Specific Vaginal Bacteria Are Associated With an Increased Risk of *Trichomonas vaginalis* Acquisition in Women

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Background. While bacterial vaginosis has been associated with an increased risk of *Trichomonas vaginalis* (TV) acquisition, it is unknown whether other characteristics of the vaginal microbiota, including the presence of key bacterial species, influence a woman's risk of TV acquisition.

Methods. The vaginal microbiota before 25 unique episodes of TV infection involving 18 women was compared to that of 50 controls who remained uninfected. TV was detected by transcription-mediated amplification. Vaginal microbiota were quantified using broad-range polymerase chain reaction analysis and taxon-specific quantitative PCR of the 16S ribosomal RNA gene.

Results. TV acquisition was significantly associated with the presence of *Prevotella amnii* (risk ratio [RR], 2.21; 95% confidence interval [CI], 1.12–4.38; *P* = .02) and *Sneathia sanguinegens* (RR, 2.58; 95% CI, 1.00–6.62; *P* = .049). When adjusted for menstrual phase, the association between *P. amnii* and TV acquisition remained similar (adjusted RR, 2.11; 95% CI, 1.03–4.33; *P* = .04), but the association between *S. sanguinegens* and TV acquisition was attenuated (adjusted RR, 2.31; 95% CI, .86–6.23; *P* = .10).

Conclusions. Key vaginal bacterial species may contribute to the susceptibility to TV acquisition. Understanding how these bacterial species increase a woman's risk of TV acquisition could help to guide the development of novel strategies to reduce women's risk of TV infection.

Keywords. *Trichomonas vaginalis*; vaginal microbiome; polymerase chain reaction; bacterial diversity.

Trichomonas vaginalis (TV) infection is the most prevalent treatable sexually transmitted infection (STI) worldwide, with a higher burden of disease among women as compared to men [1]. Trichomoniasis has been associated with an increased risk of multiple adverse outcomes in women, including pelvic inflammatory disease, preterm labor, and human immunodeficiency virus (HIV) infection [2–11]. Thus, there is a need for an improved understanding of factors associated with TV acquisition in women.

Prospective studies have demonstrated that the baseline presence of intermediate vaginal microbiota or bacterial vaginosis (BV), as defined by Nugent's criteria, is associated with increased risk of TV acquisition [12–15]. Recent cross-sectional studies suggest that specific vaginal bacteria may be driving the association between nonoptimal vaginal microbiota and increased risk of TV infection. For example, the presence of a novel *Mycoplasma* species, *Candidatus* Mycoplasma girerdii, in the vaginal microbiota is independently associated with prevalent TV [16, 17]. It is unknown whether other vaginal bacterial species or overall microbiota diversity contribute to an increased risk of TV acquisition. To explore the associations between vaginal microbiota diversity, specific vaginal bacteria, and women's risk of TV acquisition, we conducted a nested case-control study that compared women who acquired TV infection to women who remained uninfected.

METHODS

Study Population and Procedures

Case participants and controls were selected from the population of HIV-1–seronegative women enrolled in the Mombasa Cohort, a prospective open cohort study of women who exchange sex for cash or in-kind payment. Visits selected for this analysis occurred between September 2012 and May 2014. A detailed description of Mombasa Cohort procedures has been published [18]. Briefly, cohort participants completed monthly structured interviews to assess sexual and reproductive risk factors, underwent HIV-1 screening, and completed a physical examination, including a pelvic speculum examination to collect specimens for STI screening and characterization of bacteria by DNA amplification techniques.

Swab collection was avoided during menses, owing to greater variability of vaginal bacterial concentrations [19]. The start date of each woman's last menstrual period was recorded at each visit. For women whose last menstrual period was 0–28 days before their visit, the cycle phase was estimated on the basis of the assumption of a 28-day cycle. Women whose last menstrual period was >84 days before their visit were classified as having clinical amenorrhea, while those whose last menstrual period was ≥365 days before their visit were classified as menopausal. Women whose last menstrual period was 29–84 days before their visit were classified as "other." In the analyses adjusted for menstrual phase, participants classified as amenorrheic, menopausal, pregnant, or receiving depot medroxyprogesterone acetate were combined into 1 category and characterized as having "amenorrhea" (ie, no estrogen/progesterone hormonal cycle).

Participants were asked to return for monthly follow-up visits. All participants provided written informed consent. This study was approved by the human subjects committees at the University of Illinois at Chicago (Chicago, IL), the University of Washington (Seattle, WA), and Kenyatta National Hospital (Nairobi, Kenya).

Bacterial vaginosis was assessed by Gram stain, using the criteria of Nugent and Hillier [14]. Separate swabs for wet mount analysis of vaginal fluid in saline (hereafter, "wet mount") and for transcription-mediated amplification (TMA; Hologic, Bedford, MA) were collected monthly for TV detection. Wet mount was performed monthly, and TMA was performed quarterly. Untested swabs were stored at −80°C. When TV was diagnosed on the basis of wet mount findings, the TMA swab from that date was tested to confirm TV diagnosis. Stored TMA swabs from preceding months were then back-tested to identify the last visit before TV acquisition. Cases were defined as women who had a visit during which TV was detected and a visit 30–60 days earlier during which they were confirmed to be TV negative (hereafter, the "pre-TV visit"). Participants could contribute multiple cases of TV infection, provided that there was an intervening negative TV TMA result. For each case, incidence density sampling was performed to identify 2 controls who were not infected with TV on a visit date within 7 days of their matched case. Cases and controls were excluded if they had received antibiotics within 30 days of their visit date.

Characterization of the Vaginal Microbiota

Polyethylene terephthalate swabs used to collect vaginal fluid specimens were stored at −80°C and transported on dry ice to the Fred Hutchinson Cancer Research Center (Seattle, WA) for analysis. All laboratory personnel were blinded to case-control status. A MoBio BiOstic Bacteremia DNA Isolation kit (Carlsbad, CA) was used to extract and purify DNA. This protocol uses bead beating and chaotropic lysis to break apart bacterial cells and recover DNA that is free of PCR inhibitors. Blank swabs that did not contact a mucosal surface were included as negative controls to monitor for DNA contamination from extraction reagents and swabs with vaginal specimens. All samples were subjected to 2 quality control assays. First, samples were evaluated for polymerase chain reaction (PCR) inhibitors, using exogenously added DNA (aequorin plasmid) and aequorin gene quantitative PCR (qPCR) analysis [20]. Second, a broad-range 16S ribosomal RNA gene PCR assay was used to measure the total bacterial concentrations in each sample. Broad-range PCR analysis and sequencing of the V3-V4 region were then performed using the Illumina MiSeq platform to determine the relative abundance of 16S ribosomal RNA gene sequence reads from the vaginal bacterial species that were present [21].

Negative controls included any contaminating DNA from blank swabs and sterile water. These controls were processed in the same way as vaginal samples, allowing an assessment of contamination from DNA extraction or PCR reagents. DADA2 was used for quality control of and to filter, pair, and cluster the amplicon reads [22]. Sequence reads were classified using the *pplacer* phylogenetic placement tool and a curated reference set of vaginal bacterial sequences [23, 24]. Reads originating from contaminants in negative controls were removed. Sequence reads have been deposited to the National Center for Biotechnology Information Short Read Archive (PRJNA548797). Eight bacterial taxa were selected, as detailed below, for qPCR analysis using TaqMan-based assays previously available in the laboratory [25–27].

Statistical Analysis

To assess associations between the vaginal microbiota and incident TV infection, analyses used data from the pre-TV visit (cases) and from the matched visit for controls. First, the association between the total vaginal bacterial concentration and TV acquisition was determined. Because the data were highly skewed, the total vaginal bacterial concentration was $\log_{10}$ transformed. To account for some participants contributing multiple cases of TV infection, generalized estimating equations (GEEs) with a Poisson link and independent correlation structure were used to calculate relative risks (RRs) and 95% confidence intervals (CIs).

Measures of vaginal bacterial diversity and richness were compared between cases and controls, using the Shannon diversity index (SDI) and Chao-1 richness estimator, respectively. Only the 18 unique cases of TV and their 36 matched controls were used to measure these associations by log-linear regression. The SDI is a measure of diversity that accounts for both the number of different taxa and the evenness of their distribution [22]. The Chao-1 index provides an estimate of community richness, reflecting the number of different taxa [23]. Statistics of ecological diversity and richness were calculated separately for each sample by use of read numbers classified to their most specific taxonomic rank, using implementations of the R

microbiome package (available at: http://microbiome.github.io/microbiome/).

To identify potentially important species for further exploration using qPCR assays, score statistics were calculated for each bacterial taxon detected by the Illumina MiSeq system, using backward stepwise regression. Taxa were then ranked in descending order on the basis of score statistics. Starting with the taxon with the highest score statistic, estimates for the odds of TV acquisition were calculated for each ranked taxon by using unadjusted logistic regression and were not adjusted for multiple comparisons. Based on the availability of qPCR assays in the laboratory, taxa were selected for qPCR analysis if they were associated with regression estimates with a *P* value of ≤.20 (ie, *Lactobacillus crispatus*, *Prevotella amnii,* and *Sneathia* species) or if the published literature revealed that they had known or suspected associations with TV infection (ie, BV-associated bacterium 1 [BVAB1], *Dialister propionicifaciens*, *Sneathia sanguinegens*, *Mageeibacillus indolicus,* and *Prevotella* organisms) [16, 24]. Each bacterial taxon was analyzed as a dichotomous exposure (detectable vs undetectable) owing to the small sample size and multiple swab samples with undetectable concentrations. The only exception was the *Prevotella* genus assay, which had few undetectable samples and so was dichotomously modeled around the median concentration present across samples. GEEs with a Poisson link and exchangeable correlation structure were used to test the hypothesis that detectable levels of the targeted bacteria were associated with an increased or decreased risk of TV acquisition.

Sensitivity analyses were performed on the GEE models by using only the 18 unique TV cases and their 36 matched controls. An additional sensitivity analysis compared concentrations of the 8 selected bacterial taxa in cases versus controls, using Mann-Whitney *U* tests. For bacterial concentrations that were undetected in a sample, the values were set to the lower limit of detection of the qPCR assays.

Potential confounders of the association between vaginal bacterial concentrations and TV acquisition were selected on the basis of biologically plausible confounding effects. The pre-TV visit variables that were analyzed included age (continuous), contraceptive method (categorical), number of sex partners in the past week (continuous), frequency of sex in the past week (continuous), self-reported unprotected sex in the past week (categorical), menstrual phase (categorical), presence of BV (categorical), and presence of vaginal yeast (categorical). GEEs with a Poisson link and independent correlation structure were again used to explore the relationship between these potential confounders and TV acquisition for all 25 cases and 50 controls. Potential confounders for which the association with TV acquisition had a *P* value of ≤ .2 on univariate GEE analysis were considered for inclusion in a forward stepwise model-building approach. Where variables were collinear, the variable with the stronger association with TV acquisition was retained.

For all associations, the significance level was set at a *P* value of < .05. Analyses were performed using IBM SPSS Statistics, version 23 (IBM, Armonk, NY), and Stata, version 13 (StataCorp, College Station, TX).

RESULTS

There were 25 episodes of TV infection among 18 participants. These were matched to 50 controls who did not acquire TV during a similar exposure interval. Four of 18 participants with TV contributed >1 case of TV infection (1 participant contributed 4 cases, 1 participant contributed 3 cases, and 2 participants contributed 2 cases each). The mean age of participants (±SD) was 36.2 ± 9.2 years (Table 1), with no difference in age between women who acquired TV and those who did not. Women who acquired TV were less likely to be receiving hormonal contraception (3 [12%] vs 17 [34%]; *P* = .09) and less likely to be amenorrheic (5 [20%] vs 22 [44%]; *P* = .04) at their pre-TV visit. Gonorrhea was associated with an increased risk of TV acquisition, but this was based on only 1 episode of gonorrhea among the TV cases (1 [4.0%] vs 0; *P* < .001). *Chlamydia trachomatis* was not detected in any participants. The prevalence of intermediate microbiota or BV was similar among women who did and those who did not acquire TV (10 [40%] vs 17 [34%]; *P* = .62, by the χ^2 test). Similarly, there was no association between self-reported unprotected sex among women who acquired TV as compared to those who did not (6 [24%] vs 8 [16%]; *P* = .4). Of the pre-TV visit variables analyzed as potential confounders, only amenorrhea (RR, .39; 95% CI, .16–.96; *P* = .04) was significantly associated with TV acquisition. Hormonal contraception (RR, 0.39; 95% CI, .12–1.23; *P* = .1) was also associated with TV acquisition, with a *P* value of < .2. However, due to the collinearity between the use of hormonal contraception and amenorrhea, only menstrual status (luteal phase, follicular phase, amenorrhea, and other) was included in the adjusted analyses.

There was no association between the total vaginal bacterial concentration at the pre-TV visit and the risk of TV acquisition (mean, 8.80 log$_{10}$ copies/swab [95% CI, 8.37–9.24] among cases vs 8.55 log$_{10}$ copies/swab [95% CI, 8.18–8.92] among controls; RR, 1.12 [95% CI, .79–1.58]; *P* = .5). This did not change when adjusted for menstrual status (adjusted RR [aRR], 1.03; 95% CI, .74–1.44; *P* = .9). Vaginal bacterial alpha diversity as defined by the SDI was not associated with TV acquisition (mean SDI, 1.59 [95% CI, 1.18–2.00] among cases vs 1.28 [95% CI, .00–1.57] among controls; RR, 1.34 [95% CI, .84–2.15]; *P* = .2). No change in the association between SDI and TV acquisition was seen after adjustment for menstrual status (aRR, 1.14; 95% CI, .72–1.79; *P* = .6). There was higher bacterial species richness among cases with TV acquisition (mean Chao-1 index, 80.12 [95% CI, 72.56–87.68] among cases vs 69.85 [95% CI, 63.34–76.36] among controls; RR, 1.02 [95% CI, 1.00–1.04]; *P* = .02). This result was similar in the adjusted analyses (aRR, 1.02 [95% CI, 1.00–1.04]; *P* = .04).

Table 1. Association Between Baseline Characteristics and Incident *Trichomonas vaginalis* (TV) Infection in 18 Women With 25 Episodes of TV Infection (Cases) and 50 Women Matched to Each Episode Who Remained Uninfected (Controls)

Variable	Unique Participants (n = 68)[a]	Cases (n = 25)	Controls (n = 50)	RR (95% CI)	P[b]
Age, y	36.2 ± 9.2	36.4 ± 7.1	36.5 ± 10.1	1.00 (.97–1.04)	1.0
Education duration, y	8.4 ± 3.4	8.1 ± 3.0	8.6 ± 3.5	0.97 (.88–1.08)	.6
Contraceptive method					
None/condoms	38 (55.9)	17 (68.0)	27 (54.0)	Reference	
Hormonal	20 (29.4)	3 (12.0)	17 (34.0)	0.39 (.12–1.23)	.1
Other	10 (14.7)	5 (20.0)	6 (12.0)	1.18 (.50–2.80)	.7
Menstrual status					
Follicular	19 (27.9)	10 (40.0)	11 (22.0)	Reference	
Luteal	15 (22.1)	6 (24.0)	12 (24.0)	0.70 (.35–1.39)	.3
Other[c]	8 (11.8)	4 (16.0)	5 (10.0)	0.93 (.39–2.21)	.9
Amenorrheic[d]	26 (38.2)	5 (20.0)	22 (44.0)	0.39 (.16–.96)	.04
Clinical amenorrhea[e]	5 (7.4)	3 (12.0)	3 (6.0)	…	
Menopause	11 (16.2)	1 (4.0)	10 (20.0)	…	
Depot medroxyprogesterone	9 (13.2)	1 (4.0)	8 (16.0)	…	
Pregnancy	1 (1.5)	0	1 (2.0)	…	
Sex partners in past wk, no.	2.5 ± 3.5	3.0 ± 3.4	2.3 ± 3.4	1.04 (.96–1.12)	.4
Sex acts in past wk, no.	2.9 ± 3.6	3.4 ± 3.5	2.7 ± 3.4	1.04 (.96–1.12)	.4
Any unprotected sex in past wk	11 (16.2)	6 (24.0)	8 (16.0)	1.38 (.68–2.78)	.4
Genital ulceration	2 (2.9)	1 (4.0)	1 (2.0)	1.52 (.36–6.47)	.6
Nugent criteria[f]					
Normal	45 (66.2)	15 (60.0)	33 (66.0)	Reference	
Intermediate	6 (8.8)	4 (16.0)	4 (8.0)	1.60 (.72–3.55)	.2
BV	17 (25.0)	6 (24.0)	13 (26.0)	1.01 (.49–2.08)	1.0
Any gonorrhea or *Chlamydia* infection	1 (1.5)	1 (4.0)	0	3.08 (2.03–4.69)	<.001
Presence of vaginal yeast	9 (13.2)	3 (12.0)	7 (12.0)	0.89 (.34–2.30)	.8

Data are mean ± SD or no. (%) of participants, unless otherwise indicated.

Abbreviations: BV, bacterial vaginosis; CI, confidence interval; RR, risk ratio.

[a] Data are for 18 unique participants who acquired TV and 50 participants who remained uninfected. Data are from the time of the first case of TV infection for those who contributed >1 case of TV infection.

[b] Values <.05 are considered statistically significant.

[c] Data are for women whose last menstrual period was 29–84 days ago and for whom the menstrual phase could not be estimated on the basis of a 28-day cycle.

[d] Data are for clinical amenorrhea, menopause, pregnancy, or depot medroxyprogesterone acetate use.

[e] Defined as >84 days since the last menstrual period.

[f] A score of 0–3 indicates normal; 4–6, intermediate; and 7–10, BV.

Associations between TV acquisition and individual vaginal bacterial taxa were explored to determine the odds of TV acquisition associated with each 1-SD change in relative abundance of each identified taxon as defined by broad-range PCR with sequencing (Table 2). A higher relative abundance of *Bulleidia* sequence reads was associated with TV acquisition (odds ratio [OR], 2.18; 95% CI, 1.00–4.71; P = .05). This finding suggests that the odds of TV acquisition is increased by 2-fold for each 1-SD increase in the relative abundance of *Bulleidia* species, although the result was of borderline statistical significance. There was a trend toward higher relative abundances of several additional bacterial species sequence reads in the cases with TV acquisition, including *Veillonella montpellierensis* (OR, 1.89; 95% CI, .94–3.82; P = .08), *Prevotella* genogroup 3 (OR, 1.82; 95% CI, .91–3.67; P = .09), *Anaerococcus* (OR, 1.79; 95% CI, .92–3.51; P = .09), and unclassified *Bacteroidales* (OR, 1.75; 95% CI, .91–3.36; P = .09).

The 8 taxa that were compared between all 25 TV episodes and 50 controls by using qPCR are presented in Table 3. *P. amnii*

was detected in vaginal secretions from 16 pre-TV visits (64%) among cases as compared to those from 17 visits (34%) among TV-uninfected controls (RR, 2.21; 95% CI, 1.12–4.38; P = .02). This association remained similar after adjustment for menstrual phase (aRR, 2.11; 95% CI, 1.03–4.33; P = .04). Similarly, *S. sanguinegens* was present in vaginal secretions from 20 pre-TV visits (80%) among cases versus those from 25 visits (50%) among uninfected controls (RR, 2.58; 95% CI, 1.00–6.62; P = .049). This relationship was no longer statistically significant in adjusted analyses (aRR, 2.31; 95% CI, .86–6.23; P = .10). The presence of *P. amnii* and *S. sanguinegens* in the vaginal microbiota were highly correlated. Of the 33 visits with *P. amnii* detected, *S. sanguinegens* was also detected in 32 (97%). Of the 45 visits with *S. sanguinegens* detected, *P. amnii* was also detected in 32 (71%). Owing to high collinearity, the association of each species with TV infection, adjusted for the presence of the other species, could not be assessed.

To evaluate the impact of including >1 case of TV infection from some participants, a sensitivity analysis was performed

Table 2. Association Between a 1-SD Change in the Relative Abundance of Vaginal Bacteria Identified by 16S Ribosomal RNA Gene Sequencing and Incident *Trichomonas vaginalis* (TV) Infection in 18 Women Who Acquired TV (Cases) and 36 Matched Women Who Remained Uninfected (Controls)

| Bacterial Taxon | Percentage Relative Abundance, Median (Range) | | | | |
	Cases[a]	Controls	SD	OR per 1-SD Change (95% CI)	P
Bulleidia species	0 (0–1.98)	0 (0–0.20)	0.03	2.18 (1.00–4.71)	.05
Veillonella montpellierensis	0 (0–15.07)	0 (0–16.40)	1.37	1.89 (.94–3.82)	.08
Prevotella genogroup 3	0 (0–1.73)	0 (0–2.23)	0.24	1.82 (.91–3.67)	.09
Anaerococcus species	0 (0–0.20)	0 (0–0.13)	0.02	1.79 (.92–3.51)	.09
Unclassified *Bacteroidales*	0 (0–0.68)	0 (0–0.18)	0.03	1.75 (.91–3.36)	.09
Sneathia species	0 (0–17.48)	0 (0–3.33)	0.57	1.95 (.84–4.52)	.12
Prevotella amnii	0 (0–23.24)	0 (0–26.46)	3.63	1.59 (.88–2.90)	.13
Actinomyces hongkongensis	0 (0–0.23)	0 (0–0.34)	0.02	1.60 (.82–3.11)	.17
Lactobacillus crispatus/helveticus	0 (0–39.37)	0 (0–100)	34.06	0.46 (.14–1.46)	.19
Dialister micraerophilus	0.29 (0–1.09)	0.15 (0–1.32)	0.24	1.45 (.82–2.56)	.20
Mageeibacillus indolicus	0 (0–5.07)	0 (0–1.85)	0.31	1.73 (.64–4.65)	.28
Prevotella species	0 (0–5.59)	0 (0–1.02)	0.19	1.68 (.54–5.25)	.37
Howardella species	0 (0–1.37)	0 (0–0.39)	0.03	1.69 (.49–5.87)	.41
Sneathia sanguinegens	0 (0–22.98)	0 (0–17.79)	3.23	1.24 (.71–2.15)	.45
Peptostreptococcus anaerobius	0 (0–41.37)	0 (0–13.53)	0.72	1.82 (.37–9.00)	.46
Dialister propionicifaciens	0 (0–0.62)	0 (0–0.93)	0.11	0.78 (.38–1.64)	.52

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation.

[a]The following taxa were identified in the vaginal microbiota of study participants, but models for their association with TV acquisition did not converge: bacterial vaginosis–associated bacterium, *Streptococcus gallolyticus*, *Klebsiella granulomatis/pneumoniae*, *Prevotella melaninogenica*, *Anaerococcus hydrogenalis/rubeinfantis*, *Fusobacterium nucleatum*, *Klebsiella*, *Dorea formicigenerans*, *Butyrivibrio*, *Prevotella ihumii*, *Neisseria gonorrhoeae*, *Lactobacillus fermentum*, *Peptostreptococcus stomatis*, *Staphylococcus capitis/epidermidis*, *Ruminococcus faecis*, *Streptococcus infantis*, *Atopobium deltae*, *Varibaculum cambriense*, and *Bifidobacterium adolescentis*.

that included only the subset of 18 first cases of TV infection in each participant and their 36 matched controls. The associations between *P. amnii*, *S. sanguinegens,* and an increased risk of TV acquisition remained significant in both the unadjusted and adjusted analyses (Table 4). An association between the presence of BVAB1 and TV acquisition was also observed in both the unadjusted (RR, 3.69; 95% CI, 2.32–5.87; $P < .001$) and adjusted (aRR, 3.33; 95% CI, 1.96–5.67; $P = .005$) models.

Results were also similar in sensitivity analyses comparing median bacterial qPCR concentrations at the visit before infection in all 25 cases of TV to those for 50 matched controls. The median vaginal concentration of *P. amnii* in cases of TV (4.08×10^5; interquartile range [IQR], 125–5.91×10^8) was higher than that for uninfected controls (125; IQR, 125–6.63×10^6; $P = .02$). Similarly, there was a higher median concentration of *S. sanguinegens* in cases of TV (1.17×10^5; IQR, 2.42×10^3–7.82×10^6), compared with uninfected controls (median, 125; IQR, 62.5–2.67×10^5; $P = .009$). No significant associations between TV acquisition and median bacterial concentration were identified for any of the other bacterial taxa assayed by qPCR.

Table 3. Association Between Detection of Specific Vaginal Bacteria Using Targeted Quantitative Polymerase Chain Reaction Analysis and Incident *Trichomonas vaginalis* (TV) Infection in 18 Women With 25 Episodes of TV Infection (Cases) and 50 Women Matched to Each Episode Who Remained Uninfected (Controls)

Bacterial taxon	Cases (n = 25)	Controls (n = 50)	RR (95% CI)	P	Adjusted RR (95% CI)[a]	P[b]
BVAB1	6 (24)	5 (10)	1.81 (.92–3.55)	.08	1.70 (.86–3.38)	.13
Mageeibacillus indolicus (BVAB3)	14 (56)	17 (34)	1.77 (.91–3.41)	.09	1.44 (.70–2.98)	.32
Prevotella amnii	16 (64)	17 (34)	2.21 (1.12–4.38)	.02	2.11 (1.03–4.33)	.04
Prevotella species[c]	17 (68)	21 (42)	2.01 (.94–4.33)	.07	1.65 (.69–3.92)	.26
Dialister propionicifaciens	20 (80)	29 (58)	2.04 (.75–5.53)	.16	1.82 (.69–4.79)	.23
Lactobacillus crispatus	6 (24)	14 (28)	0.85 (.35–2.07)	.73	0.89 (.38–2.13)	.80
Sneathia sanguinegens	20 (80)	25 (50)	2.58 (1.00–6.62)	.049	2.31 (.86–6.23)	.10
Sneathia species	21 (84)	29 (58)	2.52 (.81–7.88)	.11	2.21 (.70 17.01)	.18

Data are no. (%) of participants.

Abbreviations: BVAB, bacterial vaginosis–associated bacterium; CI, confidence interval; RR, risk ratio.

[a]Adjusted for menstrual phase.

[b]Values <.05 are considered statistically significant.

[c]*Prevotella* species were present in almost all samples, so values for cases and controls represent proportions of samples with a *Prevotella* 16S ribosomal RNA concentration at or above the median detected concentration of 6.9 copies/mL.

Table 4. Association Between Detection of Specific Vaginal Bacteria Using Targeted Quantitative Polymerase Chain Reaction Analysis and the First Incident *Trichomonas vaginalis* (TV) Infection in 18 Women Who Acquired TV (Cases) and 36 Matched Women Who Remained Uninfected (Controls)

Bacterial taxon	Cases (n = 18)	Controls (n = 36)	RR (95% CI)	P	Adjusted RR (95% CI)[a]	P[b]
BVAB1	5 (28)	1 (3)	3.69 (2.32–5.87)	<.001	3.33 (1.96–5.67)	.005
Mageeibacillus indolicus (BVAB3)	9 (50)	10 (28)	1.79 (.86–3.73)	.12	1.48 (.66–3.33)	.34
Prevotella amnii	12 (67)	8 (22)	3.30 (1.47–7.40)	.004	3.13 (1.34–7.32)	.003
Prevotella species[c]	12 (67)	13 (37)	2.24 (.99–5.08)	.053	2.01 (.84–4.83)	.12
Dialister propionicifaciens	14 (78)	19 (53)	2.12 (.81–5.55)	.13	1.94 (.74–5.04)	.18
Lactobacillus crispatus	4 (22)	10 (28)	0.80 (.32–2.01)	.63	0.80 (.32–1.98)	.62
Sneathia sanguinegens	14 (78)	14 (39)	3.13 (1.18–8.26)	.02	2.89 (1.05–7.96)	.04
Sneathia species	15 (83)	18 (50)	3.03 (1.00–9.18)	.05	2.69 (.84–8.58)	.10

Data are mean ± SD or no. (%) of participants, unless otherwise indicated.

Abbreviations: BVAB, bacterial vaginosis–associated bacterium; CI, confidence interval; RR, risk ratio.

[a]Adjusted for menstrual phase.

[b]Values <.05 are considered statistically significant.

[c]*Prevotella* species were present in almost all samples, so values for cases and controls represent proportions of samples with a *Prevotella* 16S ribosomal RNA concentration at or above the median detected concentration of 6.9 copies/mL.

DISCUSSION

In this cohort of HIV-1–seronegative sex workers, the presence of *P. amnii* and *S. sanguinegens* were each associated with a >2-fold increased risk of TV acquisition. Moreover, the median concentrations of these 2 bacteria were >1000-fold higher in women who acquired TV as compared to uninfected controls. There was also an association between increasing vaginal bacterial species richness (Chao-1 index) and TV acquisition, with every 1 point increase in the index associated with a 2% increased risk of TV acquisition. This is the first published study to use molecular techniques to prospectively evaluate the contribution of specific vaginal bacteria to the risk of subsequent TV acquisition.

This study builds on published data that demonstrated an association between prevalent TV and detection of *Prevotella* (non-*amnii* species) and *Sneathia* species [16]. One potential mechanism by which these bacterial species may mediate TV susceptibility is through the production of metabolites that promote TV infection. For example, the metabolite nicotinamide is used by TV when grown in vitro. The precursor of nicotinamide is nicotinate, and the presence of this metabolite in vaginal secretions is positively correlated with detection of *P. amnii* and *Sneathia* species in the vaginal microbiome [25, 26]. It would be of interest to determine whether these taxa produce nicotinate in vivo and whether other metabolites used by TV, such as spermidine, may also be produced by these species [26].

The previously reported association between *Candidatus* M. girerdii and prevalent TV was not seen in this study of incident TV infection [16]. Our deep sequence analysis (Table 2) did not find an association between *Mycoplasma* species and TV acquisition. Of the reads (2543 of 4 241 387 in the data set) classified as *Mycoplasma*, most (2507 of 2543) aligned with *Candidatus* M. girerdii, highlighting that this bacterium was detected but not linked with TV acquisition.

We also did not observe an association between baseline presence of BV and risk of TV acquisition. Prior prospective studies, including an earlier analysis in this same cohort, found that women with BV at baseline were approximately 2–3 times more likely to acquire TV [12, 13, 15]. The association seen in this study between increasing Chao-1 indices and incident TV may be a proxy for and possibly mediate the relationship between BV and TV acquisition because higher Chao-1 indices have been associated with prevalent BV [27]. However, our study had a relatively small sample size as compared to earlier prospective studies and likely lacked sufficient power to detect the associations previously observed between BV and incident TV.

The lack of association between *L. crispatus* and risk of TV acquisition seen in this study parallels earlier findings in the only other prospective study to explore the association between the presence of *Lactobacillus* species and TV acquisition [13]. Both studies were in the Mombasa Cohort, but they used different techniques (culture vs nucleic acid amplification) and occurred 9 years apart with little overlap in study populations (17-month median follow-up for HIV-1–seronegative women in Mombasa Cohort) [18]. In contrast to these prospective analyses, cross-sectional studies found that *Lactobacillus* species, including *L. crispatus*, are less likely to be detected in women with prevalent trichomoniasis [24, 28]. The differences in these findings likely reflect differences in experimental design because cross-sectional studies cannot disentangle the effect of *Lactobacillus* species on the risk of TV acquisition from the effect of prevalent TV infection on *Lactobacillus* species.

Strengths of this study include the prospective design and the relatively short time frame of 30–60 days between baseline sampling of the vaginal microbiota and detection of TV infection. Minimizing the interval between these visits limits the impact of natural variability of the vaginal microbiota over time on the results [29]. In addition, use of TMA to detect and

confirm TV infection provided high sensitivity and specificity in identifying cases.

This study also had a number of limitations. First, only bacteria for which qPCR probes were available in the laboratory could be evaluated on the basis of their absolute concentrations. Additional taxa that would be of interest to explore by qPCR analysis in future studies, based on statistical trends ($P < .1$) in the relative abundance data from this analysis, include *Bulleidia* species, *Veillonella montpellierensis*, *Anaerococcus* species, and unclassified *Bacteroidales* species. Second, the modest sample size may have resulted in failure to identify associations between some vaginal bacterial species and TV infection. Third, the sample size also limited the number of variables for which adjustment was possible in multivariable analyses. Based on the literature, other factors to evaluate in larger data sets would be age and number of sex partners [30–32]. It would also be of interest to consider adjustment for the presence of human papillomavirus and herpes simplex virus type 2 infection, both of which have been associated with changes in the vaginal microbiota [33]. However, these data were not available in the present study. Last, testing the relationship between multiple bacterial taxa and TV acquisition may have led to the identification of associations between bacterial taxa and TV by chance (ie, type 1 error). In this context, the results of this study should be viewed as hypothesis generating and investigated further in future studies.

In summary, this study demonstrated that the presence and concentrations of *P. amnii* and *S. sanguinegens* in the vaginal microbiota were each associated with TV acquisition in women. Future efforts should seek to validate these findings and explore whether additional bacterial species are associated with TV acquisition. If a causal relationship exists between vaginal bacteria and TV acquisition, then novel vaginal health interventions targeting the vaginal microbiome could potentially reduce a woman's risk of trichomoniasis. Potential interventions include targeted antimicrobial therapy against select bacterial species in the vaginal microbiota and periodic presumptive antimicrobial treatment to broadly reduce the concentrations of suboptimal vaginal bacterial species [34]. If a link is confirmed between vaginal bacterial species and TV acquisition, trials of these types of interventions could provide evidence of a causal association, as well as evaluate the efficacy of strategies aimed at reducing women's risk of trichomoniasis.

Notes

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