

Behaviors Associated with Changes in The Vaginal Microbiome

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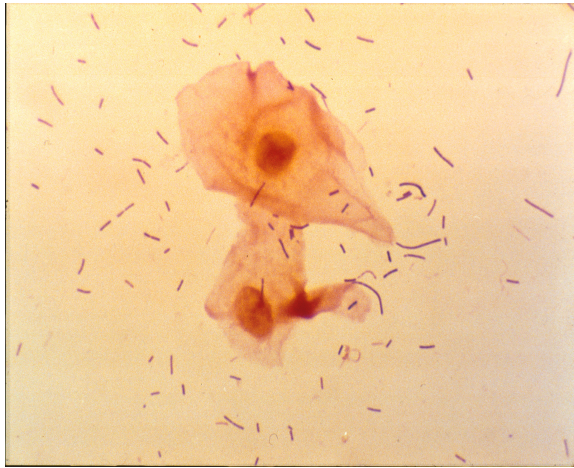
MTN Annual Meeting

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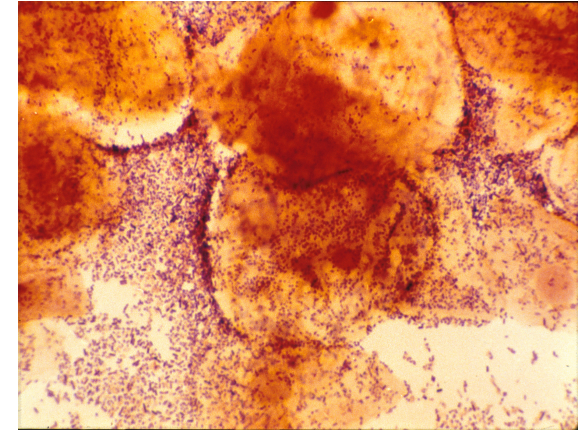
Discussion: The Healthy Vaginal Microbiome

- What defines it?
- What are the consequences of not having it?
- What disrupts it?
 - Unprotected sex
 - Relative estrogen deficiency
 - Blood (menses)
 - Hygiene practices

Spectrum of Vaginal Bacteria



pH < 4.7
Nugent = 0



pH ≥ 4.7
Nugent 7 -10
Amsel's +

- Traditional cultivation & determination of H₂O₂ production by *Lactobacilli*
- H₂O₂ - 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)

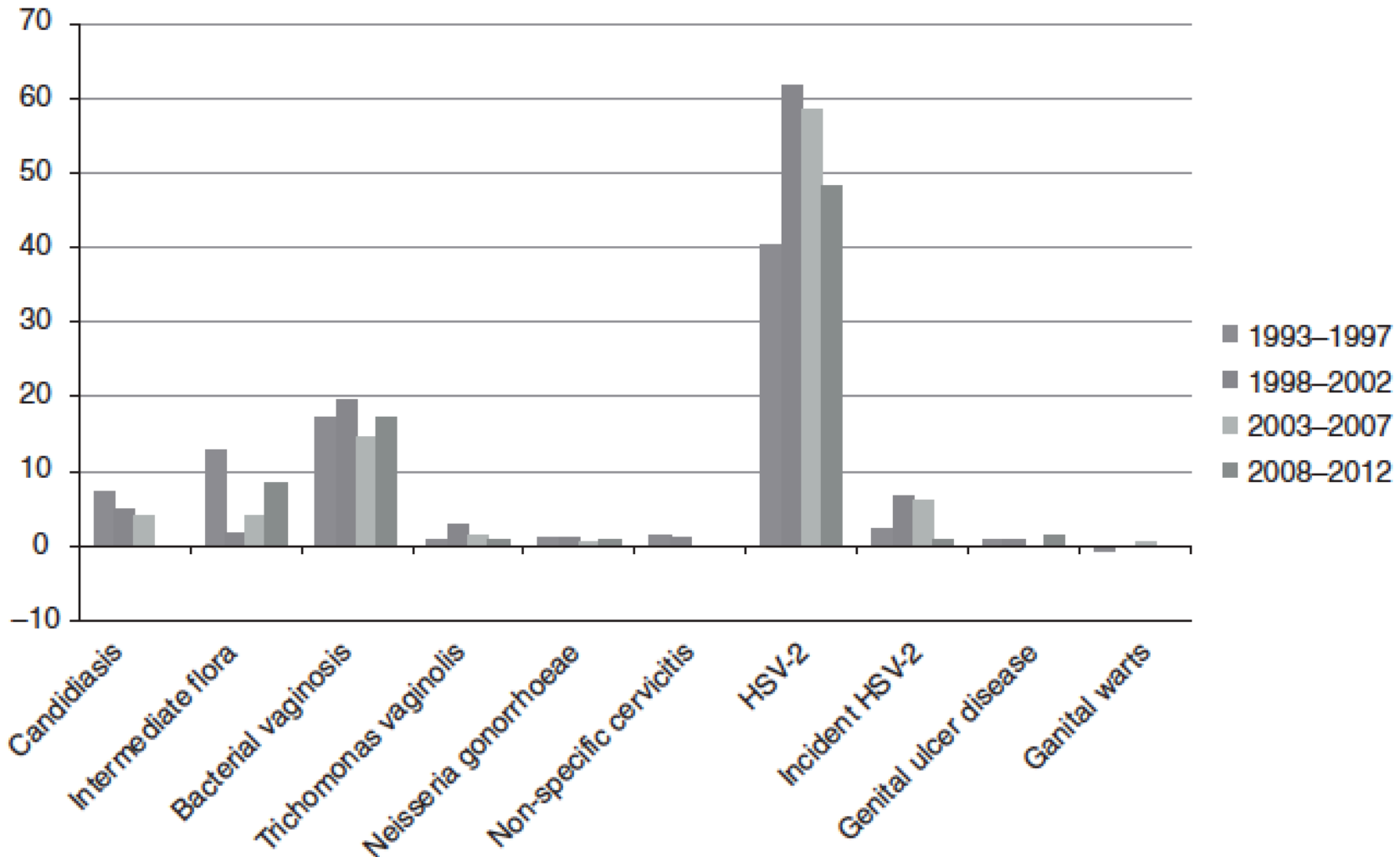
Benefits of an Optimal Vaginal Environment

Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

Maria G. Dominguez-Bello^{a,1,2}, Elizabeth K. Costello^{b,1,3}, Monica Contreras^c, Magda Magris^d, Glida Hidalgo^d, Noah Fierer^{e,f}, and Rob Knight^{b,9}

- 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

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



Role of Unprotected Sexual Activity in BV



- Well described in prospective studies
 - Condom use reduced recurrence in Peru (Sanchez 2004) and associated with normal flora over time (Schwebke 2005)
 - Women with circumcised partners had lower BV incidence (OR 0.60, 95% CI .38-.94) in subsequent year (Gray, AJOB 2008)
 - Circumcision effected change in penis microbiome: less anaerobic diversity (Price 2010) & inflammatory chemokines (Prodger 2017)
- However, sex isn't always simple...

Disruption of Optimal Vaginal Microbiome

Unprotected Sexual Activity

- Exposure site: vaginal, oral, anal sex
- Vaginal sex: re-exposure to male partner's microbiome (male genitalia as reservoir for BV-associated bacteria)
 - Exposure to other 'unfavorable factors' in partners' secretions (semen) or with sex
 - Alkaline pH of semen
 - Vaginal lubricants (Brotman 2010; MARRAZZO 2011)
- Anal sex (Bukusi 2006, Cherpes 2008, Fethers 2008)
- Oral sex and new partner with history of BV in cohort of WSW (MARRAZZO 2011; Vodstrcil 2014); women share unique LB strains through vaginal sex (MARRAZZO 2009)

BV: an STD?

Yes	No
<ul style="list-style-type: none">● Increasing no. sex partners● Concordance within female partners & related risk behaviors in WSW● Epidemiology concurrent with other STI● Rare in women with no/little sexual experience● BVAB detected in male genital tract● Lack of circumcision● Protective effect of condoms	<ul style="list-style-type: none">● Incident BV occurs in sexually experienced but abstinent women● Treatment of male partners has not improved BV-related outcomes<ul style="list-style-type: none">● Regimens may have been suboptimal

Disruption of Optimal Vaginal Microbiome

Hormonal Imbalance

- Estrogen promotes glycogen deposition into squamous epithelial cells that line the vagina
 - Supports growth of beneficial LB
- Incidence of BV was reduced by half in women who were using oral contraception vs. not (Bradshaw, CID 2012)
- Limited data to support vaginal contraceptive ring's positive effect on desirable lactobacilli (Veres 2004; Hardy R4P 2016)
- On balance, progesterone HC may effect possible reduction in BV; variable bleeding patterns of note

Changes in Vaginal Microbiota and Immune Mediators in HIV-1-Seronegative Kenyan Women Initiating Depot Medroxyprogesterone Acetate

Alison C. Roxby, MD, MSc,* David N. Fredricks, MD,*†‡ Katherine Odem-Davis, PhD,§||
 Kristjana Ásbjörnsdóttir, PhD,¶|| Linnet Masese, PhD,¶|| Tina L. Fiedler, BS,‡ Stephen De Rosa, MD,‡#
 Walter Jaoko, MBChB, MTM, PhD,** James N. Kiarie, MBChB, MMed, MPH,||¶†‡‡‡
 Julie Overbaugh, PhD,§§ and R. Scott McClelland, MD, MPH*||¶

- Monthly vaginal swabs over year before & after DMPA initiation in 15 women
- *G. vaginalis* declined, along with total bacterial load; no change in BV, pH, symptoms

TABLE 3. Baseline Levels and Estimates of Change Over Time in Inflammatory Mediator Levels After DMPA Initiation

	Baseline level, Median (IQR), log ₁₀ Scale*	Estimate (Slope Post-DMPA)†	95% CI	P	Adjusted P [#]
Log ₁₀ IL-6 (pg/mL)	1.1 (0.6–1.4)	−0.07	−0.12 to −0.01	0.03	0.08
Log ₁₀ IL-8 (pg/mL)	2.9 (2.5–3.1)	−0.06	−0.12 to −0.00	0.04	0.08
Log ₁₀ IL1- α (ng/mL)	2.6 (2.5–3)	−0.04	−0.07 to −0.02	<0.001	0.001
Log ₁₀ IP-10 (pg/mL)	1.7 (1.2–2.1)	0.01	−0.03 to 0.04	0.71	0.71
Detect RANTES (pg/mL)	—	1.07	0.85 to 1.35	0.56	0.67
Log ₁₀ SLPI (ng/mL)	2.2 (1.5–2.4)	−0.02	−0.06 to 0.02	0.35	0.53

*Adjusted using Simes method.

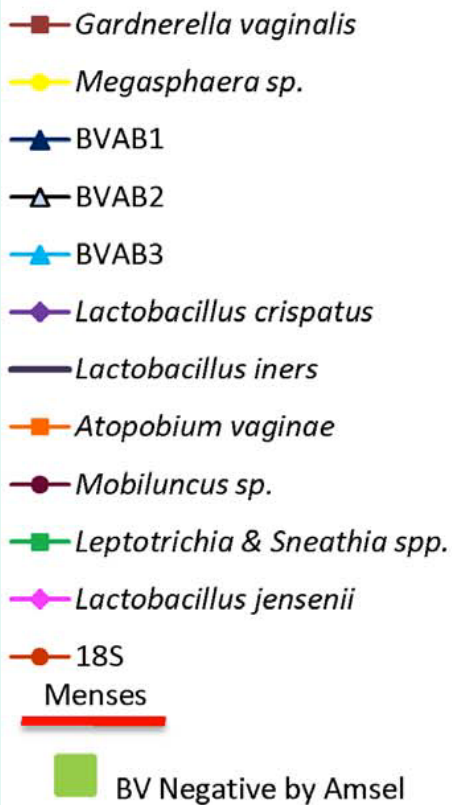
†Estimated multiplicative change per month in odds ratio of detection or arithmetic change per month in log₁₀ levels following estimated date of DMPA initiation, accounting for variability between subjects in slopes.

P values and estimates are by linear mixed effects models with linear splines pre- and post-DMPA initiation (random slopes post DMPA initiation).

Disruption of Optimal Vaginal Microbiome

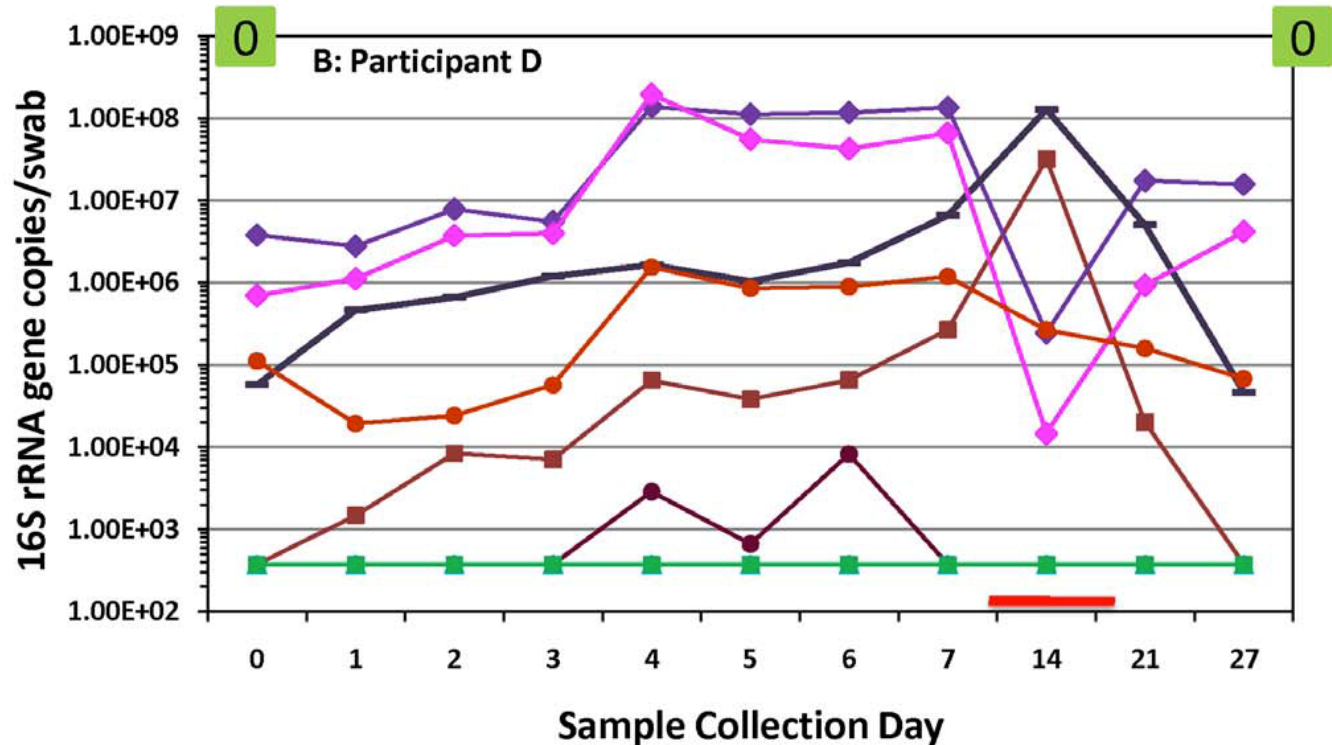
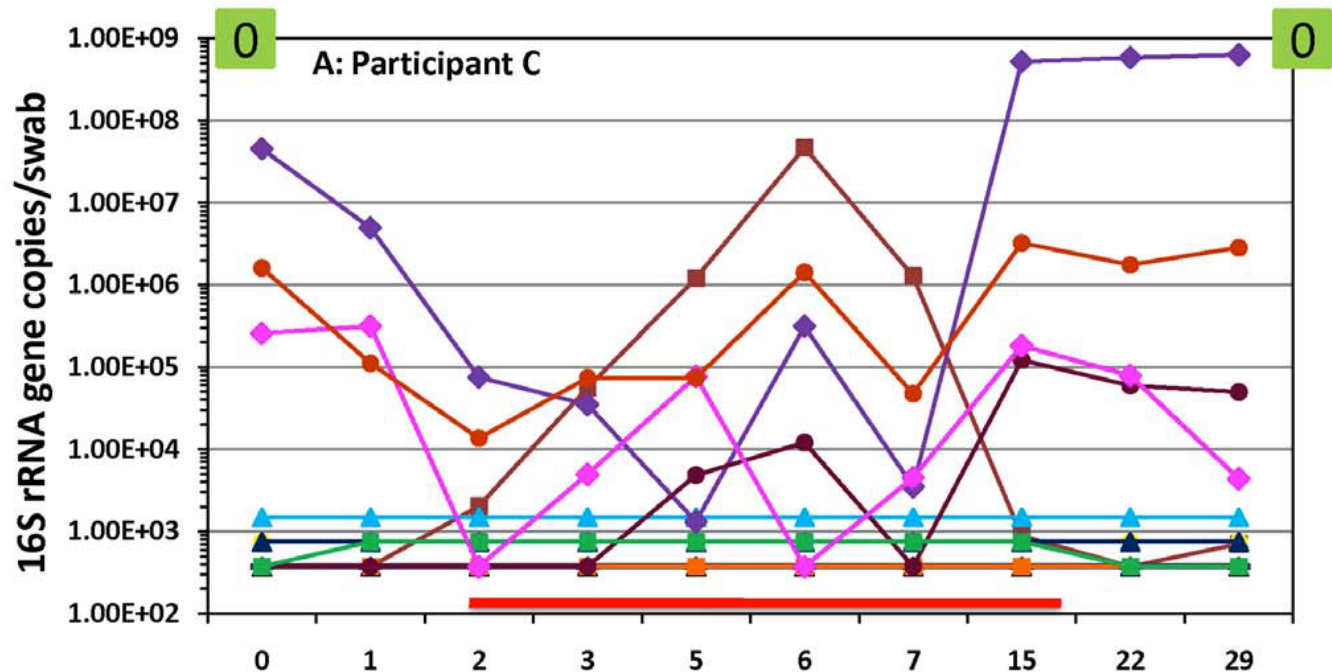
Effects of Menses

- Estrogen levels fall precipitously immediately prior to menses
- *Gardnerella vaginalis* thrives in heme-rich environment
- Daily sampling in the period leading up to menses has clarified the sequence of events, but mechanism still unclear



Effect of Menses

Two women



The Ring Plus Project

The Effect of a Contraceptive Vaginal Ring on the Vaginal Microbiota

Liselotte Hardy¹, Vicky Jespers¹, Jozefien Buyze¹, Irith De Baetselier¹, Stephen K Agaba², Vicky Cuylaerts¹, Lambert Mwamarangwe², Janneke van de Wijgert³, Tania Crucitti¹

¹Institute of Tropical Medicine, Belgium, ²Rinda Ubuzima, Rwanda, ³University of Liverpool, United Kingdom



HIV R4P 2016

- Assess impact on vaginal microbiome of CVR use, used continuously and cyclically, in women in Rwanda, and to assess biomass deposition on the CVR

qPCR (ref 1)

Lactobacillus species
Lactobacillus crispatus
Lactobacillus jensenii
Lactobacillus gasseri
Lactobacillus iners
Gardnerella vaginalis
Atopobium vaginae

Lactobacillus species
Gardnerella vaginalis
Atopobium vaginae

¹ Jespers V Obstet Gynecol 2016

Association of Sexual Debut in Adolescents
With Microbiota and Inflammatory Markers

Vicky Jespers, MD, PhD, Liselotte Hardy, MSc, Jozefien Buyze, MSc, PhD, Jasna Loos, MA, Anne Buvé, MD, PhD, and Tania Crucitti, PharmD, PhD

The Ring Plus Project

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Vaginal specimens

A high percentage (47.9%) of women was diagnosed with BV at baseline; over time the mean Nugent score decreased significantly. In the vaginal secretions we observed that over the course of the study the lactobacilli's presence and concentration increased significantly whereas the presence and concentration of Gardnerella vaginalis and the presence of Atopobium vaginae decreased significantly.

Table Longitudinal Analysis of Nugent score, Microbiota Presence and Concentration by Ring Regimen Use

Microbiota		Intermittent Use Odds Ratio or Estimate* (95%CI)	P values	Continuous Use Odds Ratio or Estimate* (95%CI)	P values
Nugent	score	-1.6 (-2.3, -0.8)	<.001	-1.9 (-2.7, -1.2)	<.001
Lactobacilli	presence	5.7 (1.2, 27.6)	.001	4.4 (1.1, 18.0)	.001
	concentration	0.8 (0.4, 1.1)	<.001	0.9 (0.5, 1.2)	<.001
<i>G. vaginalis</i>	presence	0.3 (0.1, 0.7)	.012	0.2 (0.1, 0.6)	.004
	concentration	-1.2 (-1.8, -0.6)	<.001	-1.3 (-1.9, -0.7)	<.001
<i>A. vaginae</i>	presence	0.2 (0.1, 0.5)	.001	0.2 (0.1, 0.6)	.001
Cell adherent <i>G. vaginalis</i>	presence	0.6 (0.4, 0.9)	.001	0.5 (0.3, 0.8)	.001
Molecular Composite qPCR**	score	2.1 (1.3, 2.8)	<.001	2.3 (1.6, 3.0)	<.001

Disruption of Optimal Vaginal Microbiome Intravaginal Practices

OPEN ACCESS Freely available online

PLoS MEDICINE

Intravaginal Practices, Bacterial Vaginosis, and HIV Infection in Women: Individual Participant Data Meta-analysis

Nicola Low^{1*}, Matthew F. Chersich^{2,3}, Kurt Schmidlin¹, Matthias Egger¹, Suzanna C. Francis⁴, Janneke H. H. M. van de Wijgert⁵, Richard J. Hayes⁴, Jared M. Baeten⁶, Joelle Brown^{4,7}, Sinead Delany-Moretlwe⁸, Rupert Kaul⁹, Nuala McGrath^{4,10}, Charles Morrison¹¹, Landon Myer^{12,13}, Marleen Temmerman³, Ariane van der Straten¹⁴, Deborah Watson-Jones⁴, Marcel Zwahlen¹, Adriane Martin Hilber¹

- Aimed to assess evidence in support of a causal link between practices, disruption of microbiome, and HIV acquisition

Disruption of Optimal Vaginal Microbiome

Intravaginal Practices

Box 1. Definitions of intravaginal practices used in this study.

Intravaginal Practice **Cleaning with water**

Definition

Cleaning inside the vagina, beyond the introitus, with water as the only product. Can be with or without specific mention of fingers, other materials, or douching devices to introduce water inside the vagina.

Cleaning with soap

Cleaning inside the vagina, beyond the introitus, with generic “soap” or “household soap,” or named proprietary bath soaps. Can be with or without specific mention of fingers, other materials, or douching devices to introduce soap lather inside the vagina.

Cleaning with other household products

Cleaning inside the vagina, beyond the introitus, with products that include: generic “household cleaners”; named proprietary products such as “Omo”; antiseptic solutions; vinegar; lemon juice. Can be with or without specific mention of fingers, other materials, or douching devices to introduce liquid inside the vagina.

Cloth to wipe out vagina or apply products

Use inside the vagina, beyond the introitus, of materials such as cloth, tissue, paper, cotton wool to wipe out vaginal secretions or to apply products. Includes specific practices described as “cleaning with cloth” without any other product and named products introduced with cloth or other material. Does not include use of medication, tampons, or removal of menstrual blood.

Insertion of products to dry or tighten vagina

Pushing or placing mostly nonliquid products inside the vagina (including powders, creams, herbs, tablets, sticks, stones, leaves, “traditional products”) regardless of the duration. Some questions ask specifically about the use of this practice before sexual intercourse. The intention is to achieve a sensation described as dry or tight.

Any (or no) current practice

Includes all positive (or negative) responses to general questions about the use of an intravaginal practice, or to specific questions about practices described above. Time period is that asked about at the baseline visit, usually past 1–3 mo.

Categories are not mutually exclusive. Definitions of intravaginal cleaning and insertion adapted from classification developed by the WHO Gender, Sexuality and Vaginal Practices Study Group (GSVP Study Group) [6]. Additional definitions based on specific questions used in individual studies.

Disruption of Optimal Vaginal Microbiome Intravaginal Practices

Table 4. Associations between intravaginal practices and disrupted vaginal flora in women with normal vaginal flora at baseline.

Intravaginal Practice ^a	Number in Model (Strata/Studies) ^b	Number Developing Disrupted Flora ^c	Disrupted Vaginal Flora ^b			
			Unadjusted OR (95% CI)	<i>p</i> -Value	Adjusted OR (95% CI) ^d	<i>p</i> -Value
Cleaning with soap and water	3,222 (8/7)	1,088	1.27 (1.07–1.50)	0.006	1.24 (1.04–1.47)	0.014
Cleaning with household products	2,045 (7/6)	641	0.95 (0.62–1.44)	0.797	0.89 (0.58–1.36)	0.576
Cloth to wipe out vagina or apply products	2,177 (5/4)	704	1.06 (0.85–1.32)	0.588	1.06 (0.85–1.33)	0.577
Insertion of products to dry or tighten vagina	2,264 (7/6)	735	1.26 (0.96–1.66)	0.099	1.29 (0.98–1.71)	0.072

OR from two-stage random effects meta-analysis based on ordered logistic regression.

^aBaseline category for intravaginal practices is no vaginal practice or use of water only. Intravaginal practices measured at baseline.

^bDisrupted vaginal flora as a three-level ordered categorical variable: normal vaginal flora defined as Nugent score 0–3, or Ison-Hay grade I; intermediate vaginal flora defined as Nugent score 4–6, or Ison-Hay grade II; BV defined as Nugent score 7–10, or Ison-Hay grade III. Excludes two studies that did not use Gram stain criteria [19,20].

^cNumber with normal flora at baseline who developed disrupted vaginal flora includes both women using and not using each intravaginal practice.

^dAdjusted for age, marital status, and reported number of sex partners in last 3 mo as reported at cohort entry.

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Disruption of Optimal Vaginal Microbiome

Intravaginal Practices

Table 5. Association between disrupted vaginal flora and HIV acquisition, stratified Cox regression.

Variable	Baseline Vaginal Flora Status (<i>n</i> =8,452) ^a			Vaginal Flora Status at Visit before HIV Seroconversion (<i>n</i> =8,626) ^a		
	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b	<i>p</i> -Value	Unadjusted HR (95% CI)*	Adjusted HR (95% CI) ^b	<i>p</i> -Value
Vaginal flora			<0.001			<0.001
Normal vaginal flora	1 (reference)	1 (reference)		1 (reference)	1 (reference)	
Intermediate vaginal flora	1.62 (1.27–2.08)	1.54 (1.20–1.97)		1.51 (1.19–1.91)	1.41 (1.12–1.79)	
BV	1.84 (1.48–2.28)	1.69 (1.36–2.10)		1.66 (1.35–2.05)	1.53 (1.24–1.89)	
HSV status at baseline						
Negative	1 (reference)			1 (reference)		
Positive	2.14 (1.70–2.70)	2.29 (1.80–2.90)	<0.001	2.14 (1.70–2.69)	2.31 (1.82–2.91)	<0.001
Age at cohort entry			<0.001			<0.001
>25 y	1.25 (1.04–1.50)	1.37 (1.13–1.65)		1.26 (1.05–1.52)	1.38 (1.14–1.66)	
25–34 y	1 (reference)	1 (reference)		1 (reference)	1 (reference)	
35 y or older	0.80 (0.56–1.15)	0.80 (0.56–1.15)		0.79 (0.55–1.13)	0.78 (0.54–1.12)	
Marital status			<0.001			<0.001
Currently married	1 (reference)	1 (reference)		1 (reference)	1 (reference)	
Currently unmarried	1.96 (1.46–2.64)	1.78 (1.32–2.40)		1.96 (1.46–2.62)	1.77 (1.31–2.38)	
Number of partners last 3 mo			0.034			0.023
No partner	0.97 (0.48–1.97)	0.94 (0.46–1.91)		0.96 (0.47–1.95)	0.90 (0.44–1.84)	
1 partner	1 (reference)	1 (reference)		1 (reference)	1 (reference)	
More than 1 partner	2.14 (1.47–3.12)	1.59 (1.09–2.31)		2.15 (1.48–3.13)	1.62 (1.11–2.35)	

^aIncluded in analysis are women with available vaginal flora status measured by Gram stain criteria: normal vaginal flora defined as Nugent score 0–3, or Ison-Hay grade I; intermediate vaginal flora defined as Nugent score 4–6, or Ison-Hay grade II; BV defined as Nugent score 7–10, or Ison-Hay grade III. Excludes two studies that did not use Gram stain criteria [19,20].

^bMultivariable model controls for all variables in the table.

Disruption of Optimal Vaginal Microbiome

Intravaginal Practices

- Probable link between some intravaginal practices and BV
- Strong link between BV and increased risk of HIV acquisition
- Causal pathway still to be determined

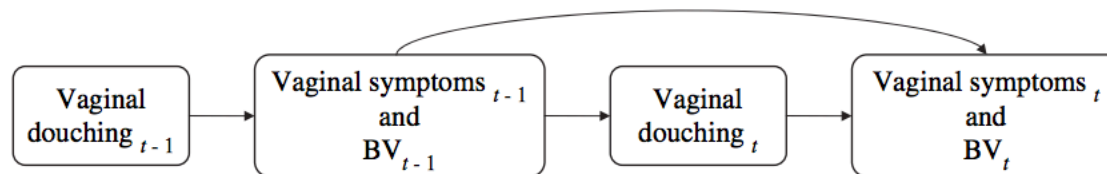


FIGURE 1. Vaginal symptoms and bacterial vaginosis (BV) represented as both confounding factors and intermediaries in relation to douching. t , time of current measurement; $t - 1$, time of previous measurement.

Summary

- Strong link between BV and increased risk of HIV acquisition
- Causal pathway still to be determined
- Many factors push the vaginal microbiome away from a state that is optimally composed to protect against HIV/STI and unfavorable reproductive outcomes
 - Many are modifiable, at least partially

Interventions, Tested or Theoretical

- Condoms
- Modification of vaginal hygiene practices
- Periodic presumptive therapy for BV (Balkus 2016) & genital herpes as a cause of chronic 'inflammation' (Johnston, UW STI CRC)
- Menstrual suppression (data pending)
- BETTER TREATMENT FOR BV

- Thank you!