

# Incubation Period and Risk Factors Support Sexual Transmission of Bacterial Vaginosis in Women Who Have Sex with Women

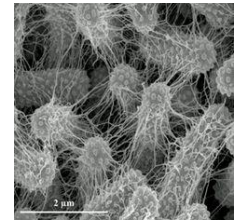
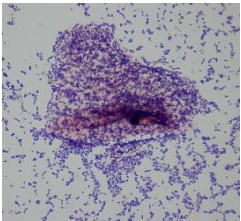
**Christina A. Muzny, MD, MSPH<sup>1</sup>, Shelly Lensing, MS<sup>2</sup>,  
Kristal J. Aaron, DrPH, MSPH<sup>1</sup>, Jane R. Schwebke, MD<sup>1</sup>**

<sup>1</sup>Division of Infectious Diseases, University of Alabama at Birmingham

<sup>2</sup>Department of Biostatistics, University of Arkansas for Medical Sciences

IUSTI World and European Congress 2018

**June 28, 2018**



# Disclosures

- R01AI097080 (NIAID) – *T. vaginalis* treatment trial
- Consultant for Lupin Pharmaceuticals

# Bacterial Vaginosis

- Most common vaginal cause of vaginal discharge in women<sup>1</sup>
  - U.S. prevalence 30% using NHANES data<sup>1</sup>
- Associated with preterm birth, low birth weight, post-operative gyn infections, and increased risk for acquisition and transmission of HIV and STIs<sup>2-3</sup>
- Characterized by depletion of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli and increases in facultative (*Gardnerella vaginalis*) and strict anaerobes (*Prevotella bivia*, *Atopobium vaginae*, BVAB1-3, *Megasphaera type I*, etc.)
  - However, etiology and incubation period remains unknown
- **Epidemiology strongly supports that BV is acquired via sexual transmission<sup>2,3</sup>**

# BV in Women Who Have Sex with Women (WSW)

- BV very common among WSW (**22%-56%**), more so than in women who have sex with men (WSM) (**23%-40%**)<sup>1-3</sup>
- Prevalent BV in WSW associated with greater numbers of female sexual partners, shared use of vaginally inserted sex toys, and oral-anal sex<sup>2</sup>
- Incident BV in WSW associated with exposure to a new sexual partner, a partner with BV symptoms, receptive oral sex, and onset of BV symptoms<sup>4</sup>
- High level of concordance of BV found in women and their female sexual partners, supportive of sexual transmission<sup>5</sup>

# Study Objective

- To examine incubation period and risk factors for incident BV in a prospective vaginal microbiome study of WSW presenting to the Jefferson County Health Department STD clinic in Birmingham, AL, USA



# Methods: WSW Recruitment Strategies

- Study recruiters posted flyers and provided word-of-mouth around Birmingham metro area
- Study advertisements: UAB campus/local newspapers, local radio stations
- Walk-ins to the JCDH STD clinic
- Participation in local LGBT events
  - Community DISH events
  - Birmingham Funk Fest
  - Annual Central Alabama Pride Festivals
- Alliances with local non-profit organizations that assist sexual minority women
  - Magic City Acceptance Project
  - Project HOPE
  - Housing Authority of the Birmingham District



**HOUSING AUTHORITY**  
BIRMINGHAM DISTRICT

**UAB** THE UNIVERSITY OF  
ALABAMA AT BIRMINGHAM

Knowledge that will change your world

# Methods: Screening

- Screening inclusion criteria: Female, ages 18-45, history of sex (oral, vaginal, and/or anal) with a female partner during past year, current female sexual partner
- Screening exclusion criteria: Use of antimicrobials within the past 14 days, known HIV, pregnancy, currently on menstrual period
- Urine pregnancy test performed
- Sexual history and self-collected vaginal swabs obtained for Amsel criteria<sup>1</sup> and Nugent score<sup>2</sup> (interpreted by a research clinician in the STD clinic)

# Methods: Enrollment

- Enrollment inclusion criteria: no Amsel criteria, Nugent score 0-3 with no *G. vaginalis*
- Enrollment exclusion criteria: pregnancy, trichomoniasis on wet mount, symptomatic vaginal yeast infection
- Study questionnaire completed and pelvic exam performed with specimens obtained for gonorrhea, chlamydia, and trichomonas NAAT
- Women practiced completion of day 1 of a daily diary and 2 self-collected vaginal swabs for Nugent score and future vaginal microbiota sequencing
- Exclusion after initial enrollment: duplicate baseline Nugent score >3 (read by research lab), STI diagnosis (+ gonorrhea, chlamydia, trichomonas NAAT)
- Daily self-collected vaginal swabs and diaries were obtained until development of incident BV (Nugent score 7-10 on at least 2-3 consecutive days) or for 90 days (participants dropped off and picked up new supplies weekly at the study site)



STUDYNO:

**K23 Vaginal Flora  
 Daily Study Diary**

Date Form Completed:

/   / 2 0 1

Form not completed:

**PLEASE MARK ACTIVITIES IN THE PAST 24 HOURS**

No sexual activity today *SKIP to Daily Check List*  
**Partner gender(s):**  M  F  
**Partner Type:**  Regular  Occasional  New  
**Race of Partner:**  African-American  Caucasian  Other

	Y N		Who performed? P Me	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mouth/tongue on vagina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mouth/tongue on anus	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Finger or hand on vagina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Finger or hand inside vagina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Finger or hand inside anus	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Shared sex toy	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Direct genital contact (clit to clit)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vaginal lubrication	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dental dams	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
			<b>Y N</b>	
Penile-Vaginal Sex			<input type="radio"/>	<input type="radio"/>
Multiple female partners at the same time			<input type="radio"/>	<input type="radio"/>
Multiple male partners at the same time			<input type="radio"/>	<input type="radio"/>
Sex during period (either)			<input type="radio"/>	<input type="radio"/>
<b>Daily check list:</b>			<input type="radio"/>	<input type="radio"/>
Douched			<input type="radio"/>	<input type="radio"/>
Vaginal odor			<input type="radio"/>	<input type="radio"/>
Vaginal irritation			<input type="radio"/>	<input type="radio"/>
Vaginal itching			<input type="radio"/>	<input type="radio"/>
Vaginal discharge			<input type="radio"/>	<input type="radio"/>
Having menstrual period			<input type="radio"/>	<input type="radio"/>
Oral medication			<input type="radio"/>	<input type="radio"/>
Intra-vaginal medication			<input type="radio"/>	<input type="radio"/>
<b>Daily check list:</b>			<b>Y N</b>	
2 Swabs			<input type="radio"/>	<input type="radio"/>
1 slide			<input type="radio"/>	<input type="radio"/>
Collection time:	<input type="text"/>	:	<input type="text"/>	<input type="radio"/> AM <input type="radio"/> PM

Place sticker here

Where?  Vagina  Anus  
 Tow washed between uses?  Yes  No  
 Condom used on toy?  Yes  No

**TO BE COMPLETED BY NURSE**

Mon  Fri  
 Tue  Sat  
 Wed  Sun  
 Thurs

Clinician initials:

# Methods – Statistical Analysis

- Kaplan-Meier estimates were computed for time to incident BV from study entry
- Accounting for differing lengths of follow-up, rates of sexual activities and douching (per 100 person-days) were compared according to incident BV status using Poisson calculations
- Days with menstruation were analyzed as a time-varying covariate in a Cox proportional hazard model
- Analyses performed using SAS 9.4

# Results

- Study enrollment occurred from October 2014 – March 2018
- **204 WSW** screened; **162/204** (79.4%) were screen failures; **42 (20.6%)** women enrolled

Reason for Screen Failure	N (%)
<b>Baseline Nugent score &gt;3</b>	105 (64%)
<b>No current female sexual partner</b>	13 (8%)
<b>In a hurry</b>	11 (7%)
<b>Recent antibiotic use</b>	5 (3%)
<b>Menses</b>	5 (3%)
<b>Trichomoniasis on wet mount</b>	4 (2%)
<b>Symptomatic vaginal yeast infection</b>	2 (2%)
<b>Unable to make weekly visits</b>	2 (2%)
<b>HIV-positive</b>	2 (2%)
<b>Miscellaneous reasons</b>	13 (8%)

- Of 42 enrolled, 2 later withdrawn due to enrollment Nugent score >3 at second read, 1 withdrawn due to *G. vaginalis* morphotypes on enrollment Gram stain at second read, and 3 withdrawn due to positive CT (n=2) or TV (n=1) NAAT at enrollment → **36 participants**

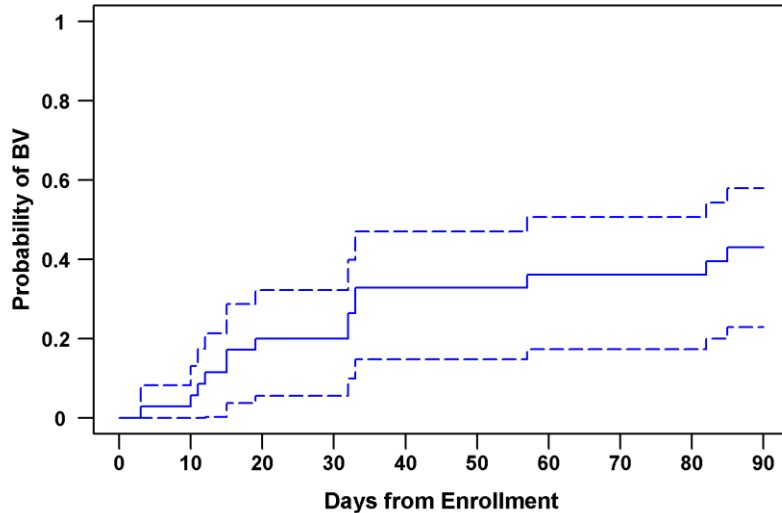
# Results

**Table 1. Characteristics of Women Who Have Sex with Women Participating in a BV Pathogenesis Study in Birmingham, AL (n=36)\***

Characteristic	Incident BV n=14	No Incident BV N=22	Total n=36	p-value <sup>€</sup>
Age, years				0.571
Mean ± SD	29 ± 8	31 ± 8	30 ± 8	
Range	19-44	20-48	19-48	
African American Race	13 (93)	20 (91)	33 (92)	0.999
Education				0.703
Some high school	1 (7)	3 (14)	4 (11)	
High school / GED	5 (36)	9 (41)	14 (39)	
Some college / Degree	8 (57)	9 (41)	17 (47)	
Sexual Orientation				>0.999
Homosexual	9 (64)	13 (59)	22 (61)	
Bisexual	5 (36)	9 (41)	14 (39)	
Sexual Behavior History				0.478
Women only	9 (69)	11 (52)	20 (59)	
Women and Men	4 (31)	10 (48)	14 (41)	
Smoked 30+ Packs in Lifetime	9 (64)	9 (41)	18 (50)	0.305
History of STIs <sup>§</sup>	13 (93)	17 (77)	30 (83)	0.371
Trichomoniasis	8 (57)	9 (41)	17 (47)	0.500
Chlamydia	6 (43)	6 (27)	12 (33)	0.472
Gonorrhea	3 (21)	3 (14)	6 (17)	0.658
History of BV	3 (21)	9 (41)	12 (33)	0.293
Current Contraception Use	1 (7)	4 (18)	5 (14)	0.628

\*Data presented as n (column %) unless otherwise specified; numbers in subgroups may not equal sums in columns because of missing data on selected variables; <sup>€</sup>T-test (age) or Fisher's exact test; <sup>§</sup>No woman had a history of syphilis, genital herpes, genital warts, HIV, or pelvic inflammatory disease; STIs=sexually transmitted infections; BV=bacterial vaginosis

# Time to Incident BV



At **10 days**, the probability of incident BV was **5.7%** (95% CI, 1.5-21.0%)

At **30 days**, the probability was **20.0%** (95% CI, 10.1-37.4%)

At **60 days**, the probability was **36.2%** (95% CI, 22.3-55.0%)

At **90 days**, 14/36 women (**39%**) developed incident BV

# Results

Table 2. Rate of Sexual Activities and Douching per 100 Days of Follow-Up (95% CI)

Sexual Activity or Symptoms	Incident BV <sup>€</sup> n=14	No Incident BV <sup>€</sup> N=22	Relative Incidence	p-value <sup>*</sup>
Any sexual activity	19.2 (15.4-23.8)	14.9 (13.1-16.9)	1.29 (1.00-1.66)	0.048
Sex with a woman	13.8 (10.7-17.8)	10.4 (8.9-12.1)	1.32 (0.98-1.78)	0.063
Sex with a man	3.0 (1.8-5.2)	3.4 (2.6-4.4)	0.89 (0.49-1.63)	0.709
Sex with a new female partner	0.2 (0.1-0.7)	0	-	-
Sex with a new male partner	0.7 (0.2-2.2)	0.4 (0.2-0.8)	1.89 (0.47-7.54)	0.370
Receptive oral sex	7.5 (5.3 (10.6)	5.9 (4.9-7.3)	1.26 (0.84-1.88)	0.263
Direct genital-genital contact	3.5 (2.1-5.8)	5.5 (4.5-6.8)	0.64 (0.37-1.10)	0.104
Sex Toy Use	3.3 (1.9-5.5)	3.1 (2.3-4.1)	1.06 (0.58-1.91)	0.857
Inconsistent dental dam use	13.3 (10.3-17.3)	11.5 (10.0-13.3)	1.16 (0.86-1.55)	0.339
Inconsistent condom use	1.4 (0.6-3.1)	1.3 (0.8-2.0)	1.08 (0.43-2.67)	0.872
Receptive digital-vaginal sex	13.1 (10.1-17.0)	9.5 (8.1-11.2)	1.37 (1.01-1.86)	0.043
Receptive digital-anal sex	3.7 (2.3-6.1)	0.6 (0.3-1.1)	6.7 (3.00-15.20)	<0.001
Finger or hand on vagina	15.7 (12.3-19.9)	12.6 (11.0-14.5)	1.24 (0.94-1.63)	0.129
Douching	0	0.2 (0.1-0.7)	-	-

\*p-values calculated from a Poisson model; <sup>€</sup>There were 428 days of follow-up in the incident BV group and 1614 days of follow-up in the no incident BV group.

# Results

- The rate of incident BV was 3.5 times higher (HR=3.5, 95% CI: 1.2-10.6, p=0.024) for women who had menses in the prior 2 days vs. those who did not
- Sexual activity of any kind occurred in 93% of WSW with incident BV at a median of 4 days (95% CI, 2-6) prior to incident BV
- The mean relative abundance of *P. bivia*, *G. vaginalis*, *A. vaginae*, and *Megasphaera* type I became significantly higher in women with incident BV 4 days before (*P. bivia*), 3 days before (*G. vaginalis*), and on the day of (*A. vaginae* and *Megasphaera* type I) incident BV, respectively (data not shown)<sup>1</sup>
- The mean relative abundance of other common BV-associated bacteria was not significantly different between groups prior to incident BV (data not shown)<sup>1</sup>

# Limitations

- Small sample size
- Survey questionnaire and daily diaries were based on self-report and subject to social-desirability and recall bias
- Despite the use of experienced Nugent score readers in this study (CAM and JRS), Nugent score sensitivity can be as low as 65%<sup>1</sup>, and some cases of incident BV may have been missed



# Conclusions

- Incident BV was common in this sample (39%) and associated with sexual activity and onset of menses
  - Menses known to promote the temporary expansion of BV-associated bacteria present in low levels<sup>1</sup>
    - Heme favors the growth of proteolytic organisms, including *G. vaginalis*<sup>2</sup>
- If BV is sexually transmitted, these data suggest that the incubation period may be around 4 days, which is consistent with other bacterial STIs such as *Neisseria gonorrhoeae*<sup>3</sup>

# Acknowledgements

## UAB STI Research Team

Jane Schwebke, MD

Debasish Chattopadhyay, PhD

Hanne Harbison, CRNP, MSN

Cyndi Poore, CRNP

Saralyn Richter, RN

Rhonda Whidden, RN

Meghan Whitfield, CRNP

Angela Pontius, CRNP

Christen Press, CRNP

Cheri Aycock, Keonte Graves

Joy Lewis, Jesie Martinez

Kristal Aaron, Carmen Mitchell

Maranda Weathers

## UAB Vaginal Microbiome Research Team

William Van Der Pol

Ranjit Kumar, PhD

Elliot Lefkowitz, PhD

Casey Morrow, PhD

Xiangqin Cui, PhD



## LSU Vaginal Microbiome Research Team

David H. Martin, MD

Gene Blanchard, BS

Meng Luo, PhD

David Welsh, MD

**NIAID K23AI106957**



**NCATS: UL1TR001417: CCTS Multidisciplinary Network Pilot Program Award**

## University of Mississippi Medical Center

Rajesh Talluri, PhD

Mike Griswold, PhD

