Bacterial species colonizing the vagina of healthy women are not associated with race

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ABSTRACT

The vaginal microbiota of 36 white versus 25 black asymptomatic women were compared using both cultivation-dependent and -independent identification. Significant differences by race were found in colonization and density of bacterial species. However, exclusion of 12 women with bacterial vaginosis by Nugent criteria resulted in no significant differences by race.

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Some studies evaluating the microbiome of reproductive age women have reported that black and white women have significant differences in the predominant microbial communities. In these studies, the term "healthy" was used to describe women without genital tract symptoms [1–3]. A limitation of this approach is that "healthy" populations of asymptomatic women can harbor genital tract infections which can alter their microbiome [4–6]. Additionally, there is a racial disparity with bacterial vaginosis (BV) being significantly more common among black versus white women (51% vs 23%), and only 16% of women with BV in the United States report symptoms [7].

Ravel et al. [1] described 5 bacterial communities using 454 pyrosequencing in 98 white, 104 black, 97 Asian, and 97 Hispanic healthy women recruited from Baltimore and Atlanta. These sexually active asymptomatic women were not screened for sexually transmitted infections (STIs) or evaluated for BV using the Amsel criteria, but women reporting vaginal discharge symptoms in the past 48 h were excluded. The study reported that 90% of white women compared to 60% of black women had one of the four *Lactobacillus*-dominant vaginal communities. The most prevalent vaginal community for white women (45%) was the *L. crispatus*–dominant community group I, which was found in only 22% of black women [1]. Whereas, community group IV, which was characterized by a diverse microbiome including *Gardnerella vaginalis*, *Lactobacillus* species at non-predominant quantities, *Aerococcus*, *Prevotellaceae* family, *Sneathia*, *Megasphera*, *Atopobium*, *Porphyromonas*, *Peptoniphilus*, *Mobiluncus*, *Dialister*, *Prevotella* and others, was the most prevalent among black women (40%), but found in only 10% of white women [1]. In a similar study comparing vaginal microbiomes, Fettweiss and colleagues also did not screen for asymptomatic infections of "healthy" non-pregnant women [2]. In their analysis of 243 women of European ancestry versus 419 African American women, women of European ancestry were more likely to be colonized by *L. crispatus, L. jensenii, L. gasseri,* and *Staphylococcus,* while African American women were more likely to be colonized by *Mycoplasma hominis*, *L. iners*, *Aerococcus*, *Anaerococcus*, BVAB1, BVAB2, *Dialister*, *Peptoniphilus*, *Coriobacteriaceae*, *Parvimonas*, *Megasphaera*, *Sneathia*, *Prevotella amnii*, *Atopobium* and *G. vaginalis* [2]. Both studies concluded that healthy black and white women have vastly different vaginal microbiomes.

The objective of this secondary analysis was to evaluate differences in vaginal microbiota in black versus white women after excluding women having asymptomatic chlamydial or gonococcal infections, as well as *Trichomonas vaginalis* and BV, as diagnosed using Amsel criteria. We hypothesized that many of the reported differences in vaginal microbiota for black versus white women are attributable to the inclusion of women having asymptomatic BV or STIs. In this study, we compared the cultivable microbiota of black

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and white women after excluding all genital infections, and before and after excluding BV based on Nugent criteria. Quantitative PCR (qPCR) was also performed to determine differences in detection of five vaginal microbes.

The study population included the baseline data of a clinical trial of an investigational product [8]. The protocol was approved by the University of Pittsburgh Institutional Review Board, and all women provided written informed consent. Of the 121 participants recruited and screened from gynecology clinics at Magee-Womens Hospital of UPMC and the surrounding community, 61 HIV-negative women were enrolled between September 2012 and July 2013. Inclusion criteria included healthy women aged 18–45, without symptoms. Exclusion criteria included pregnancy, hysterectomy, sexually transmitted infection diagnosed by nucleic acid amplification tests for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, BV based on 3 of 4 Amsel criteria, or *Trichomonas vaginalis* by positive rapid antigen test. Women were also excluded if they had an abnormal complete blood count or complete metabolic panel, or had used antibiotics or antifungals in the 14 days preceding enrollment.

Swabs were used to obtain samples from the lateral vaginal wall. One Dacron swab was used to prepare a slide that was Gram stained and scored using the Nugent criteria [9]. A flocked swab was collected for qPCR and placed in a 2-mL cryovial for storage at $\leq -70\ ^\circ C$. For qPCR, specific primers developed previously were utilized to target *L. crispatus, L. iners*, *G. vaginalis*, *Atopobium vaginae*, and *Megasphaera*-like bacterium type I and detected with SYBR green technology [10]. For quantitative culture, two Dacron swabs were placed into a solid anaerobic transport medium (BBL Port-A-Cul tube, Becton Dickinson, Sparks, MD), transported to the laboratory within 24 h, and serially diluted as previously described [11].

Isolated microorganisms were subcultured for purity and stored at $\leq -70\ ^\circ C$.

Group B *Streptococcus*, *Enterococcus faecalis*, *Escherichia coli* or other enteric bacteria, *Candida albicans* or other yeast, *Staphylococcus aureus,* and *G. vaginalis* were identified using Gram stain and colony morphology and biochemical tests [12]. For other isolates, Gram stain and colony morphology and aerobic growth were used for preliminary identification. Identification algorithms based on paired 16S rRNA gene sequencing and restriction fragment length polymorphism (RFLP) analysis were generated [13–16]. Restriction enzymes utilized were *Hpy*CH4V (New England Biolab, Ipswich, MA), FastDigest *Bsu*RI (Thermo Scientific) or *Hae*III (Promega, Madison, WI), Hinf1 (Promega), or *Taq*I (Promega). Repetitive-sequence PCR [17], which was also used for *Lactobacillus* species identification, and RFLP patterns were evaluated independently by at least 2 readers. Equivocal samples were prepared for direct sequencing. Sequencing was performed by The Genomics Research Core Sanger Sequencing Facility at University of Pittsburgh. Sequencing chromatograms were evaluated and considered acceptable for ≥700 base pairs. Sequences were compared to GenBank using the Basic Local Alignment Search Tool and Ribosomal Database Project using Sequence Match.

Fisher's exact tests were used to compare the prevalence of organisms between black and white women. Mann-Whitney U tests were used to compare quantities among colonized women.

The study population had a mean age of 26.7 years with a range of 18–44 years. More than half (56%) of the women were single, and most (70%) were employed. Half of the women had a single sexual partner, and the remainder did not report being sexually active. In our analysis of bacterial colonization of all 61 asymptomatic women, we found that by cultivation, *Prevotella amnii* was more

Table 1
Frequency and density of microorganisms detected by cultivation and qPCR from 61 asymptomatic women.

Vaginal isolates	Frequency of detection (N = 61)			Quantity median (range)[a] 10^x cfu or copies/mL[b]		
	White (n = 36)	Black (n = 25)	P value	White (n = 36)	Black (n = 25)	P value
Cultivation						
Any *Lactobacillus* species	34 (94.4%)	24 (96.0%)	>0.99	8.3 (4.1, 9.1)	7.5 (6.1, 8.9)	0.012
Lactobacillus crispatus	27 (75.0%)	14 (56.0%)	0.17	8.3 (6.1, 8.9)	7.8 (2.3, 8.7)	0.07
Lactobacillus jensenii	20 (55.6%)	12 (48.0%)	0.61	7.1 (5.1, 9.1)	7.3 (5.5, 8.9)	0.55
Lactobacillus iners	9 (25.0%)	12 (48.0%)	0.10	7.3 (6.5, 7.1)	7.0 (4.1, 7.7)	0.60
Lactobacillus gasseri	6 (16.7%)	5 (20.0%)	0.75	6.4 (5.1, 8.3)	6.1 (2.7, 7.1)	0.79
E.coli	6 (16.7%)	6 (24.0%)	0.53	3.8 (2.1, 5.1)	3.1 (2.5, 4.1)	0.39
Group B *Streptococcus*	7 (19.4%)	6 (24.0%)	0.76	2.1 (1.1, 5.1)	2.7 (1.1, 6.6)	0.53
Enterococcus	12 (33.3%)	9 (36.0%)	>0.99	2.7 (1.1, 4.7)	3.3 (1.1, 6.1)	0.60
Gardnerella vaginalis	16 (44.0%)	14 (56.0%)	0.44	6.7 (2.1, 8.7)	8.3 (7.1, 9.3)	0.005
Any *Atopobium* species	4 (11.1%)	7 (28.0%)	0.17	6.6 (5.1, 8.5)	7.1 (3.1, 8.3)	>0.99
Atopobium vaginae	3 (8.3%)	5 (20.0%)	0.25	7.1 (5.1, 8.5)	7.1 (5.1, 8.3)	>0.99
Mycoplasma hominis or *Ureaplasma* spp	16 (44.4%)	14 (56.0%)	0.44	5.1 (3.9, 8.1)	5.6 (1.1, 7.1)	0.38
Any *Prevotella* species	18 (50.0%)	16 (64.0%)	0.31	4.2 (2.1, 8.3)	5.1 (2.5, 8.1)	0.30
P. amnii	0	4 (16.0%)	0.02	–	6.3 (5.7, 7.5)	–
P. timonensis	4 (11.1%)	7 (28.0%)	0.17	5.1 (3.7, 8.3)	4.3 (2.1, 8.1)	0.65
P. bivia	14 (38.9%)	10 (40.0%)	>0.99	4.1 (2.1, 6.9)	4.6 (2.1, 7.7)	0.63
Porphyromondaceae family	6 (16.7%)	10 (40.0%)	0.07	3.8 (3.1, 8.1)	3.4 (2.1, 8.5)	0.71
Porphyromonas uenonis	2 (5.6%)	6 (24.0%)	0.054	5.8 (3.5, 8.1)	4.9 (2.3, 8.5)	>0.99
Dialister microaerophilus	3 (8.3%)	6 (24.0%)	0.14	2.6 (2.1, 3.1)	5.8 (2.1, 7.9)	0.10
Bacteroides species	12 (33.3%)	11 (44.0%)	0.43	3.1 (2.1, 4.1)	3.1 (2.3, 7.7)	0.07
Any *Peptoniphilus* species	16 (44.4%)	11 (44.0%)	>0.99	4.1 (2.1, 6.5)	4.3 (3.1, 7.9)	0.29
Peptoniphilus harei	13 (36.1%)	8 (32.0%)	0.79	4.1 (2.1, 5.5)	4.6 (3.1, 7.1)	0.50
Anaerococcus species	9 (25.0%)	5 (20.0%)	0.76	5.1 (2.1, 6.3)	3.3 (2.9, 7.7)	0.90
Streptococcus anginosus	12 (33.3%)	11 (44.0%)	0.43	4.1 (2.1, 4.8)	4.1 (3.1, 5.1)	0.35
Quantitative PCR						
Lactobacillus crispatus	35 (97.2%)	25 (100%)	>0.99	7.8 (2.9, 8.5)	6.4 (2.9, 8.4)	0.059
Lactobacillus iners	29 (80.6%)	24 (96.0%)	0.12	7.7 (3.3, 8.8)	8.0 (3.5, 9.1)	0.26
Gardnerella vaginalis	26 (74.3%)	21 (84.0%)	0.53	5.0 (2.8, 8.3)	6.5 (3.2, 8.7)	0.04
Atopobium vaginae	11 (30.6%)	12 (48.0%)	0.19	5.1 (3.1, 7.2)	6.7 (3.3, 7.5)	0.051
Megasphaera-like bacterium-type I	4 (11.1%)	7 (28.0%)	0.17	7.6 (3.0, 7.9)	7.9 (2.9, 8.5)	0.41

[a] Quantity assessed among women colonized with microorganism.
[b] Cfu = colony forming units for cultivation; copies for qPCR.