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Cervicovaginal bacterial communities in reproductive-aged Tanzanian women with *Schistosoma mansoni*, *Schistosoma haematobium*, or without schistosome infection

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Abstract
Schistosome infection is recognized as a potentially modifiable risk factor for HIV in women by the World Health Organization. Alterations in cervicovaginal bacteria have been associated with HIV acquisition and have not been studied in schistosome infection. We collected cervical swabs from Tanzanian women with and without *S. mansoni* and *S. haematobium* to determine effects on cervicovaginal microbiota. Infected women were treated, and follow-up swabs were collected after 3 months. 16S rRNA sequencing was performed on DNA extracted from swabs. We compared 39 women with *S. mansoni* with 52 uninfected controls, and 16 with *S. haematobium* with 27 controls. *S. mansoni*-infected women had increased abundance of *Peptostreptococcus* ($p = 0.026$) and presence of *Prevotella timonesis* ($p = 0.048$) compared to controls. High-intensity *S. haematobium* infection was associated with more diverse cervicovaginal bacterial communities than uninfected controls ($p = 0.0159$). High-intensity *S. mansoni* infection showed a similar trend ($p = 0.154$). At follow-up, we observed increased alpha diversity in *S. mansoni* (2.53 vs. 1.72, $p = 0.022$) and *S. haematobium* (2.05 vs. 1.12, $p = 0.066$) infection groups compared to controls. Modifications in cervicovaginal microbiota, particularly increased diversity and abundance of taxa associated with bacterial vaginosis and HIV (*Peptostreptococcus, Prevotella*), were associated with schistosome infection.

Keywords Schistosomiasis · Microbiome · Cervicovaginal microbiota · Tanzania · HIV

Introduction

Increased prevalence and incidence of HIV in African women with schistosome infections is supported by studies

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of both *S. haematobium* [1–3], which predominantly affects the urogenital tract, and *S. mansoni* [4, 5], which concentrates in the gastrointestinal tract. One longitudinal study reported that *S. mansoni*-infected women had a subsequent 2.8-fold increased risk for HIV acquisition [5]. Another noted a 1.4-fold increased hazard of HIV acquisition in women with *S. haematobium* infection, and a 1.3-fold increased hazard in women with *S. mansoni* infection that

did not reach significance ($p = 0.12$) [6]. Though not all studies have confirmed these associations [7, 8], schistosome infection has been declared a potentially modifiable risk factor for HIV acquisition by both the World Health Organization and the Joint United Nations Program on HIV/AIDS [9, 10].

The mechanism for increased risk of HIV acquisition in schistosome-infected women is not fully understood. Increased risk in schistosome-infected women but not men [1, 5, 6, 11], suggests that increased HIV susceptibility may be attributable to genital mucosal effects of schistosome infection. Previously, we showed that women with *S. haematobium* had altered cervical mucosal gene expression and lower cervicovaginal interleukin-15 levels compared to schistosome-uninfected women, whereas women with *S. mansoni* did not have these alterations [12]. Possible genital tract alterations by which *S. mansoni* may increase risk for HIV acquisition remain unclear.

Increased cervicovaginal bacterial diversity is a putative risk factor for HIV acquisition in African women [13–15] and to our knowledge has not been investigated in schistosome-infected women. There are, however, schistosome-associated alterations in gut and urinary bacterial communities. *S. mansoni* infection has been associated with lower intra- individual gut bacterial diversity in mice and children [16, 17], and *S. haematobium* infection is associated with gut microbiota suggestive of dysbiosis [18, 19] and with immune-stimulatory urinary bacterial taxa [20]. Therefore, it is plausible that schistosome infections may affect cervicovaginal bacterial communities given that parasite eggs concentrate in the genital tract in *S. haematobium*, and to a lesser extent in *S. mansoni* [21].

Our objective was to investigate the effects of *S. haematobium*, *S. mansoni* and praziquantel treatment on cervicovaginal bacterial communities. We hypothesized that schistosome-infected women would have altered cervicovaginal microbiota compared to uninfected women, and that these alterations would vary by schistosome species and after anti-schistosome treatment. We therefore compared women with and without *S. haematobium* and women with and without *S. mansoni* separately. In addition, we compared microbiota of women who were infected with *S. haematobium* or *S. mansoni*, treated with praziquantel, and confirmed to be schistosome-uninfected at follow-up. A deeper understanding of the relationship between schistosome infections and cervicovaginal bacterial communities could suggest additional treatment strategies by which the sequelae of schistosome infections in women could be managed, with the ultimate goal of decreasing morbidity and preventing HIV infections in this vulnerable population.

Methods

Ethics

All participants provided written informed consent, which was obtained in private by a nurse in Kiswahili. Those with schistosome and other genital tract infections received free treatment for themselves and their partners if indicated. Women with newly diagnosed HIV were referred to the nearest HIV treatment center for free ongoing care.

Ethical approval for this study was provided by the Joint Research Ethics Committee of Bugando Medical Centre/the Catholic University for Health and Allied Sciences in Mwanza, Tanzania (CREC/171/2017), the National Institute for Medical Research (NIMR) in Dar es Salaam (NIMR/HQ/R.8a/Vol.IX/2446), and Weill Cornell Medicine in New York (1612017800).

Eligibility screening

We invited reproductive-aged women living in rural Tanzanian villages with high prevalence of *S. haematobium* or *S. mansoni* infection to undergo screening for HIV and schistosome infections, as previously described [12, 22]. Women from *S. mansoni*-endemic villages were screened between July 2017 and January 2018 and women from *S. haematobium*-endemic villages were screened between June and September 2018. *S. mansoni*- and *S. haematobium*-endemic villages were >60 km apart. In this community-based sample, women were apparently healthy and had no obvious clinical conditions. HIV counseling and testing was performed by a trained nurse using the Determine HIV-1/2 test (Alere, MA), with positive results confirmed by Uni-Gold HIV-1/2 test (Trinity BioTech, Ireland), per Tanzanian national guidelines. Urine samples were collected, filtered with a single syringe (two filtrations of 10 mL each to increase sensitivity), and examined microscopically for *S. haematobium* ova by trained parasitologists. Five slides per stool sample [23] were examined for *S. mansoni* ova by Kato-Katz technique. Serum was stored at −20 °C within 10 h of collection and schistosome circulating anodic antigen (CAA) was quantified at the NIMR laboratory in Mwanza using up-converting phosphor technology with CAA values >30 pg/mL considered positive [4, 24, 25].

Study procedures

Women received a follow-up card to return for results in 1–2 weeks. Each received schistosome test results privately, with free praziquantel treatment if infected. Women confirmed positive for *S. haematobium* or *S. mansoni* by both microscopy and CAA, or those confirmed to be negative both by microscopy and CAA, were eligible for cohort

participation. For every schistosome-infected woman enrolled, the following uninfected woman from her village was also invited to participate in the cohort. Thus, infected and uninfected women were recruited from the same schistosome-endemic villages. Pregnant women were screened but not eligible for cohort participation because endocervical brushing would be performed.

Cohort-eligible women participated in a structured private interview a nurse in Kiswahili, followed by gynecologic examination with specimen collection. For this study, a sealed Copan swab was touched straight to the cervical face and vaginal fornix, and then placed immediately back into the transport container, which was resealed and placed on ice (ThermoScientific™ COPAN ESwab™, U.S.). All women were tested for trichomoniasis, chlamydia and gonorrhea and screened for cervical cancer using acetic acid, as per Tanzanian national guidelines, with follow-up referrals provided to Bugando Medical Centre for free ongoing cervical cancer screening and care as needed [26]. Women who tested positive for trichomoniasis were provided same-day treatment free of charge and women positive for gonorrhea or chlamydia were provided free treatment several weeks later, after PCR testing in the central laboratory.

Participants provided 1–2 phone numbers to allow study nurses to maintain contact with them for the study's duration. Participants returned after 3 months, at the midpoint in their menstrual cycles. At follow-up, women provided urine, blood, stool, and gynecologic samples, and were again tested for STIs and treated as indicated. Women who tested positive for schistosomiasis by urine, stool, or serum at any follow-up received directly observed praziquantel treatment.

Sample processing and testing

Point-of-care *Trichomonas* testing was performed during the gynecologic examination (Osom Rapid Test, Sekisui Diagnostics, U.S.). For chlamydia and gonorrhea testing at NIMR, DNA was extracted from cervicovaginal swab specimens by QIAamp DNA mini-kit (Qiagen, Germany) for quantitative polymerase chain reaction analysis using the Artus CT/NG RGQ kit.

Cervical swabs were placed on ice and stored at −80 °C within four hours of collection at the NIMR laboratory until shipment to Weill Cornell Medicine on dry ice.

16S rRNA sequencing on vaginal swab

Minor adaptions were made to the previously described phenol/chloroform/isoamyl extraction for vaginal swabs [27]. After thawing to room temperature, 500 µl of extraction buffer (200 mM Tris, pH 8.0, 200 mM NaCl, and 20 mM EDTA) was added to the tube containing the swab and vortexed to ensure sufficient binding. Liquid containing DNA extraction buffer and sample was then suspended in 200 µl of 20% SDS, 500 µl of phenol/chloroform/isoamyl alcohol (24:24:1), and 500 µl of 0.1-mm-diam zirconia/silica beads (BioSpec Products, US). Cells were lysed by mechanical disruption by bead beater (FisherScientific, U.S.) for 2 min. Two rounds of phenol/chloroform/isoamyl alcohol extraction were performed. DNA was precipitated with 40 µl sodium acetate and 880 µL ethanol, incubated at −80 °C for 20 min, and pelleted at max speed for 20 min. The supernatant was removed and pellet resuspended in 200 µl TE buffer with 100 µg/ml RNase (ThermoScientific). Isolated DNA was further purified with QIAamp Mini Spin Columns (Qiagen).

The V4-V5 region of the 16S rRNA gene was amplified using primers 563F (5′-nnnnnnnn-NNNNNNNNNNNN-AYTGGGYDTAAAGNG-3′) and 926R (5′-nnnnnnnn-NNNNNNNNNNNN-CCGTCAATTYHTTTRAGT-3′) as previously described, with extraction blanks serving as controls [28]. Amplicons were purified using the Qiaquick PCR Purification kit (Qiagen) and quantified with the Agilent 2200 tape station (Agilent Technologies, Germany). PCR products were then pooled at equimolar amounts. Libraries were prepared with TruSeq DNA library preparation kit (Illumina, US), per the manufacturer's instructions, and sequenced on the Illumina MiSeq platform using a paired-end 250 × 250-bp kit.

16S rRNA phylogenetic analysis

Paired 16S rRNA (V4-V5) end reads were merged and demultiplexed. The DADA2 pipeline [29] was used for maximum expected error filtering (Emax = 10) [30], with amplicon sequence variant (ASV) grouping (by unique 16S sequence). Singleton sequences were removed. Nucleotide BLAST of representative sequences from each ASV was used for taxonomic classification, with NCBI RefSeq as the reference database. Minimum threshold for *E* values was 1e−10. A de novo neighbor-joining phylogenetic tree of 16S ASV sequences was constructed using MUSCLE [29].

Statistical analysis

We calculated that enrolling 15 women with schistosome infection and 25 without would provide >86% power to detect a difference in alpha diversity of 1.4 versus 1.8, assuming a standard deviation of 0.4 [30].

For univariate analysis to identify differently abundant and present taxa in *S. mansoni*-infected vs. uninfected women from *S. mansoni*-endemic villages and *S. haematobium*-infected vs. uninfected women from *S. haematobium*-endemic villages, we first filtered out extremely low

prevalent taxa from downstream analysis in order to reduce problems induced by spurious operational taxonomic units (OTUs) [31, 32]. In order to deal with excessive zeros in the data, we then classified taxa as either prevalent or less prevalent and formulated statistical tasks adaptively to the prevalence of taxa. There was a natural separation of taxa clusters based on the prevalence with a cutoff near one third [33]. Therefore, for prevalent taxa, defined as taxa present in at least one third (≥32.6%) of women, we focus on identifying taxa that show differential abundance by infection status using rank-sum tests. For less prevalent taxa, actual composition is largely determined by zero counts. Instead of comparing small and zero abundance in most cases, we aim to detect presence/absence of taxa patterns [34] that were associated with infection status using logistic regression (or Fisher's Exact test if any cell count was <5). As per multiple previous studies [13, 14, 35], we did not correct for multiple statistical tests when comparing bacterial taxa between infection groups. Instead, we used follow-up data to validate baseline findings.

Alpha diversity was calculated using the Shannon index measuring within-sample bacterial community diversity and compared using rank-sum test by infection intensity. Beta diversity was measured using principal coordinate analysis with the Bray-Curtis distance metric [36, 37]. Infection intensity was categorized as low (CAA 30-3,000 pg/mL) and high (CAA > 3,000 pg/mL) [38]. Analyses were performed using R version 3.6.1.

Results

In *S. mansoni*-endemic villages, 41 women with *S. mansoni*, one with *S. haematobium-S. mansoni* coinfection, and 58 uninfected women consented to cohort participation. The woman with *S. haematobium–S. mansoni* coinfection was excluded from analysis. Sequencing data were successfully obtained from 39 *S. mansoni*-infected (22 with low-intensity and 17 with high-intensity infection) and 53 uninfected cervical swabs at baseline; the remaining seven samples did not amplify after DNA extraction.

In *S. haematobium*-endemic villages, 47 women consented to cohort participation. Of these, 18 had *S. haematobium* infection and 29 were uninfected at enrollment. Sequencing data were successfully obtained from 17 *S. haematobium*-infected (12 with low-intensity and five with high-intensity infection) and 27 uninfected baseline cervical swabs; three samples did not amplify after DNA extraction and one did not generate sufficient sequencing depth.

Meta data, OTUs, taxa, and phylogenetic trees are included for *S. mansoni* and *S. haematobium* baseline and follow-up in Supplementary Data Files 1–16.

Overall bacterial composition

Lactobacillus was the most common taxa at genus level across all samples, with a mean relative abundance of 0.28, followed by *Gardnerella*, *Megasphaera*, and *Sneathia*. In addition, 27.2% (37/136) of samples had *Lactobacillus* encompassing >0.5 of their relative abundance. Mean alpha diversity across all samples was 1.94.

S. mansoni baseline

S. mansoni-infected women had similar numbers of sexual partners in the last year, rates of HIV infection, frequencies of vaginal cleansing, and rates of current sexually transmitted infections (STIs) compared to schistosome-uninfected women from *S. mansoni*-endemic villages (Table 1). Women with *S. mansoni* more frequently experienced food suffrage (71.8% vs. 42.3%) and were slightly younger at first sexual encounter (17 vs. 17.5 years) ($p < 0.05$ for all).

Neither beta diversity ($p = 0.515$, Fig. 1a, Supplementary Fig. 1a) nor alpha diversity differed between infected and uninfected women at baseline (2.03 vs. 1.88, $p = 0.320$, Fig. 2a). Those with high-intensity *S. mansoni* infection tended towards increased alpha diversity compared to uninfected women (2.04 vs. 1.88, $p = 0.154$, Fig. 2a).

Overall microbial composition of *S. mansoni*-infected and uninfected women is shown at the genus level (Fig. 3). Relative abundance and presence/absence of bacteria at different phylogenetic levels were compared between infected and uninfected women from *S. mansoni*-endemic villages at baseline. *Prevotella timonensis* was more often detected in *S. mansoni*-infected than uninfected women ($p = 0.048$). *Peptostreptococcus anaerobius* was more abundant in *S. mansoni*-infected women ($p = 0.040$), at both genus and family levels (Fig. 4). *Peptoniphilaceae, Tissierellales,* and *Tissierella* were more abundant in infected women at the family, order and class levels ($p = 0.046$, $p = 0.046$, $p = 0.045$, respectively). *Lactobacillus* and *Lactobacillus iners* were both reduced in women with high-intensity *S. mansoni* infection compared to uninfected and low intensity infected women, though this did not reach significance ($p = 0.273$ and $p = 0.141$, respectively).

S. mansoni follow-up

Sequencing data were successfully obtained from 59 women from *S. mansoni*-endemic villages at 3-month follow-up. Of the 34 women uninfected at baseline, 33 remained uninfected at follow-up, while one had acquired *S. mansoni* infection. Of the 25 women infected with *S. mansoni* at baseline, 18 were cured with

Table 1 Demographic characteristics at baseline.

	S. mansoni infected N = 39 number (%)/median [IQR]	Uninfected from S. mansoni village N = 52 number (%)/median [IQR]	S. haematobium infected N = 16 number (%)/median [IQR]	Uninfected from S. haematobium village N = 27 number (%)/median [IQR]
Age (years)	29 [23, 37]	32 [24, 41]	34 [22, 41]	27.5 [23, 40]
Food insufficiency	28 (71.8)**	22 (42.3)**	7 (43.8)	5 (18.5)
Years of school	7 [4, 7]	7 [4, 7]	7 [2, 7]*	7 [4, 7]*
Number of pregnancies	3 [2, 5]	3 [2, 6]	5 [3, 6]	4 [2, 5]
Children birthed	3 [1, 5]	3 [2, 6]	4.5 [3, 6]	4 [2, 5]
Experienced infertility	8 (20.5)	12 (23.1)	3 (21.4)	4 (14.8)
Sexual partners (last year)	1 [1, 1]*	1 [1, 1]*	1 [1, 1.5]*	1 [1,1]*
Accepted money for sex (last year)	9 (23.1)	7 (13.5)	5 (33.3)*	2 (7.4)*
Husband has children outside of marriage	29 (55.8)	28 (71.8)	8 (53.3)	17 (63.0)
Vaginal cleansing	37 (94.9)	47 (90.2)	16 (100.0)	25 (92.6)
Cleanse with water	37 (94.9)	46 (88.5)	16 (100.0)	25 (92.6)
Cleanse with soap	27 (69.2)	42 (80.8)	15 (93.8)	23 (85.2)
Cleanse with other	7 (18.0)	6 (11.5)	4 (25.0)	9 (33.3)
Vaginal insertion of herbs, leaves, roots or other substances	4 (10.3)	2 (3.9)	1 (6.3)	1 (3.7)
Family planning use (last 3 months)	11 (28.2)	17 (32.7)	5 (31.3)	9 (33.3)
Days since last menstrual period	14 [12, 17]*	12.5 [10, 15]*	11 [9, 13]	14 [11, 18]
Currently Breastfeeding	8 (20.5)	9 (17.0)	3 (18.8)	10 (37.0)
Menopause	4 (10.3)	1 (1.9)	0 (0.0)	2 (7.4)
Previous HIV test	39 (100.0)*	46 (88.5)*	13 (86.7)	24 (88.9)
HIV infected	3 (7.7)	3 (5.7)	0 (0.0)	0 (0.0)
CAA (pg/mL)	2091 [179, 10468][a]	4.0 [0, 8.9][a]	718 [368.9, 7266][a]	0.3 [0, 3.5][a]
Prior treatment for schistosome infection	7 (18.0)	16 (30.8)	8 (53.3)	8 (29.6)
Within past 5 years	2 (5.1)	3 (5.8)	NA	NA
More than 5 years ago	5 (12.8)	13 (25.0)	NA	NA
Current laboratory-confirmed STI	13 (33.3)	15 (28.9)	5 (31.2)	8 (29.6)
Trichomoniasis	9 (24.3)	7 (14.0)	5 (31.3)	4 (14.8)
Chlamydia	2 (5.1)	4 (7.8)	0 (0.0)	2 (7.4)
Gonorrhea	2 (5.1)	3 (5.7)	0 (0.0)	2 (7.4)
Treatment for STI (last year)	3 (7.7)	5 (9.6)	2 (12.5)	1 (3.7)

HIV human Immunovirus, *CAA* circulating anodic antigen.

*p < 0.1; **p < 0.05.

[a]Not compared because CAA value was part of the study group definition.

praziquantel treatment, while seven remained infected. Therefore, sequencing data from 8 *S. mansoni*-infected and 51 uninfected women were used in follow-up analysis.

Women infected with *S. mansoni* at follow-up had significantly higher alpha diversity compared to uninfected women (2.53 vs. 1.72, *p* = 0.022, Fig. 2b). Of note, all infected women at follow-up had low-intensity infections.
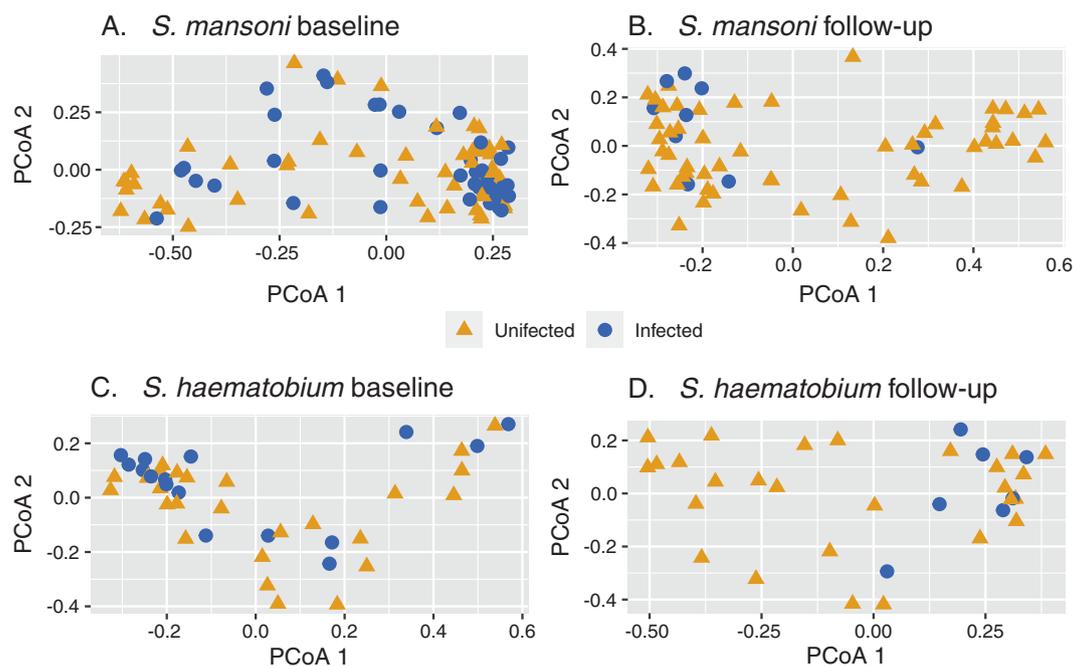
Fig. 1 Beta diversity between *Schistosome* infected and uninfected women at baseline and follow-up. Principal coordinate analysis using Bray-Curtis distance metric on schistosome infected and uninfected women at baseline and 3-month follow-up. Women infected with *S. haematobium* at 3-month follow-up (**d**) are more clustered compared to uninfected women (**c**), or to women with *S. mansoni* infection at baseline (**a**) or 3-month follow-up (**b**). This indicates that infected women have cervicovaginal microbiomes that are more similar to each other than uninfected women.

There was no significant difference in beta diversity between infected and uninfected women at follow-up ($p = 0.359$, Fig. 1b, Supplemental Fig. 1b). Differences in diversity at the genus level between infected and uninfected groups are shown in Fig. 5a.

Taxa that were significantly different at baseline were tested at follow-up for validation of baseline findings. *Peptostreptococcus* was more abundant among infected women at the genus level ($p = 0.049$) and trended towards higher abundance at the family level ($p = 0.063$) (Fig. 4). *Prevotella timonesis* was more often detected in women infected at follow-up ($p = 0.008$). At the family, order, and class levels, *Peptoniphilaceae, Tissierellales,* and *Tissierella* were more abundant in the infected group ($p = 0.042$ for all), consistent with baseline findings.

S. haematobium baseline

S. haematobium-infected women had similar numbers sexual partners in the last year, and practices of vaginal cleansing compared to uninfected women from *S. haematobium*-endemic villages (Table 1).

Baseline alpha diversity (1.83 vs. 1.36, $p = 0.390$, Fig. 2c) and beta diversity ($p = 0.604$, Fig. 1c, Supplemental Fig. 1c) were not different. Women with high-intensity *S. haematobium* infections had significantly higher alpha diversity than uninfected women (2.43 vs. 1.37, $p = 0.016$)

and than women with low-intensity *S. haematobium* infections (2.43 vs. 1.83, $p = 0.038$, Fig. 2c).

Overall microbial composition of *S. haematobium*-infected and uninfected women is shown at the genus level in Fig. 3b. In contrast to *S. mansoni* findings, at all phylogenetic levels, the relative abundance and presence or absence of bacteria were not significantly different between *S. haematobium*-infected and uninfected women at baseline. Women with high-intensity *S. haematobium* infection had reduced abundance of *Lactobacillus iners* compared to women with low-intensity infection, though this did not reach significance ($p = 0.393$).

S. haematobium follow-up

Sequencing data were successfully obtained from 34 women from *S. haematobium*-endemic villages at follow-up. Of the 20 women uninfected at baseline, 16 remained uninfected at follow-up, while four had acquired *S. haematobium*. Of the 12 women infected with *S. haematobium* at baseline, nine were cured with praziquantel treatment, while three remained infected. Therefore, sequencing data from seven *S. haematobium*-infected and 27 uninfected women were used in follow-up analysis.

Women with *S. haematobium* infection at follow-up showed a trend towards higher alpha diversity than uninfected women (2.05 vs. 1.12, $p = 0.066$, Fig. 2d). Of note,
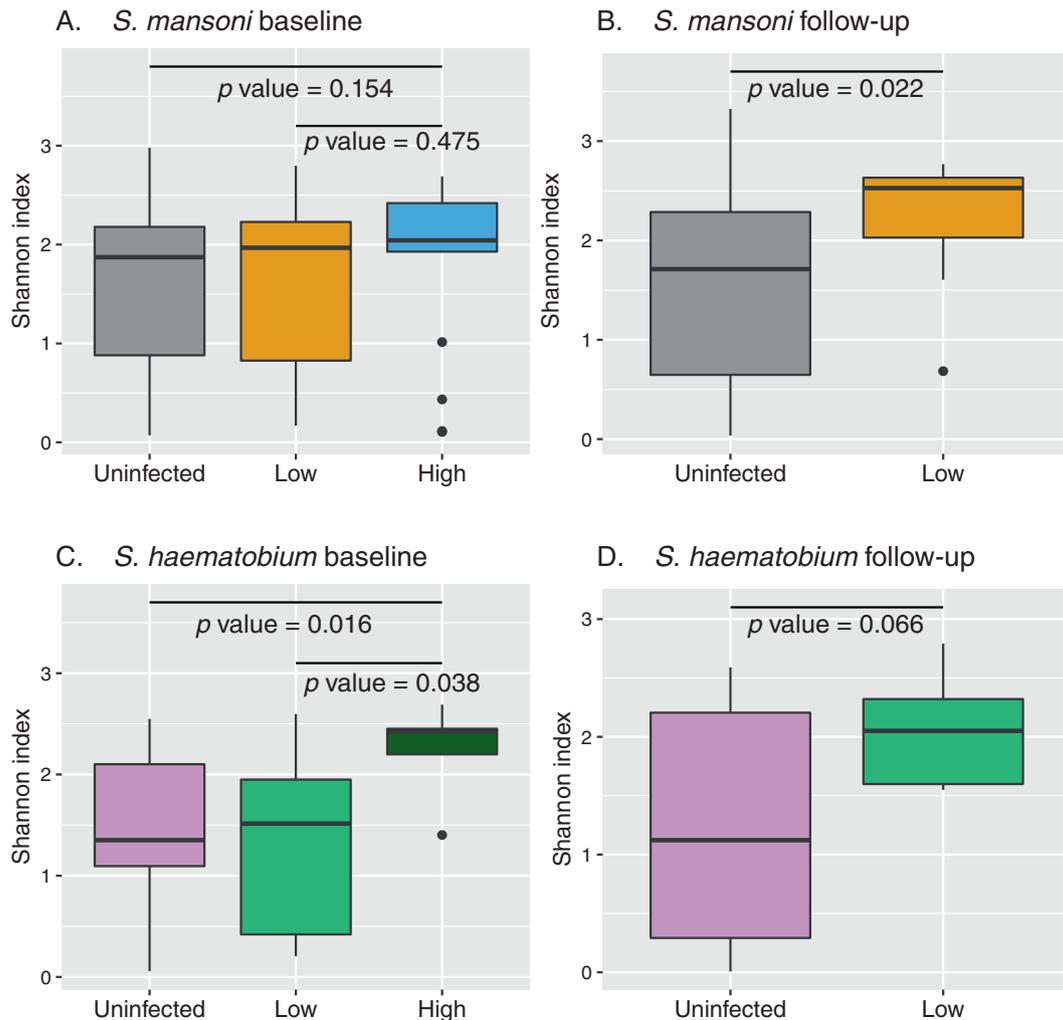
Fig. 2 *S. mansoni* **and** *S. haematobium* **alpha diversity at baseline and 3-month follow-up, by infection intensity.** Differences in alpha diversity, as shown by Shannon Diversity Index, between uninfected, low intensity infected, and high intensity infected women at baseline and 3-month follow-up from *S. mansoni* and *S. haematobium*-endemic villages. The plots display medians (dark horizontal bars) and interquartile ranges (boxes), with error bars representing 1.5 times the interquartile range or minimum/maximum values. Women with high-intensity *S. mansoni* infection had a trend towards increased diversity compared to uninfected women at baseline ($p = 0.154$). Women with high-intensity *S. haematobium* infection had significantly increased diversity compared to their uninfected counterparts at baseline ($p = 0.016$). Women with low-intensity infection *S. mansoni* or *S. haematobium* infection had increased diversity compared to their uninfected counterparts at follow-up ($p = 0.022$ and $p = 0.066$).

all infected women at follow-up had low-intensity infections. Women with *S. haematobium* had significantly different beta diversity than uninfected women, demonstrating that women with *S. haematobium* had microbiota more similar to each other than uninfected women at follow-up ($p = 0.017$, Fig. 5d, Supplementary Fig. 1d).

Discussion

Our novel study investigated the effects of schistosome infections on the cervicovaginal bacterial communities of African women to explore whether differences in microbiota could be a plausible and potentially modifiable mechanism by which schistosome infection may increase risk of HIV. We report that women with high-intensity *S. mansoni* or *S. haematobium* infections had increased cervicovaginal bacterial diversity compared to controls. *S. mansoni* and *S. haematobium*-infected women who had become infected or remained infected at follow-up also had higher diversity than uninfected women. In addition, women with *S. mansoni* infection, but unexpectedly not *S. haematobium* infection, had modified abundance and presence of specific bacterial taxa linked with increased vaginal pH compared to uninfected women.

Existing literature suggests that high-diversity, low *Lactobacillus* cervicovaginal microbiota are associated with increased risk of HIV acquisition [13, 39], and that African
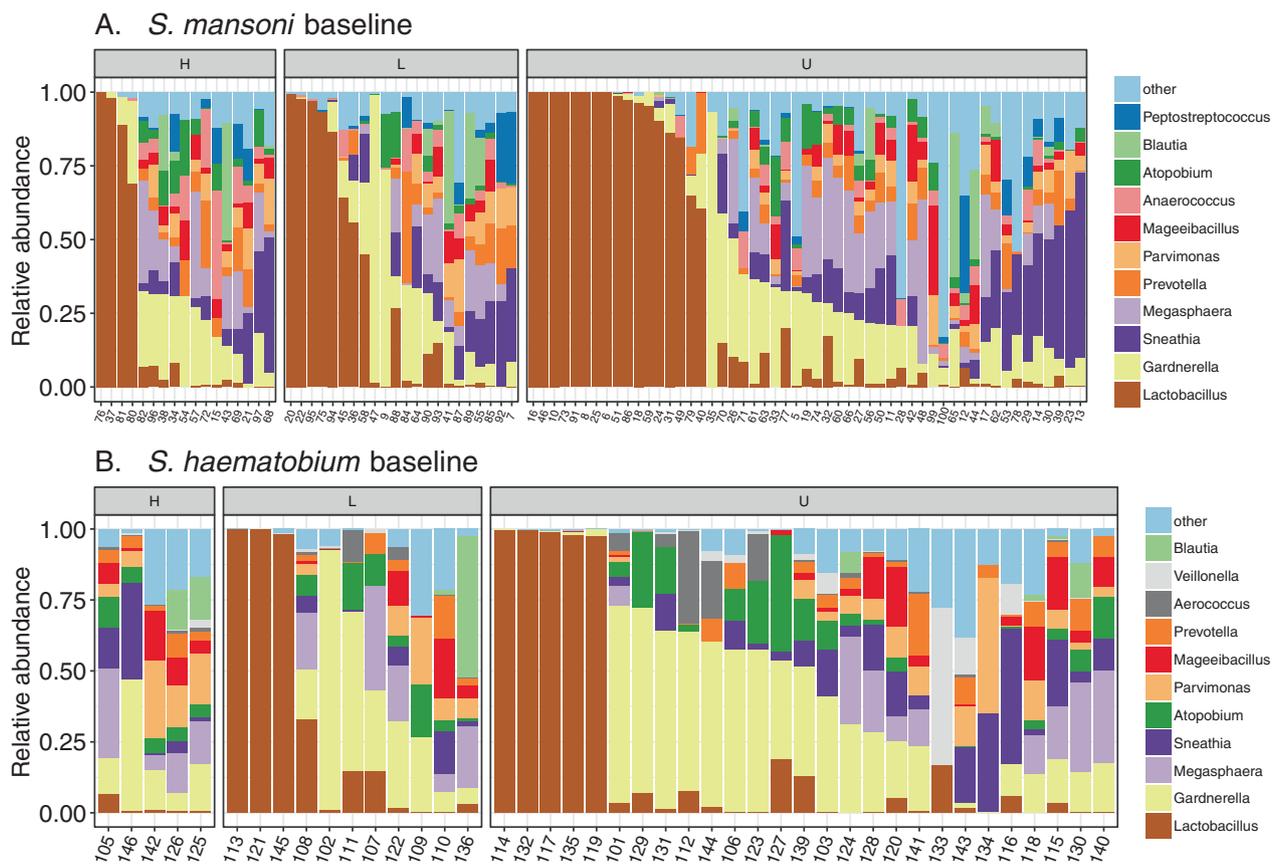
A. *S. mansoni* baseline

B. *S. haematobium* baseline

Fig. 3 Relative abundances of 11 most abundant cervicovaginal microbiota by genus in *S. mansoni* and *S. haematobium* groups at baseline. Relative abundance of the 11 most abundant cervicovaginal microbiota at the genus level for uninfected, low intensity infected, and high intensity infected women with *S. mansoni* (upper horizontal band) and *S. haematobium* (lower horizontal band) at baseline. Each vertical bar represents a successfully sequenced cervicovaginal sample. Labels above each bar indicate infection status: "U" for uninfected, "L" for low-intensity infection, and "H" for high-intensity infection. Women with *S. mansoni* infection had increased *Peptostreptococcus* (shown in dark blue, $p = 0.026$) and women with high-intensity *S. mansoni* infection showed a trend toward decreased *Lactobacillus* ($p = 0.273$). No significant differences were observed in individual taxa between *S. haematobium* infected and uninfected women.

women have lower *Lactobacillus* and higher diversity than white European women [13, 14]. We report that women with high-intensity schistosome infection have increased cervicovaginal bacterial diversity compared to uninfected women. We also report a trend toward decreased *Lactobacillus* species and *Lactobacillus iners* in high-intensity *S. mansoni* infection, which did not reach significance likely because of sample size limitations. Both these results indicate that those with high-intensity schistosome infection may be at increased risk for acquiring HIV and align with our prior finding that women with high-intensity schistosome infection had a higher prevalence of HIV than women with low-intensity or without infections [4]. Our findings concur with studies reporting *Lactobacillus* dominance in <30% of African women, compared to 90% of European women [13].

Our findings also indicate that *Peptostreptococcus anaerobius* and *Prevotella timonesis* were more abundant and present, respectively, among *S. mansoni* infected

compared to uninfected women. *Peptostreptococcus* and *Prevotella* are associated with increased vaginal pH [40] and bacterial vaginosis [41], both of which have been associated with increased HIV acquisition [41–45]. Thus, increased presence and abundance of *Peptostreptococcus* and *Prevotella* species could either increase or result from high vaginal pH and thereby contribute to increased HIV acquisition in *S. mansoni*-infected women. *Prevotella* has also been associated with genital inflammation and HIV acquisition [13], suggesting that increased *Prevotella* among *S. mansoni*-infected women could either contribute to or reflect increased mucosal inflammation in the genital tract.

Despite the the presence of eggs in the urogenital tract, *S. haematobium*-infected women had no significant differences in cervicovaginal microbiota compared to uninfected women. This contrast with *S. mansoni*-infected women could be due to distinct species differences, leading to different pathogenic and immunogenic effects in the host and
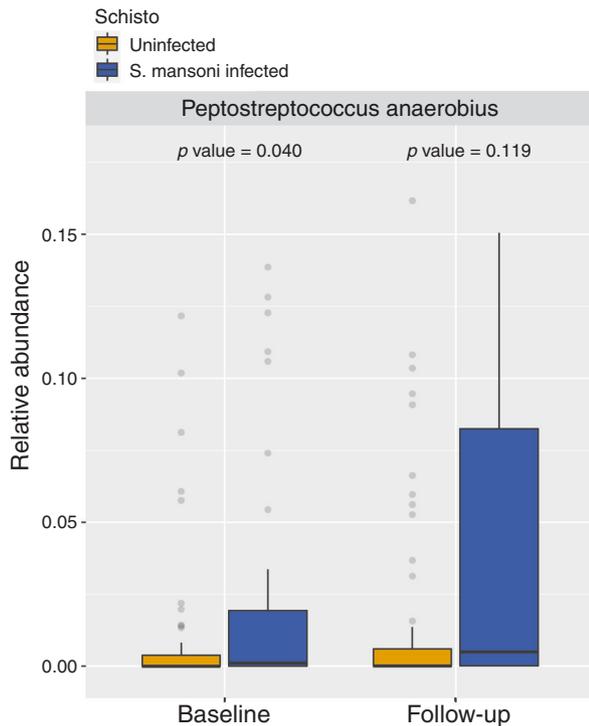
Fig. 4 Differences in abundance of *Peptostreptococcus anaerobius* between *S. mansoni*. Infected and uninfected at baseline and 3-month follow-up. Differences in relative abundance of *Peptostreptococcus anaerobius* between *S. mansoni* infected and uninfected women at baseline and 3-month follow-up. The plots display medians (dark horizontal bars) and interquartile ranges (boxes), with error bars representing 1.5 times the interquartile range or maximum values. Women with *S. mansoni* infected had increased abundance of *Peptostreptococcus anaerobius* at baseline and at follow-up compared to uninfected women ($p = 0.040$ and $p = 0.119$, respectively).

different impacts of host immunity on the parasites. *S. mansoni* female worms produce approximately 10-fold more eggs than *S. haematobium* worms and maintain high egg production regardless of host age, while *S. haematobium* egg production decreases in older hosts [46–48]. Human autopsy studies have demonstrated large numbers of calcified *S. haematobium* eggs lodged in host tissues, while lower numbers of retained *S. mansoni* eggs suggests more efficient egg excretion [47]. *S. mansoni* egg excretion is facilitated by the parasite's exploitation of vascular Peyer's Patch gut lymphoid tissue, which enhances transit of eggs to the intestinal lumen while also damaging Peyer's Patch structure and lymphoid cellularity [49]. An analogous lymphoid tissue does not exist for *S. haematobium* eggs migrating to the lumen of the bladder and may have differential systemic immune ramifications. In addition, both *Peptostreptococcus* and *Prevotella* are commonly found not only in the gut but also in the vagina, particularly in women with anaerobe-dominated cervicovaginal microbiota, and effects of *S. mansoni* could be more easily enhanced in the vaginal tract in which these populations are already present

[13, 39]. Together, this body of data suggests multiple differential effects of *S. mansoni* on host tissue and host immunity, which may explain why it altered cervicovaginal microbiota while *S. haematobium* did not.

While ours is the first to report alterations in cervicovaginal microbial communities in women with and without schistosome infections, previous studies documented increased *Prevotella* in the gut microbiota of children and adolescents with *S. haematobium* infection [18, 19]. Studies investigating the relationship between *S. mansoni* and gut microbiota in mice [16] and children [17] reported subtle differences between infected and uninfected groups, though expectedly these differences do not match differences identified in cervicovaginal microbiota. Both these studies also found decreased alpha diversity in *S. mansoni*-infected individuals, consistent with gut dysbiosis and inflammation.

In contrast, increased diversity is associated with inflammation in cervicovaginal microbiota [13, 14] and women who were persistently schistosome-infected at follow-up had higher diversity. We hypothesize that this may be explained by immune changes caused by schistosome reinfection. Because praziquantel treatment was observed, women who were infected at baseline were likely cured and re-infected, or had juvenile worms that matured, before follow-up. The first six weeks of schistosome infection are characterized by a dynamic Th1-predominant dominant immune environment, which switches to Th2 predominance when egg-laying begins [50]. We hypothesize that this immune shift could affect microbial diversity, causing women who have been recently infected or reinfected to have increased diversity compared to those with chronic schistosome infections.

Our study was limited by small sample size, precluding our ability to control for differences between infected and uninfected groups and our ability to conduct a paired analysis, given that some baseline and follow-up sample pairs did not amplify. Multiple papers report that cervicovaginal bacterial communities are not associated with STIs, hormonal contraceptive use, or sexual behavior [13, 14]. In addition, women with schistosome infection in our study were more likely to experience food insecurity, which may have affected our findings. Further, the aforementioned potential confounders were similar between *S. mansoni* infected and uninfected groups and *S. haematobium* infected and uninfected groups, so these factors likely did not skew our results. Other limitations include that we did not determine vaginal pH and we could not examine the role of schistosome infection stage at baseline.

In conclusion, we have demonstrated longitudinally that *S. mansoni* and *S. haematobium* infections differentially modify cervicovaginal microbiota, and that both intensity of infection and stage of infection could affect these
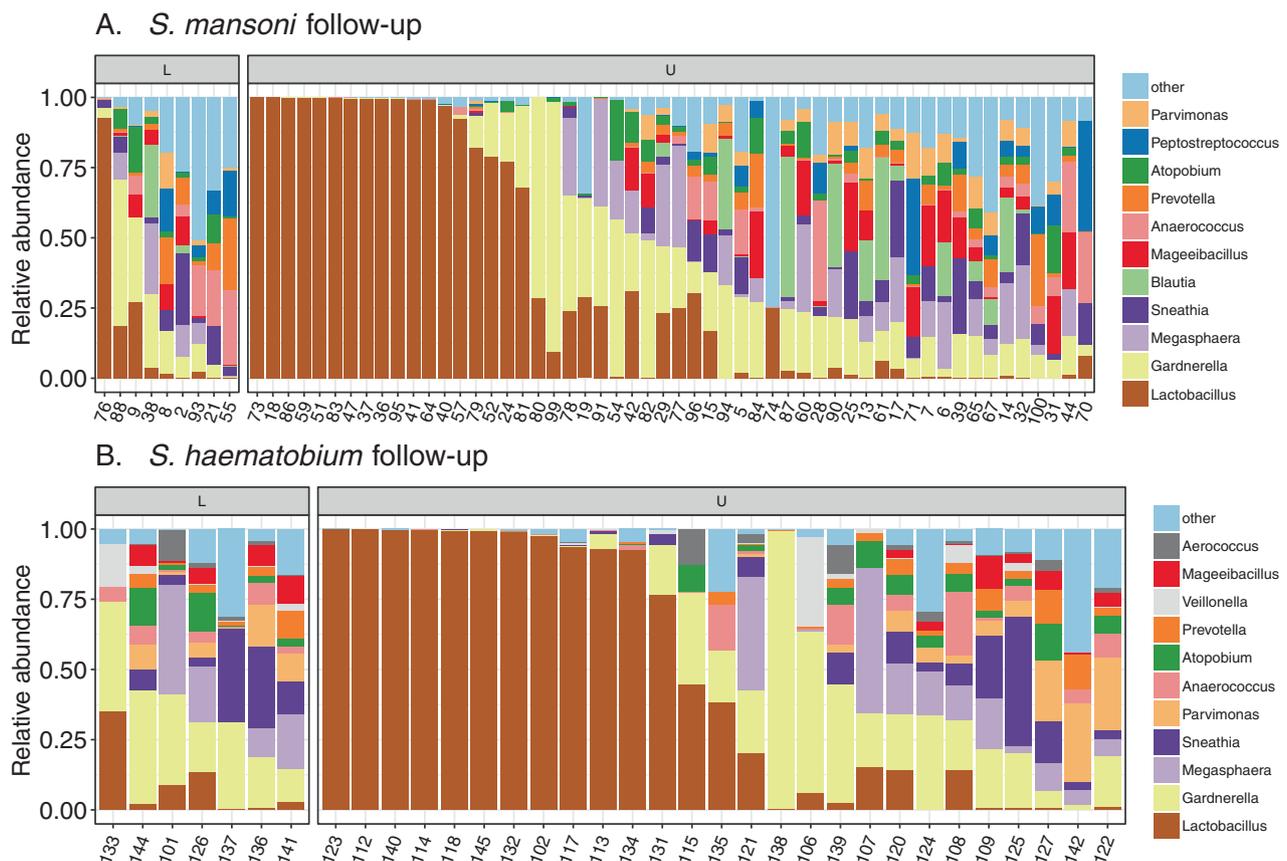
A. *S. mansoni* follow-up

B. *S. haematobium* follow-up

Fig. 5 *S. mansoni* and *S. haematobium* **microbiota by genus at 3-month follow-up.** Relative abundance of cervicovaginal microbiota at the genus level for uninfected, low intensity infected, and high intensity infected women with *S. mansoni* (upper band) and *S. haematobium* (lower band) at 3-month follow-up. Each vertical bar represents a successfully sequenced cervicovaginal sample. Labels above each bar indicate infection status: "U" for uninfected and "L" for low-intensity infection. Women with *S. mansoni* infection had increased *Peptostreptococcus* (as shown in dark blue, $p = 0.049$) and increased alpha diversity ($p = 0.022$). Women infected with *S. haematobium* infected also had increased alpha diversity ($p = 0.017$).

modifications. Changes observed in *S. mansoni*-infected women, including increased *Peptostreptococcus* and *Prevotella timonesis*, offer potential mechanisms for increased HIV incidence in *S. mansoni*-infected women. Observations of increased bacterial diversity in high-intensity infected and potentially newly-infected women with either schistosome species suggest that women with intense or recent schistosome infections may be more susceptible to HIV. These data warrant future studies to investigate the role of schistosome infection stage on cervicovaginal microbiota, as well as the potential to decrease HIV risk by normalizing altered cervicovaginal microbial communities in schistosome-infected women.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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