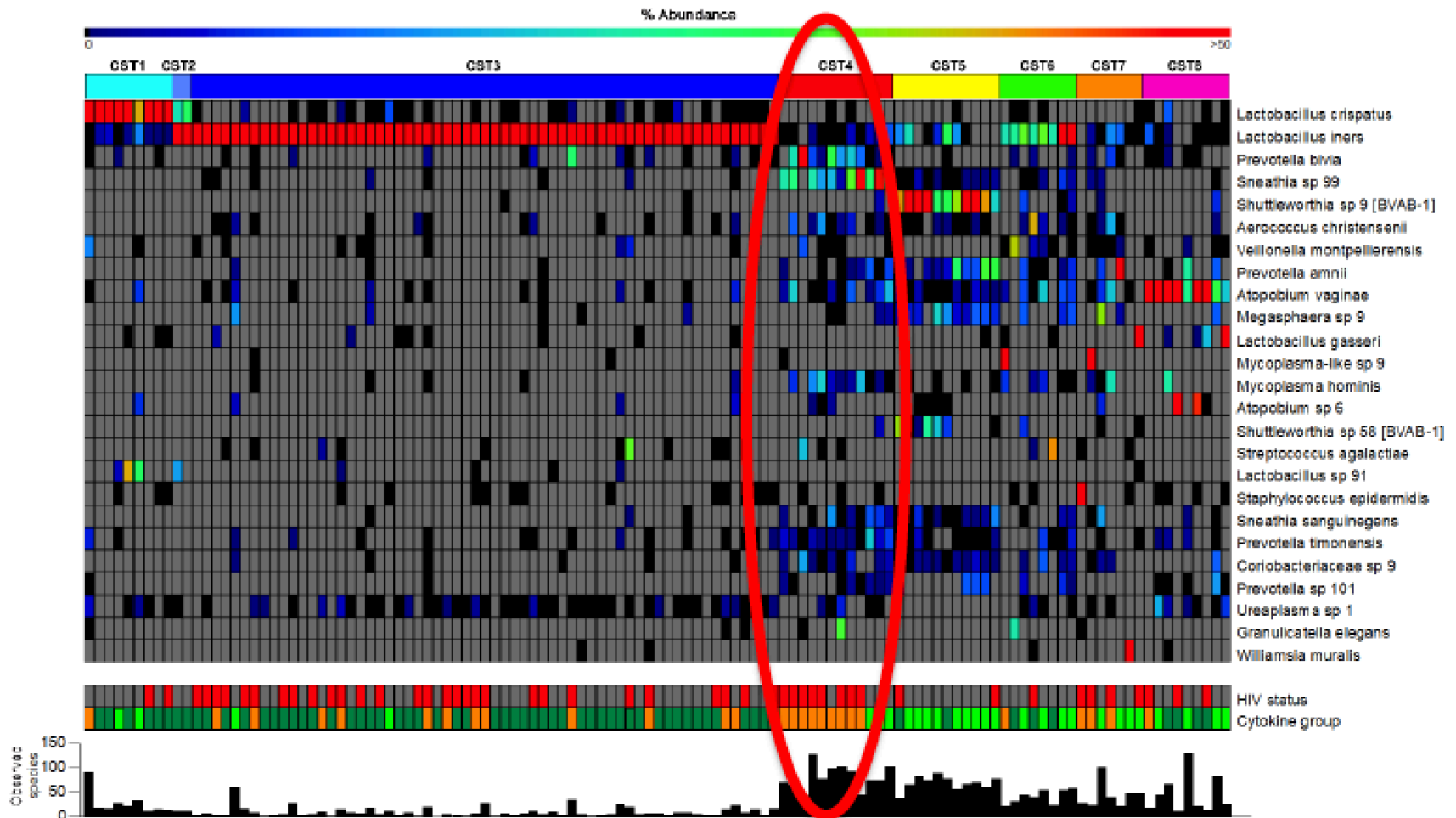
**p-value**

**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 75<sup>th</sup> 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**

	<b><i>P. bivia</i>+ OR*</b>	<b>P value</b>
HC	19.2 (95% CI: 4.0-92.4)	p<0.001
HIV+	12.7 (95% CI: 2.1-77.8)	p=0.006

*\*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

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*McClelland RS, Lingappa J, John-Stewart G, Kinuthia, Yuhus K, Jaoko W, Srinivasan S, Mandaliya K, Fiedler T, Munch M, Richardson BA, Overbaugh J, Fredricks DN*

Disclosure: RSM has a research grant from Hologic Corp, paid



# Vaginal bacteria associated with increased risk of HIV acquisition in African women

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

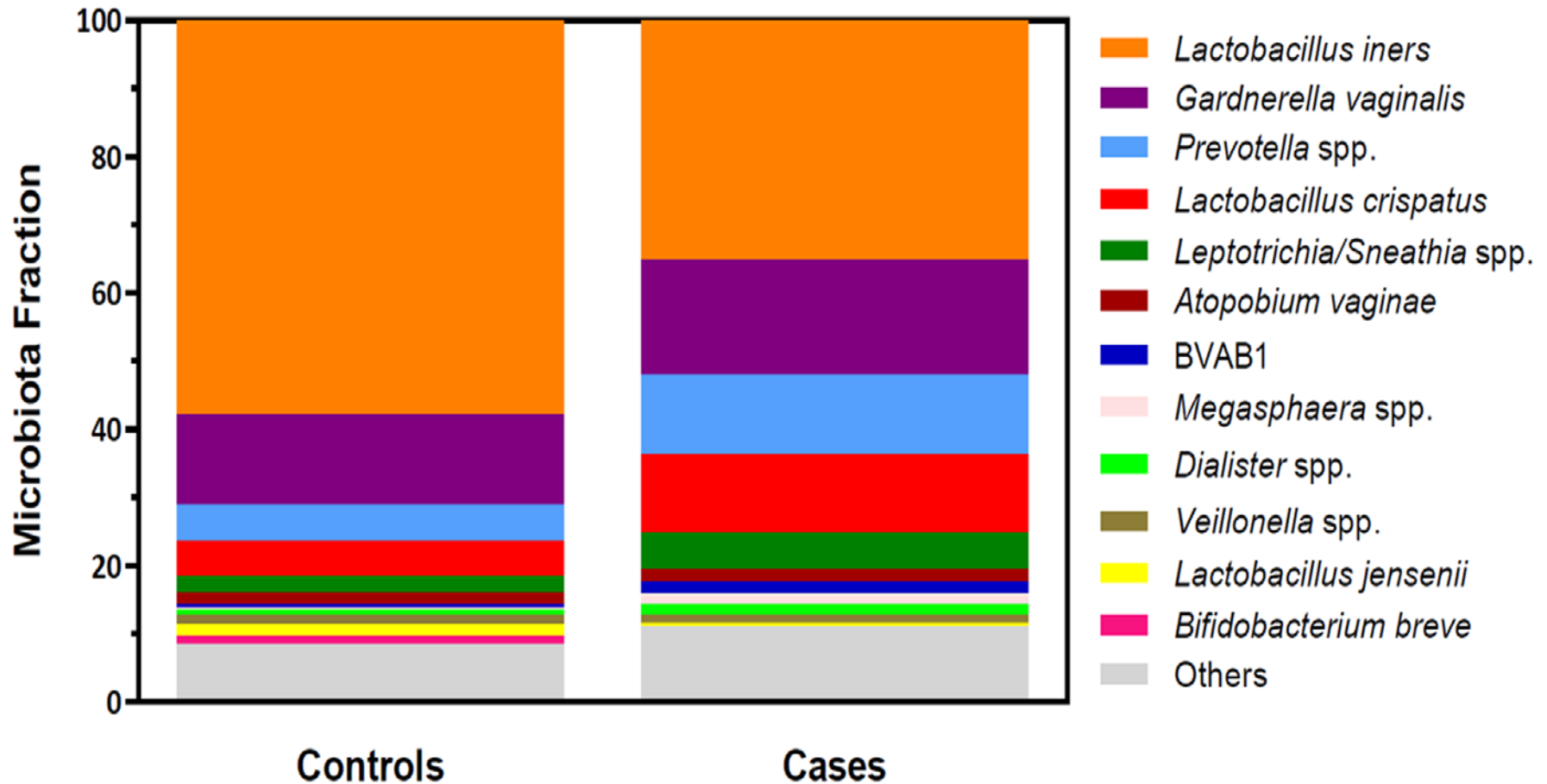
## Methods

- 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 Median (IQR) or N (%)

	Controls (N=262)	Cases (N=87)
Age	29 (23-36)	26 (22-30)
Married	199 (76%)	66 (76%)
DMPA	37 (14%)	18 (21%)
Pregnant	57 (22%)	20 (23%)
BV	67 (29%)	32 (42%)

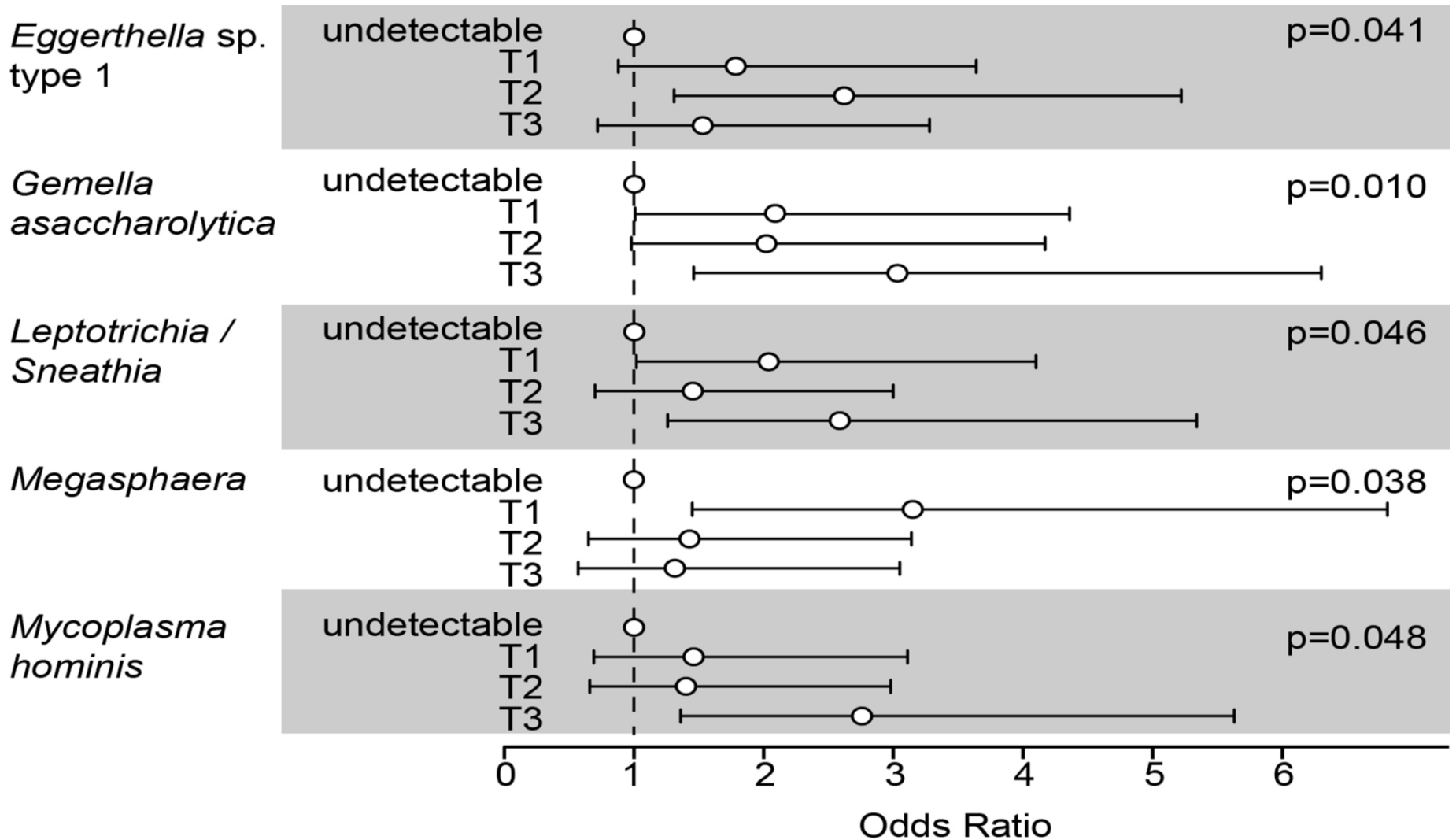
# Overall vaginal bacterial community diversity in 57 cases versus their 57 matched controls



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



N=87 cases + 262 controls; undetectable compared to 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> 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

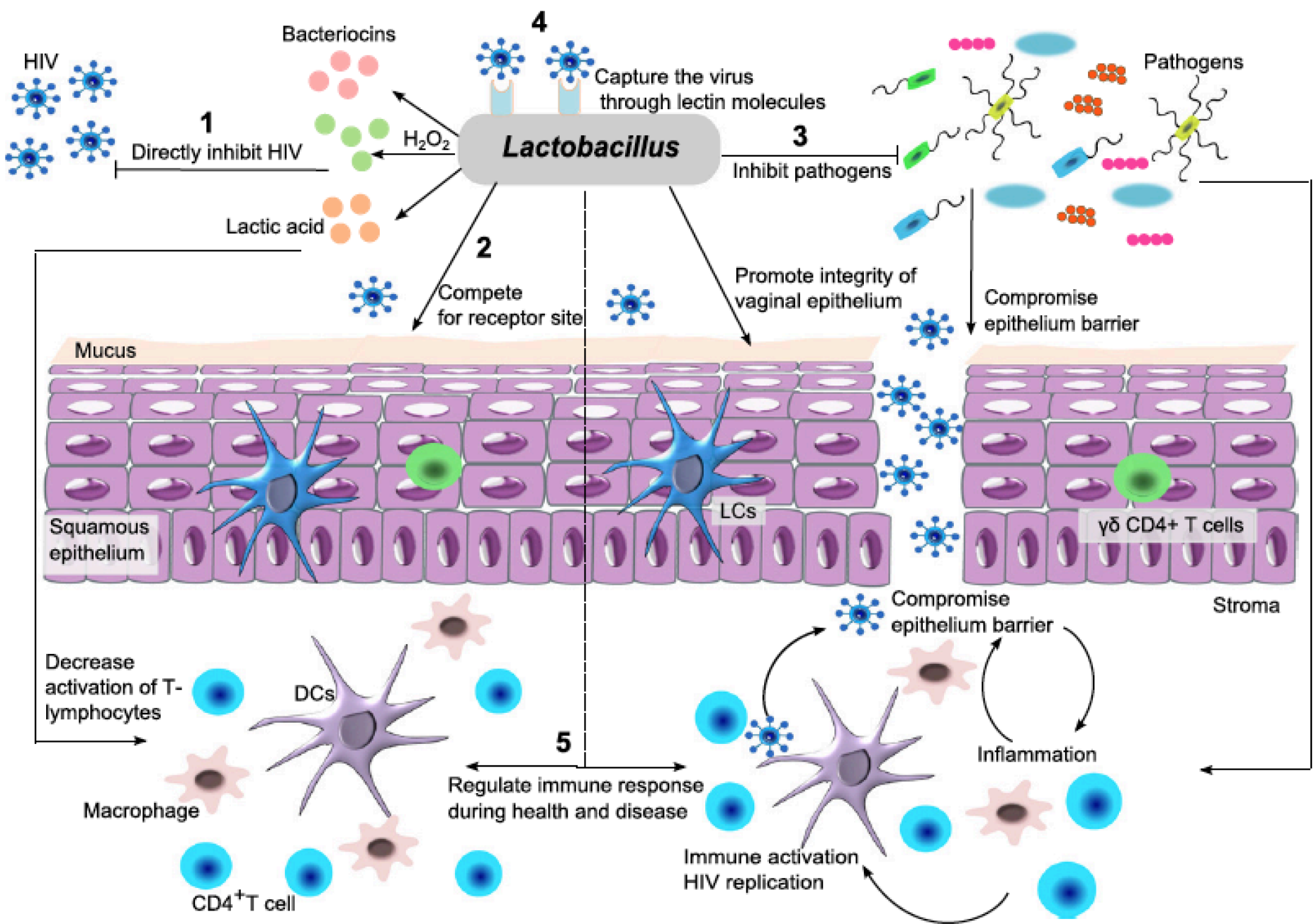


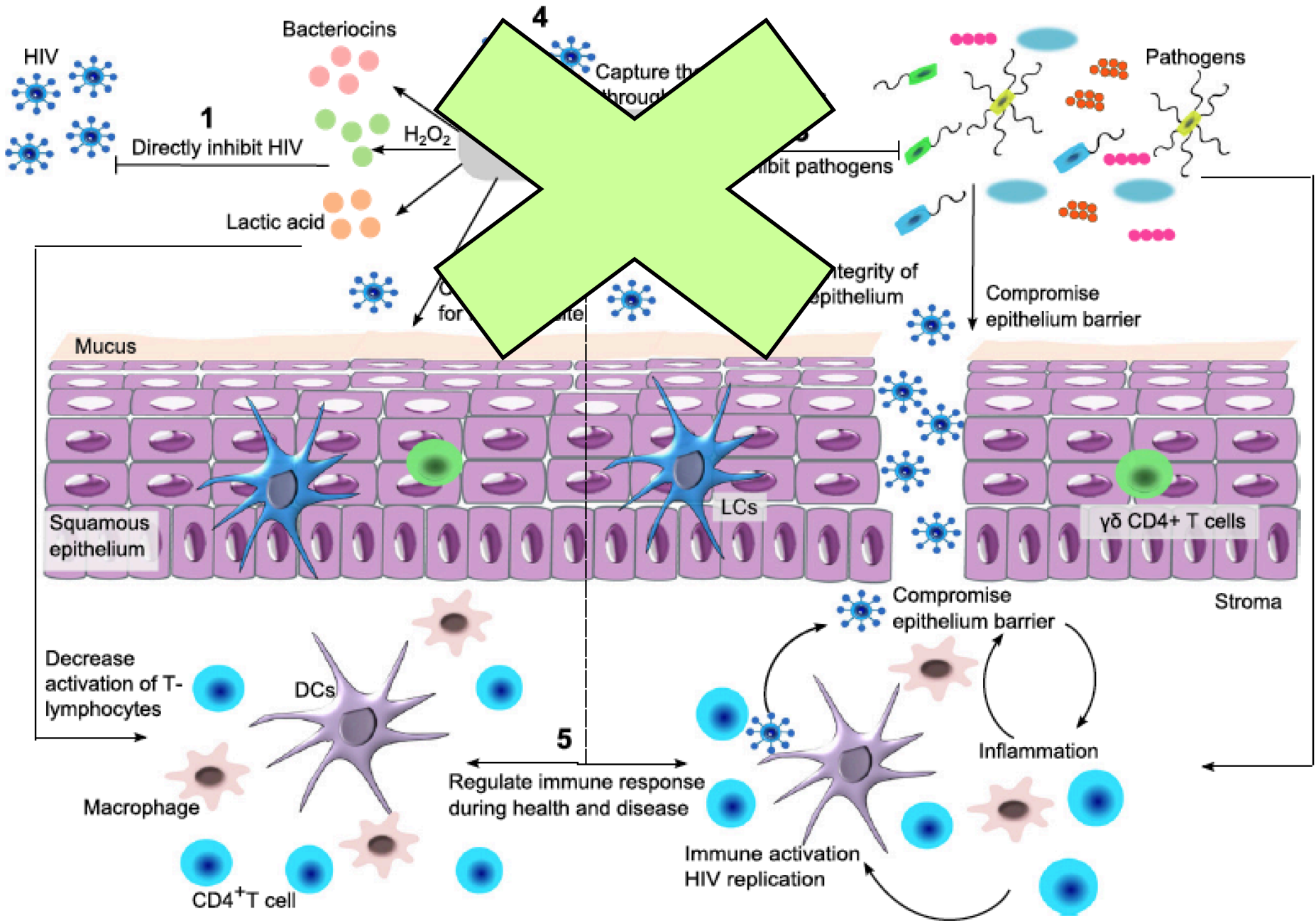
# Conclusions

- 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





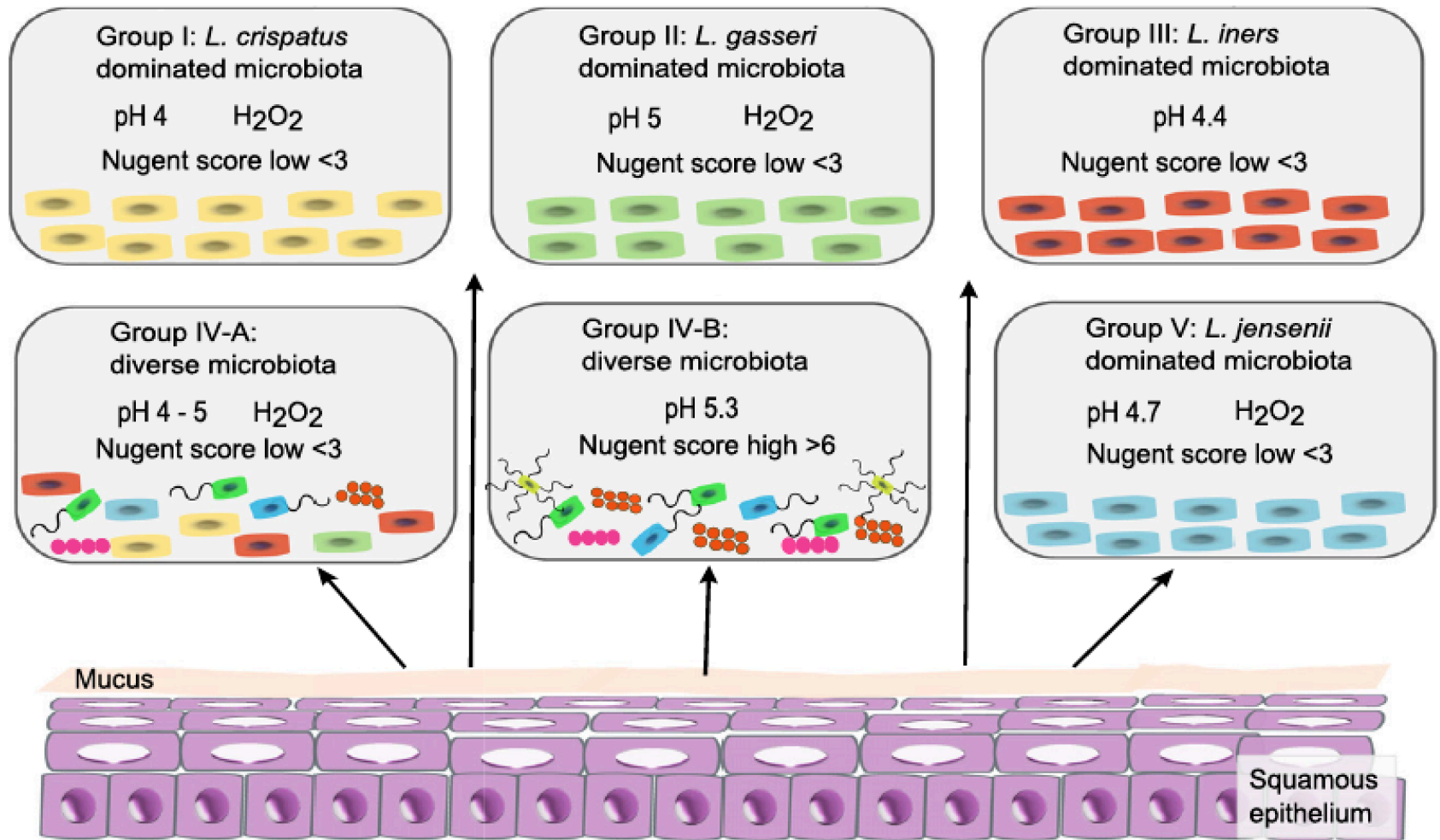


Figure from

# BV & Increased HIV Acquisition

- Loss of H<sub>2</sub>O<sub>2</sub> (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 Wachih, MChB,†  
Christopher Pettengell, BSc,\* Sherzana Sunderji, BSc,\* Sanja Huibner, BSc,\* T. Blake Ball, PhD,‡  
Francis A. Plummer, MD,‡ Walter Jaoko, PhD, MChB,† and Rupert Kaul, MD, PhD\*†§*

Vaginal Microbiota and Sexually Transmitted Infections That May Influence Transmission of Cell-Associated HIV

# 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; Mrazek 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

Condition	Bacteria	References
Cervicitis	<i>M. indolicus</i> <i>L. crispatus</i>	Gorgos 2015
Urethritis	<i>Sneathia spp</i>	Manhart 2013
Endometritis	<i>Sneathia spp</i> BVAB-1 <i>Atopobium vaginae</i>	Haggerty 2016

