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Mycoplasma genitalium and Bacterial Vaginosis-Associated Bacteria in a Non-Clinic-Based Sample of African-American Women

Kristen R. Moore, PhD[1], **Meena Tomar, MS**[2], **Brandie D. Taylor, PhD**[3], **Scott E. Gygax, PhD**[2,4], **David W. Hilbert, PhD**[2,5], **Donna D. Baird, PhD**[1]

[1]Epidemiology Branch A3-05, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

[2]Femeris Women's Health Research Center, Medical Diagnostic Laboratories, A Division of Genesis Biotechnology Group, Hamilton, New Jersey, USA[a]

[3]Department of Epidemiology and Biostatistics, College of Public Health, Temple University, Philadelphia, Pennsylvania

[4]Current address: Department of Medical Laboratory Sciences and Biotechnology, Jefferson College of Health Professions, Thomas Jefferson University, 130 S. 9th St., Philadelphia, PA 19017, USA[a]

[5]Current address: Merck, 770 Sumneytown Pike, West Point, PA 19486

Abstract

Background—*Mycoplasma genitalium* is associated with adverse reproductive problems. Yet, prevalence estimates from studies that screen women not seeking care are rare. Studies have reported co-occurrence of *M. genitalium* with bacterial vaginosis (BV), but no prior study of specific BV-associated bacteria has been conducted in African-Americans whose reproductive tract infection burden is high.

Methods—Using qPCR we screened vaginal swabs for *M. genitalium,* nine BV-associated bacteria, and four *Lactobacillus* species from 200 participants drawn from a cohort of African-Americans, 23–35-years-old. Sexual history, herpes serostatus, and Nugent score had been assessed. Prevalence of *M. genitalium* was computed. The associations of other vaginal bacteria with *M. genitalium* were examined with binomial regression.

Results—*M. genitalium* prevalence was 18%. Detection and quantity of two BV-associated bacteria were significantly associated with higher prevalence of *M. genitalium:* [*Leptotrichia/ Sneathia* - detection PR: 2.9 95% CI (1.1–7.7) and quantity PR: 1.2 95% CI (1.0–1.3), *Megasphaera* phylotype 1 - detection PR: 2.2 95% CI (1.2–4.2) and quantity PR: 1.1 95% CI (1.0–1.2)]. Increased quantity of *L. iners* was also positively associated with *M. genitalium* [PR: 1.3

Correspondence to: Kristen R. Moore, PhD, Epidemiology Branch A3-05, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 [PH: 984-287-3751; FAX: 301-480-3290] kristen.moore@nih.gov)].

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95% CI (1.0–1.8)]. Nugent ≥7, herpes serostatus, and lifetime number of sex partners were not associated with *M. genitalium*.

Conclusions—Specific BV-associated microbes and *L. iners* were associated with *M. genitalium*, but Nugent ≥7 was not. Studies are needed to confirm a high prevalence of *M. genitalium* in African-Americans and to understand its interactions with other vaginal bacteria.

SHORT SUMMARY

In a non-clinical study of African-American women, *M. genitalium* prevalence was 18%. Presence/increased quantity of *Leptotrichia/Sneathia, Megasphaera* phylotype 1, and increased quantity of *L. iners* were associated with *M. genitalium*.

Keywords

Mycoplasma genitalium; bacterial vaginosis; qPCR; vaginal microbiota

INTRODUCTION

The emergent reproductive tract pathogen *Mycoplasma genitalium* is common with estimated prevalence similar to chlamydia and higher than gonorrhea. (1) However, the majority of studies on *M. genitalium* have been among men to assess risk of urethritis. (2) In women, *M. genitalium* has been associated with cervicitis, pelvic inflammatory disease (PID), infertility, preterm birth, and spontaneous abortion, (3) although robust prospective evaluations are limited. Additionally, *M. genitalium* may increase transmission of human immunodeficiency virus (HIV). (4)

The public health burden of *M. genitalium* has been found to be highest among those of Black race/ethnicity, but majority of data are from clinic-based studies or general population studies outside the US. In a recent meta-analysis, (5) only one of the population-based studies included Black American women. (6) Prior research on other sexually transmitted infections (STIs) has shown that a higher prevalence among Blacks is not due to ethnicity or heritage, but social conditions that affect sexual health. (7) These social conditions may not necessarily be the same in the US as other countries. Also, studies have shown that co-occurring risk factors for STIs differ by gender and race/ethnicity, which has implications for developing interventions. (8) Thus, additional estimates of *M. genitalium* prevalence in non-clinic-based African-American women and its potential risk factors would be beneficial to better understand the burden of *M. genitalium* in this population. This is especially important given that African-American women also tend to experience the reproductive health outcomes associated with *M. genitalium* more frequently than other race/ethnicities. (9)

Given the higher burden of STIs among African-American women (10) and the potential for increased susceptibility and long-term sequalae, it is also important to better understand how these microbes co-occur. Bacterial vaginosis (BV) has been reported to co-occur with *M. genitalium* (11) and is associated with the risk of many of the same adverse outcomes (PID, spontaneous abortion, and preterm birth, and increased risk of HIV). (12) Bacterial vaginosis, a shift from the dominant vaginal flora of *Lactobacillus* species to a mixed

vaginal flora with large numbers of anaerobic bacteria, is very common among African-American women (13) and often chronic and recurring, (14) but studies of *M. genitalium* and BV in African-American women are rare.

The studies previously conducted on BV and *M. genitalium* evaluated the association using mostly Nugent scoring (0–10; 7+ indicative of BV) or Amsel criteria (based on signs and symptoms) in mostly clinic-based or high-risk populations and found inconsistent results. (15–25) However, one study among low-risk Russian women evaluated two BV-associated bacteria using qPCR, which targets and quantifies specific bacteria. (26) Only a few studies included African-American women but were clinical or high-risk samples (16, 20, 24, 25), and none utilized qPCR. This may be especially important because African-Americans are more likely to have a diverse microbiota dominated by non-*Lactobacillus* spp., regardless of BV status or symptomatology. (27)

Thus, the aim of this non-clinic-based study of young African-American women was two-fold; 1) to investigate the prevalence of *M. genitalium*, and 2) to examine the association of both presence and quantity of specific BV-associated bacteria with *M. genitalium* using qPCR.

MATERIALS AND METHODS

We used baseline data from the Study of Environment, Lifestyle, and Fibroids (SELF), an ultrasound-based, prospective study of fibroid incidence among a community-based volunteer sample of 1,693 23–35-year-old African-American women in the Detroit, Michigan area recruited from 2010–2012. Women were ineligible for SELF if they had a prior clinical diagnosis of uterine fibroids; had a hysterectomy; had ever taken medication to treat lupus, Grave's disease, Sjogren's scleroderma, or multiple sclerosis; or ever had any type of cancer treated with radiation or chemotherapy. At the clinic visit, participants completed self-administered computer-assisted web questionnaires (CAWI) and telephone interviews (CATI) and provided non-fasting blood samples and self-collected vaginal swabs. Swabs were placed in a biohazard bag and into a cooler with frozen gel packs and were transported to the lab daily at 4°C. The swabs were stored dry and frozen at −80°C and sent to the NIEHS biorepository for storage at −80°C. Detailed study methods have been described previously. (28) Nugent scoring for BV was measured by the laboratory of Dr. Jane Schwebke at the University of Alabama at Birmingham using Gram-stained slides prepared from vaginal swabs at Medical Diagnostics Laboratory where qPCR was conducted. Serostatus of IgG antibodies to herpes simplex virus type 2 (HSV-2) had been measured previously (29) using the highly sensitive and specific HerpeSelect® 2 Enzyme-Linked Immunosorbent Assay (Focus Diagnostics, Cypress, California) per package instructions. (30)

Study Sub-Sample

This study was conducted as a secondary data analysis from 200 women selected for a nested case-control study from SELF which was primarily designed to investigate the relationship between BV-associated bacteria and incident fibroids. Briefly, from a subset of fibroid-free women at baseline, 100 fibroid cases and 100 non-cases were frequency

matched by age. Further details of the case-control design have been described in the supplemental text.

qPCR Analysis of Microorganisms

After the vaginal swabs were smeared on slides for Gram staining, DNA was extracted using mechanical disruption and the QIAamp minikit (Qiagen). We chose nine bacterial species based on their association with BV in previous literature (31, 32) and the resources/expertise of the Medical Diagnostics Laboratory who performed the testing. qPCR assays quantified *Gardnerella vaginalis*, *Atopobium vaginae*, *Leptotrichia/Sneathia*, BVAB1, BVAB2, *Mageeibacillus indolicus* (previously BVAB3), *Megasphaera* phylotype 1 and 2, and four *Lactobacillus* spp. (*L. crispatus*, *L. gasseri*, *L. jensenii* and *L. iners*) as previously described. (32) Assays were developed for *M. genitalium* and *Prevotella spp.* group (*P. bivia*, *P. disiens*, *P. intermedia* and *P. melaninogenica*) by Medical Diagnostics Laboratory, and were validated according to Clinical Laboratory Improvement Amendments methodology. (33) These two assays have not been previously published. All assays are sensitive to 10 template copies/reaction, are highly specific for the target organism within a panel of common vaginal organisms and generate a linear signal across template concentrations ranging from 10^1 – 10^9 copies/reaction. The concentration of targets was determined by comparing the reaction to a standard curve that was generated using 10^3 to 10^7copies of a plasmid encoding the amplification target. Each reaction plate was run with multiple negative controls to ensure no template DNA contamination of reagents. Bacterial quantity represented the mean concentration of two assays and was expressed as the $\log_{10}$ transformed number of copies/μl of template DNA. We coded non-detects as 0 and detects as 1 in presence/absence analyses. For continuous analyses, given that the limit of detection (LOD) for all of the assays was 10 copies/reaction, we substituted $10/\sqrt{2}=7.1$ for non-detects. (34)

Statistical Analysis

We computed the prevalence and 95% confidence interval (CI) for *M. genitalium* and described the sample using descriptive statistics by *M. genitalium* prevalence for each of the following demographic and behavioral variables: age in years (23–29, 30–35); education (<Bachelors, ≥Bachelors); current smoking at least 1 cigarette/day (yes, no); body mass index, kg/m^2 (15–29, 30+ [obese]) calculated from measurements systematically taken at the study visit; alcohol use in the past year (low/none, moderate/heavy); current marital status (married, not married); current douching at least once/year (yes, no), number of lifetime sex partners (0–5, 6–10, 11+), age at first intercourse (≤16, 17+), and current hormonal contraceptive use [yes (pill, ring, patch, hormonal intrauterine device), no (includes non-hormonal intrauterine device users)]. Binomial regression [prevalence ratios (PRs) and 95% CIs] (35) was used to quantify the association of each factor with *M. genitalium* detection (yes/no). For the BV-associated bacteria, we assessed both detection (yes, no) and continuous quantity with the association of *M. genitalium* detection (yes/no). The quantity PR is interpreted as an x-fold increase in the prevalence of *M. genitalium* for each additional $\log_{10}$ copies per μl of BV-associated bacteria. Post-hoc, we also did a converse analysis to see how *M. genitalium* quantity varied with the detection of the specific bacteria that had positive associations with *M. genitalium* detection.

Because of frequency matching in the case-control design, we did adjust for fibroid case status, (36) but the results were very similar, thus only the unadjusted are presented. All analyses were conducted with SAS version 9.4.

RESULTS

qPCR results were inconclusive for three women, leaving a sample of 197. Overall, the mean age of the sample was 29 (SD: 3.6), 64% were obese, 72% had less than a Bachelor's degree, 18% were current smokers, 29% were current douchers, and 22% were current hormonal contraceptive users. The prevalence of *M. genitalium* was 18% (95% CI: 13–24; n=36/197). The median (25^{th}-75^{th} percentile) *M. genitalium* concentration was 2.5 (2.2–2.7) $\log_{10}$ copies/μl. The prevalence of HSV-2 and Nugent ≥7 were 42% and 52%, respectively.

There tended to be fewer current smokers [PR 95% CI: 0.41 (0.13–1.25)] and more hormonal contraceptive users [PR 95% CI: 1.74 (0.95–3.19)] among those with *M. genitalium* (Table 1), but broad confidence intervals for both. *M. genitalium* was not positively associated with number of sex partners, age at first sex, or HSV-2 serostatus (Table 1) or Nugent ≥7 (Table 1).

The prevalence of the BV-associated microbes ranged from 24% (*Megasphaera* phylotype 2) to 76% (*G. vaginalis*) (Table 2). Among those with detected BV-associated bacteria, the median (25^{th}-75^{th} percentile) $\log_{10}$ copies/μl were lowest for *Prevotella spp.* (4.1 (3.2–4.9)) and highest for *Megasphaera* phylotype 1 (6.9 (6.5–7.1)) (Table 3). The prevalence of *L. iners* was 82% and between 10% and 31% for the *Lactobacillus* spp. considered markers of vaginal health. The percent detection of BV-associated bacterial species did not differ by *M. genitalium* status for most species (Table 2). However, both the detection and quantity of *Leptotrichia/Sneathia* (detection PR: 2.9 95% CI (1.1–7.7); quantity PR: 1.2 95% CI (1.0–1.3)) and *Megasphaera* phylotype 1 (detection PR: 2.2 95% CI (1.2–4.2); quantity PR: 1.1 95% CI (1.0–1.2)) were associated with *M. genitalium* (Table 3). The presence of the two BV bacteria were also associated with an increase in the quantity of *M. genitalium*, though the associations were statistically marginal (*Leptotrichia/Sneathia,* p=0.10 and *Megasphaera* phylotype 1, p=0.05). *Lactobacillus* spp. reflective of vaginal health were not significantly associated with *M. genitalium* (*gasseri*, *crispatus*, and *jensenii*) though prevalence of *M. genitalium* tended to be lower in women with these bacteria. In contrast, quantity of *L. iners* was positively associated with *M. genitalium* and borderline significant, quantity PR: 1.3 95% CI (1.0–1.8) [p-value = 0.06] Table 3.

DISCUSSION

In this non-clinic-based sample of young African-American women the prevalence of *M. genitalium* was 18%. The detection and quantity of two BV-associated bacteria, *Leptotrichia/Sneathia* and *Megasphaera* phylotype 1, were positively associated with *M. genitalium*. Also, the quantity of *L. iners* was positively associated with *M. genitalium.*

We found a much higher prevalence of *M. genitalium* than the 4% reported for African-Americans (compared to 0.4% among Whites) in the only other non-clinic-based US study where African-Americans comprised 23% of the study population. (6) However, they

conducted PCR of urine samples which are less sensitive than vaginal swabs (37) and had a 10-year earlier enrollment period. Our results are higher than the 12% reported for African-American women in a recent multi-center clinic-based study (38) and similar to the 16% reported for an urban OBGYN clinic-based study of young women, predominantly African-American. (39) Some recent clinic-based studies that included women with STIs or at high risk for STIs reported prevalence of *M. genitalium* among African-American women ranging from 15–24% compared to 3–16% among White women. (25, 40–42) Studies among high-risk groups have not found associations between typical STI risk factors (age, number of sex partners, age at first sex, other STIs) and *M. genitalium*, similar to our results. (19, 25, 41)

In our study, although Nugent ≥7 was not associated with *M. genitalium*, two BV-associated bacteria were, *Leptotrichia/Sneathia* and *Megasphaera* phylotype 1*. L. iners* which has previously been found to be associated with a diverse microbiota, (27) was also associated with *M. genitalium.* The one other study that conducted qPCR examined low-risk Russian women and only included *G. vaginalis* and *A. vaginae* in their multiplex panel along with *Lactobacillus* spp. (26) They found an increased odds of *M. genitalium* among women with high numbers of the two BV-associated bacteria, and a protective association with *M. genitalium* for microbiota dominated by *L. crispatus, L. jensenii and L. gasseri*. They also found that an *L. iners*-dominated microbiota was associated with increased prevalence of *M. genitalium* although not significantly, similar to our results. They did not conduct Nugent scoring for comparison.

Regarding the null association we found between Nugent ≥7 and *M. genitalium,* only a few previous studies evaluated this relationship in populations that included African-American women; however, none reported results specifically for African-American women and all were among women seeking care or high-risk. (16, 20, 24, 25) One study that was 86% African-American (16) and another that was 22% African-American (20) found that those with BV diagnosed by Amsel criteria, were less, rather than more, likely to have *M. genitalium,* although not significantly. The other two studies reported positive, yet non-significant associations between BV and *M. genitalium.* (24, 25) Nugent-identified bacteria are non-specific (31) which has led to the examination of fastidious BV-associated bacteria in reproductive health. With the more diverse micriobiota including anaerobic bacteria that is commonly seen in African-American women regardless of symptoms or Nugent score (43), an association with Nugent ≥7 may be undetectable, but associations with specific BV bacteria may be informative. The two BV-associated bacteria that were associated with *M. genitalium* in our study, *Sneathia* spp. and *Megasphaera* spp*.,* have been implicated in both PID and infertility. (44)

Studies that have not included African-Americans were mostly cross-sectional, and they have found null (17) or positive associations for the co-occurrence of BV and *M. genitalium.* (15, 18, 21–23) Two studies with prospective data found positive associations between baseline Nugent ≥7 and incident *M. genitalium*. (19, 22) A third study with prospective data found an inverse relationship between *M. genitalium* and incident Nugent ≥7 although they had found a positive association for co-occurrence of "*M. genitalium or C. trachomatis"*

(associations for the two were not reported separately) and Nugent ≥7 at baseline. However, both prospective and cross-sectional associations were not statistically significant. (15)

Our study had limitations, as well as strengths. It was a cross-sectional analysis; thus, temporality of infection is unknown. Although information on clearance of *M. genitalium* is very limited, one study found a median time from incidence to untreated clearance of 1.5 months. (24) We did not have data on Amsel criteria, condom use, recent sexual activity (number of partners/vaginal-penile acts), reporting of vaginal symptoms, or diagnoses for concurrent STIs (i.e. chlamydia, gonorrhea, or *Trichomonas vaginalis*), cervicitis, or PID. These would not impact our prevalence estimates or the relationships we found between the BV-associated bacteria and *M. genitalium* but would provide more insight on the study sample and the factors associated with *M. genitalium* in this group of women. This was a relatively small sample of women in one geographic location, so results need broader replication. The strengths of this study are: qPCR measurement of *M. genitalium*, its non-clinic-based design, measurement of other factors of interest, including serological measurement of HSV-2, Nugent scoring for BV, and especially qPCR-based measurement of BV-associated bacteria.

In conclusion, our sample of young African-American women had a higher prevalence of *M. genitalium* than other non-clinic-based populations. Nugent ≥7 and the STI risk factors available were not predictive of prevalence. Clinicians should be aware that African-American women may have a higher prevalence of *M. genitalium* than initially thought and should refer to Centers for Disease Control and Prevention guidelines for testing and treatment. (45) Further research is needed to 1) evaluate the prevalence of *M. genitalium* in the US, 2) identify the necessary and sufficient factors that allow *M. genitalium* to invade and thrive in the reproductive tract, and 3) investigate its role in reproductive tract morbidities. Such work is important for shaping future guidelines to limit reproductive and pregnancy morbidities among *M. genitalium* positive women, especially African-Americans, who bear a high burden of both exposure and adverse sequelae.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Associations of Factors with Prevalent *M. genitalium* among African-American Women Aged 23–34 Years (n = 197), Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012

	Prevalent *M. genitalium*		PR (95% CI)
Covariate	**No n=161 (82%) n (%)**	**Yes n=36 (18%) n (%)**	
Age 30–35[a]	68 (42)	15 (42)	0.98 (0.54–1.79)
Obese (BMI 30+[b])	106 (66)	20 (56)	0.73 (0.39–1.38)
Less than Bachelor's Degree	118 (73)	23 (64)	0.70 (0.38–1.29)
Alcohol Use[c]	119 (74)	26 (72)	0.93 (0.48–1.80)
Not Currently Married	120 (75)	28 (78)	1.16 (0.57–2.37)
Currently Smoke	33 (21)	3 (8)	0.41 (0.13–1.25)
Currently Douche[d] (missing=1)	49 (31)	8 (22)	0.70 (0.34–1.44)
Current Hormonal Contraception Users	32 (20)	12 (33)	1.74 (0.95–3.19)
Number of Lifetime Sex Partners (missing=4)			
0–5	43 (27)	12 (35)	reference
6–10	47 (30)	12 (35)	0.93 (0.46–1.90)
≥ 11	69 (43)	10 (29)	0.58 (0.27–1.25)
1st Intercourse ≤ 16 Years (missing=4)	101 (64)	17 (50)	0.64 (0.35–1.17)
HSV-2 Seropositive (missing=2)	67 (42)	15 (42)	0.98 (0.54–1.79)
Bacterial Vaginosis (Nugent score 7+)	84 (52)	18 (50)	0.93 (0.52–1.68)
Nugent Score 4+	96 (60)	22 (61)	1.05 (0.57–1.93)

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2; PR, prevalence ratio

Numbers may not add to totals due to missing

[a] No persons over 34 years of age were recruited, but some 34-year-olds had turned 35 by the time they had their ultrasound examination; referent group 23–29-year-olds.

[b] Body mass index was calculated as weight (kg)/height (m)^2; referent group 15–29.

[c] Low alcohol consumption was defined as having had less than 10 alcoholic drinks in last year.

Heavy drinkers were those who usually consumed 6 or more drinks on days when they imbibed alcohol or who consumed 4 or more drinks per sitting at least 2–3 times a month. All others were considered moderate drinkers. Referent group is low/no consumption.

[d] Referent is participants who douched less than 10 times in their life or currently less than once per year.

Table 2.

Detection of Bacterial Species and Prevalence Ratios for *M. genitalium*

Bacterial Species	**Total N % Detected (n=197)**	***M. genitalium***		***M. genitalium* PR (95% CI)**
		Yes N% Detected (n=36)	***No N% Detected (n=161)***	
Gardnerella vaginalis	150 (76)	28 (78)	122 (76)	1.1 (0.5–2.2)
Atopobium vaginae	135 (69)	24 (67)	111 (69)	0.9 (0.5–1.7)
Leptotrichia/Sneathia	**145 (74)**	**32 (89)**	**113 (70)**	**2.9 (1.1–7.7)**
BVAB1	107 (54)	21 (58)	86 (53)	1.2 (0.7–2.2)
BVAB2	112 (57)	92 (57)	20 (56)	1.0 (0.5–1.7)
Mageeibacillus indolicus	87 (44)	71 (44)	16 (44)	1.0 (0.6–1.8)
***Megasphaera* phylotype 1**	**93 (47)**	24 (67)	69 (43)	**2.2 (1.2–4.2)**
Megasphaera phylotype 2	48 (24)	10 (28)	38 (24)	1.2 (0.6–2.3)
Prevotella species group	129 (65)	24 (67)	105 (65)	1.1 (0.6–2.0)
Lactobacillus iners	161 (82)	30 (83)	131 (81)	1.1 (0.5–2.5)
Lactobacillus gasseri	20 (10)	3 (8)	17 (11)	0.8 (0.3–2.4)
Lactobacillus jensenii	55 (28)	6 (17)	49 (30)	0.5 (0.2–1.2)
Lactobacillus crispatus	61 (31)	9 (25)	52 (32)	0.7 (0.4–1.5)

Abbreviations: BVAB, bacterial vaginosis associated bacteria, CI, confidence interval; PR, prevalence ratio

Table 3.

Concentrations of Bacterial Species Among those with Detectable Levels and Quantity Prevalence Ratios for *M. genitalium*[a,b]

Bacterial Species	Overall Log_{10} copies/μl Median (25^{th}–75^{th} percentile)[c]	*M. genitalium*		*M. genitalium* PR (95% CI) Per log_{10} copies/μl of BVAB[a,b]
		Log_{10} copies/μl Median (25^{th}–75^{th} percentile)[c]	Log_{10} copies/μl Median (25^{th}–75^{th} percentile)[c]	
Gardnerella vaginalis	4.9 (4.3–5.6)	5.2 (4.3–5.9)	4.9 (4.3–5.6)	1.1 (0.9–1.2)
Atopobium vaginae	5.7 (5.0–6.0)	5.8 (5.5–6.4)	5.6 (5.0–6.0)	1.0 (0.9–1.2)
Leptotrichia/Sneathia	**5.7 (3.8–6.5)**	**5.7 (3.3–7.4)**	**5.7 (4.4–−6.4)**	**1.2 (1.0–1.3)**
BVAB1	5.5 (2.8–6.6)	3.7 (2.7–6.4)	5.7 (2.9–6.6)	1.0 (0.9–1.1)
BVAB2	4.6 (3.9–5.2)	5.0 (4.2–5.4)	4.6 (3.8–5.1)	1.0 (0.9–1.2)
Mageeibacillus indolicus	4.6 (4.0–5.4)	5.0 (4.4–5.5)	4.5 (3.9–5.4)	1.0 (0.9–1.2)
***Megasphaera* phylotype 1**	**6.9 (6.5–7.1)**	**6.8 (6.6–7.1)**	**6.9 (6.5–7.2)**	**1.1 (1.0–1.3)**
Megasphaera phylotype 2	6.6 (6.1–6.9)	6.9 (5.8–7.1)	6.6 (6.1–6.9)	1.0 (0.9–1.2)
Prevotella species group	4.1 (3.2–4.9)	3.8 (3.2–5.0	4.1 (3.1–4.9)	1.0 (0.9–1.2)
Lactobacillus iners	4.0 (3.6–4.4)	4.4 (4.1–4.8)	3.9 (3.4–4.3)	1.3 (1.0–1.8)
Lactobacillus gasseri	3.7 (2.8–4.3)	3.7 (3.3–9.4)	3.7 (2.6–4.1)	1.0 (0.8–1.3)
Lactobacillus jensenii	3.4 (3.0–3.8)	4.1 (3.8–4.2)	3.3 (3.0–3.7)	0.9 (0.7–1.2)
Lactobacillus crispatus	4.3 (3.7–4.7)	4.9 (4.5–5.1)	4.2 (3.7–4.6)	1.0 (0.8–1.2)

Abbreviations: BVAB, bacterial vaginosis associated bacteria, CI, confidence interval; PR, prevalence ratio

[a] For the prevalence ratios we substituted a value for non-detects using the limit of detection /√2 (10/√2=7.1)

[b] As an example, the PR for *Leptotrichia/Sneathia* is interpreted as a 1.2-fold increase in the prevalence of *M. genitalium* for each additional log_{10} copies per μl of *Leptotrichia/Sneathia* bacteria

[c] Among those with the bacteria detected as shown in Table 2