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# Changes in the Vaginal Microbiome During Pregnancy and the Postpartum Period in South African Women: a Longitudinal Study

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## Abstract

Pregnant women in sub-Saharan Africa have high rates of maternal morbidity. There is interest in the impact of the vaginal microbiome on maternal health, including HIV and sexually transmitted infection (STI) acquisition. We characterized the vaginal microbiota of South African women  $\geq 18$  years with and without HIV in a longitudinal cohort over two visits during pregnancy and one visit postpartum. At each visit, we obtained HIV testing and self-collected vaginal swabs for point-of-care testing for STIs and microbiota sequencing. We categorized microbial communities and evaluated changes over pregnancy and associations with HIV status and STI diagnosis. Across 242 women (mean age 29, 44% living with HIV, 33% diagnosed with STIs), we identified four main community state types (CSTs): two lactobacillus-dominant CSTs (dominated by *Lactobacillus crispatus* and *Lactobacillus iners* respectively) and two diverse, non-lactobacillus-dominant CSTs (one dominated by *Gardnerella vaginalis* and one by diverse facultative anaerobes). From the first antenatal visit to the third trimester (24–36 weeks gestation), 60% of women in the *Gardnerella*-dominant CST shifted to lactobacillus-dominant CSTs. From the third trimester to postpartum (mean 17 days post-delivery), 80% of women in lactobacillus-dominant CSTs shifted to non-lactobacillus-dominant CSTs with a large proportion in the facultative anaerobe-dominant CST. Microbial composition differed by STI diagnosis (PERMANOVA  $R^2 = 0.002$ ,  $p = 0.004$ ), and women diagnosed with an STI were more likely to be categorized as *L. iners*-dominant or *Gardnerella*-dominant CSTs. Overall, we found a shift toward lactobacillus dominance during pregnancy and the emergence of a distinct, highly diverse anaerobe-dominant microbiota profile in the postpartum period.

**Keywords** Vaginal microbiome · Microbiome in pregnancy · South Africa

## Abbreviations

ART	Anti-retroviral therapy	MG	Milligrams
CST	Community state type	NG	<i>Neisseria gonorrhoea</i>
CT	<i>Chlamydia trachomatis</i>	SD	Standard deviation
G	Grams	STI	Sexually transmitted infection
HIV	Human immunodeficiency virus	TV	<i>Trichomonas vaginalis</i>

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## Introduction

Sub-Saharan Africa faces significant challenges in maternal and infant health. In 2020, 70% of all maternal deaths worldwide occurred in sub-Saharan Africa [1], and there were an estimated 50 infant deaths for every 1000 live births in 2020 [2]. Many of these adverse birth outcomes are caused by preventable or curable infectious diseases. HIV has been estimated to account for 24% of pregnancy-related mortality in sub-Saharan Africa and is a leading cause of death among reproductive-aged cisgender women [3, 4]. The risk of HIV acquisition is increased during pregnancy and postpartum, which then also increases the risk of vertical transmission [5]. HIV incidence remains high in pregnant and breastfeeding women in South Africa; in a recent study in Cape Town, postpartum HIV incidence was 1.86/100 person-years (95% CI 0.88–3.89), and incidence was highest during the first 6 months postpartum (2.71/100 person-years, 95% CI 1.13–6.51) [6]. Sub-Saharan Africa also has the highest incidence rate of bacterial STIs in the world, with 60 million new infections every year [7]. Untreated STIs in pregnancy lead to adverse pregnancy outcomes including preterm birth, stillbirth, and infant death [8]. In South Africa specifically, the prevalence of bacterial STIs during pregnancy ranges from 30 to 40% [9, 10].

There is growing interest in the role of the vaginal microbiome in the health of reproductive-aged women and their infants, particularly with respect to HIV and STI acquisition during pregnancy. Studies from North America and Europe have shown that an optimal vaginal microbiome is dominated by lactobacillus species, including *Lactobacillus crispatus*, *L. gasseri*, and *L. jensenii* [11], which modulate vaginal pH predominantly through the production of D-lactic acid [12]. Conversely, microbiomes dominated by facultative anaerobes including *Gardnerella*, *Prevotella*, and *Atopobium* spp. are linked to bacterial vaginosis (BV). BV and anaerobe-dominant vaginal microbial states may increase the risk of HIV and STI acquisition [13–16] and have been shown to be associated with adverse pregnancy outcomes including preterm birth [17–19].

Subsequent work has demonstrated that the vaginal microbiome varies significantly with geography, ethnicity, and socioeconomic status [11, 20, 21]. Studies have reported that African women have diverse vaginal microbiota with high levels of non-lactobacillus anaerobes, including *Gardnerella*, *Prevotella*, and other BV-associated species [13, 22, 23]. They also have a higher relative abundance of *Lactobacillus iners* which is more likely to coexist with rather than inhibit the growth of anaerobic bacteria [24, 25]. These microbial patterns are not fully understood but may be related to a variety of social, economic, and behavioral factors [26, 27]. Observational studies have shown that differences in microbial composition may

increase the risk of acquiring HIV and STIs [15, 25, 28], and some studies have suggested that this may contribute to the disproportionately high burden of HIV and STIs among African women [13, 29, 30]. However, many *L. iners* and anaerobe-dominant profiles also occur in healthy African women and may represent normal variants that behave differently from lactobacillus-dominant profiles [22, 28, 31]. There is a need to better characterize the vaginal microbiome to understand what constitutes optimal and identify patterns associated with the risk of disease acquisition.

Pregnancy is a normal physiologic state that both influences and is influenced by the composition of the vaginal microbiome. Over the course of pregnancy, there is a gradual increase in lactobacillus species (including *L. iners*) and decreased microbial diversity, followed by a rapid increase in diversity and growth of anaerobic species in the postpartum period [17, 20, 23, 28, 32–34]. This is thought to be mediated by estrogen, which promotes glycogen deposition in the vaginal epithelium and supports lactobacillus proliferation [34, 35]. Given the associations between lactobacillus abundance and an optimal microbiome, studies have suggested that pregnancy induces a favorable change in the microbiome to prevent maternal genital infection and adverse birth outcomes [20]. Conversely, the rapid increase in bacterial diversity in the postpartum period [32, 34] may contribute to increased HIV risk during the postpartum period. Shifts in microbial composition during pregnancy may be particularly pronounced in women with diverse microbiome profiles at baseline [20]. Several cross-sectional studies performed in African countries have shown that pregnant women with HIV have more anaerobe-predominant microbiome profiles [14, 25, 31, 36], and a study from Kenya described species differences in pregnant women with *Chlamydia trachomatis* (CT) or *Trichomonas vaginalis* (TV) infection compared to uninfected women. However, there are few longitudinal studies of the vaginal microbiome in pregnant African women, and it is not fully understood whether these microbial transitions during pregnancy and postpartum have any impact on maternal and neonatal outcomes.

This study aims to characterize the vaginal microbiota over the course of pregnancy and in the immediate postpartum period in a cohort of women in Cape Town, South Africa. We also evaluated associations of microbial composition with HIV serostatus and STI diagnosis across the peripartum period.

## Methods

### Recruitment and Visit Procedures

The STI in pregnancy (STIP) study was conducted at a public sector antenatal clinic (ANC) in Cape Town, South Africa, as described previously [10]. Briefly, from

November 2017 to July 2018, we enrolled pregnant women  $\geq 18$  years of age with and without HIV presenting to the ANC for prenatal care. Gestational age was estimated based on the date of the last menstrual period. Women participated in two visits over the course of their pregnancy and one in early postpartum: first visit to the ANC (Visit A), third-trimester visit (Visit B), and postpartum visit (Visit PPt). Women who presented for Visit A at gestational age  $> 28$  weeks did not have a Visit B.

## Data Collection

At each study visit, a trained study counselor collected data from a study survey on demographics, sexual behavior, health data (including HIV status and treatment), and any symptoms of STIs. Each woman then self-collected vulvovaginal swabs using Xpert® CT/NG Vaginal/Endocervical Specimen Collection kits (Cepheid, Sunnyvale, CA). These swabs underwent on-site Xpert nucleic acid amplification testing for *Neisseria gonorrhoea* (NG) and *Chlamydia trachomatis* (CT) and an Xpert TV assay for *Trichomonas vaginalis* (Cepheid). Afterwards, swabs were sent to UCLA for microbiome analysis. Women were given same-day results for CT, NG, and TV testing if the results were available before they left the clinic. Women with a positive STI test result based on the Xpert® result or who reported symptoms and did not report previously receiving treatment for their STI at the same visit were given treatment in accordance with South African national guidelines [37]. CT infections were treated with 1 g azithromycin orally via directly observed therapy, NG with an intramuscular injection of 250 mg ceftriaxone plus 1 g azithromycin orally (or 2 g azithromycin in case of significant penicillin allergy), and TV with 400 mg metronidazole orally every 12 h for 7 days. Women who presented with symptoms including dysuria, unusual vaginal discharge, or vaginal itching were given all three antibiotics as syndromic treatment [37]. As per the national STI guidelines, women were given counselling, provided with condoms, and given a partner notification/referral letter [37].

At enrollment, pregnant women with unknown or negative HIV serostatus were tested for HIV according to the South African National testing guidelines [37]. These women received a Toyo® Anti-HIV ½ rapid assay, and those who tested positive received a confirmatory Determine™ HIV Ag/Ab Combo rapid test (Abbott, Chicago, IL). Women with known HIV reported whether they were taking antiretroviral therapy (ART). Women who tested negative for HIV received repeat rapid HIV testing at each subsequent visit. At the postpartum visit, women were asked about their delivery details and infant outcomes, and

this was verified against their antenatal clinical records. World Health Organization guidelines were used to categorize adverse pregnancy and birth outcomes [38].

## Microbiota Profiling

Profiling of the bacterial microbiota of the collected vaginal swab samples was performed by sequencing the V4 (515F/806R) region of the 16S rRNA gene, as previously described [39]. Briefly, samples were transferred to Lysing Matrix E tubes (MP Biomedicals, Burlingame, CA, USA) with RLT lysis buffer (Qiagen, Hilden, Germany) and bead-beated on a TissueLyser (Qiagen). Following manufacturer protocol, the AllPrep DNA/RNA/Protein kit (Qiagen) was used to extract DNA. In addition to negative controls from the DNA extraction and PCR steps used to identify contaminant sequences, independent aliquots of a bacterial mock community were processed together with samples to evaluate extraction, amplification, and the expected relative abundance of bacteria [40].

## Data Processing and Statistical Analysis

DADA2 was used for exact sequence inference and chimera removal, followed by contaminant sequence removal using the “decontam” R package [41]. Species-level taxonomic labels were assigned using BLASTn against the SILVA database (release 138). We assigned vaginal microbial community state types (CSTs) using the VALENCIA nearest centroid classification method for ease of comparing CSTs across studies [42].

Statistical analyses were performed using the “phyloseq,” “vegan,” “lmerTest,” “glmmTMB,” and “emmeans” packages in the R statistical computing environment (version 4.1.3) [43–47]. Data were stratified by study visit and clinical variables of interest: HIV status, STI diagnosis at any time point, and pregnancy and birth outcomes. The Shannon diversity and Bray-Curtis dissimilarity were used for the analysis of alpha and beta diversity, respectively. Permutational multivariate analysis of variance (PERMANOVA) as implemented in the “adonis2” R function was used to identify drivers of overall microbiota variation. Differences in CST composition and stability were assessed using a chi-squared test or Z-test of equal proportions as appropriate. Alpha diversity was compared using a mixed effects linear model with a subject-level random effect, and data are presented as estimated marginal means. Differential abundance testing at the species level was performed using a zero-inflated negative binomial model. *p*-values for alpha diversity and species-level differential abundance testing were adjusted for multiple comparisons using the Benjamini-Hochberg

false discovery rate (FDR) method. All other *p*-values were reported without adjustment.

## Results

### Population Characteristics

We recruited and enrolled 242 women, of which 107 (44%) were living with HIV (Table 1). The mean age was 29.5 years (SD 6.1), and 101 (42%) participants reported to be married or cohabitating. Most participants had completed secondary school (91%), and most were unemployed or students (69%). The mean gestational age at Visit A (first ANC visit) was 18.6 weeks (SD 6.3), with a range of 6–30 weeks (Supplement S1). The gestational

age range at Visit B (third trimester visit) was 24–36 weeks. The mean gestational age at the postpartum visit was 17 days after delivery (SD 28 days). The majority of women (98.8%) reported a single partner in the 3 months prior to Visit A.

Of the women living with HIV, 76 (71%) were already on ART at the first ANC visit (Visit A), 12 had previously been on ART but were not taking it at the time of Visit A, and 24 were newly diagnosed and had not yet initiated ART. Of the 76 women taking ART, the majority (75%) reported taking a combination of tenofovir disoproxil, emtricitibine, and efavirenz. Six women who initially tested HIV-negative at Visit A were seroconverted by Visit Ppt. A total of 80 participants (33%) tested positive for any STI at any time point, with 52 (22%) positive for CT, 14 (6%) positive for NG, and 38 (16%) positive

**Table 1** Characteristics of study participants (*n* = 242)

		<i>n</i> (%)
Age (mean ± SD)		29.5 ± 6.1
Education	Degree/diploma	10 (4.1)
	Primary	11 (4.5)
	Secondary	221 (91.3)
Employment	Attending school/college	19 (7.9)
	Formal employment	73 (30.2)
	Informal employment	2 (0.8)
	Unemployed	148 (61.2)
Gestational age at Visit A (initial antenatal visit, mean ± SD)		18.6 ± 6.3
HIV status	Not living with HIV	135 (55.8)
	Living with HIV	107 (44.2)
Already on ART at baseline	No	31 (29.0)
	Yes	76 (71.0)
Had vaginal sex during pregnancy	No	17 ( 7.0)
	Yes	225 (93.0)
Number of partners in last 3 months	1	239 (98.8)
	2	3 (1.2)
Any STI diagnosis at any visit	No	162 (66.9)
	Yes	80 (33.1)
CT diagnosis at any visit	No	190 (78.5)
	Yes	52 (21.5)
NG diagnosis at any visit	No	228 (94.2)
	Yes	14 ( 5.8)
TV diagnosis at any visit	No	204 (84.3)
	Yes	38 (15.7)
Term delivery	Full term	220 (90.9)
	Premature (born before 37 weeks)	22 (9.1)
Pregnancy outcome		6 ( 2.5)
	Live birth	222 (91.7)
	Miscarriage	7 ( 2.9)
	Neonatal death	2 ( 0.8)
	Stillbirth	4 ( 1.7)
	Termination	1 ( 0.4)

for TV. The majority of STI diagnoses were made during Visit A (Supplement S2). Overall, 90.9% of participants delivered full-term, and 91.7% had live births without complications.

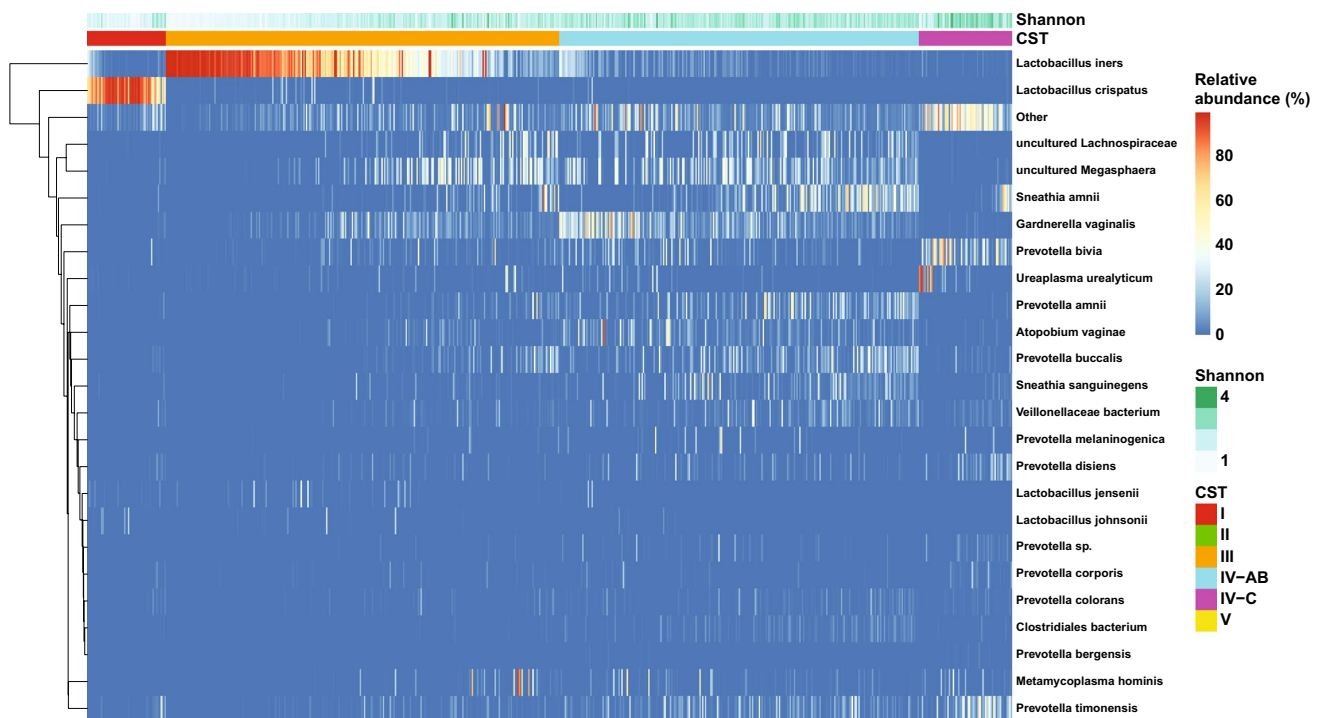
## Bacterial Community Composition

Based on a previously defined set of vaginal community types [42], we identified five CSTs: CST I, dominated by *L. crispatus*; CST III, dominated by *L. iners*; CST IV-A, dominated by *Candidatus lachnovcurva vaginae* and *Gardnerella vaginalis* with moderate *Atopobium vaginae*; CST IV-B, with high *G. vaginalis* and low *Ca. l. vaginae* and moderate *A. vaginae*; and CST IVC, low *Lactobacillus*, *G. vaginalis*, and *A. vaginae* with high proportions of facultative anaerobes including *Prevotella* species (Supplement S3). CST IV-A was relatively small and did not appear distinct from CST IV-B on the two major axes of the principal coordinates analysis (PcoA, Supplement S3), so these two were grouped into CST IV-AB for all remaining analyses (Fig. 1). The most prevalent CSTs were CST III (43% of all samples) and CST IV-AB (39% of all samples). Lactobacillus-dominant CSTs were the majority (51%), but only 9% of women were assigned to CST I. There were no samples assigned to CST

II (*L. jensenii* dominant) or CST V (*L. gasseri* dominant). Approximately 10% of samples were categorized as CST IV-C, which was highly diverse and had large proportions of *Prevotella bivia*, *Prevotella timonensis*, and *Ureaplasma urealytica*.

## Changes in Vaginal Microbiota During Pregnancy

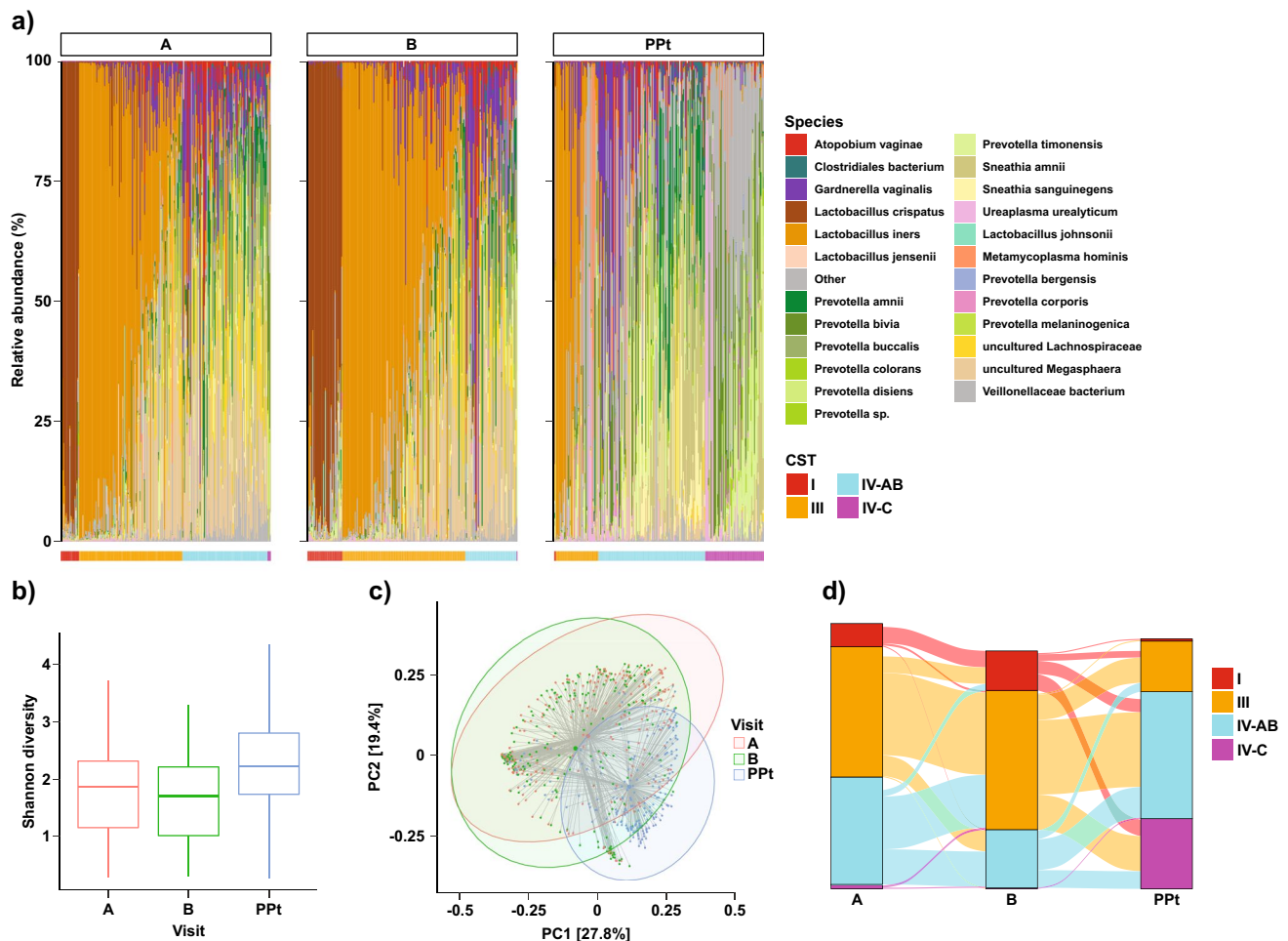
We next looked at the microbiota across Visits A (6–30 weeks), B (24–36 weeks), and postpartum (mean 17 days after delivery) (Fig. 2). Compared to Visit A, women at Visit B had higher relative abundances of *L. crispatus* and *L. iners* and higher proportions of CST I and CST III (Fig. 2a). At the postpartum visit, women were more likely to have CST IV-AB and CST IV-C. Shannon's diversity was significantly increased at the postpartum visit compared to Visit A ( $p < 0.001$ , Fig. 2b). Interestingly, very few samples belonged to CST IV-C at Visit A and Visit B, but a sizable proportion of CST IV-C emerged postpartum. Overall community composition differed significantly by visit or trimester (PERMANOVA  $R^2 = 0.04$ ,  $p < 0.001$ , Fig. 2c). Since Visit A varied widely in gestational age, we examined taxa plots from Visit A by week of gestation (Supplement S4), which did not significantly differ.



**Fig. 1** Heatmap of bacterial taxa identified by 16S sequencing of cervical swabs collected from 242 women across three visits during pregnancy. CSTs determined by the VALENCIA classifier; CST I dominated by *L. crispatus*; CST III dominated by *L. iners*; CST IVA dominated by *Candidatus lachnovcurva vaginae* and *Gardnerella*

*vaginalis* with moderate *Atopobium vaginae*; CST IVB with high *G. vaginalis* and low *Ca. l. vaginae* and moderate *A. vaginae*; and CST IVC, low *Lactobacillus*, *G. vaginalis*, and *A. vaginae* with high proportions of facultative anaerobes including *Prevotella* species. The Shannon diversity index for each sample is depicted across the top





**Fig. 2** Composition of the vaginal microbiome across three visits during pregnancy. Visit A = initial visit (gestational age ranging from 6 to 30 weeks); Visit B = second-trimester visit (GA 28–36 weeks); Visit PPt = postpartum visit (mean 17 days after delivery). **a** Relative proportions of bacterial taxa at each visit across pregnancy. CSTs are shown across the bottom. **b** Shannon's diversity between visits, presented as box plots with mean and interquartile range for each visit. The Shannon diversity was higher at Visit PPt compared to Visit A ( $p = 2.0E-11$ ). **c** PCoA of samples across visits using the Bray-Curtis index (PERMANOVA  $R^2 = 0.04$ ,  $p < 0.001$ ). Shaded ellipses denote

95% confidence intervals. **d** Transitions between CSTs across visits. Moderate changes in CST distribution were observed from Visit A to B, with decreased CST IV-AB at Visit B, as a result of shifting to CST I or CST III. CST IV-AB was more likely to transition to CST III than to CST I (chi-squared  $p < 1E-10$ ). From Visit B to Visit PPt, a majority of CST III samples converted to CST IV-AB, while some members of CST I, CST III, and CST IV-AB all shifted to CST IV-C. CST III was more stable and less likely to transition than CST I (Table 2, chi-squared  $p = 0.048$ )

Moderate transitions in CST distribution were observed at an individual level from Visit A to B, with women with CST IV-AB at Visit A predominantly shifting to CST III (Fig. 2d, Table 2, chi-squared  $p < 0.001$ ).

During pregnancy, women with CSTs I and III had a more stable vaginal microbiota than those with IV-AB, whereas CST IV-AB was more stable during the transition period from pregnancy to postpartum (Supplement S5). From Visit B to postpartum, drastic CST transitions were observed, with a majority of CST III samples shifting to CST IV-AB, while a high proportion of women with CST I, CST III, and CST IV-AB all shifted to CST IV-C in similar proportions (Fig. 2d, Table 2). In this transition, very few women in both

CST I and CST III remained in the same CST, but women in CST III were more likely than women in CST I to remain in the same CST at the postpartum visit ( $p = 0.048$ ).

### Vaginal Microbial Associations with HIV Serostatus

We next assessed whether HIV infection and any STI diagnosis during pregnancy were associated with differences in vaginal microbiota. CST distribution did not differ by HIV status at any visit (Fig. 3a, Table 3), although community composition differed significantly between women with and without HIV (PERMANOVA  $R^2 = 0.002$ ,  $p = 0.006$ , Fig. 3b). Shannon's diversity was not significantly

**Table 2** Transitions across CSTs from (a) Visit A (initial antenatal visit) to Visit B (third trimester) and (b) Visit B (third trimester) to Visit PPt (postpartum visit)

(a)		To CST (at Visit B)			
		I	III	IV-AB	IV-C
From CST (at Visit A)*	I	15 (83.3%)	2 (11.1%)	1 (5.6%)	0 (0.0%)
	III	15 (13.6%)	75 (68.2%)	19 (17.3%)	1 (0.9%)
	IV-AB	6 (7.0%)	48 (55.8%)	32 (37.2%)	0 (0.0%)
	IV-C	0 (0.0%)	2 (66.7%)	1 (33.3%)	0 (0.0%)
(b)		To CST (at Visit PPt)			
		I	III	IV-AB	IV-C
From CST (at Visit B)#^	I	1 (2.9%)	6 (17.6%)	12 (35.3%)	15 (44.1%)
	III	1 (0.8%)	23 (18.5%)	68 (54.8%)	32 (25.8%)
	IV-AB	0 (0.0%)	8 (15.4%)	28 (53.8%)	16 (30.8%)
	IV-C	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)

\*Women in CST IV at Visit A were more likely to transition to CST III than CST I at Visit B (chi-squared  $p < 0.001$ )

#Compared to CST III, women in CST I at Visit B were less likely to remain in the same CST at Visit PPt (chi-squared  $p = 0.048$ )

^At Visit B, there was no significant difference in the likelihood of women of CSTs I, III, or IV-AB to transition to IV-C at Visit PPt ( $p = 0.1177$ )

different between women with and without HIV (Fig. 3c). With respect to individual species, differential abundance testing showed that HIV infection was associated with a lower relative abundance of *L. jensenii* at Visit B, a lower relative abundance of *P. corporis* and *bergensis* at the postpartum visit, and higher *Metamycoplasma hominis* and *A. vaginae* at the postpartum visit (Supplement S6,  $p < 0.01$ ). Transitions in CSTs did not appear to differ by HIV status (Fig. 3d). Specifically, there was no significant difference between women with and without HIV in the likelihood of transitioning from CST IV to CST I or III from Visit A to Visit B, and no significant difference in transition from CST I or III from Visit B to CST IV at Visit PPt, nor any differences in CST stability (Supplement S5).

Overall, 29% of women living with HIV had not yet initiated ART at Visit A. A higher proportion of women living with HIV who had not yet initiated ART had vaginal microbiota characterized by CST IV-AB, though Shannon's diversity was non-significantly higher in this group (Supplement S7, unadjusted  $p = 0.06$ ). The six women who seroconverted to HIV-positive over the course of the study were all categorized as CST IV-AB or CST IV-C at Visit PPt (Supplement S8), though this difference was not statistically significant.

### Vaginal Microbial Associations with STI Diagnosis During the Peripartum Period

CST distribution differed between women with and without an STI diagnosis (Fig. 4a). At Visit A, women with an STI diagnosis were more likely to be categorized as CST IV-AB or CST III (Fig. 4a, Table 3, Fisher's exact  $p = 0.013$ ). This pattern appeared to hold for each individual STI, though it did not reach significance for NG (Supplement S9, Table 3).

Only three women categorized as CST I at Visit A were diagnosed with STIs at any visit. Postpartum, there was a trend for women with an STI diagnosis to be more likely to have vaginal microbiota characterized as CST IV-C compared to any other CSTs (Table 3,  $p = 0.07$ ).

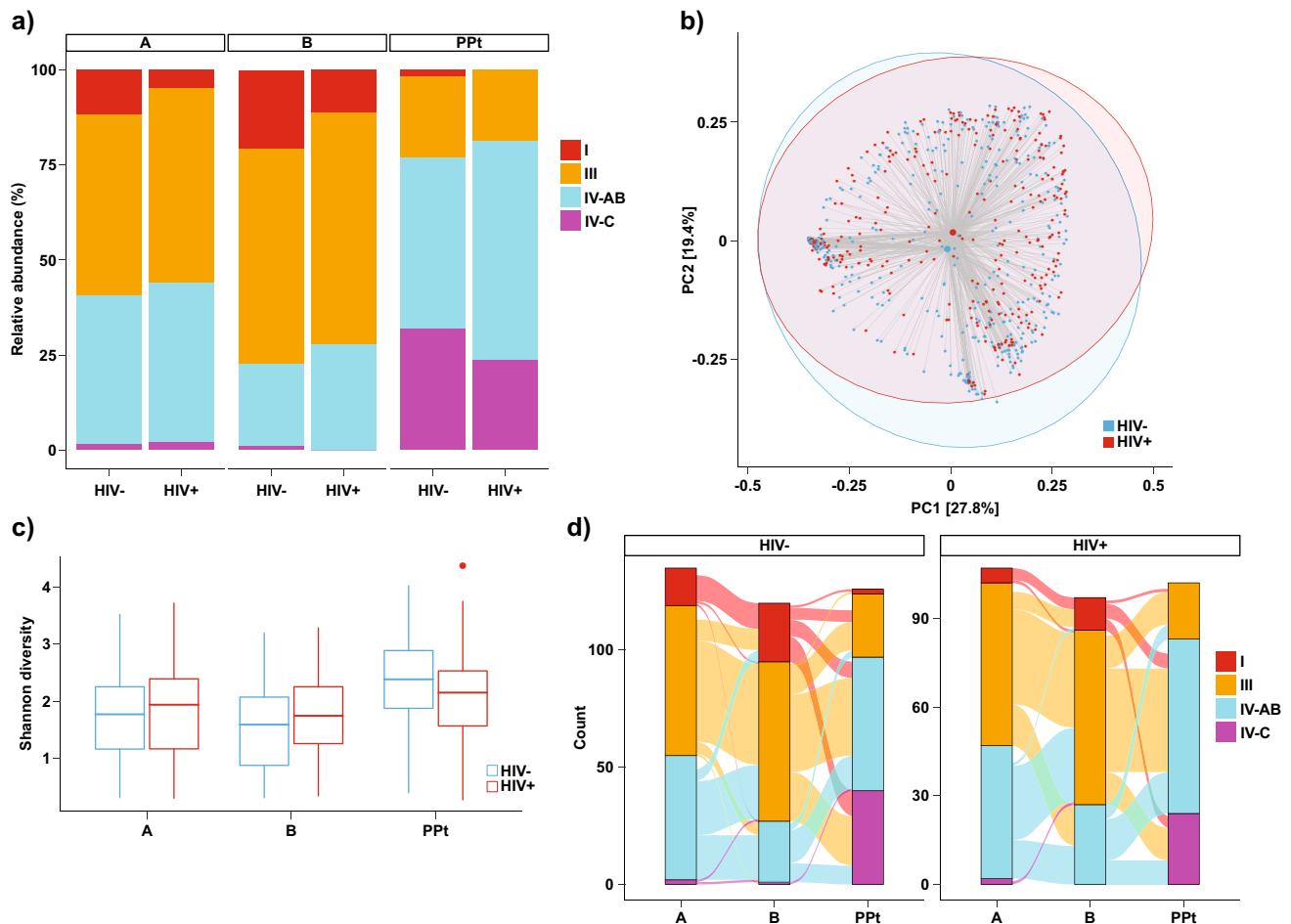
Overall microbiota composition also differed between women with and without an STI diagnosis (PERMANOVA  $R^2 = 0.002$ ,  $p = 0.004$ , Fig. 4b). Shannon's diversity was increased in women who had an STI diagnosis compared to women who did not at Visit A (Fig. 4c,  $p = 0.004$ ) and at Visit B (Fig. 4c,  $p = 0.05$ ). Individual taxa associated with STI diagnosis included *P. bivia*, *colorons*, *amnii*, and *bucallis*, *Metamycoplasma hominis*, and *Sneathia amnii* at Visit A (Supplement S10,  $p < 0.05$ ), as well as *P. bucallis* at Visit B and other *Prevotella* species at Visit PPt (Supplement S10,  $p < 0.05$ ). Most of these associations appeared to be driven by *C. trachomatis* (Supplement S9 and S10). STI diagnosis did not appear to have any impact on CST stability and transition probabilities over the course of pregnancy (Fig. 4d, Supplement S5).

Fifty-nine women reported possible STI-related symptoms at Visit A and 14 at Visit B. Visually, CST distribution showed increased CST IV-C at Visit PPt in women who reported symptoms (and thus received antibiotics) at a prior visit, but PCoA and Shannon's diversity index showed no significant differences (Supplement S11).

### Vaginal Microbial Associations with Adverse Pregnancy and Birth Outcomes

In addition to HIV and STI status, we also examined birth outcomes and other variables for associations with microbiota composition. Overall, 36 participants (15%) reported adverse





**Fig. 3** CST distribution across visits with respect to HIV status. **a** Relative proportions of CSTs across visits, stratified by HIV status. **b** Shannon's diversity at each visit by HIV status. **c** PCoA by HIV status across all visits using the Bray-Curtis index (PERMANOVA  $R^2 =$

0.002,  $p = 0.006$ ). Shaded ellipses denote 95% confidence intervals. **d** Transitions between CSTs across visits, stratified by HIV status. No differences in transition probabilities were observed

pregnancy or birth outcomes including miscarriage, neonatal death, stillbirth, or preterm birth. We included these outcomes as a composite adverse birth or pregnancy outcome variable. Visually, women who had adverse pregnancy or birth outcomes were more likely to be categorized in CST IV-A at Visit A or Visit B and less likely to be in CST IV-C at Visit PPt, as compared to women who delivered full-term live infants, but the result was not statistically significant (Supplement S12). We did find a relationship between age and CST, with younger women being more likely to be in CST IV-C at both Visit A ( $p = 0.044$ ) and Visit PPt ( $p = 0.035$ , Table 3).

## Discussion

We profiled the vaginal microbiota in a cohort of South African women over three visits during pregnancy and in the immediate postpartum period. We confirmed the transition

of diverse microbial signatures toward lactobacillus-dominant signatures during pregnancy and identified the emergence of a facultative-anaerobe-rich, diverse signature in the early postpartum period which appears distinct from the vaginal microbial signatures of women early in pregnancy. We did not find a significant difference in CST distribution in women with and without HIV, but we found that women diagnosed with STIs were more likely to have microbial signatures belonging to CST III or CST IV-A at the first ANC visit. To our knowledge, this is one of the few longitudinal studies of the vaginal microbiota in pregnant South African women.

We categorized vaginal 16S rRNA gene sequences according to the VALENCIA nearest-centroid classification model for ease of comparison across populations. Consistent with prior studies among women of African descent [11, 22], about half of all samples belonged to lactobacillus-dominant CSTs, whereas the other half were dominated by diverse

**Table 3** Differences in participant characteristics across CSTs at Visit A ( $n = 242$ )

		CST				<i>p</i> -value
		I	III	IV-AB	IV-C	
Median age (IQR)		32 (26–37)	29 (24–35)	30 (26–33)	22.5 (20.5–24.5)	0.044
Having vaginal sex at Visit A	Yes	20 (95.2%)	112 (94.1%)	89 (90.8%)	4 (100%)	0.77
	No	1 (4.8%)	7 (5.9%)	9 (9.2%)	0 (0%)	
HIV/ART status	Not living with HIV at baseline	16 (76.2%)	64 (53.8%)	53 (54.1%)	2 (50%)	0.256
	Living with HIV, not on ART at baseline	2 (9.5%)	12 (10.1%)	16 (16.3%)	1 (25%)	
	Living with HIV and on ART at baseline	3 (14.3%)	43 (36.1%)	29 (29.6%)	1 (25%)	
STI diagnosis at any visit	Yes	3 (14.3%)	35 (29.4%)	41 (41.8%)	3 (75%)	0.013
	No	18 (85.7%)	84 (70.6%)	57 (58.2%)	1 (25%)	
CT diagnosis at any visit	Yes	1 (4.8%)	21 (17.6%)	30 (30.6%)	1 (25%)	0.019
	No	20 (95.2%)	98 (82.4%)	68 (69.4%)	3 (75%)	
NG diagnosis at any visit	Yes	1 (4.8%)	7 (5.9%)	7 (7.1%)	2 (50%)	0.068
	No	20 (95.2%)	112 (94.1%)	91 (92.9%)	2 (50%)	
TV diagnosis at any visit	Yes	2 (9.5%)	18 (15.1%)	14 (14.3%)	3 (75%)	0.037
	No	19 (90.5%)	101 (84.9%)	84 (85.7%)	1 (25%)	
Birth outcome	Normal	18 (85.7%)	94 (79%)	72 (73.5%)	3 (75%)	0.794
	Adverse event	3 (14.3%)	24 (20.2%)	24 (24.5%)	1 (25%)	
	Missing outcome	0 (0%)	1 (0.8%)	2 (2%)	0 (0%)	

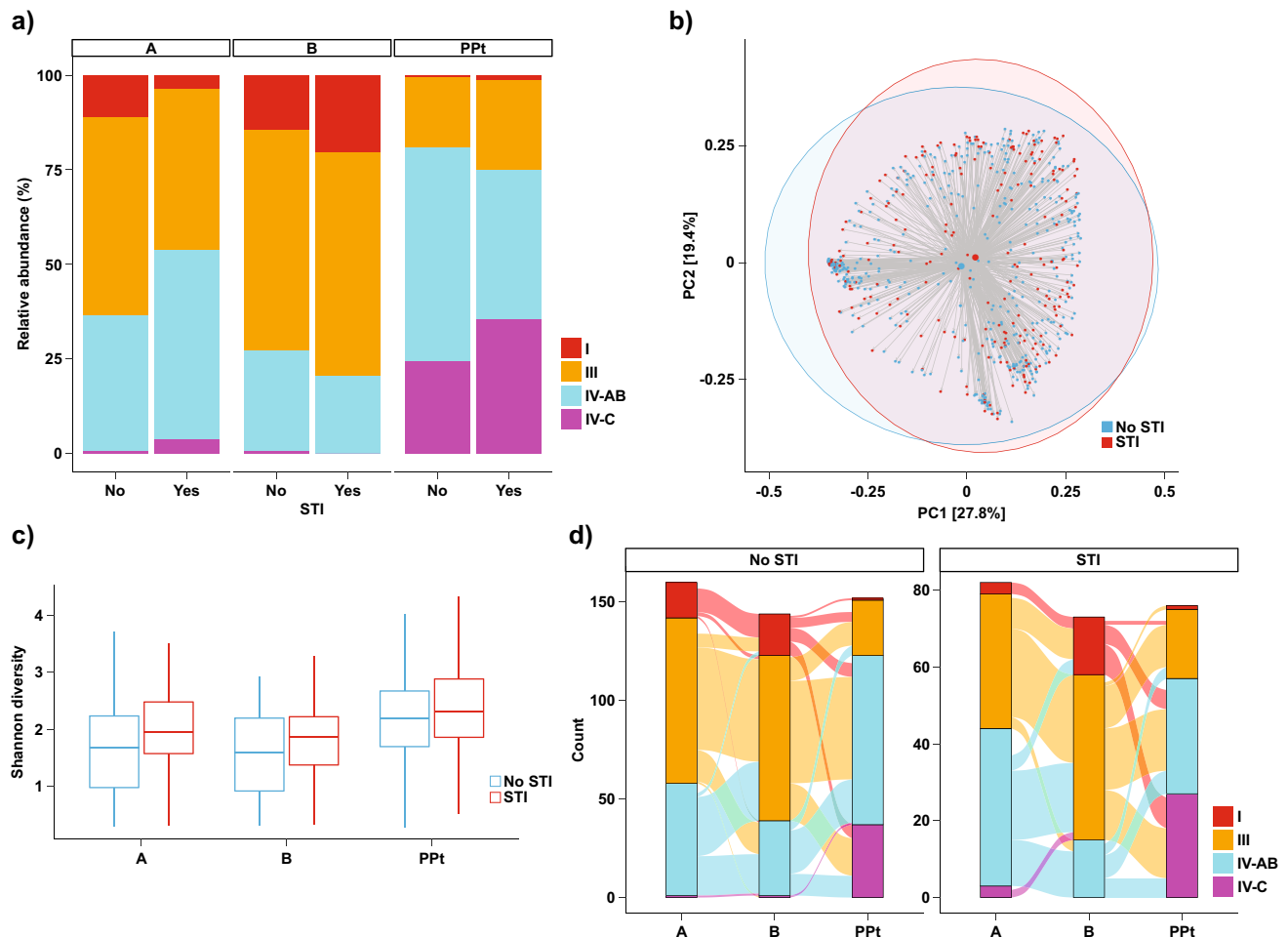
communities. Among the diverse communities, we found distinct and substantial populations of CST IV-AB and CST IV-C. Other studies performed in African populations have also identified distinct anaerobe-dominant CSTs [15, 25, 31, 36], though this may vary with CST clustering methods [48]. It is unclear whether these two non-lactobacillus-dominant CSTs are clinically significant. However, previous studies in non-pregnant women in the FRESH and CAPRISA cohorts also identified a distinct, facultative anaerobe-dominant cervicotype which showed a significantly increased risk of HIV acquisition, in addition to a *Gardnerella*-dominant cervicotype which did not show this same increased risk [15, 49].

During pregnancy, we noted a significant shift away from CST IV toward lactobacillus-dominant CSTs, which is consistent with prior studies ([20, 28, 33, 34]. Serrano et al. showed that American women of African descent had a decrease in diversity and transition to lactobacillus species early in pregnancy, as early as the second trimester [20]. Our data is limited by the variability of gestational age at the first visit and the absence of a microbiome sample before pregnancy, but there was a clear shift toward lactobacillus species even into the third trimester (Visit B). Like Serrano et al., we noted that vaginal microbial profiles from CST IV were more likely to switch to CST III as compared to CST I [20].

In the postpartum period, we observed an increase in facultative anaerobic taxa and alpha diversity with a significant number of women categorized as CST IV-C, which was not present in high proportions during pregnancy. We noted that both CST I and CST III were similarly likely

to transition to CST IV-AB and CST IV-C postpartum. Unlike Goltsman et al., who found that CST I was more stable than CST III from the third trimester into the postpartum period, our data showed the opposite [50]. Previous studies have documented a sharp increase in diversity and anaerobic taxa during the postpartum period [32–34, 36], in part from a combination of decreased estrogen and changes in cervical remodeling during delivery [32]. Altogether, these results support a model by which pregnancy acts as a selective pressure toward a more optimal, lactobacillus-dominant vaginal microbiome, possibly mediated by estrogen. Understanding the process by which this occurs may facilitate the development of therapeutics, such as topical hormones, for beneficially altering the vaginal microbiome during or after pregnancy [51, 52].

We found differences in community composition, but not alpha diversity or CST distribution in the vaginal microbiota of women with and without HIV. Prior studies show increased alpha diversity in women living with HIV versus women without HIV [14, 16, 31], though some of these results may be confounded by socioeconomic factors. Price et al. found that compared with women on ART, women not on ART have more diverse vaginal microbiomes with a higher proportion of anaerobes. Chehoud et al. showed no difference in vaginal microbiota by HIV status when all women with HIV women were virally suppressed on ART [53]. In our study, a subset of women who were not on ART at the first ANC visit trended toward more CST IV-AB at Visit A compared to those established on ART, suggesting some of this discrepancy is explained



**Fig. 4** CST distribution across visits with respect to STI diagnosis at any visit. **a** Relative proportions of CSTs across visits, stratified by STI diagnosis. **b** Shannon's diversity by STI diagnosis across all visits. At Visit A, Shannon's diversity was higher among women who had been diagnosed with an STI ( $p = 0.004$ ). **c** PCoA by STI diagno-

sis across all visits using the Bray-Curtis index (PERMANOVA  $R^2 = 0.002$ ,  $p = 0.004$ ). Shaded ellipses denote 95% confidence intervals. **d** Transitions between CSTs across visits, stratified by STI diagnosis. No differences in transition probabilities were observed

by ART use or degree of immunodeficiency. Unfortunately, our results are limited by the absence of viral load and CD4 data from the time of enrollment.

At the first ANC visit, women who were diagnosed with any STIs (CT, NG, or TV) were more likely to be categorized as CST IV-AB or CST III than CST I. Our findings are consistent with previous studies noting fewer STI diagnoses in *L. crispatus*-dominant profiles, and more STI diagnoses in both *L. iners* and anaerobe-dominant profiles [25, 54], and supports the hypothesis that *L. crispatus* is more protective against pathogens than *L. iners* and anaerobic species. More research is needed to understand whether and how *L. crispatus* protects against pathogens, and whether there are distinctions between *L. iners* and anaerobe-dominant microbiome profiles with respect to STI susceptibility, as STIs are a significant contributor to peripartum morbidity in sub-Saharan Africa. We did not find any significant differences in the

microbiota with respect to adverse birth outcomes including preterm birth. However, our study was not powered to detect this difference, and only 36 women in our sample had adverse pregnancy or birth outcomes.

Strengths of our study include its longitudinal design across pregnancy and into the postpartum period. In addition to the limitations already mentioned, others include small numbers of women living with HIV who are not on ART. For STI testing, as all women were treated, it is unclear if any subsequent changes in their microbiota were mediated by antibiotics. We did not collect data on inflammatory markers, metabolomics, or other vaginal biomarkers. Additionally, our study took place among pregnant women at one antenatal clinic in Cape Town, and the results may not be generalizable across other regions or populations. Finally, this study was observational and cannot make any causal inferences regarding pregnancy and vaginal microbiota.

## Conclusions

Our study confirms a shift toward lactobacillus dominance during pregnancy and the rapid emergence of distinct, highly diverse anaerobe-dominant vaginal microbial communities in the postpartum period. More work is needed to better understand the impact of the vaginal microbiome on perinatal outcomes and STI, HIV acquisition, and vertical transmission. Researchers have suggested that the shift toward lactobacillus during pregnancy, which is mediated by estrogen and other physiologic changes, might foster a more optimal vaginal environment to prevent infection during pregnancy, which is then lost during the postpartum period. If this is the case, then there may be a role for hormonal and other therapies mimicking the physiologic changes of pregnancy to manipulate the vaginal microbiome toward an optimal state.

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**Author Contributions** DJD, HJ, and KL conceived the study. KL wrote the first version of the manuscript. FL provided statistical support for the design of the manuscript and performed the sequencing and statistical analysis. GA supervised the laboratory analytical procedures and validated the laboratory results. DN, DJD, and LM performed the study design, coordination, and data collection for the study. All authors contributed to the editing and approved the final paper.

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**Data Availability** The datasets generated and/or analyzed during the current study will be available in the NCBI SRA at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA894713>.

**Code Availability** Available upon request.

## Declarations

**Ethics Approval** This study was part of the STIP (STI in pregnancy) study done in Cape Town, South Africa. Ethical approval and oversight were obtained from the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town (#454/2017) and the University of California Los Angeles (#19-000237). All experiments were performed in accordance with the Declaration of Helsinki.

**Consent to Participate** Written informed consent was obtained from all participants before enrollment. Study data were collected and managed using Research Electronic Data Capture (REDCap). All study data used a unique participant identifier allocated at study enrolment, and

all electronic communications were done through password-protected, encrypted files.

**Consent for Publication** All participants provided informed consent for the publication of de-identified data.

**Competing Interests** The authors declare no competing interests.

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