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Altered Vaginal Microbiota Are Associated With Perinatal Mother-to-Child Transmission of HIV in African Women From Burkina Faso

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Abstract

Background: Mother-to-child transmission (MTCT) of HIV remains a significant problem in resource-limited settings, despite the advent of antiretroviral therapies. Because perturbations in vaginal microbial communities are associated with sexual transmission of HIV, we determined whether perinatal MTCT is associated with the vaginal microbiotas of HIV-infected mothers.

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Methods: We conducted a retrospective analysis of cervicovaginal microbiotas by pyrosequencing of bacterial 16S rRNA genes (median 350 sequences per sample) from 10 transmitters and 54 nontransmitters during a perinatal MTCT prevention clinical trial of azidothymidine and the microbicide benzalkonium chloride. Logistic regression was performed adjusting for multiple covariates, including CD4⁺ T-cell numbers and treatment group, to correlate abundances of microbial taxa with perinatal MTCT.

Results: The vaginal microbiotas of these subjects were dominated by several lactobacilli species, although a subset of subjects was colonized by diverse anaerobic species. MTCT of HIV was associated with significantly greater relative abundances of several groups of microorganisms. Most notably, among the abundant bacterial species, *Gardnerella vaginalis* was significantly enriched in cases of antepartum transmission, compared with nontransmission (odds ratio 1.7; $P=0.004$). Neither azidothymidine nor benzalkonium chloride treatment was associated with shifts in microbial distributions compared with the placebo control group.

Conclusions: These data suggest that alterations in vaginal microbial communities are associated with an increased risk for perinatal MTCT, consistent with results with horizontal transmission of HIV. Therefore, determining the mucosal features associated with alterations in vaginal microbial communities may guide efforts to modulate the risk for HIV MTCT.

Keywords

microbiota; rRNA; phylogenetic analysis; microbial ecology; HIV/AIDS

INTRODUCTION

Mother-to-child transmission (MTCT) of HIV remains a significant risk in the developing world. Without medical intervention, ~25% of infants of HIV-infected mothers become infected before weaning.^{1,2} Short-course antiretroviral regimens are effective,^{3–6} but only half of HIV-infected mothers in resource-limited countries receive antiretroviral therapy during pregnancy.^{2,7} Thus, additional modalities for prevention and treatment are urgently needed to reduce the incidence of MTCT. Moreover, determining why ~75% of infants of HIV-infected mothers are not infected, despite viral exposure in utero, at birth, and during breastfeeding may direct development of novel interventions.

Vaginal microbial communities influence the rates of both horizontal HIV acquisition^{8–12} and subsequent transmission.¹³ Bacterial vaginosis, characterized by imbalances in microbial community composition (dysbiosis) within the vagina, is significantly associated with HIV acquisition [eg, odds ratio (OR) 2.0].⁹ The mechanism(s) by which the defining micro-biological features of bacterial vaginosis, loss of *Lactobacillus* spp. and gain of gram-negative facultative anaerobes (eg, *Gardnerella vaginalis*), enhance the infectivity of HIV remain unknown. Lactobacilli may create a physical environment that is inhospitable to viral propagation through production of lactic acid or H₂O₂.¹⁴ Alternatively, lactobacilli could induce an antiinflammatory state within the vaginal mucosa that lessens the abundance of cellular targets of HIV (dendritic cells and activated CD4⁺ T lymphocytes). In contrast, microorganisms enriched in bacterial vaginosis could either directly disrupt the vaginal mucosal barrier,¹⁵ thus increasing local HIV loads, or induce adverse sequelae such as

premature rupture of membranes (PROM) or preterm labor¹⁶ that compromise the integrity and sterility of the amniotic cavity.

Analogous to sexual transmission of HIV,^{8,9,11,12,17} we hypothesized that particular microbial communities may also correlate with the frequency of MTCT. Mechanistically, vaginal microbes could modify HIV transmission rates either in utero¹⁸ or during delivery.¹⁹ To test this hypothesis, we retrospectively characterized the indigenous vaginal microbial communities of mothers with HIV infection in Burkina Faso, West Africa, who either did or did not transmit HIV to their infants. Microbial communities were surveyed by broad-range amplification of bacterial 16S rRNA genes and pyrosequencing. We found that the characteristics of the vaginal microbiota among African women with HIV infection was comparable with that described from women in other settings and geographical venues. Of note, altered vaginal microbial communities were associated with an increased risk for perinatal MTCT, consistent with results with horizontal transmission of HIV.

MATERIALS AND METHODS

Study Design

This is a case-cohort study nested within the DITRAME prospective cohort of HIV-1–infected pregnant women and their live-born children from the ANRS 049a and 049b trials. The DITRAME ANRS 049a and 049b phase 2 multicenter, double-blind, randomized placebo-controlled trials evaluated the tolerance and prophylactic efficacy of azidothymidine (AZT; ANRS 049a^{3,20}) and of the topical antiseptic benzalkonium chloride (CdB; ANRS 049b^{21,22}) on MTCT among HIV-seropositive women. The DITRAME ANRS 049a trial was conducted in 2 large cities of West Africa (Abidjan, Côte d’Ivoire, and Bobo-Dioulasso, Burkina Faso) from September 1995 to May 1997.³ Briefly, eligible HIV-1–infected pregnant women were randomized at 36–38 weeks’ gestation to receive either oral zidovudine (250–300 mg twice a day) or a matching placebo, until the beginning of labor; then a single oral dose of 500/600 mg until delivery; and a 7-day postpartum treatment of 500/600 mg per day. No treatment was given to the neonate. In the DITRAME ANRS 049b trial,²¹ women testing positive for HIV-1 infection in prenatal care units in Abidjan (Côte d’Ivoire) and Bobo-Dioulasso (Burkina Faso) from November 1996 to April 1997 were eligible, with their informed consent. Women self-administered daily a vaginal suppository of 1% CdB or matched placebo from 36 weeks of pregnancy, plus a single dose during labor. Only vaginal samples from Burkina Faso are analyzed in this report.

A single cervicovaginal lavage (CVL; 3 mL of sterile saline) fluid specimen was obtained from each study participant by gentle aspiration at 36–38 weeks of gestation. After low-speed centrifugation, the fluid supernatants were frozen at –80°C on the same day and shipped in liquid nitrogen dry shippers. All women for whom CVL specimens were available (n = 70) were included in this study, although 6 were excluded due to failure to produce polymerase chain reaction (PCR) products (see following). The protocols were approved by the Centre Muraz Ethical Committee and Ministry of Health of Burkina Faso, and the institutional review boards at the University of Minnesota and the University of Colorado (protocol 10–0708). Written informed consent was obtained from all participants.

HIV transmission was evaluated by measuring HIV RNA in infant plasma with Amplicor v1.5 Roche HIV RNA kits (Hoffman-La Roche Ltd, Basel, Switzerland) within 72 hours after birth and at day 45. Infants with positive results by 72 hours were defined as cases of antepartum MTCT, those with initial negative test for HIV (72 hours) followed by a positive test by day 45 as intrapartum MTCT, and those who were negative at day 45 were classified as controls.²³

16S rRNA Gene Pyrosequencing

One hundred microliters of CVL supernatant in 500 μ L of Buffer B²⁴ was heated 10 minutes at 70°C to inactivate viral particles. Five hundred microliters of buffer-saturated phenol (pH 8; Sigma-Aldrich, St Louis, MO) and 100 mg of 0.1-mm zirconium beads (Biospec Products Inc, Bartlesville, OK) were added and specimens agitated 3 minutes in a Mini-Beadbeater-8 (Biospec Products Inc). Samples were chloroform-extracted and DNA-precipitated after addition of 0.5 volume 7.5 M NH_4OAc and 1 volume 100% isopropanol. Pellets were rinsed in 80% ethanol, dried, resuspended in 20 μ L TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and stored at -80°C. Bacterial rRNA gene concentrations were determined by quantitative PCR (QPCR).²⁵ Aliquots of genomic DNAs were diluted to 250,000 rRNA templates per microliter.

Multiplexed broad-range 16S amplicon libraries were constructed for pyrosequencing on a 454 Life Sciences GS FLX instrument (Branford, CT) using barcoded primers.²⁵ Samples were amplified through 30 PCR cycles (early log phase for 250,000 templates/ μ L), and if necessary, additional cycles were performed to produce sufficient amplicons for sequencing (33, 36, or 38 cycles maximum). Bacterial 16S rRNA DNA was successfully amplified from 64 of 70 (91%) subjects (10 transmitters, 54 nontransmitters); these 64 subjects comprise the study population. No statistical differences in clinical parameters were observed between the 64 PCR-amplified and the 6 nonamplified specimens. Eighteen specimens were subjected to replicate DNA extraction (2–3 per specimen) and replicate PCR reactions (2–6 per specimen). Median within-subject Morisita–Horn community similarity values (C_{MH}) of 0.99 [interquartile range (IQR) 0.94–1.0] for extraction replicates and 0.99 (IQR 0.99–1.0) for PCR replicates indicate that nearly identical distributions of operational taxonomic units (OTUs) were observed in replicate samples from the same subject.

Sequence Analysis

Pyrosequences were polished, deconvoluted,²⁶ and excluded by the following criteria: (a) trimmed length <150 nucleotides, (b) >0 ambiguous base(s), (c) absence of barcode, and/or (d) SINA²⁷ quality score <75. Taxonomies were assigned by BLAST search of a database extracted from the All-Species Living Tree Project (version LTP_S95)²⁸ and corroborated using the Ribosomal Database Project Naïve Bayesian classifier²⁹ as described.²⁵ On the basis of high BLAST percent identity scores (median 99%, IQR 98%–99%), most of the vaginal microorganisms could be identified to the species level (96% of sequences were 97% identical to LTP_S95 sequences). Biodiversity indices^{30,31} were estimated through 10,000 bootstrap replicates.³²

Enumeration of Selected Microorganisms

Quantification of total bacteria, the 2 most prominent sequence types (*Gardnerella vaginalis* and *Lactobacillus* spp.) was performed by QPCR using standard curves derived from cloned 16S genes,²⁴ using the following primers: (a) total bacteria³³: 8F (5' AGAGTTTGATCCTGGCTCAG) and 338R (5' CTGCTGCCTCCCGTAGGAGT), (b) *G. vaginalis*³⁴: GardnF (5' GACTGAGATACGGCCCAGAC) and GardnR (5' ATTCGAAAGGTACACTCACC), and (c) *Lactobacillus*³⁴: LactoF (5' TGGAAACAGRTGCTAATACCC) and LactoR (5' GYCCATTGTGGAAGATTCCC). The cycling protocol was as follows: (a) denaturation at 95°C (10 minutes) and (b) 40 cycles of 95°C (15 seconds), 56°C (15 seconds), and 60°C (30 seconds followed by fluorescence plate read).

Statistical Analyses

Analyses used the R statistical software package (v.2.8.1).³⁵ Differences in mean values of continuous variables (eg, age) between pairs of categorical variables (eg, treatment group) were assessed by Student *t* test. Percent abundances of bacterial groups, estimated by 16S rRNA sequences, were logit transformed into continuous variables using the “car” package of R.³⁶ The association between HIV transmission and genus-level clustering of subjects were assessed by Fisher exact test. Associations between the main outcome variables (eg, HIV transmission, treatment arm) and the abundances of individual species- and phylum-level taxa were evaluated by logistic regression using the glm function in R with a quasibinomial distribution (to accommodate over-dispersion) and the logit link function.³⁵ Because univariate analyses indicated potential associations between vaginal microbiotas and age, CD4⁺ T-cell count and CD8⁺ T-cell count, results were adjusted for these covariates [no associations were observed between microbiota and mode of delivery, low birth weight (<2.5 kg birth weight), PROM, or prematurity (<37 weeks' gestation³⁷)]. Adding treatment as a covariate in the analysis of associations between microbiome and MTCT did not appreciably change the statistical results, so treatment arm was not included in the reported analysis of MTCT. Because of the exploratory nature of this study, *P* values were not adjusted for multiple comparisons. Rather, results are interpreted as indicating candidate microbes potentially associated with HIV transmission. Morisita–Horn community similarity indices (C_{MH}) were calculated using the vegdist function of the R package “vegan”³⁸ and compared between categories by Wilcoxon rank-sum test. Heatmaps were generated with the “heatmap.2” function of the R package “gplots.”³⁹ Complete-linkage hierarchical clustering of subjects was performed using the default options of the “hclust” and “dist” functions of the R package “stats.”³⁵ Euclidean dissimilarity matrices were calculated using logit-transformed OTU abundance data.

DNA Sequence Accession Numbers

Sequences were deposited in GenBank with the accession numbers JF461543–JF487783.

RESULTS

Clinical Characteristics

All CVL specimens available from women enrolled in the ANRS 049a or 049b study in Burkina Faso were included in this substudy (N = 70). Six subjects were excluded because the CVL specimens failed to produce bacterial 16S PCR products. Of the 64 HIV-infected mothers remaining, 10 transmitted HIV to their infants [4 (6%) antepartum and 6 (9.3%) intrapartum; Table 1]. Transmitters were characterized by significantly lower circulating CD4⁺ T-cell numbers compared with nontransmitters ($P < 0.024$; Table 1), as previously reported,³ whereas neither age nor CD8⁺ T-cell counts differed significantly between cases and controls. No significant differences in age, CD4⁺ T-cell counts, or CD8⁺ T-cell counts were observed between treatment arms (Table 1). HIV RNA levels were not routinely available in infant or maternal plasma or CVL in this cohort.

Characterization of Vaginal Microbial Ecology

High-throughput pyrosequencing of 16S rRNA genes amplified by PCR with pan-bacterial primers generated a data set (Table 2) consisting of 49,301 polished sequences, with a median length of 245 nucleotides (IQR 224–257 nucleotides) and a median of 350 sequences per specimen (IQR 255–600 reads/subject). Good's coverage indices for species-level OTUs ranged from 94.2% to 100% per subject (median 99.5%), indicating that this depth of sequencing reflected the true biodiversity of each specimen.

Analysis of vaginal rRNA sequences revealed that the study population as a whole contained 198 species-level bacterial OTUs from 111 genera and 9 phyla (Table 2 and Fig. 1). The majority of the sequences belonged to only 2 phyla, the Firmicutes (low G + C gram positives; 66.2% of sequences) and the Actinobacteria (high G + C gram positives; 17.3% of sequences). Members of the phyla Proteobacteria (7.9%), Fusobacteria (3.7%), Bacteroidetes (2.7%), and Tenericutes (eg, mycoplasmas and ureaplasmas; 2.1%) also were detected in CVL specimens at lower abundances (Table 2 and Fig. 1).

Substantial between-individual differences were observed in vaginal microbiotas (Fig. 1). Hierarchical clustering of subjects based on similarity of vaginal microbiotas defined 4 broad groups of subjects. The microbiotas of 30 of 64 subjects, of whom 4 were intrapartum HIV transmitters, were dominated by the genus *Lactobacillus* (Fig. 1, cluster 1), which included the species *L. iners* (77% of sequences in cluster 1), *L. crispatus* (11%), *L. fornicalis* (3.9%), *L. gasseri* (3.2%), and *L. vaginalis* (0.5%). A second cluster of 8 individuals (Fig. 1, cluster 2) was dominated by coagulase-negative staphylococci (eg, *S. haemolyticus* and *S. epidermidis*), with lesser quantities of lactobacilli, and did not include any HIV transmitters. The vaginal communities of 24 individuals in cluster 3 (Fig. 1) contained more complex mixtures of a variety of genera, dominated by *Gardnerella* spp. (eg, *G. vaginalis*, 36.8% of sequences in cluster 3) and *Lactobacillus* spp. (*L. iners*, 18.7% abundance). Other genera in this cluster of individuals included *Sneathia* (9.5%), *Atopobium* (6.0%), *Prevotella* (6.3%), *Anaerococcus* (4.4%), and *Mycoplasma* (4.3%), all of which have been associated with bacterial vaginosis.^{40,41} All 4 antepartum and 2 of 6 intrapartum HIV transmitters belonged to cluster 3, suggesting a weak association between membership in

this group and antepartum MTCT ($P=0.09$). The fourth cluster consisted of only 2 nontransmitting subjects characterized by low *Lactobacillus* spp. and diverse anaerobic groups (eg, *Prevotella*, *Sneathia*, *Peptostreptococcus*).

Vaginal Microbiota and MTCT

HIV transmission, either antepartum or intrapartum, was significantly associated with the abundances of several individual bacterial phyla and species-level OTUs (Table 2). Most notably, the relative abundances of *G. vaginalis* [36.7% vs 15.1% of sequences/subject; OR 1.7; 95% confidence interval (CI) 1.2 to 2.4; $P=0.004$] and its phylum Actinobacteria (38.1% vs 17.3% of sequences/subject; OR 1.6; 95% CI 1.1 to 2.3; $P=0.009$) were elevated by >2-fold in antepartum cases compared with nontransmitters. The OR values were adjusted for multiple covariates, including CD4⁺ and CD8⁺ T-cell counts and maternal age (univariate analyses suggested no associations between microbiota and mode of delivery, low birth weight, PROM, premature birth, or treatment, so these variables were not included in the analysis). Although the treatment group did not significantly affect vaginal microbial communities, *G. vaginalis* sequences tended to be more abundant in treatment groups (23.3% for AZT and 18.5% for CdB) compared with controls (7.8%; $P=0.32$ and $P=0.14$, respectively), as were actinobacterial sequences (28.2% and 20.8% vs 9.5%; $P=0.24$ and $P=0.26$, respectively). Several *Prevotella* spp. also were significantly enriched in antepartum cases compared with controls, although the increase in the phylum Bacteroidetes was not significant. In contrast, the phylum Firmicutes, of which the lactobacilli are prominent vaginal constituents, was reduced in relative sequence abundance in antepartum cases, compared with those in nontransmitting control mothers (52.8% vs 72.3%; OR 0.69; 95% CI 0.48 to 0.99; $P=0.05$). Although the species *L. iners*, typically among the most common vaginal species,⁴² was also present in antepartum cases at somewhat lesser frequencies than controls (26.7% vs 42.0% sequence abundance), the difference was not statistically significant ($P=0.37$). Only 2 bacterial species-level OTUs, *Brevibacterium casei* and *L. gasseri*, were significantly associated with intrapartum transmission (Table 2). Thus, MTCT is associated with differential abundances of several bacterial groups and in the diversity of these groups.

Quantification of Specific Microbial Groups

We sought to independently corroborate pyrosequencing results by targeted quantification of candidate microbial groups. Because of their relatively high abundances and suggested association with HIV transmission, both *G. vaginalis* and lactobacilli were enumerated in CVL specimens by QPCR. To normalize results between specimens, total bacterial PCR using pan-bacterial 16S rRNA primers was used to enumerate total bacteria in each specimen. Therefore, results are presented as logit-transformed percentages of total bacterial populations occupied by the particular bacterial groups (Figs. 2A, B).

Specific QPCR (Fig. 2A) confirmed that antepartum HIV transmitters carried significantly higher relative abundances of *G. vaginalis* compared with nontransmitters (32.4% vs 8.8% of total bacterial load; $P=0.009$), but differences between antepartum and intrapartum transmitters were not significant. In contrast, QPCR of lactobacilli did not differ significantly in abundance between transmitters or controls (Fig. 2A). In general, the QPCR

results matched those of pyrosequencing with Pearson correlation coefficients of 0.82 and 0.76 for *G. vaginalis* and lactobacilli, respectively.

DISCUSSION

We characterized for the first time the vaginal microbial communities of HIV-infected expectant mothers in sub-Saharan Africa. Consistent results for the vaginal bacteria identified by 16S rRNA pyrosequencing confirm that although 198 different species were identified in individual mothers, *Lactobacillus* spp. predominate, accounting for 41% of all bacteria identified. These results were similar to those reported in North America,^{40,41,43,44} South America,⁴⁵ Africa,⁴⁶ Asia,⁴⁷ and Europe,⁴⁸ confirming the existence of a core vaginal microbiota, despite geographic separation and ethnic and immunologic variations.

To our knowledge, these are also the first in-depth, culture-independent, high-throughput molecular analyses of the vaginal microbiological correlates of MTCT. After adjusting for maternal age, CD4⁺ and CD8⁺ T-cell counts, and treatment arm (AZT vs CdB vs placebo), several vaginal microbial groups were positively and significantly correlated with perinatal MTCT. The most striking association observed was between antepartum HIV transmission and increased relative abundance of *G. vaginalis* (OR 1.7; no significant difference in prevalence was observed between transmitters and nontransmitters). Although a prominent member of the vaginal microbiota of many study subjects, *G. vaginalis* was doubled in relative abundance in the broad-range 16S rRNA sequence libraries of antepartum transmitters (37% of sequences), relative to nontransmitters (15% of sequences). Species-specific PCR quantification of *G. vaginalis* in CVL specimens, normalized to total bacteria, confirmed this result.

Based on similarities in vaginal microbiota, 6 of 10 (60%) MTCT cases and 20 of 54 (37%) nontransmitter controls formed a microbiological cluster (cluster 3; Fig. 1) that was distinguished by increased abundances and/or prevalences of multiple genera, including *Gardnerella*, *Sneathia*, *Atopobium*, *Prevotella*, and *Mycoplasma*. Furthermore, lactobacilli, the primary constituents of the “normal” vaginal microbiota,⁴⁰ typically were present at reduced abundances in these subjects in cluster 3. Although bacterial vaginosis was not evaluated or diagnosed during the ANRS 049 trial, these vaginal microbiotas in cluster 3 are similar to those reported for cases of vaginosis in developed countries.^{40,41} These results are consistent with a trend toward higher risk for MTCT in women with the microbiological hallmarks of bacterial vaginosis. Because of the complex relationships between bacterial vaginosis, herpes simplex virus 2 infection, and HIV acquisition, it is possible that bacterial vaginosis is a mediator between herpes simplex virus 2 and HIV infections.⁴⁹ Neither treatment with AZT nor with CdB was associated with altered vaginal microbiotas (Figs. 1, 2 and data not shown).

Limitations of this case-control study include reduced statistical power due to a small number of cases, absence of clinical diagnosis of vaginal disorders (such as bacterial vaginosis and other sexually transmitted infections), and lack of data on HIV RNA viral load in CVL. The similarity of microbial communities reported here to those described previously^{40,41,43,44} indicates that long-term storage at -80°C since sampling did not

compromise the specimens analyzed. Because of the study design, the causal relationships between vaginal HIV loads, alterations in the vaginal microbiota, and MTCT could not be addressed. For instance, independent of its effects on MTCT risk, compromised mucosal immunity in the reproductive tract may have indirectly altered the vaginal microbiota. Nonetheless, the statistically significant positive correlations between microbial groups such as *G. vaginalis* and MTCT justify prospective follow-up investigations to establish the generalizability of the observed associations between vaginal microbiota and risk for MTCT in the context of biologically relevant cofactors.

Determining the biological factors that naturally protect some neonates from perinatal HIV transmission may lead to novel strategies for preventing infections. Few in-depth culture-independent studies of the human vaginal microbiota have been reported among individuals with HIV infection,⁴⁴ particularly in the context of clinical outcomes. Nonetheless, vaginal dysbiosis and/or acquisition of specific pathogens (eg, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*) are well-established risk factors for sexual transmission of HIV^{8,9,11,12,17} and have been associated with higher HIV RNA burden in the genital tract.^{50,51} The results of this study suggest that vaginal microbial communities also may influence the risk for prenatal MTCT. The loss of normally protective function(s) provided by commensal vaginal microbes could disrupt cervicovaginal mucosal barrier integrity and thereby lead to increased local infiltration of immune effector cells, including HIV-infected leucocytes along with free virus, into cervicovaginal tissues. Under this model, the effects of increased HIV virion loads in the genital mucosa could raise the risk for both in utero and intrapartum transmission.¹⁹ Indeed, vaginal dysbiosis is associated with an increased risk for premature delivery. Because the risk for in utero MTCT is also significantly correlated with the occurrence of premature rupture of membranes,^{18,52} the impact of vaginal microbes on the upper genital tract also could contribute indirectly to the risk for both prenatal and perinatal MTCT.¹⁶ Alternatively, such local infection and inflammation may promote increased local HIV replication or enhanced transfer of virus from the systemic compartment. Indeed, ongoing studies are directed to confirm these results and to establish a more direct link between cervicovaginal microbial ecology, including the presence or absence of H₂O₂-producing lactobacilli, local HIV RNA and DNA levels, mucosal inflammation and epithelial integrity, and the rate of MTCT of HIV.

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FIGURE 1.

Vaginal microbial diversity and MTCT. Distribution of vaginal bacterial genera in study participants. Color levels denote logit-transformed abundances of 16S rRNA sequences in patient CVL samples. The top 2 rows designate the HIV transmission (“none” = no transmission) and treatment (“AZT” = azidothymidine arm; “CdB” = benzalkonium chloride arm; “PLB” = placebo arm) categories to which subjects were assigned. Remaining rows designate bacterial genera identified by 16S rRNA phylogenetics. Labels “1,” “2,” “3,” and “4” denote large-scale clusters of subjects suggested by hierarchical clustering of logit-transformed sequence abundance data.

FIGURE 2.

PCR quantification of specific microbial groups. A, Association of MTCT with selected groups of vaginal microorganisms. *x* axes designate HIV transmission categories: none, no transmission; ante, antepartum transmission; intra, intrapartum transmission. Panels labeled “16S sequences” display logit-transformed percent abundances of 16S rRNA pyrosequences of the phyla Actinobacteria and Firmicutes, and of *Gardnerella vaginalis* and *Lactobacillus* spp. Panels labeled “QPCR” display independent results of QPCR enumeration of these groups in cervicovaginal fluids, relative to QPCR enumeration of total bacteria using universally conserved primers. *P* values are from logistic regression analyses that adjusted for treatment arm, age, CD4⁺ T-cell count, and CD8⁺ T-cell count. B, Association of study treatment arms with selected groups of vaginal microorganisms. *x* axes designate treatment arms: AZT, azidothymidine arm; CdB, benzalkonium chloride arm; PLB, placebo arm. Remaining labels are as described for panel A.

TABLE 1.

Clinical and Demographic Characteristics of Subjects

	All	HIV Transmission			Treatment		
		No	Antepartum	Postpartum	PLB	AZT	CdB
Subjects	64	54	4	6	21	25	18
HIV transmitter	10	0	4	6	3	5	2
Maternal age (yr) (IQR)	23.0(21.0–27.0)	23.0 (21.0–26.5)	25.0 (23.0–26.5)	23.5 (21.0–27.5)	23.0 (20.0–28.0)	25.0 (21.0–28.0)	23.0(21.3–25.8)
CD4 (cells/mL) (IQR)	520 (383–704)	535 (411–724)	454 (358–524)	385 (300–498)	528 (470–673)	524 (389–729)	433 (311–632)
CD4 <200 (%)	4.5 (3/66)	5.6 (3/54)	0 (0/4)	0 (0/6)	0 (0/21)	4.0 (1/25)	11 (2/18)
CD4 <350 (%)	18 (12/66)	17 (9/54)	25 (1/4)	33 (2/6)	4.8 (1/21)	20 (5/25)	33 (6/18)
CD8 (cells/mL) (IQR)	873 (658–1304)	893 (643–1328)	801 (667–948)	835 (712–1177)	858 (676–983)	1008 (703–1436)	800 (534–1119)
Caesarean (%)	3.3 (2/61)	2.0 (1/51)	25 (1/4)	0 (0/6)	5 (1/20)	0 (0/25)	6 (1/16)
Low birth weight* (%)	14 (9/63)	11 (6/53)	50 (2/4)	17 (1/6)	10 (2/20)	16 (4/25)	17 (3/18)
PROM (%)	15 (8/52)	11 (5/44)	50 (2/4)	25 (1/4)	18(3/17)	14 (3/21)	14 (2/14)
Premature [†] (%)	3.5 (2/57)	2.0 (1/49)	33 (1/3)	0 (0/5)	10 (2/20)	0 (0/21)	0 (0/16)

* Birth weight <2.5 kg.

[†] Birth <37 weeks' gestational age.

PLB, placebo.

TABLE 2.
Phylogenetic Distribution of 16S rRNA Sequences Associated With HIV Transmission

Phylogenetic Classification [*]	Nontrans (n = 54)		Antepartum (n = 4)		Intrapartum (n = 6)	
	% [†]	OR[‡]	% [†]	OR[‡]	% [†]	OR[‡]
Actinobacteria	17.3	38.0	1.6 (1.1 to 2.3)	0.009	31.7	
<i>Gardnerella vaginalis</i>	15.1	36.7	1.7 (1.2 to 2.4)	0.004	19.5	
<i>Brevibacterium casei</i>	0.1	0.0			10.2	2.0 (1.0 to 3.8)
Other Actinobacteria	2.1	1.4			2.0	
Bacteroidetes	2.6	7.9			0.0	
<i>Prevotella bivia</i>	0.4	1.3	1.9 (1.0 to 3.4)	0.05	0.0	
<i>P. timonensis</i>	0.5	1.2	1.7 (0.93 to 3.3)	0.09	0.0	
<i>P. denticola</i>	0.1	5.1	2.9 (1.3 to 6.7)	0.02	0.0	
Other Bacteroidetes	1.6	0.4			0.0	
Cyanobacteria	0.0	0.0			0.0	
Deferribacteres	0.0	0.0			0.0	
Deinococcus	0.0	0.0			0.0	
Firmicutes	72.3	52.8	0.69 (0.5 to 0.99)	0.05	64.7	
<i>Lactobacillus gasseri</i>	1.8	1.3	1.8 (1.2 to 2.7)	0.003	2.8	1.9 (1.2 to 2.9)
<i>Dialister microaerophilus</i>	0.2	2.1	3.0 (1.6 to 5.9)	0.002	0.0	
<i>Lactobacillus delbrueckii</i>	0.1	1.0	2.3 (1.1 to 4.8)	0.04	0.0	
<i>Acetivibrio cellulolyticus</i>	0.0	0.2	3.2 (0.9 to 12)	0.09	0.0	
Other Firmicutes	70.1	48.3			62.0	
Fusobacteria	2.6	0.6			0.0	
Proteobacteria	2.9	0.0			1.6	
Tenericutes	2.3	0.6			2.0	
Pyrosequencing reads [§]	38,387	2289			8625	
Observed richness (S_{obs})	5.6	8.8			5.1	
Estimated richness (S_{chaol})	7.6	12.1			6.3	
Shannon diversity (H)	1.0	1.6			0.9	
Evenness (%)	35.1	56.2			38.9	

The table summarizes most abundant species/genera of 16S rRNA sequences in CVL.

* Highest bit score in BLAST query. Blast % IDs <97 are named only to the genus level.

[†] 16S sequence abundances of nontransmitting (nontrans), antepartum, or intrapartum HIV-transmitting women as percentage of total sequence in category. Values are averages for subjects in a category and sum to 100% for phyla (bold) or species/genera (italics).

[‡] ORs and P values for logistic regression of MTCT of antepartum or intrapartum cases versus controls, adjusted for other demographic/clinical data. Only species-level OTUs with *P* values <0.1 are noted; 95% CIs are in parentheses.

[§] Sequences analyzed per category.

^{||} Biodiversity and OTU richness calculated for species-level OTUs. Values are means for all subjects in category.