Associations between Vaginal Microbiota and Cervicitis

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Abstract

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Background: Cervicitis is defined as the inflammation of the cervix. Many cases of cervicitis are caused by sexually transmitted infections (STIs); however, a substantial portion of cervicitis cases have no known etiology. Cervicitis is also known to be associated with bacterial vaginosis (BV), a clinical condition characterized by the transition of the vaginal microbiota from being lactobacilli dominated to having higher bacterial diversity. In this analysis, we investigated the relationship between specific vaginal bacterial species and cervicitis.

Methods: For this cross-sectional analysis, we utilized data from participants in the placebo arm of the Preventing Vaginal Infections trial. During bimonthly pelvic exams, cervicovaginal swabs were collected, and quantitative polymerase chain reaction assays were used to measure the concentrations of specific taxa. For the exposure, concentrations for each bacterial taxon were categorized into tertiles; samples with concentrations below the lower limit of quantitation were classified as not detectable and served as the reference group. Cervicitis was defined as having 30 or more polymorphonuclear leukocytes per high power field on microscopy on cervical Gram stain. We used Poisson GEE models to evaluate the association between bacterial concentration, categorized as tertiles, and cervicitis prevalence and estimate prevalence ratios.

Results: In the analysis adjusting for sexually transmitted infections, vaginal washing, and condom use, certain BV-associated bacteria had one or more tertiles that were associated with an increased detection of cervicitis, including BVAB1, *Megasphaera* species, and *Mageeibacilus indolicus.* Only the highest tertiles of *Lactobacillus iners* and *Lactobacillus jensenii* were associated with an increased detection of cervicitis.

Conclusions: Presence of certain BV-associated species and *Lactobacillus* species in the vagina may contribute to cervicitis. This analysis was limited to Kenya and the US, and given differences of vaginal microbiota by region, additional studies are needed to understand if these associations are present in other regions. Further study is also needed to explore the role of lactobacilli in cervicitis.

Background

Cervicitis involves inflammation of the cervix, and this condition is typically diagnosed based on the presence of cervical mucopus and/or cervical bleeding when touched with a swab.[1] A laboratory diagnosis of cervicitis relies on cervical Gram stain with varying thresholds of 10 to 30 polymorphonuclear leukocytes (PMNs) per high power field on microscopy. Complications related to cervicitis include pelvic inflammatory disease and increased susceptibility to HIV infection. Cervicitis is most often caused by a sexually transmitted infection (STI), such as chlamydia or gonorrhea.[1,2] The prevalence of cervicitis varies globally due to differences in clinical definitions of cervicitis and prevalence of STIs. Since cervicitis is not a reportable clinical condition and requires a speculum exam for diagnosis, the understanding of prevalence comes predominantly from cross-sectional studies that include populations at varying degrees of risk for cervicitis. Several studies conducted in the US have reported cervicitis prevalence ranging from a low 23% amongst participants being recruited for a clinical trial and 45% amongst patients presenting to STI clinics. [3,4,5]

Data also suggest that bacterial vaginosis (BV) is associated with cervicitis. [6] Evidence of this relationship includes the lack of a typical pathogen for cervicitis detected when BV is present and treatment of BV leading to the resolution of cerivicitis.[1, 7, 8] Bacterial vaginosis is a clinical condition that occurs when the vaginal microbiota transitions from being *Lactobacillus* dominated, which is generally considered healthy, to having high bacterial diversity and an abundance of anaerobic and Gram-negative species.[9] This transition in the vaginal microbiota can lead to symptoms such as vaginal discharge and odor.[9] Previous studies have identified a number of bacteria associated with the presence of the clinical condition BV; however, individuals can have BV-associated bacteria present in their vagina without reporting symptoms.[10]

While there is an association between the clinical condition of BV and cervicitis, it is unclear which specific bacterial taxa or groups of taxa may drive this association, assuming there is a causal relationship. Evidence indicates that BV-associated bacteria can cause the breakdown of the cervical mucus barrier, which can lead to bacteria colonizing the cervix.[11] Previous studies have also assessed associations between specific vaginal bacteria and cervicitis; however, evidence is mixed. One study reported an association of cervicitis with *Mageeibacillus indolicus*, a BV-associated species, and other studies have found no association or an inverse association between cervicitis and *Lactobacillus* species.[12,13] To address this gap, we used data from the Preventing Vaginal Infections (PVI) trial, a randomized controlled trial of an intervention to improve vaginal health, to assess the relationship between specific vaginal microbial taxa and prevalent cervicitis.[14,15]

Methods

Study design and setting

The PVI trial was a randomized, double-blind, placebo-controlled trial that evaluated the efficacy of monthly periodic presumptive treatment with metronidazole and miconazole intravaginal suppositories for reducing BV and vulvovaginal candidiasis over the course of one year.[14,15] The PVI trial was approved by human subjects research committees at all participating institutions, and participants provided written consent at screening and enrollment. In addition, participants were asked to provide written informed consent for future use of stored samples. This analysis utilized data from the placebo arm of the PVI trial to assess the association between vaginal microbiota and cervicitis. The PVI trial recruited participants from three research clinics in Kenya (two in Nairobi and one in Mombasa) and one in Alabama, USA. Two of the Kenyan clinics recruited from people reporting transactional sex, and the other two clinics recruited from the general population.

Study participants

To be eligible to participate in the PVI trial, potential participants had to be 18-45 years old, negative for HIV, not pregnant or breastfeeding, sexually active, and have one or more of the following vaginal infections diagnosed at screening: BV by Nugent score, *Trichomonas vaginalis*, and/or vulvovaginal candidiasis. Enrolled participants were assigned female sex at birth; however, no specific data on gender identity was collected at the time. Potential participants with symptomatic BV or vulvovaginal candidiasis (VVC) or *T. vaginalis* (TV) regardless of symptoms were treated and could enroll 7 to 14 days later. If eligible and willing to participate, participants were randomized to the placebo or treatment arm. For this analysis, data from participants randomized to the placebo arm and who provided consent for further testing of specimens were included.

Data collection & laboratory procedures

A detailed description of data collection procedures for the PVI trial has been presented.[15] In brief, at enrollment, structured interviewer administered questionnaires were used to collect data on sexual behaviors, demographics, and medical history. At monthly follow-up visits, the same approach was used to collect data on sexual behaviors, use of the intravaginal suppositories, and genital tract symptoms. At enrollment and months 2, 4, 6, 8, 10, and 12, participants had a pelvic examination, and cervicovaginal swabs were used to collect samples for genital tract infections testing including BV, TV, vaginal yeast, *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG). A Gram stain was performed on the cervicovaginal swabs, and greater than or equal to 30 polymorphonuclear leukocytes per high power field on microscopy indicated cervicitis. An additional swab of vaginal fluid was collected and stored at -80 C for future testing of the vaginal microbiota.

Following the completion of the trial, vaginal swabs were shipped to the Fredricks Laboratory in Seattle, USA for microbial analysis.[14] The MoBio Biostic Bacteremia DNA isolation kit was used to get purified DNA. Sham extraction controls were utilized to ensure no contamination, and samples underwent two quality control assays. Bacterium-specific quantitative PCR (qPCR) assays were performed using purified DNA and generated bacterial concentrations for *Fannyhessea vaginae* (previously characterized as *Atopobium vaginae*), BVAB1, BVAB2, *Gardnerella vaginalis, Mageeibacillus indolicus, Megasphaera* species, *Leptotrichia/Sneathia* species, *Lactobacillus crispatus, L. iners, and L. jensenii*.

Statistical analysis

Our primary exposures were the $\log_{10}$ transformed concentrations of BV-associated bacteria and lactobacilli obtained from the qPCR analysis of vaginal swabs. For each taxon, values below the lower limit of quantification (LLOQ), which was 1.98 $\log_{10}$ copies/swab for the assay, were classified as "not detectable" (referent group); values above the LLOQ were categorized into tertiles. Our primary outcome was the presence of cervicitis based on having greater than or equal to 30 polymorphonuclear leukocytes (PMNs) per high-powered microscopy field on cervical Gram stain.

We used Poisson GEE models to evaluate the association between bacterial concentration and cervicitis prevalence, estimating prevalence ratios.[16] Due to the longitudinal design, we used an independent correlation structure to account for repeated measures and the different number of measurements. We adjusted for the following confounders decided *a priori* in our analysis: STI detection (CT, NG, TV, MG), condom use in the past week (categorical), and vaginal washing in the past month (binary) (Figure 1). After removing participants that did not consent to further testing or were missing data for qPCR, cervicitis, STIs, condom use, or vaginal washing, the 110 participants contributed a total of 732 visits (Figure 2). We planned to repeat the above analyses stratified by country based on prior PVI trial microbiome analyses that demonstrated differences in species prevalence by country, however, there were insufficient outcomes among the participants from Kenya.[14] As a result, country specific analyses were only performed for participants from the United States. In addition, we performed a sensitivity analysis removing visits with concurrent STI presence. Statistical analyses were performed using R (version 4.2.3).

Results:

Overall, cervicitis was detected in 34 of 110 participants at some point during the study and at 16.3% of visits included in this analysis (median visits per participant: 7; IQR: 7 - 7). Baseline characteristics among participants who experienced cervicitis at any point in the study versus those that did not are summarized in Table 1. Participants were around 29 years old, and 76% of participants were from the Kenya cohort. Based on Nugent score, 40 participants (36%) had BV.

The majority were sexually active in the past week (82%), and among those reporting sex, approximately a third used condoms as their only contraceptive method. Of participants with cervicitis detected at some point in the study, 71% were from the US cohort. Vaginal washing was more common in the no cervicitis group and was reported predominantly among Kenyan participants. Gonorrhea, chlamydia, or trichomoniasis was detected in less than 10% of samples at each visit (Table 2). However, *Mycoplasma genitalium* detection ranged from 8% to 16% per visit.

BV by Nugent score was present among 47.9% of samples in the cervicitis group compared to only 30.2% of samples in the no cervicitis group (adjusted prevalence ratio (aPR): 1.75, 95% confidence interval (CI): 1.22, 2.50). Figure 3 shows the histograms of log_{10} concentration of each bacterial species with the corresponding tertiles. For each bacterial species, we summarized the number of samples for each tertile by cervicitis presence in Table 3 & 3A. The most commonly detected taxa were *Gardnerella vaginalis*, *Fannyhessea vaginae* and *Lactobacillus iners*. Amongst those with cervicitis, BVAB1 was detected in 57.1% of samples compared to only 17% of samples amongst those without cervicitis. For *Megasphaera* species, 55.5% of samples had detectable concentrations in the cervicitis group compared to 42.3% of samples in the no cervicitis group. *L. jensenii* was detected in 38.7% of samples in the cervicitis group and 22.5% of samples in the no cervicitis group.

In assessing the relationship between BV-associated bacteria and cervicitis detection, in unadjusted models, each BVAB1 tertile was associated with an increased likelihood of cervicitis detection compared to samples where BVAB1 was not detected (Table 3). After adjusting for

STIs, condom use, and vaginal washing, individuals in the highest tertile of BVAB1 were 4.69 times more likely to have cervicitis than those with no detection of BVAB1 (95% CI: 3.05, 7.22; Table 3). The magnitude of the association was generally similar for the first (aPR: 4.11, 95% CI: 2.63, 6.43) and second (aPR: 3.47, 95% CI: 2.08, 5.78) tertiles. Similarly, all tertiles of the *Megasphaera* spp. and the two highest tertiles of *M. indolicus* were associated with an increased likelihood of cervicitis detection in both unadjusted and adjusted models. (Table 3). All other BV-associated species had similar trends towards an association with cervicitis but did not reach statistical significance. For the *Lactobacillus* species, only the highest tertiles of *L. iners* (aPR: 2.81, 95% CI: 1.36, 5.80) and *L. jensenii* (aPR: 2.71, 95% CI: 1.72, 4.28) were found to be positively associated with cervicitis (Table 3A). While we planned to perform a sensitivity analysis using the cervicitis outcome definition of symptoms and 10 or more PMNs, there were not sufficient outcomes (n=19).

When the analysis was repeated among US participants only, the magnitudes of the associations were similar; however, given the much smaller sample size, the associations were no longer statistically significant (Table 4 & 4A). In a sensitivity analysis where visits with STIs were removed, all tertiles of BVAB1 and the highest tertiles of *M. indolicus*, *L. iners*, and *L. jensenii* were associated with increased detection of cervicitis; the magnitudes of the associations were similar to those in the primary findings (Table 5 & 5A).

Discussion:

This secondary analysis of data from the PVI trial sought to explore the relationship between vaginal bacteria detected by qPCR and cervicitis. BV was associated with an increased likelihood of cervicitis. For BV-associated bacterial species, each tertile of BVAB1 and *Megasphaera* species and only higher tertiles of *Mageeibacilus indolicus* were associated with an increased detection of cervicitis. For *Lactobacillus* species, *L. iners*, and *L. jensenii* were associated with an increased detection of cervicitis. The magnitude of the associations were similar in the US-only analysis but did not reach statistical significance due to limited power. Since CT, GC, TV and MG are also associated with cervicitis, we conducted a sensitivity analysis to remove visits where these STIs were detected. Our findings were consisitent with the analysis including STIs as cervicitis was associated with BVAB1, *Mageeibacilus indolicus*, *L. iners*, and *L. jensenii*.

Most participants with cervicitis at any point in the study were from the US cohort. Cervicitis is known to vary by country due to behavioral factors, prevalence of STIs, and diagnostic practices. Given that many factors potentially associated with BV and cervicitis were similar between the Kenyan and US participants, it was unexpected that most cases of cervicitis were amongst the US participants. Differences in bacterial detection at enrollment by country for the PVI trial were previously noted with the US participants having a higher prevalence of of *L. jensenii*, *L. iners*, BVAB1, and *M. indolicus*.[14] Concentrations of bacterial species in the vagina can also vary by country and behavioral factors.[17,18,19] In addition, more recent findings indicate that genetic markers may influence bacterial composition in the vagina, which could also vary within populations.[20] In a study by Gorgos *et al.* amongst women presenting to clinics in Seattle and Kenya, no association between BVAB1 and cervicitis was idenitifed.[12] Given the association of each tertile of BVAB1 with cervicitis in this study, the relationship between BVAB1 and cervicitis should be studied further. BVAB1 has been previously identified as contributing to pelvic and genital inflammation, so presence in the vagina may influence cervical inflammation.[21,22] As for *Megasphaera* species, Gorgos *et al*. found a trend towards an association of *Megasphaera* species type 2 with cervicitis in the Seattle cohort, although this did not reach statistical significance. [12] With similar findings from this study, the role of *Megasphaera* species in cervicitis should be evaluated further. *Megasphaera elsdensii* was previously found to induce an inflammatory response and may contribute to cervicitis through this mechanism.[23] The association of *M. indolicus* with cervicitis found in this study also aligns with the Gorgos study.[12] However, this association was not observed in the US participants for this study nor the Kenya-only analysis in the Gorgos *et al*. study, which could be attributed to small sample sizes.

Contrary to the findings of two previous studies that showed the *Lactobacillus* genus or *L. jensenii* detected in cervical fluid samples being associated with a reduced likelihood of cervicitis, this study found that *L. iners* and *L. jensenii* detected in vaginal fluid were associated with an increased detection of cervicitis. [12,13] In this study, *L. iners* was highly prevalent, and there were few samples below the LLOQ. This could have decreased statistical power and made results unreliable. While *L. jensenii* and *L. crispatus* are known to be associated with vaginal health, the relationship of *L. iners* with vaginal health is contested.[24,25,26] Therefore, the association of *L. iners* with cervicitis may indicate that this species contributes to inflammation. As for *L. jensenii*, this is one of the most common species to colonize the vagina among women worldwide along with *L. crispatus*.[27] Further analysis is needed to elucidate whether there is a mechanistic relationship between *L. jensenii* and cervicitis, or if our observed association is due to chance. While *L. crispatus* is considered to be a key marker of vaginal health, there was no association between *L. crispatus* and cervicitis identified in this study, which could be attributed to the low number of visits with *L. crispatus* detection.[25, 26]

Other studies have examined the relationship between vaginal bacteria and cervicitis, but this study had significantly more cervicitis outcomes due to longitudinal follow-up and repeated sampling. A limitation of this study was that some tertiles of bacterial concentrations had relatively few samples and spanned wide ranges. Therefore, the US-only analysis had reduced power, which impacted the ability to detect a significant difference. Further analysis by country is also needed to isolate the relationship of vaginal bacteria with cervicitis from differences in study populations. Additional studies may consider looking at the association of vaginal bacteria and a different definition of cervicitis that includes symptoms.

Conclusion:

Given that BV is associated with cervicitis and a proportion of cervicitis cases have no known etiology, BV-associated species may contribute to cervical inflammation as shown with these findings. While most treatment and diagnosis guidelines for cervicitis target STIs, larger studies identifying which vaginal microbiota taxa are associated with cervicitis could change treatment options or indication for screening for cervicitis.

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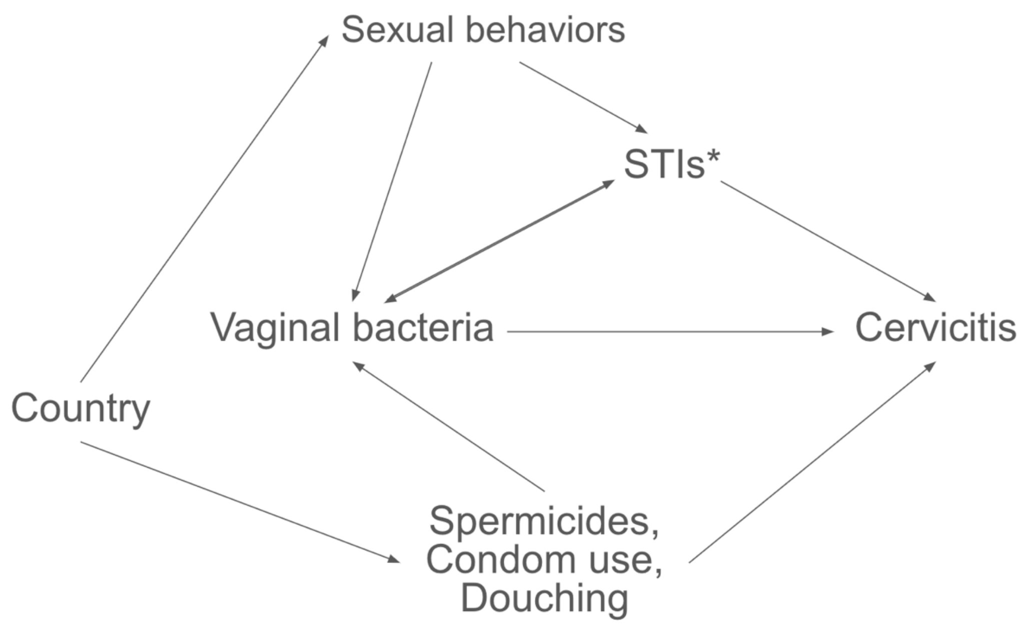
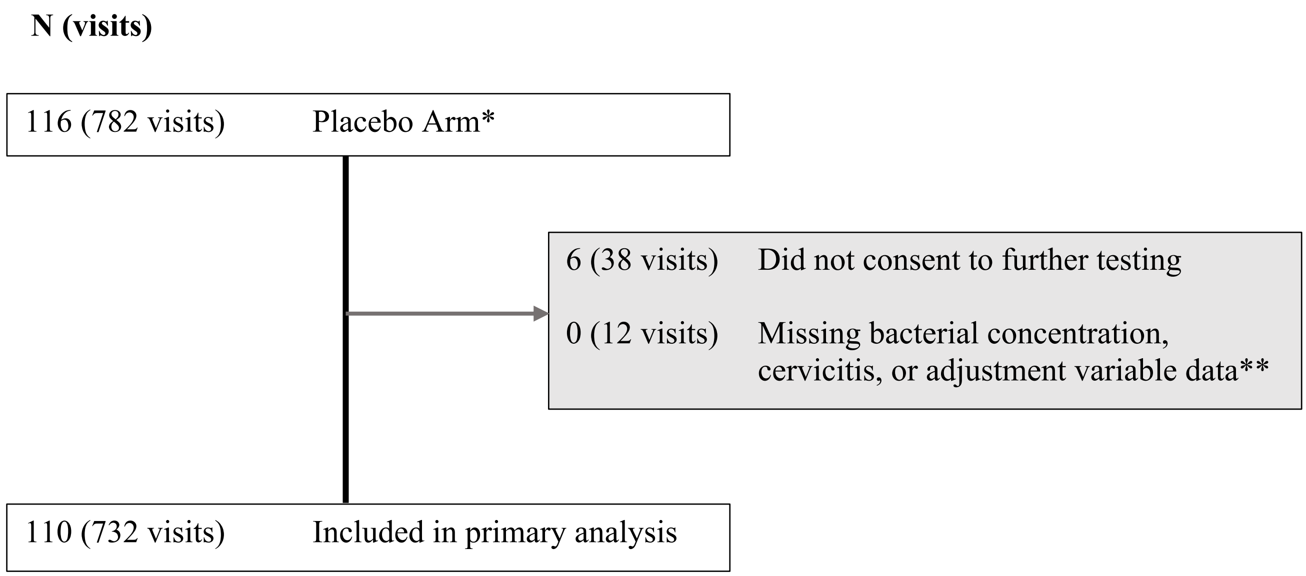
Figure 1. Conceptual Model for Factors influencing the Relationship of Vaginal Bacteria and Cervicitis



Lines in the figure indicate hypothesized relationships between factors.

* Sexually transmitted infections (STIs) include gonorrhea, chalmydia, HSV-2, Trichomoniasis, and *Mycoplasma genitallium*. Gonorrhea and chlamydia have a stronger relationship with cervicitis, but evidence has shown a relationship of cervicitis with the other STIs as well.

Figure 2. Flowchart of Participants included in the secondary analysis of BV-associated Bacteria and Cervicitis



*Even visits only

**Adjustment variables are vaginal washing, condom use, MG, TV, CT, GC

Table 1. Demographic and Clinical Characteristics at Enrollment for Participants Randomized to the Placebo Arm in the PVI trial

Baseline Characteristic	**Placebo arm (N = 110)**		**Cervicitis* (N = 34)**		**No cervicitis** (N = 76)**	
	n or median	% or IQR	n or median	% or IQR	n or median	% or IQR
Demographic factors						
Study Site						
Kenya	84	(76)	10	(29)	74	(97)
US	26	(24)	24	(71)	2	(3)
Age	29	(23-34)	30	(23-35)	29	(24-33)
Clinical factors						
Nugent Score						
Normal (0-3)	50	(45)	13	(38)	37	(49)
Intermediate (4-6)	20	(18)	5	(15)	15	(20)
Bacterial vaginosis (7-10)	40	(36)	16	(47)	24	(32)
Contraceptive Use						
None	16	(15)	5	(15)	11	(14)
Condoms only	30	(27)	7	(21)	23	(30)
Oral contraceptive	12	(11)	5	(15)	7	(9)
Injectable contraceptive	25	(23)	6	(18)	19	(25)

Intrauterine device	10	(9)	4	(12)	6	(8)
Implant	9	(8)	2	(6)	7	(9)
Tubal ligation	5	(5)	4	(12)	1	1
Other	3	(3)	1	(3)	2	(3)
Behavioral Factors						
Vaginal washing in past month	55	(50)	13	(38)	42	(55)
Any vaginal sex in the past week	90	(82)	31	(91)	59	(78)
Among those reporting sex						
No condom use	26	(24)	12	(35)	14	(18)
Intermittent condom use	11	(10)	1	(3)	10	(13)
100% condom use	53	(48)	18	(53)	35	(46)
New sex partner in the past week	23	(21)	4	(12)	19	(25)
Number of vaginal sex acts in the past week	2	(1-4)	2	(1-4)	2	(1-3)
Number of different vaginal sex partners in the past week	1	(1-3)	1	(1-1)	2	(1-3)

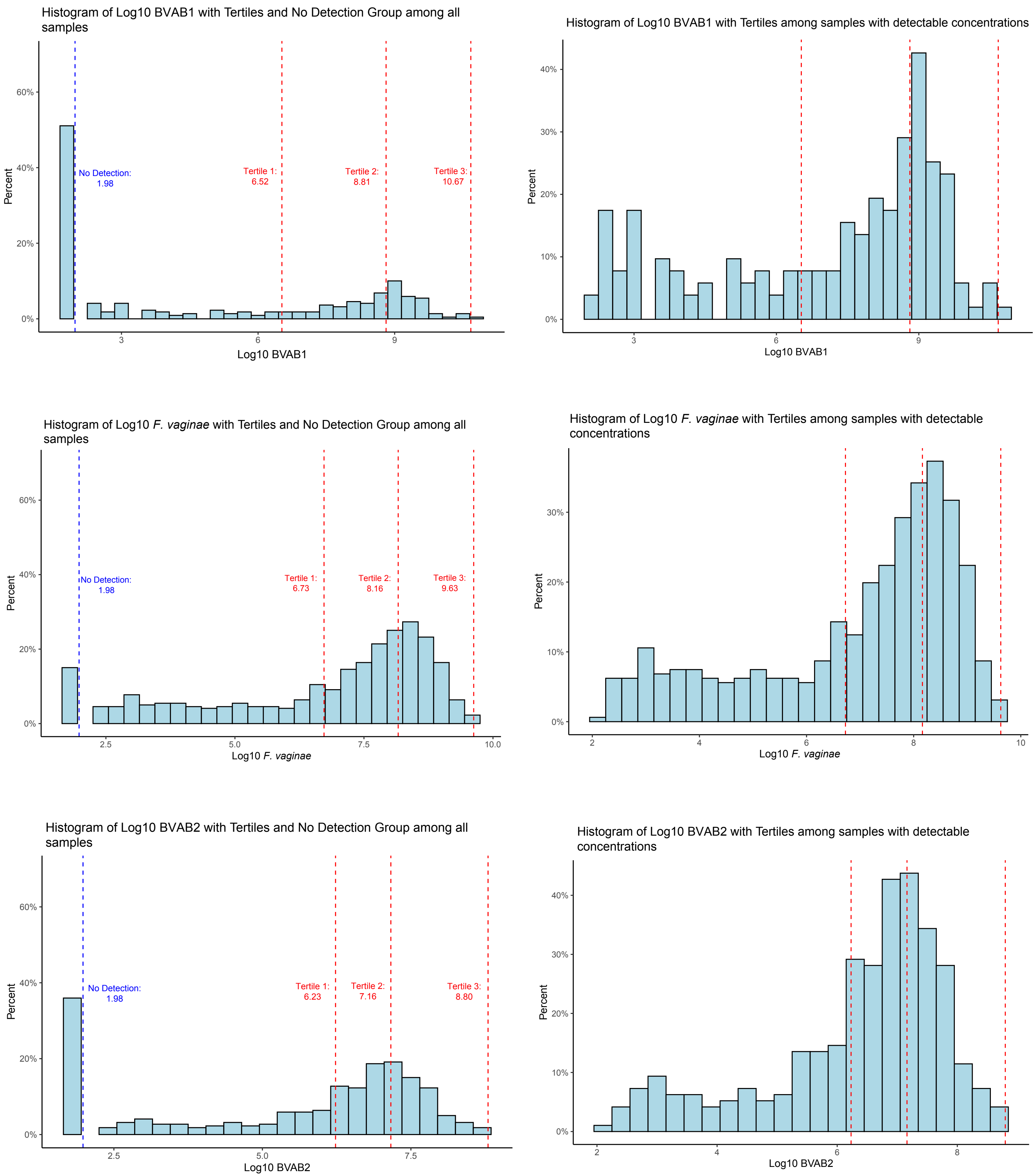
*Among participants who had cervicitis at any time during follow-up
**Among participants who did not have cervicitis at any time during follow-up

Table 2. Number of Individuals with an STI detected by Visit

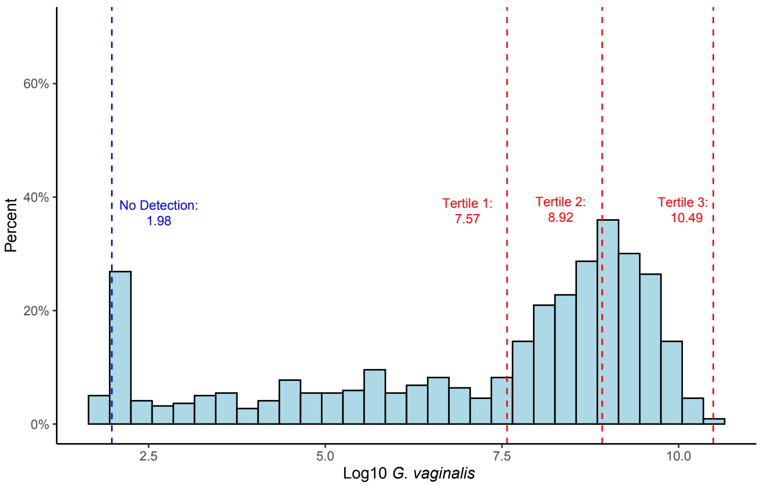
Sexually Transmitted Infections	**Visit 0**		**Visit 2**		**Visit 4**		**Visit 6**		**Visit 8**		**Visit 10**		**Visit 12**	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Gonorrhea	0	(0)	3	(2.7)	3	(2.7)	2	(1.8)	2	(1.8)	2	(1.8)	0	(0)
Chlamydia	8	(7.3)	5	(4.5)	5	(4.5)	5	(4.5)	8	(7.3)	2	(1.8)	5	(4.5)
Trichomoniasis	6	(5.5)	10	(9.1)	10	(9.1)	7	(6.4)	10	(9.1)	10	(9.1)	10*	(9.1)
Mycoplasma genitalium	9	(8.2)	10	(9.1)	18	(16.4)	14	(12.7)	16	(14.5)	16	(14.5)	11	(10.0)

*2 individuals missing TV data for visit 12 after excluding visits missing cervicitis or qPCR data

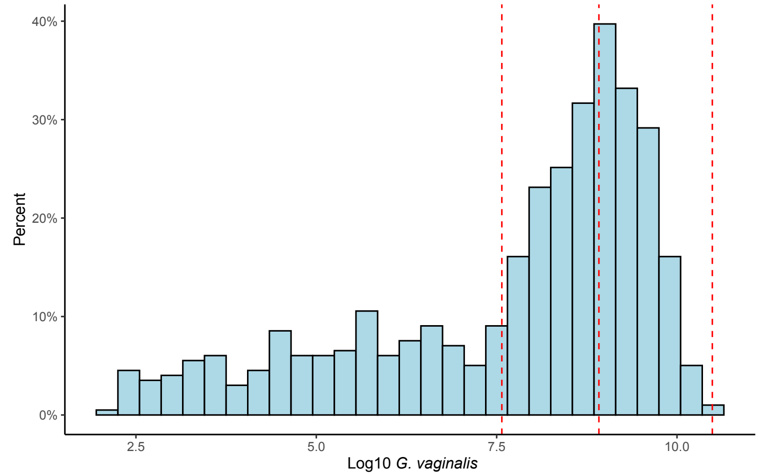
Figure 3. Histograms of Bacterial Concentrations with Tertiles



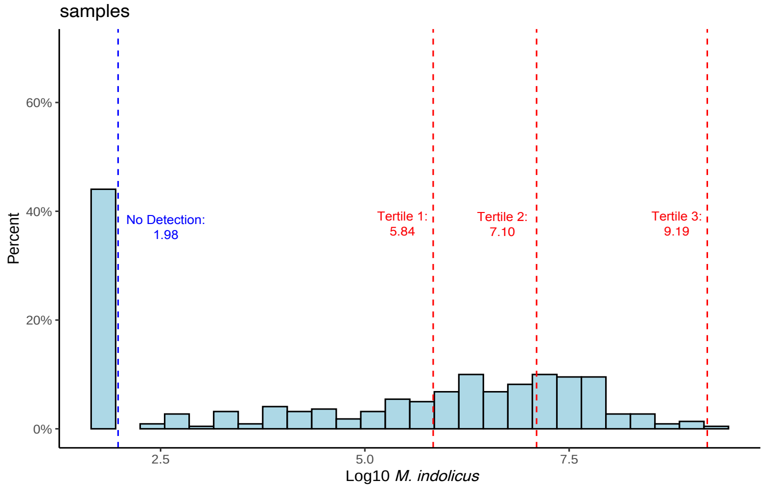
Histogram of Log10 *G. vaginalis* with Tertiles and No Detection Group among all samples



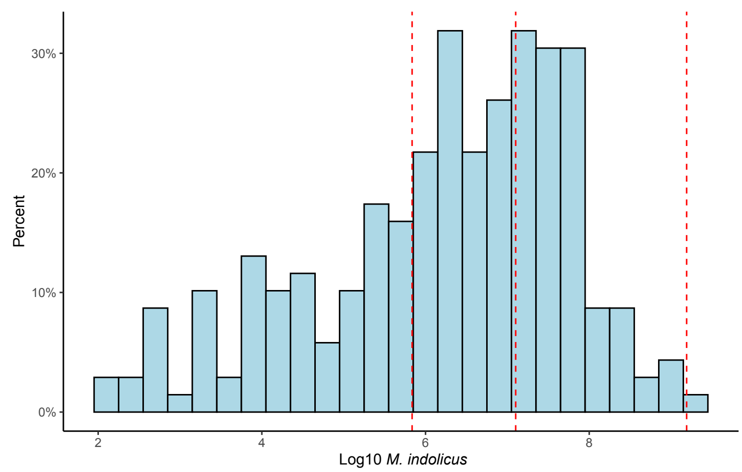
Histogram of Log10 *G. vaginalis* with Tertiles among samples with detectable concentrations



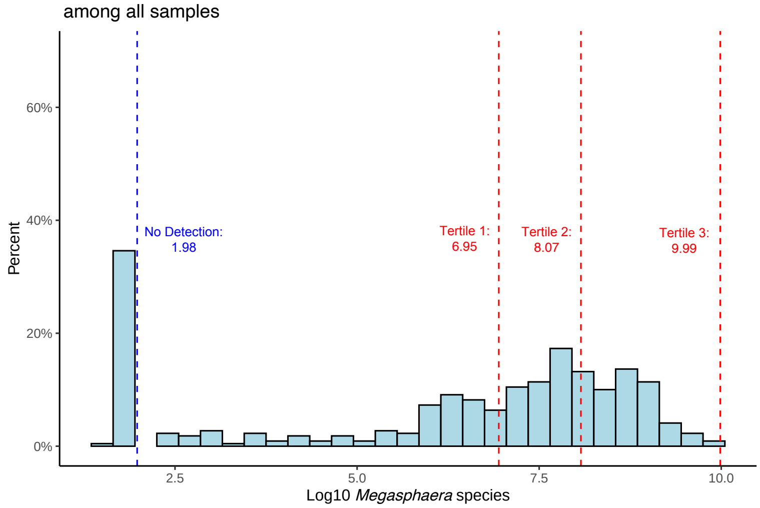
Histogram of Log10 *M. indolicus* with Tertiles and No Detection Group among all samples



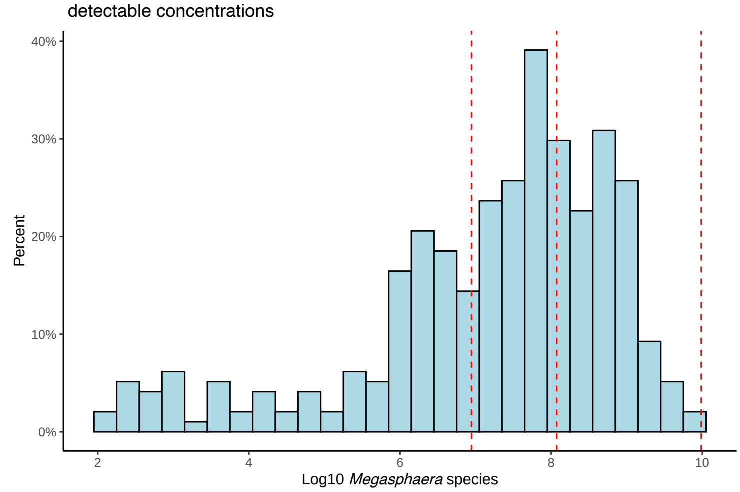
Histogram of Log10 *M. indolicus* with Tertiles among samples with detectable concentrations

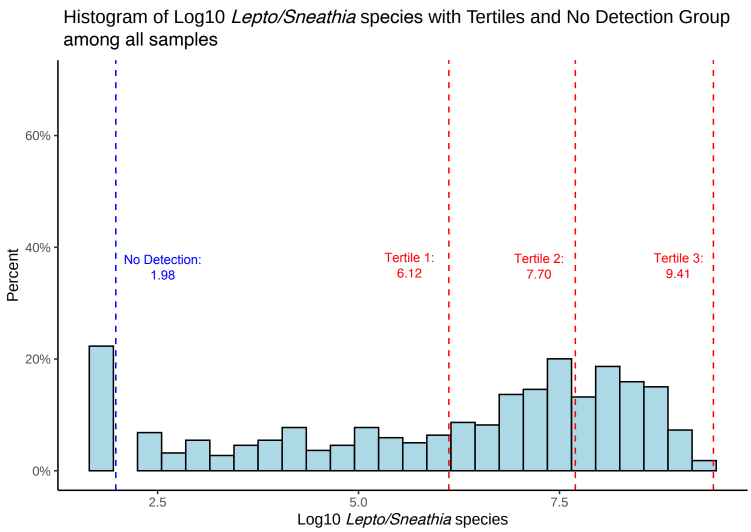


Histogram of Log10 *Megasphaera* species with Tertiles and No Detection Group among all samples

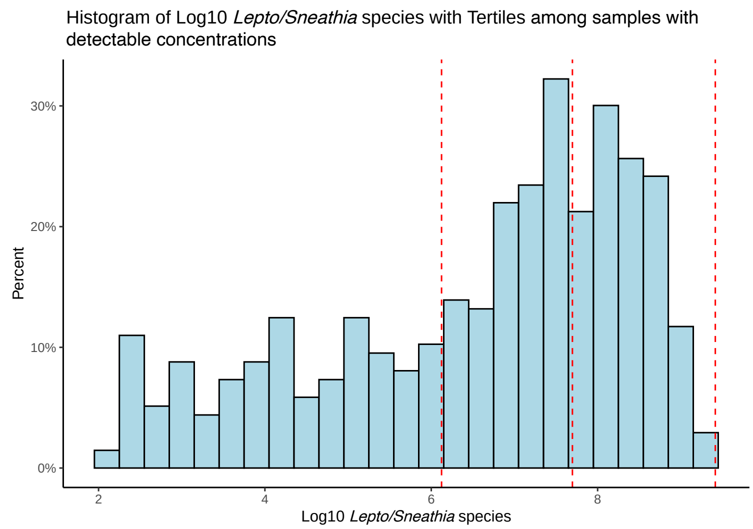


Histogram of Log10 *Megasphaera* species with Tertiles among samples with detectable concentrations

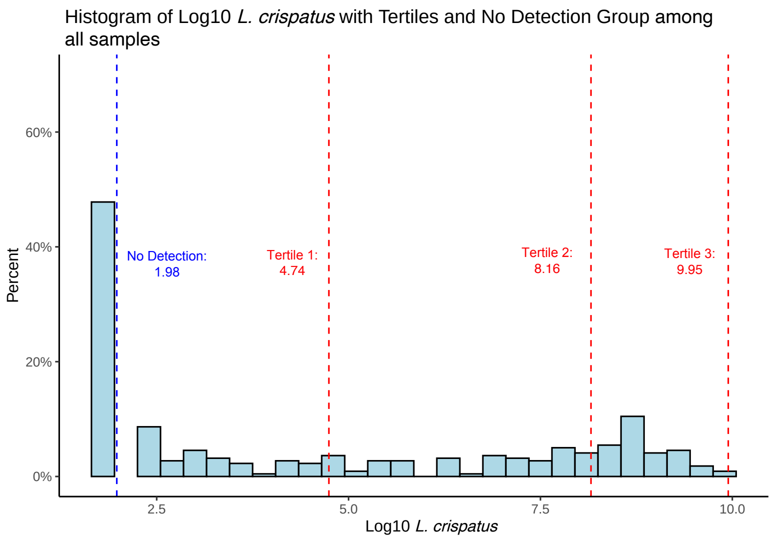


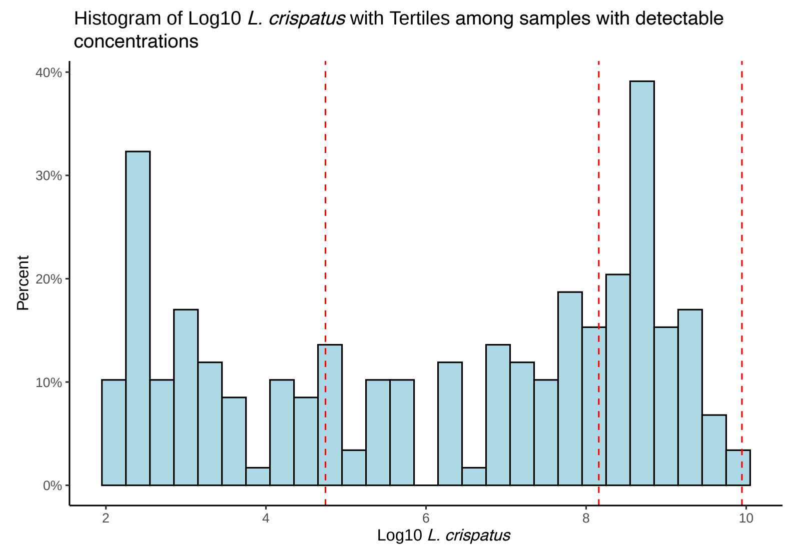


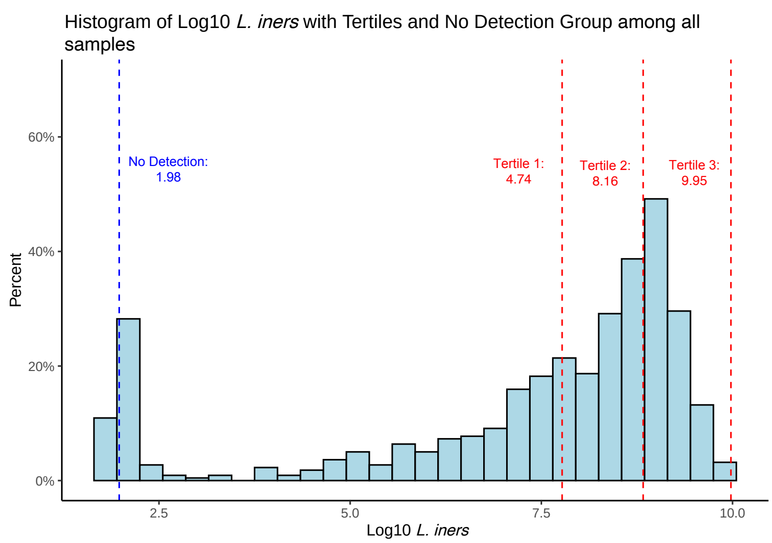
Histogram of Log10 *Lepto/Sneathia* species with Tertiles and No Detection Group among all samples
Percent
60%
40%
20%
0%
No Detection: 1.98
Tertile 1: 6.12
Tertile 2: 7.70
Tertile 3: 9.41
2.5
5.0
7.5
Log10 *Lepto/Sneathia* species

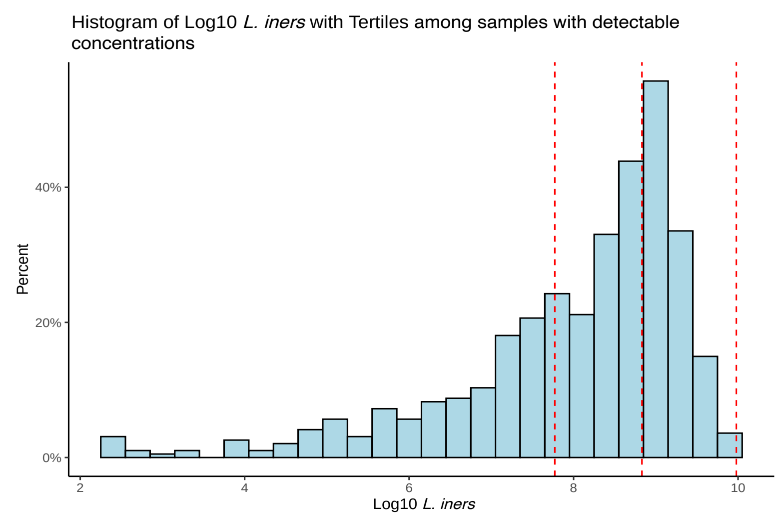


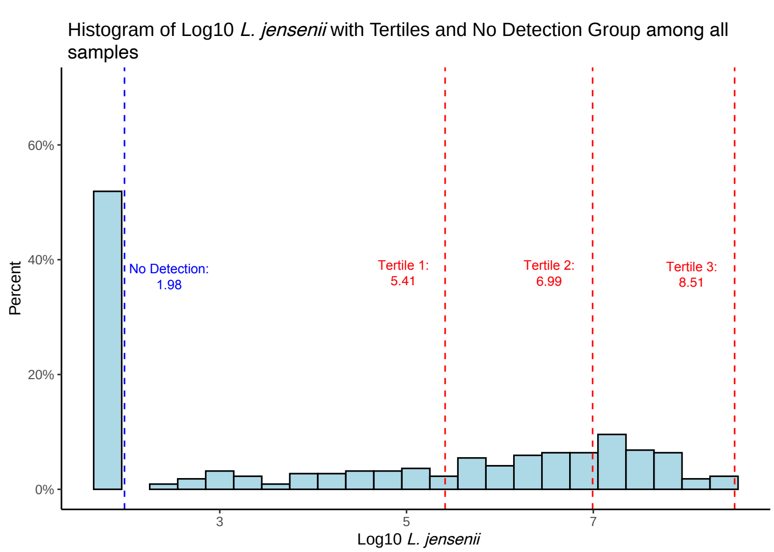
Histogram of Log10 *Lepto/Sneathia* species with Tertiles among samples with detectable concentrations
Percent
30%
20%
10%
0%
2
4
6
8
Log10 *Lepto/Sneathia* species


Histogram of Log10 L. crispatus with Tertiles and No Detection Group among all samples
Percent
Log10 L. crispatus
No Detection: 1.98
Tertile 1: 4.74
Tertile 2: 8.16
Tertile 3: 9.95
0%
20%
40%
60%
2.5
5.0
7.5
10.0


Histogram of Log10 L. crispatus with Tertiles among samples with detectable concentrations
Percent
Log10 L. crispatus
0%
10%
20%
30%
40%
2
4
6
8
10


Histogram of Log10 L. iners with Tertiles and No Detection Group among all samples
Percent
Log10 L. iners
No Detection: 1.98
Tertile 1: 4.74
Tertile 2: 8.16
Tertile 3: 9.95
0%
20%
40%
60%
2.5
5.0
7.5
10.0


Histogram of Log10 L. iners with Tertiles among samples with detectable concentrations
Percent
Log10 L. iners
0%
20%
40%
2
4
6
8
10


Histogram of Log10 L. jensenii with Tertiles and No Detection Group among all samples
Percent
Log10 L. jensenii
No Detection: 1.98
Tertile 1: 5.41
Tertile 2: 6.99
Tertile 3: 8.51
0%
20%
40%
60%
3
5
7

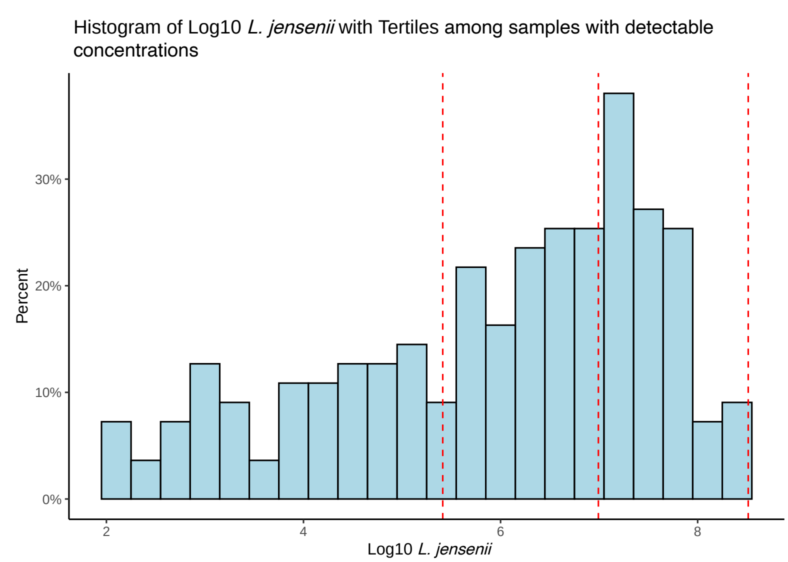

Histogram of Log10 L. jensenii with Tertiles among samples with detectable concentrations
Percent
Log10 L. jensenii
0%
10%
20%
30%
2
4
6
8

Table 3. Number of Visits with Cervicitis Present and Absent by Tertile of each BV-associated Bacterial Species & Prevalence Ratios for Each Species present with Cervicitis

		Detection at visit of diagnosis (N = 732) *				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 119)		Cervicitis absent (N = 613)		Unadjusted	Adjusted**
		n	%	n	%	PR (95% CI)	PR (95% CI)
BVAB1	*ND*	51	(42.9)	509	(83.0)	*Ref*	*Ref*
	First	23	(19.3)	35	(5.7)	**4.35 (2.77, 6.84)**	**4.11 (2.63, 6.43)**
	Second	17	(14.3)	40	(6.5)	**3.27 (1.98, 5.42)**	**3.47 (2.08, 5.78)**
	Third	28	(23.5)	29	(4.7)	**5.39 (3.53, 8.23)**	**4.69 (3.05, 7.22)**
Fannyhessea vaginae	*ND*	28	(23.5)	168	(27.4)	*Ref*	*Ref*
	First	29	(24.4)	150	(24.4)	1.13 (0.70, 1.83)	1.20 (0.74, 1.95)
	Second	26	(21.8)	152	(24.7)	1.02 (0.63, 1.67)	1.17 (0.71, 1.92)
	Third	36	(30.3)	143	(23.3)	1.41 (0.89, 2.21)	1.39 (0.88, 2.22)
BVAB2	*ND*	58	(48.7)	354	(57.7)	*Ref*	*Ref*
	First	12	(10.1)	95	(15.5)	0.80 (0.45, 1.41)	0.83 (0.46, 1.50)
	Second	28	(23.5)	78	(12.7)	**1.88 (1.24, 2.84)**	**1.97 (1.30, 3.01)**
	Third	21	(17.6)	86	(14.0)	1.39 (0.88, 2.20)	1.44 (0.90, 2.29)
	ND	9	(7.6)	60	(9.8)	*Ref*	*Ref*

Gardnerella vaginalis	First	36	(30.3)	185	(30.2)	1.25 (0.64, 2.44)	1.23 (0.62, 2.43)
	Second	25	(21.0)	196	(31.9)	0.87 (0.43, 1.75)	0.92 (0.46, 1.87)
	Third	49	(41.2)	172	(28.1)	1.70 (0.89, 3.26)	1.70 (0.88, 3.31)
Mageeibacillus indolicus	*ND*	65	(54.6)	437	(71.3)	*Ref*	*Ref*
	First	14	(11.8)	63	(10.3)	1.40 (0.83, 2.39)	1.67 (0.97, 2.86)
	Second	18	(15.1)	58	(9.5)	**1.83 (1.13, 2.95)**	**2.01 (1.24, 3.27)**
	Third	22	(18.5)	55	(9.0)	**2.21 (1.42, 3.44)**	**2.07 (1.32, 3.24)**
Megasphaera species	*ND*	53	(44.5)	355	(57.9)	*Ref*	*Ref*
	First	22	(18.5)	86	(14.0)	1.57 (0.99, 2.47)	**1.71 (1.08, 2.71)**
	Second	20	(16.8)	88	(14.4)	1.43 (0.89, 2.29)	**1.62 (1.00, 2.64)**
	Third	24	(20.2)	84	(13.7)	**1.71 (1.10, 2.66)**	**1.64 (1.04, 2.58)**
Leptotrichia/ Sneathia species	*ND*	42	(35.3)	235	(38.3)	*Ref*	*Ref*
	First	22	(18.5)	130	(21.2)	0.95 (0.59, 1.53)	0.93 (0.57, 1.52)
	Second	29	(24.4)	122	(19.9)	1.27 (0.82, 1.96)	1.39 (0.88, 2.18)
	Third	26	(21.8)	126	(20.6)	1.13 (0.72, 1.77)	1.13 (0.71, 1.79)
Nugent score	Normal (0-3)	51	(42.9)	320	(52.2)	*Ref*	*Ref*
	Intermediate (4-6)	11	(9.2)	108	(17.6)	0.67 (0.37, 1.22)	0.73 (0.40, 1.34)
	Bacterial vaginosis (7-10)	57	(47.9)	185	(30.2)	**1.71 (1.21, 2.42)**	**1.75 (1.22, 2.50)**

*This includes all visits regardless of STI detection.
**Adjusted for STI detection (CT, NG, TV, MG), condom use (categorical), and vaginal washing in the past month (binary)

Table 3A. Number of Visits with Cervicitis Present and Absent by Tertile of each Lactobacillus Species & Prevalence Ratios for Each Species present with Cervicitis

		Detection at visit of diagnosis (N = 732) *				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 119)		Cervicitis absent (N = 613)		Unadjusted	Adjusted**
		n	%	n	%	PR (95% CI)	PR (95% CI)
L. crispatus	ND	92	(77.3)	444	(72.4)	*Ref*	*Ref*
	First	12	(10.1)	54	(8.8)	1.06 (0.61, 1.84)	1.25 (0.71, 2.20)
	Second	7	(5.9)	57	(9.3)	0.64 (0.31, 1.29)	0.57 (0.28, 1.17)
	Third	8	(6.7)	58	(9.4)	0.71 (0.36, 1.37)	0.75 (0.38, 1.47)
L. iners	ND	7	(5.9)	79	(12.9)	*Ref*	*Ref*
	First	29	(24.4)	187	(30.5)	1.65 (0.77, 3.52)	1.95 (0.91, 4.18)
	Second	36	(30.3)	178	(29.0)	2.07 (0.98, 4.34)	2.04 (0.98, 4.26)
	Third	47	(39.5)	169	(27.6)	2.67 (1.29, 5.54)	**2.81 (1.36, 5.80)**
L. jensenii	ND	73	(61.3)	475	(77.5)	*Ref*	*Ref*
	First	12	(10.1)	49	(8.0)	1.48 (0.84, 2.59)	1.47 (0.84, 2.58)
	Second	13	(10.9)	49	(8.0)	1.57 (0.92, 2.70)	1.55 (0.89, 2.68)
	Third	21	(17.6)	40	(6.5)	**2.58 (1.66, 4.03)**	**2.71 (1.72, 4.28)**

Table 4. Number of Visits with Cervicitis Present and Absent by Tertile of each BV-associated Bacterial Species & Prevalence Ratios for Each Species present with Cervicitis among US participants

		Detection at visit of diagnosis (N = 169) *				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 108)		Cervicitis absent (N = 61)		Unadjusted	Adjusted**
		n	%	n	%	PR (95% CI)	PR (95% CI)
BVAB1	*ND*	41	(38.0)	28	(45.9)	*Ref*	*Ref*
	First	21	(19.4)	12	(19.7)	1.07 (0.78, 1.47)	1.11 (0.80, 1.55)
	Second	23	(21.3)	11	(18.0)	1.14 (0.83, 1.55)	1.17 (0.84, 1.63)
	Third	23	(21.3)	10	(16.4)	1.17 (0.86, 1.60)	1.13 (0.82, 1.56)
Fannyhessea vaginae	*ND*	23	(21.3)	14	(23.0)	*Ref*	*Ref*
	First	27	(25.0)	17	(27.9)	0.99 (0.70, 1.38)	1.06 (0.74, 1.51)
	Second	24	(22.2)	20	(32.8)	0.88 (0.62, 1.24)	0.89 (0.62, 1.28)
	Third	34	(31.5)	10	(16.4)	1.24 (0.90, 1.71)	1.29 (0.71, 1.80)
BVAB2	*ND*	50	(46.3)	26	(42.6)	*Ref*	*Ref*
	First	12	(11.1)	19	(31.1)	**0.59 (0.40, 0.86)**	**0.59 (0.40, 0.88)**
	Second	22	(20.4)	9	(14.8)	1.08 (0.80, 1.46)	1.09 (0.79, 1.50)
	Third	24	(22.2)	7	(11.5)	1.18 (0.88, 1.58)	1.17 (0.85, 1.59)
Gardnerella vaginalis	*ND*	8	(7.4)	5	(8.2)	*Ref*	*Ref*

	First	33	(30.6)	19	(31.1)	1.03 (0.64, 1.65)	1.07 (0.66, 1.75)
	Second	28	(25.9)	23	(37.7)	0.89 (0.55, 1.44)	0.95 (0.58, 1.56)
	Third	39	(36.1)	14	(23.0)	1.20 (0.75, 1.90)	1.23 (0.70, 1.77)
Mageeibacillus indolicus	*ND*	57	(52.8)	34	(55.7)	*Ref*	*Ref*
	First	14	(13.0)	12	(19.7)	0.86 (0.60, 1.23)	0.87 (0.58, 1.31)
	Second	19	(17.6)	7	(11.5)	1.17 (0.85, 1.60)	1.16 (0.83, 1.63)
	Third	18	(16.7)	8	(13.1)	1.11 (0.80, 1.53)	1.08 (0.78, 2.05)
Megasphaera species	*ND*	45	(41.7)	30	(49.2)	*Ref*	*Ref*
	First	19	(17.6)	13	(21.3)	0.99 (0.71, 1.37)	1.02 (0.73, 1.44)
	Second	18	(16.7)	11	(18.0)	1.03 (0.74, 1.44)	1.00 (0.71, 1.42)
	Third	26	(24.1)	7	(11.5)	1.31 (0.98, 1.76)	1.32 (0.96, 1.83)
Leptotrichia/ Sneathia species	*ND*	38	(35.2)	24	(39.3)	*Ref*	*Ref*
	First	21	(19.4)	15	(24.6)	0.95 (0.69, 1.32)	1.00 (0.71, 1.41)
	Second	23	(21.3)	12	(19.7)	1.07 (0.78, 1.47)	1.08 (0.77, 1.52)
	Third	26	(26.9)	10	(16.4)	1.18 (0.87, 1.60)	1.20 (0.87, 1.66)
Nugent score	Normal (0-3)	45	(41.7)	28	(45.9)	*Ref*	*Ref*
	Intermediate (4-6)	7	(6.5)	11	(18.0)	0.63 (0.39, 1.02)	0.62 (0.37, 1.03)
	Bacterial vaginosis (7-10)	56	(51.9)	22	(36.1)	1.16 (0.92, 1.48)	1.16 (0.90, 1.50)

*This includes all visits regardless of STI detection.

**Adjusted for STI detection (CT, NG, TV, MG), condom use (categorical), and vaginal washing in the past month (binary)

Table 4A. United States - Number of Visits with Cervicitis Present and Absent by Tertile of each Lactobacillus Species & Prevalence Ratios for Each Species present with Cervicitis

		Detection at visit of diagnosis (N = 169) *				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 108)		Cervicitis absent (N = 61)		Unadjusted	Adjusted**
		n	%	n	%	PR (95% CI)	PR (95% CI)
L. crispatus	ND	86	(79.6)	37	(60.7)	*Ref*	*Ref*
	First	8	(7.4)	7	(11.5)	0.76 (0.49, 1.18)	0.78 (0.49, 1.24)
	Second	13	(12.0)	17	(27.9)	**0.62 (0.43, 0.88)**	**0.60 (0.42, 0.88)**
	Third	1	(0.9)	0	(0)	1.43 (0.43, 4.74)	1.20 (0.72, 1.81)
L. iners	ND	7	(6.5)	1	(1.6)	*Ref*	*Ref*
	First	31	(28.7)	23	(37.7)	0.67 (0.40, 1.08)	0.66 (0.39, 1.13)
	Second	39	(36.1)	14	(30.0)	0.84 (0.52, 1.37)	0.86 (0.51, 1.45)
	Third	31	(28.7)	23	(37.7)	0.67 (0.40, 1.08)	0.69 (0.78, 1.99)
L. jensenii	ND	66	(61.1)	44	(72.1)	*Ref*	*Ref*
	First	17	(15.7)	3	(4.9)	**1.42 (1.02, 1.96)**	**1.47 (1.04, 2.08)**
	Second	10	(9.3)	9	(14.8)	0.88 (0.59, 1.31)	0.86 (0.56, 1.30)
	Third	15	(13.9)	5	(8.2)	1.25 (0.89, 1.76)	1.23 (0.85, 1.78)

Table 5. Number of Visits with Cervicitis Present and Absent by Tertile of each BV-associated Bacterial Species & Prevalence Ratios for Each Species present with Cervicitis and Excluding Visits with an STI detected (CT, GC, TV or MG)

		Detection at visit of diagnosis (N = 543)				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 97)		Cervicitis absent (N = 446)		Unadjusted	Adjusted*
		n	%	n	%	PR (95% CI)	PR (95% CI)
BVAB1	*ND*	44	(45.4)	382	(85.7)	*Ref*	*Ref*
	First	19	(19.6)	20	(4.5)	**4.72 (2.89, 7.69)**	**4.40 (2.69, 7.19)**
	Second	13	(13.4)	26	(5.8)	**3.23 (1.84, 5.67)**	**3.14 (1.79, 5.52)**
	Third	21	(21.6)	18	(4.0)	**5.21 (3.25, 8.37)**	**4.28 (2.63, 6.99)**
Fannyhessea vaginae	*ND*	27	(27.8)	143	(32.1)	*Ref*	*Ref*
	First	24	(24.7)	100	(22.4)	1.22 (0.74, 2.01)	1.20 (0.72, 1.99)
	Second	22	(22.7)	104	(23.3)	1.10 (0.66, 1.83)	1.16 (0.69, 1.96)
	Third	24	(24.7)	99	(22.2)	1.23 (0.75, 2.03)	1.10 (0.67, 1.83)
BVAB2	*ND*	51	(52.6)	284	(63.7)	*Ref*	*Ref*
	First	12	(12.4)	57	(12.8)	1.14 (0.64, 2.02)	1.15 (0.64, 2.05)
	Second	20	(20.6)	49	(11.0)	**1.90 (1.19, 3.05)**	**1.80 (1.12, 2.89)**
	Third	14	(14.4)	56	(12.6)	1.31 (0.77, 2.25)	1.29 (0.75, 2.23)
	ND	9	(9.3)	57	(12.8)	*Ref*	*Ref*

Gardnerella vaginalis	First	30	(30.9)	129	(28.9)	1.38 (0.70, 2.72)	1.21 (0.60, 2.41)
	Second	21	(21.6)	138	(30.9)	0.97 (0.48, 1.97)	0.93 (0.45, 1.89)
	Third	37	(38.1)	122	(27.4)	1.71 (0.88, 3.31)	1.47 (0.75, 2.87)
Mageeibacillus indolicus	*ND*	57	(58.8)	336	(75.3)	*Ref*	*Ref*
	First	10	(10.3)	40	(9.0)	1.38 (0.75, 2.54)	1.60 (0.87, 2.96)
	Second	12	(12.4)	38	(8.5)	1.65 (0.94, 2.92)	1.69 (0.96, 2.96)
	Third	18	(18.6)	32	(7.2)	**2.48 (1.53, 4.02)**	**2.10 (1.29, 3.42)**
Megasphaera species	*ND*	46	(47.4)	279	(62.6)	*Ref*	*Ref*
	First	23	(23.7)	50	(11.2)	**2.23 (1.41, 3.51)**	**2.27 (1.43, 3.59)**
	Second	10	(10.3)	62	(13.9)	0.98 (0.53, 1.83)	1.13 (0.60, 2.12)
	Third	18	(18.6)	55	(12.3)	**1.74 (1.06, 2.86)**	1.59 (0.96, 2.64)
Leptotrichia/ Sneathia species	*ND*	39	(40.2)	204	(45.7)	*Ref*	*Ref*
	First	16	(16.5)	84	(18.8)	1.00 (0.59, 1.69)	0.96 (0.56, 1.64)
	Second	23	(23.7)	77	(17.3)	1.43 (0.90, 2.29)	1.43 (0.88, 2.30)
	Third	19	(19.6)	81	(18.2)	1.18 (0.72, 1.95)	1.09 (0.66, 1.81)
Nugent score	Normal (0-3)	46	(47.4)	258	(57.8)	*Ref*	*Ref*
	Intermediate (4-6)	8	(8.2)	66	(14.8)	0.71 (0.36, 1.41)	0.71 (0.36, 1.41)
	Bacterial vaginosis (7-10)	43	(44.3)	122	(27.4)	**1.72 (1.18, 2.51)**	**1.63 (1.11, 2.39)**

*Adjusted for condom use (categorical) and vaginal washing in the past month (binary)

Table 5A. STIs Removed - Number of Visits with Cervicitis Present and Absent by Tertile of each Lactobacillus Species & Prevalence Ratios for Each Species present with Cervicitis

		Detection at visit of diagnosis (N = 543)				Prevalence Ratios for Each Species with Cervicitis Present	
Bacteria	Tertile	Cervicitis present (N = 97)		Cervicitis absent (N = 446)		Unadjusted	Adjusted*
		n	%	n	%	PR (95% CI)	PR (95% CI)
L. crispatus	ND	76	(78.4)	310	(69.5)	*Ref*	*Ref*
	First	9	(9.3)	43	(9.6)	0.88 (0.47, 1.65)	1.01 (0.54, 1.91)
	Second	5	(5.2)	48	(10.8)	0.48 (0.21, 1.09)	0.47 (0.21, 1.06)
	Third	7	(7.2)	45	(10.1)	0.68 (0.34, 1.38)	0.76 (0.37, 1.54)
L. iners	ND	7	(7.2)	60	(13.5)	*Ref*	*Ref*
	First	23	(23.7)	136	(30.5)	1.38 (0.64, 2.99)	1.63 (0.75, 3.53)
	Second	32	(33.0)	126	(28.3)	1.94 (0.92, 4.08)	2.02 (0.97, 4.23)
	Third	35	(36.1)	124	(27.8)	**2.11 (1.01, 4.41)**	**2.40 (1.15, 5.00)**
L. jensenii	ND	58	(59.8)	336	(75.3)	*Ref*	*Ref*
	First	13	(13.4)	37	(8.3)	**1.77 (1.02, 3.05)**	**1.78 (1.03, 3.07)**
	Second	10	(10.3)	39	(8.7)	1.39 (0.75, 2.55)	1.39 (0.76, 2.55)
	Third	16	(16.5)	34	(7.6)	**2.17 (1.31, 3.60)**	**2.68 (1.60, 4.48)**