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Vaginal microbiome, antiretroviral concentrations, and HIV genital shedding in the setting of hormonal contraception initiation in Malawi

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Abstract

Objective.—To understand how vaginal microbiota composition affects antiretroviral concentrations in the setting of hormonal contraception initiation.

Methods.—Cervicovaginal fluid (CVF) concentrations of tenofovir, lamivudine, and efavirenz from 73 Malawian women living with HIV were compared before and after initiation of depot-medroxyprogesterone acetate (DMPA) or levonorgestrel implant. We evaluated antiretroviral concentrations and vaginal microbiota composition/structure in the context of contraception

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initiation and predicted genital shedding using multivariable repeated measurements models fit by generalized estimating equations.

Results.—Mean lamivudine CVF concentrations decreased 37% 1 month after contraception initiation. Subgroup analyses revealed a 41% decrease in women 1 month after initiating levonorgestrel implant, but no significant difference was observed in DMPA group alone. Tenofovir, lamivudine, and efavirenz CVF concentrations were positively correlated with anaerobic bacteria associated with non-optimal vaginal microbiota. Risk of genital HIV shedding was not significantly associated with tenofovir or lamivudine CVF concentrations (tenofovir RR: 0.098, p=0.75; lamivudine RR: 0.142, p=0.54). Lack of association between genital HIV shedding and efavirenz CVF concentrations did not change when adjusting for vaginal microbiota composition, and lamivudine/tenofovir CVF concentrations (RR: 1.33, p=0.531).

Conclusion.—No effect of hormone initiation on genital shedding provides confidence that women with HIV on either DMPA or levonorgestrel implant contraception will not have compromised ART efficacy. The unexpected positive correlation between antiretroviral CVF concentrations and certain bacterial taxa relative abundance requires further work to understand the mechanism and clinical relevance.

Keywords

HIV; Microbiome; Progestin contraception; Antiretroviral Concentrations; Genital Shedding

Introduction

The effect of progestin contraceptives on antiretroviral concentrations are unknown in the female genital tract (FGT), which has implications for HIV treatment and prevention. In 2020, approximately 15.4 million women of childbearing age (15 – 49 years old) were living with HIV, with 50% in Sub-Saharan Africa (SSA), and many were on both contraceptives and antiretrovirals [1]. Studies examining female to male HIV transmission in the context of hormonal contraception have been mixed with adjusted hazard ratios from 0.46 to 1.76. [2,3] We have previously shown no association between HIV genital shedding and initiation of two progestin contraceptives, depot medroxyprogesterone acetate (DMPA) or levonorgestrel implant up to 33 months post contraception initiation in Malawian women living with HIV (WLWH), [4,5]

Non-optimal vaginal microbiota have been associated with a decrease in tenofovir efficacy, which may have implications for HIV genital shedding. Identifying vaginal microbiota effect on antiretroviral integrity in the vaginal microenvironment would provide insight for characterizing dose-response relationships.

Here, we sought to characterize associations between CVF antiretroviral exposure and the vaginal microbiota. We also examined correlations between CVF tenofovir and lamivudine concentrations and genital shedding of HIV in the setting of DMPA and levonorgestrel implant contraception initiation.

Methods

Study recruitment, enrollment, and visits

The study procedures for this randomized trial have been previously described [6] and primary results published. [5] Detailed methods can be found in the Supplemental Methods. Under a protocol approved by University of North Carolina and U.S. Centers for Disease Control and Prevention IRB, the Malawi National Health Sciences Research Committee, and the Malawi Pharmacy Medicines and Poisons Board, participants were recruited between May 2014- April 2015 from patients desiring contraception at Bwaila Maternity Hospital in Lilongwe, Malawi. Enrolled participants provided informed consent to be randomized to either DMPA or levonorgestrel implant. Samples were collected during first 14 days of next anticipated menstrual cycle to capture the follicular phase, and then again between day 15 from onset of last menses until start of next menses to capture the luteal phase. Women then initiated DMPA or levonorgestrel implant during first seven days after start of next menses. Women returned for repeat sample collection three days and one month after contraceptive initiation.

Weck-Cell/ vaginal swab collection and cervicovaginal fluid ART analysis

Efavirenz, tenofovir, and lamivudine were quantified in Weck-Cel sponges by the UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core (CPAC) using validated HPLC-MS/MS methods (Supplemental Methods and [4]). HIV-1 RNA quantification was performed on the Abbott m2000 System after RNA extraction from TearFlo strips by the UNC Center for AIDS Research HIV/STD Laboratory Core [5].

Microbiome Sequencing and Community State Type Assignment

16S ribosomal RNA gene amplicon sequencing was performed on DNA extracted from vaginal swabs as previously published. [7] Community state types (CSTs) describe the vaginal bacterial communities present and were determined using Bray-Curtis dissimilarity measure and Ward linkage hierarchical clustering [8].

Statistical Analysis

Cervicovaginal concentrations of tenofovir, lamivudine, and efavirenz were log transformed for all analyses. CVF concentrations of tenofovir, lamivudine, and efavirenz were compared between follicular and luteal phase visits to test antiretroviral differences throughout the menstrual cycle using a Kruskal Wallace test. We compared CVF drug concentrations before and after contraceptive initiation to test changes due to contraception using a multivariable generalized linear model with repeated measurement fits with generalized estimating equations (GEE).

Differences in CVF antiretroviral concentrations between assigned CST groups independent of time were evaluated using a multivariable generalized linear model with GEE. CST I was the reference as it is considered an optimal CST due to high relative abundance of *L. crispatus*. [8] We also compared changes in mean tenofovir, lamivudine, and efavirenz CVF concentrations between CSTs, dependent and independent of time.

The 20 most prevalent bacterial taxa, each with a relative abundance greater than 0.1%, were included in this analysis. The rest of the present taxa were added into “other” category. Single variable generalized linear model with repeated measurement fits with GEE was performed to examine significant associations between CVF antiretroviral concentrations and individual bacterial taxa.

Associations of detectable (≥200 copies/ml) genital tract HIV-1 RNA with antiretroviral CVF drug concentrations were evaluated using a multivariable log-binomial model with repeated measurements fit with GEE adjusted for CST and relative abundance.

Results

Participant Characteristics

We included all 73 WLWH in the trial (37 randomized to DMPA and 36 to levonorgestrel implant). Sixty-two women had data from all four visits; 11 women had only three visits. Supplemental Figure 1 depicts vaginal bacterial community states and the corresponding CSTs for all 281 samples. Supplemental Table 1 shows the number of women in each CST group at each visit. Supplemental Figure 2 depicts changes in CST classification over time for each participant.

Associations between cervicovaginal fluid antiretroviral concentrations and hormonal contraception initiation

CVF concentrations of tenofovir, lamivudine, and efavirenz were not significantly different between follicular and luteal phases (TFV, p = 0.31; 3TC, p = 0.92; EFV, p = 0.92). Therefore, we pooled pre-initiation visits when comparing changes in antiretroviral concentrations between pre-contraception initiation, three days, and one month post-contraception initiation.

Tenofovir and efavirenz CVF concentrations were not significantly affected by contraception use up to one month after initiation. (Figure 1A, p=0.75; Figure 1C, p=0.23) Lamivudine CVF concentrations decreased by 37% one month after contraception initiation (Figure 1B, p=0.014). Specifically, women who initiated levonorgestrel implant had a 41% decrease in CVF lamivudine concentrations while women on DMPA had no effect (levonorgestrel, p=0.036; DMPA, p=0.085).

Associations between cervicovaginal fluid antiretroviral concentrations and structure of the microbiota

Six CSTs were defined for this study: CST I, III-A, III-B, IV-A, IV-B, and IV-C. CST I and III are dominated by *L. crispatus* and *L. iners,* respectively, whereas CST IVs comprise diverse sets of anaerobic bacteria and little *Lactobacilli*. No women with available drug concentrations were in CST II. Drug concentrations within CSTs were not affected by visit (Supplemental Figure 3 A-C), suggesting that contraception initiation did not affect the relationship between CSTs and antiretrovirals. Additionally, Haddad *et al.* found no effects of DMPA and levonorgestrel on vaginal microbiota in the same cohort of women.[7] Therefore, visits were pooled for analyses.

Cervicovaginal concentrations for all three drugs were associated with specific CSTs which varied by drug (Table 1). Timepoints with vaginal swabs assigned to CST III-B had 60% lower tenofovir CVF concentrations than CST I (p=0.026). Lamivudine CVF concentrations were significantly lower in samples in CSTs III-A, IV-A, and IV-B compared to CST I. (III-A: 41%, p=0.01; IV-A: 46%, p=0.019; IV-B: 48%, p=0.016). Efavirenz CVF concentrations in samples from CST IV-C were 62% lower than CST I (p=0.0094).

For all three drugs, there was a positive correlation between CVF drug concentrations and relative abundance of anaerobic taxa (Supplemental Table 2). CVF tenofovir concentrations were positively correlated with *Prevotella bivia*, *L. crispatus*, and the phylum *Proteobacteria*. CVF lamivudine concentrations were positively correlated with phylum *Proteobacteria*, genus *Megasphaera*, *L. crispatus*, and *P. bivia*. CVF efavirenz was positively correlated with *Atopobium vaginae*. CVF efavirenz concentrations were negatively correlated with “other” bacteria (all bacteria with relative abundance <0.1%) but had a high standard error (SE = 32.23). *G. vaginalis* was not significantly associated with CVF drug concentrations (TFV: p=0.98; 3TC: p=0.66; EFV: p=0.48).

Risk of genital shedding

In 12 out of the 73 women, genital HIV shedding was detectable at one or more of visits included in this analysis. Neither tenofovir nor lamivudine CVF concentrations were associated with genital HIV shedding, even after adjusting for CST and bacterial load (RR: 0.35, p=0.31; RR: 0.34, p=0.48). Here, we found no significant improvement over our previously published analysis with CVF efavirenz [4] for the prediction of risk of genital HIV shedding after adjusting for vaginal microbiota with efavirenz, tenofovir, and lamivudine. (EFV, RR: 1.35, p=0.064; TFV, RR: 0.22, p=0.93: 3TC, RR: 0.13, p=0.73).

Discussion

Hormonal contraception and vaginal microbiota may affect antiretroviral disposition and efficacy, such as genital HIV shedding, in the FGT. While we found no significant changes in tenofovir or efavirenz CVF concentrations due to the initiation of contraception, lamivudine concentrations decreased one month after levonorgestrel implant initiation, with no significant changes in women on DMPA. Notably, contraception did not influence viral shedding. Further decreases in lamivudine concentrations more than a month after contraceptive initiation are possible and may warrant further study.

While CST III-B was associated with lower tenofovir concentrations compared to CST I, tenofovir concentrations were not correlated with *L. iners* relative abundance, dominating species in CST III-B. This pattern was also seen with lamivudine and efavirenz, suggesting not one bacterial taxon is responsible for changes in CVF antiretroviral concentration, and differences may be more driven by functional profiles. Functional differences exist between strains of the same species and these cannot be accounted for in our analyses. [9]

Findings of no correlation between any drug in CVF and *G. vaginalis* contrasts with Klatt *et al.* who showed a decrease in tenofovir concentrations in a co-culture with *G. vaginalis* and *P. bivia in vitro*, attributed to bacterial metabolism of tenofovir to adenine. [10]

However, our findings are consistent with two Phase 1 studies of a tenofovir/levonorgestrel intravaginal ring showing no long term correlations between tenofovir CVF concentrations and *G. vaginalis*. [11,12]

There are two possible mechanisms behind the unexpected positive correlations between CVF antiretroviral concentrations and anaerobic bacteria taxa associated with non-optimal vaginal microbiota: 1) *Lactobacillus* species sequester these drugs, and 2) changes in pH with anaerobic bacteria affecting drug partitioning. Tavena *et al.* 2018 showed a decrease in tenofovir being transported and metabolized by *L. crispatus in vitro* and showed as pH increased, tenofovir uptake decreased. [13] It is unclear how these findings would affect HIV transmission risk, and further studies are needed.

We previously reported no association between genital shedding and CVF concentrations of efavirenz, but tenofovir and lamivudine CVF concentrations were not included. Here we confirmed that adjusting for the composition or structure of the vaginal microbiota when evaluating associations between efavirenz, tenofovir, lamivudine and genital HIV shedding did not improve our prediction of risk of genital HIV shedding. These findings further support previous evidence that progestin contraception does not impact the risk of HIV transmission from women on effective ART to their partners. [4,5,14-16]

The results of our study are limited by small sample size and the nature of sub-group analyses. Furthermore, we were only able to measure CVF antiretroviral concentrations, not genital tissue concentrations, though tissue concentrations may be a better efficacy surrogate.

In conclusion, we found hormone initiation did not affect genital shedding, providing confidence that WLWH on either DMPA or levonorgestrel implant contraception will not have compromised ART efficacy. Tenofovir, lamivudine, and efavirenz CVF concentrations had a positive correlation with several anaerobic bacteria taxa. Identification of a mechanism would increase our understanding of the complex environment of the FGT providing insights into improving ART and viral suppression in this compartment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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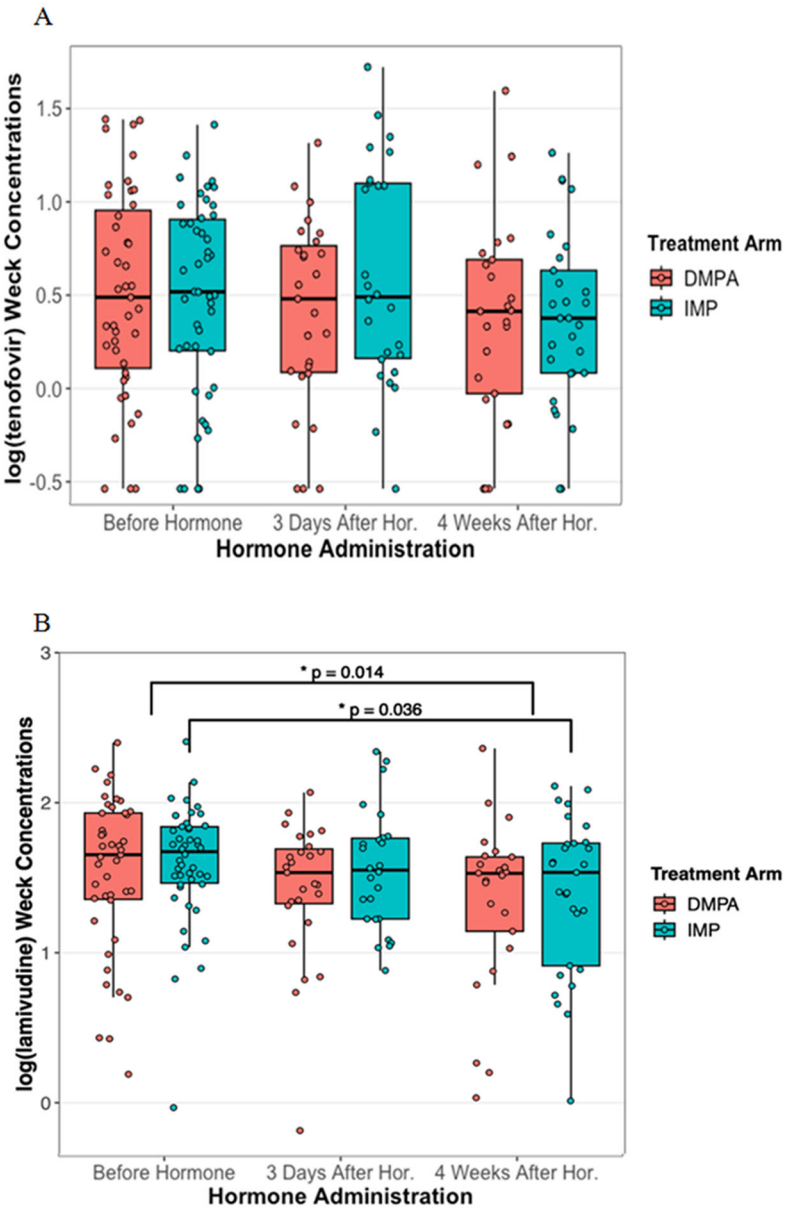
References

1. UNAIDS. Epidemiological Estimates, 2021. ; 2021. https://www.unaids.org/en/resources/documents/2021/global-commitments-local-action
2. Polis CB, Phillips SJ, Curtis KM. Hormonal contraceptive use and female-to-male HIV transmission: a systematic review of the epidemiologic evidence. AIDS 2013; 27:493–505. [PubMed: 23079808]
3. Phillips SJ, Polis CB, Curtis KM. The safety of hormonal contraceptives for women living with HIV and their sexual partners. Contraception 2016; 93:11–16. [PubMed: 26515194]
4. Kourtis AP, Wiener J, Hurst S, Nelson JAE, Cottrell ML, Corbett A, et al. Brief Report: HIV Shedding in the Female Genital Tract of Women on ART and Progestin Contraception: Extended Follow-up Results of a Randomized Clinical Trial. J Acquir Immune Defic Syndr 2019; 81:163–165. [PubMed: 31095006]
5. Chinula L, Nelson JAE, Wiener J, Tang JH, Hurst S, Tegha G, et al. Effect of the depot medroxyprogesterone acetate injectable and levonorgestrel implant on HIV genital shedding: a randomized trial. Contraception 2018; 98:193. [PubMed: 29746813]
6. Kourtis AP, Haddad L, Tang J, Chinula L, Hurst S, Wiener J, et al. A randomized clinical trial on the effects of progestin contraception in the genital tract of HIV-infected and uninfected women in Lilongwe, Malawi: Addressing evolving research priorities HHS Public Access. Contemp Clin Trials 2017; 52:27–34. [PubMed: 27836505]
7. Haddad LB, Tang JH, Davis NL, Kourtis AP, Chinula L, Msika A, et al. Influence of Hormonal Contraceptive Use and HIV on Cervicovaginal Cytokines and Microbiota in Malawi. mSphere Published Online First: 21 February 2023. doi:10.1128/MSPHERE.00585-22
8. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, Mcculle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci 2011; 108 Suppl:4680–7. [PubMed: 20534435]
9. Ma B, France MT, Crabtree J, Holm JB, Humphrys MS, Brotman RM, et al. A comprehensive non-redundant gene catalog reveals extensive within-community intraspecies diversity in the human vagina. Nat Commun 2020; 11:940. [PubMed: 32103005]
10. Klatt NR, Cheu R, Birse K, Zevin AS, Perner M, Noël-romas L, et al. Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. Science (80-) 2017; 945:938–945.
11. Thurman A, Schwartz JL, Ravel J, Gajer P, Marzinke MA, Yousefieh N, et al. Vaginal microbiota and mucosal pharmacokinetics of tenofovir in healthy women using tenofovir and tenofovir/levonorgestrel vaginal rings. PLoS One 2019; 14:e0217229. [PubMed: 31107913]
12. Thurman AR, Ravel J, Gajer P, Marzinke MA, Ouattara LA, Jacot T, et al. Vaginal Microbiota and Mucosal Pharmacokinetics of Tenofovir in Healthy Women Using a 90-Day Tenofovir/Levonorgestrel Vaginal Ring. Front Cell Infect Microbiol 2022; 12:204.
13. Taneva E, Sinclair S, Mesquita PM, Weinrick B, Cameron SA, Cheshenko N, et al. Vaginal microbiome modulates topical antiretroviral drug pharmacokinetics. JCI insight 2018; 3. doi:10.1172/JCI.INSIGHT.99545
14. Low AJ, Konate I, Nagot N, Weiss HA, Kania D, Vickerman P, et al. Cervicovaginal HIV-1 Shedding in Women Taking Antiretroviral Therapy in Burkina Faso: A Longitudinal Study. J Acquir Immune Defic Syndr 2014; 65.www.jaids.com
15. Day S, Graham SM, Masese LN, Richardson BA, Kiarie JN, Jaoko W, et al. A prospective cohort study of the effect of depot medroxyprogesterone acetate on detection of plasma and cervical HIV-1 in women initiating and continuing antiretroviral therapy. J Acquir Immune Defic Syndr 2014; 66:452–456. [PubMed: 24798764]

16. King CC, Ellington SR, Davis NL, Coombs RW, Pyra M, Hong T, et al. Prevalence, Magnitude, and Correlates of HIV-1 Genital Shedding in Women on Antiretroviral Therapy. J Infect Dis J Infect Dis ® 2017; 216:1534–1574. [PubMed: 29240922] ®



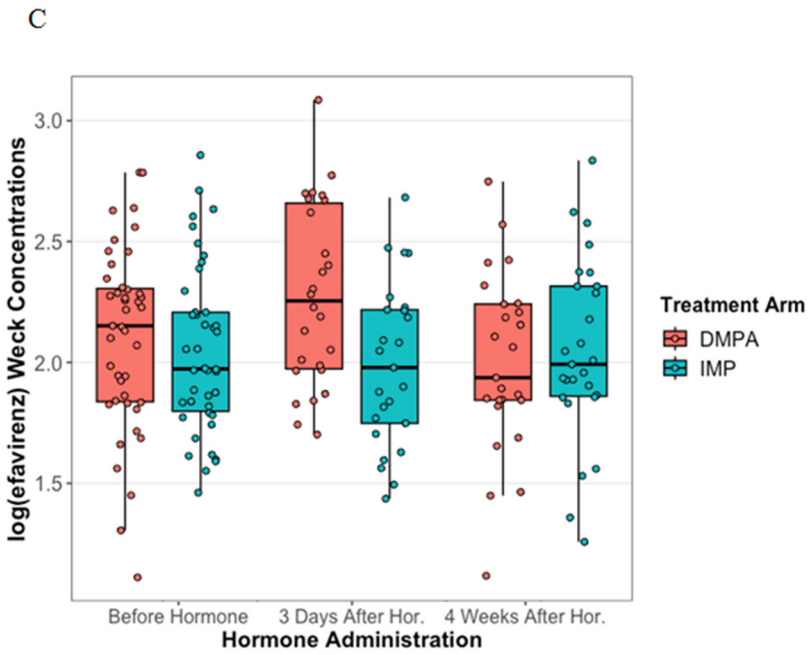


Figure 1. Antiretroviral concentrations affected by Progestin contraception initiation.

Cervicovaginal fluid concentrations of tenofovir, lamivudine and efavirenz were measured from Wek-Cel sponges using LC-MS/MS. The log transformed tenofovir (**A)**, lamivudine (**B**), and efavirenz (**C**) concentrations were compared before, 3 days after, and 4 weeks after contraception initiation using general estimating equations. Concentrations between women on depot medroxyprogesterone acetate injectable (**red**) and levonorgestrel implant (teal) were compared at each visit. There were no significant statistical differences for tenofovir or efavirenz. Mean lamivudine concentrations significantly decreased 37% 1 month after contraception initiation. Mean lamivudine CVF concentrations decreased 41% in women on the levonorgestrel implant post-contraception initiation compared to pre-contraception initiation, but no significant difference for DMPA.

Antiretroviral concentrations in the cervicovaginal fluid before, 3 days after, and 1 month after contraceptive hormone initiation for A) tenofovir, B) lamivudine, and C) efavirenz.

Table 1.

Multivariable General Estimating Equation Models Predicting tenofovir, lamivudine, and efavirenz cervicovaginal fluid concentrations with Community State Types.

Community State Type*	Estimate	Standard Error	P-value
Tenofovir			
I	REF	REF	REF
III-A	−0.21	0.12	0.078
III-B	−0.34	0.15	0.026*
IV-A	−0.18	0.13	0.15
IV-B	−0.18	0.14	0.19
IV-C	0.0287	0.13	0.83
Lamivudine			
I	REF	REF	REF
III-A	−0.25	0.09	0.010*
III-B	−0.33	0.17	0.052
IV-A	−0.29	0.12	0.019*
IV-B	−0.28	0.11	0.016*
IV-C	−0.11	0.11	0.34
Efavirenz			
I	REF	REF	REF
III-A	−0.25	0.15	0.10
III-B	−0.050	0.16	0.75
IV-A	−0.23	0.14	0.10
IV-B	−0.27	0.17	0.11
IV-C	−0.42	0.16	0.0094*

*Community state types describe the different vaginal bacterial communities present in the participants of the current study. They were defined using Bray-Curtis dissimilarity measure and Ward linkage hierarchical clustering. CST I and III are dominated by L. crispatus and L. iners respectively, whereas CST IVs comprise of diverse sets of anaerobic bacteria and low to no lactobacilli.