**Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women**


Antiretroviral-based strategies for HIV prevention have shown inconsistent results in women. We investigated whether vaginal microbiota modulated tenofovir gel microbicide efficacy in the CAPRISA (Centre for the AIDS Program of Research in South Africa) 004 trial. Two major vaginal bacterial community types—one dominated by *Lactobacillus* (59.2%) and the other where *Gardnerella vaginalis* predominated with other anaerobic bacteria (40.8%)—were identified in 688 women profiled. Tenofovir reduced HIV incidence by 61% (P = 0.013) in *Lactobacillus*-dominant women but only 18% (P = 0.644) in women with non-*Lactobacillus* bacteria, a threefold difference in efficacy. Detectable mucosal tenofovir was lower in non-*Lactobacillus* women, negatively correlating with *G. vaginalis* and other anaerobic bacteria, which depleted tenofovir by metabolism more rapidly than target cells convert to pharmacologically active drug. This study provides evidence linking vaginal bacteria to microbicide efficacy through tenofovir depletion via bacterial metabolism.

More than 1 million women are infected with HIV annually, and the majority of these new infections occur in young women in sub-Saharan Africa, with South Africa having among the highest incidence rates (1, 2). Antiretroviral-based clinical trials in men who have sex with men have consistently demonstrated effectiveness in preventing HIV infection (3–5); however, studies in women have produced widely varying results. In clinical trials of women, the efficacy of antiretroviral drugs to prevent HIV infection ranged from ~49% [VOICE (Vaginal and Oral Interventions to Control the Epidemic) study] to 75% [TDF2 (Tenofovir Disoproxil Fumarate—Oral Interventions to Control the Epidemic) study] to assess whether vaginal bacteria modulate the efficacy of antiretroviral-based prevention technologies, especially those that are topically applied to the vaginal surface.

Characterization of the vaginal microbiome using unbiased metagenomic, metatranscriptomic, and metaproteomic approaches represents a potential paradigm shift in understanding host-microbial interactions at the mucosal surface in vivo. We recently used metaproteomics to gain insight into host-bacterial interactions in vaginal microbial dysbiosis (20). This method simultaneously collects unbiased information on microbial and host proteins, thus providing systems-level information on microbial communities and mucosal surfaces not available with other techniques.

In this study, we used a metaproteomic approach to assess whether vaginal bacteria modulate the efficacy of the topical microbicide tenofovir in preventing HIV infection, and we also used in vitro systems to determine mechanisms of microbiome influence on tenofovir.

**Vaginal microbial diversity in women using tenofovir or placebo microbicide gels**

Samples from 888 HIV-negative women that were assigned to either the tenofovir or the placebo-gel arm were analyzed by protein mass spectrometry, as outlined in the materials and methods (fig. S2). Proteomic analysis identified 3334 distinct bacterial proteins from 188 different species in the cervicovaginal lavage (CVL) samples of 688 women. Two major vaginal bacterial community groups were identified: one in which *Lactobacillus* was the predominant genus (group I) (n = 423 women, 61.2%) and the other dominated by non-*Lactobacillus* microbiota (group II) (n = 265 women, 38.5%) (Fig. 1). Approximately 11% of individuals had no single dominant species (defined as >50% community composition), and the majority of these individuals fell into group II.

In comparing the mass spectrometry proteomic approach with 16S ribosomal RNA (rRNA) sequencing, we found concordance with respect to classifying women into groups I and II (91.5% agreement) and measurements of bacterial abundance, including major taxa *Lactobacillus*, *G. vaginalis*, *Prevotella*, and others (P < 0.001) (fig. S3). A limitation of this study was that clinical BV data was not collected during the CAPRISA 004 trial.
thus precluding comparisons to Nugent score or other BV criteria.

Group I showed the lowest diversity [Shannon diversity index (H-index) median: 0.05], with the majority (70.5%) having \textit{L. iners} as the dominant species, followed by \textit{L. crispatus} (15.1%) and other lactobacilli, such as \textit{L. jensenii} and \textit{L. gasseri} (3.0%). In Fig. 1B, the first subgroup (a) was homogeneous, with clear dominance of \textit{Lactobacillus} (54.7%) with very low diversity (H-index median: 0.035), whereas a minority of these individuals (subgroup b, 6.8%) showed an intermediate amount of diversity (H-index median: 0.87) where small amounts of \textit{G. vaginalis}, \textit{Prevotella}, and \textit{Mobiluncus} were detected. A subanalysis of group I at the \textit{Lactobacillus} species level clearly illustrates \textit{L. iners} as predominant (fig. S4).

Women in group II had higher overall bacterial diversity (H-index: 0.78) with several distinct subgroups. The largest subgroup (c) was dominated by \textit{G. vaginalis} (n = 163, 23.7%, H-index: 0.66) and contained multiple taxa, including \textit{Prevotella} and minor amounts of \textit{Lactobacillus} and \textit{Mobiluncus} (Fig. 1B). The second-largest subgroup (d) was the most diverse and had no one clearly dominant taxa (n = 78, 11.3%, H-index: 1.15); \textit{G. vaginalis}, \textit{Prevotella}, and \textit{Mobiluncus} were predominant. Finally, the smallest subgroup (e) was low in diversity, containing either \textit{Pseudomonas} or \textit{Escherichia} (n = 24, 3.5%, H-index: 0.14) (Fig. 1B). Grouping subgroups a and b, which showed relative homogeneity in a single group (group I), with the remaining three subgroups (c, d, and e), which had more variability and diversity into a single group (group II), was supported by principal components analysis (Fig. 1C). Overall, the majority (96.2%, n = 407) of women in group I had >50% \textit{Lactobacillus} by abundance.

For downstream comparisons of topical tenofovir efficacy, we chose a \textit{Lactobacillus}-dominant (>50%) (LD) or non-\textit{Lactobacillus}-dominant (≤50%) (non-LD) classification, as >50% \textit{Lactobacillus} at

Fig. 1. Bacterial profiling by mass spectrometry using cervicovaginal lavage samples from 688 women from the CAPRISA 004 trial. (A) Overall bacterial diversity plot of the major genera of all women profiled. (B) Average bacterial community group structure for each of the two major profiles: group I (subgroups a and b) and group II (subgroups c, d, and e). (C) Principal components analysis of 688 women using bacterial proportion data showing the five subgroups.
Microbial profiles and tenofovir adherence

The proteome level corresponds with a practical clinical marker of vaginal pH below 4.5 [interquartile range pH value of 4.0 to 4.5 (20)] and accurately identified 96.2% of group I individuals. The use of LD and non-LD definitions did not appreciably change any outcomes reported on the basis of group I versus group II comparisons.

Both LD and non-LD women had similar baseline clinical, behavioral, and demographic characteristics, as well as sexual behavior and gel adherence during the trial (table S1). The presence of a sexually transmitted infection (STI)—which included Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoea, Mycoplasma genitalium, Treponema pallidum, and herpes simplex virus 2 (HSV-2)—was comparable between groups. Although not statistically significant, women within the non-LD group were marginally younger (mean age 23.6 versus 24.1 years, \( P = 0.069\)). Within each of the groups of LD and non-LD women, the number of individuals assigned to tenofovir gel and placebo gel was similar (table S2).

Vaginal microbial profiles and tenofovir gel efficacy

In the LD group, the HIV incidence rate was 61% lower [95% confidence interval (CI): 0.16 to 0.89] in women assigned to tenofovir gel compared with those assigned to the placebo gel (2.7 versus 6.9 per 100 women-years; \( P = 0.089\)) (Fig. 2A). In contrast, in non-LD women, the HIV incidence rate was only 18% lower (95% CI: 0.37 to 1.77) in those assigned to tenofovir gel compared with those assigned to placebo gel (6.4 versus 7.8 per 100 women-years; \( P = 0.82\)). Adjusting for STIs (including HSV-2 infection), antibiotic usage, depot medroxyprogesterone acetate (DMPA) usage, and sexual behaviors (frequency of sex, number of partners, and condom usage) did not affect these findings (table S3).

Tenofovir, particularly \( L. \) crispatus, has been associated with reduced HIV infection. Although the HIV incidence rate of 4.8 per 100 women-years in LD women was 32% lower (95% CI: 0.4 to 1.2) than the 7.1 per 100 women-years in non-LD women, this was not statistically significant (\( P = 0.127\)) (fig. S5). Comparing the subgroup of all 63 \( L. \) crispatus–dominant (>50% abundance) to non-LD women yielded a 57% lower HIV incidence (95% CI: 0.13 to 1.41) that was not statistically significant (0.1 versus 7.1 per 100 women-years; \( P = 0.167\)) (fig. S6). Comparison of \( L. \) crispatus–dominant women to all others as a single group (\( L. \) iners–dominant and non-LD women) produced similar findings (50% lower HIV incidence; 3.1 versus 6.0 per 100 women-years; 95% CI: 0.16 to 1.60, \( P = 0.237\)). Similarly, within the placebo gel group, the 51 \( L. \) crispatus–dominant women compared with non-LD women showed a 48% lower HIV incidence (95% CI: 0.12 to 1.27) that was not statistically significant (4.17 versus 7.8 per 100 women-years; \( P = 0.837\)).

This is likely attributed to insufficient power due to the low numbers of women with \( L. \) crispatus dominance. However, because the proportion of women with \( L. \) crispatus dominance was similar in women assigned to tenofovir gel and placebo gel (53% versus 49%), the HIV incidence differences between these two groups of women are not due to \( L. \) crispatus. In addition, considering just \( L. \) iners–dominant women, the efficacy of tenofovir was maintained, where the HIV incidence rate was 67% lower (95% CI: 0.13 to 0.83) in those assigned to tenofovir gel compared with those assigned to placebo gel (2.5 versus 7.7 per 100 women-years in the tenofovir and placebo arms, respectively; \( P = 0.0118\)) (fig. S8).

Microbicide gel adherence and tenofovir efficacy in LD and non-LD women

Gel adherence, as assessed by monthly empty applicator returns (22), was similar in both groups: 60.0% [244 of 407 (244/407)] of LD women compared to 61.4% (172/280) of non-LD women had >50% gel adherence (where >50% of sex acts were covered by two applications of the gel, as recommended in the trial (Table 1)). Stratifying LD and non-LD women separately on adherence demonstrates that gel adherence >50% was associated with higher efficacy in preventing HIV in LD women than in non-LD women (Table 1). The efficacy of tenofovir gel in preventing HIV infection in the subgroup of women with >50% adherence was 78% (95% CI: 29%, 95%; \( P = 0.003\)) in the LD group but only 26% (95% CI: 98%, 75%; \( P = 0.558\)) in the non-LD group.

Vaginal tenofovir concentrations are lower in non-LD versus LD women

Tenofovir concentrations (\( n = 270\)) were assessed in a random sample of CVLs from HIV-negative women and from the first postinfection visit CVL from HIV seroconvertors. Although gel adherence was not different between the LD and non-LD groups and time since the last gel application was similar (\( P = 0.558\)) (table S1), tenofovir was only detectable in CVL samples in 29.8% (34/114) of non-LD women compared with 46.2% (72/156) of LD women (\( P = 0.008\)). Genital tenofovir concentrations were also significantly lower in non-LD (upper quartile = 24.3 ng/ml) compared with LD women (upper quartile = 8020 ng/ml).
Metabolism by vaginal bacteria affects uptake and conversion of tenofovir to active drug in target cells

We next assessed whether tenofovir metabolism by bacteria affects the kinetics or ability of target cells to uptake tenofovir and convert to pharmacologically active tenofovir diphosphate. We performed cocultures of Jurkat cells (HIV targets) in the presence of tenofovir, with G. vaginalis, L. iners, L. crispatus, or abiotic controls (both tenofovir alone or tenofovir plus Jurkat cells), to assess overall tenofovir depletion and uptake in culture. In these Jurkat cell cultures, we found that tenofovir is most rapidly depleted in the presence of G. vaginalis (Fig. 3E). Concomitantly to tenofovir loss, intracellular tenofovir concentrations rose sharply in G. vaginalis but not in L. iners or L. crispatus cultures (P < 0.0001) (Fig. 3B). Predicted metabolites at mass/charge ratios (m/z) of 136.06, 206.10, 159.07, and 59.05 showed a sharp increase at 136.06 m/z in G. vaginalis cultures, indicating adenine production via cleavage of oxy-methylphosphonic acid (Fig. 3C), the side-chain component of tenofovir (P < 0.0001 compared with L. crispatus, L. iners, and abiotic). Residual tenofovir plus the intracellular metabolite adenine made up >80% of recovered products, indicating that adenine is the major metabolite of tenofovir metabolism by G. vaginalis (Fig. 3D). Finally, to determine whether other major bacterial species in non-LD women were capable of metabolizing tenofovir, we tested the ability of P. amnii, P. bivia, Mobiluncus mulieris, and Escherichia coli to deplete tenofovir. We found that both Prevotella species and M. mulieris significantly depleted tenofovir compared with abiotic (Wilkins-Chalgren media) controls (P = 0.0007 for all at 24 hours), and E. coli trended toward depletion (P = 0.100 as compared to abiotic tryptic soy media), though not to the same extent or as rapidly as G. vaginalis (Fig. 3E).

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14.0% with L. iners (P < 0.0001) and 9.4% with L. crispatus (P < 0.0001) (Fig. 3A). Of interest, G. vaginalis used here was a subtype C strain (ATCC type strain 14018), but repeating these methods with G. vaginalis with Kenyan clinical isolates from three different subtypes demonstrated that all subtypes of G. vaginalis metabolized tenofovir (P < 0.005) (Fig. 3B). Concomitantly to tenofovir loss, intracellular tenofovir concentrations rose sharply in G. vaginalis but not in L. iners or L. crispatus cultures (P < 0.0001) (Fig. 3B). Predicted metabolites at mass/charge ratios (m/z) of 136.06, 206.10, 159.07, and 59.05 showed a sharp increase at 136.06 m/z in G. vaginalis cultures, indicating adenine production via cleavage of oxy-methylphosphonic acid (Fig. 3C), the side-chain component of tenofovir (P < 0.0001 compared with L. crispatus, L. iners, and abiotic). Residual tenofovir plus the intracellular metabolite adenine made up >80% of recovered products, indicating that adenine is the major metabolite of tenofovir metabolism by G. vaginalis (Fig. 3D). Finally, to determine whether other major bacterial species in non-LD women were capable of metabolizing tenofovir, we tested the ability of P. amnii, P. bivia, Mobiluncus mulieris, and Escherichia coli to deplete tenofovir. We found that both Prevotella species and M. mulieris significantly depleted tenofovir compared with abiotic (Wilkins-Chalgren media) controls (P = 0.0007 for all at 24 hours), and E. coli trended toward depletion (P = 0.100 as compared to abiotic tryptic soy media), though not to the same extent or as rapidly as G. vaginalis (Fig. 3E).

**Metabolism by vaginal bacteria affects uptake and conversion of tenofovir to active drug in target cells**

We next assessed whether tenofovir metabolism by bacteria affects the kinetics or ability of target cells to uptake tenofovir and convert to pharmacologically active tenofovir diphosphate. We performed cocultures of Jurkat cells (HIV targets) in the presence of tenofovir, with G. vaginalis, L. iners, L. crispatus, or abiotic controls (both tenofovir alone or tenofovir plus Jurkat cells), to assess overall tenofovir depletion and uptake in culture. In these Jurkat cell cultures, we found that tenofovir is most rapidly depleted in the presence of G. vaginalis, relative to Jurkat cells alone (P = 0.0001) or those with L. iners (P = 0.0022) or L. crispatus (P = 0.0238) (Fig. 4A). To assess microbial tenofovir metabolism, we measured adenine levels in the cell pellets and found that adenine was created only in cultures with G. vaginalis (P = 0.0022 relative to all conditions) (Fig. 4B). A

**Fig. 3. Metabolism of tenofovir by G. vaginalis and BV-associated bacteria.** (A) Tenofovir fold change in supernatants after 1 mg/ml tenofovir was added to G. vaginalis, L. iners, and L. crispatus cultures or abiotic controls in NY3II media. Tenofovir levels were measured by mass spectrometry at 0, 4 and 24 hours. Data show average ± SEM (error bars) of 18 replicate experiments for L. iners and G. vaginalis cultures compared with 15 replicates of abiotic controls. (B) Total intracellular tenofovir detected in cell pellets from cultures. (C) Predicted tenofovir metabolite adenine was measured in cultures. (D) Total drug recovery of tenofovir and adenine metabolite. (E) Tenofovir fold change in supernatants after 1 mg/ml tenofovir was added to cultures of P. amnii, P. bivia, M. mulieris, E. coli, or abiotic controls in Wilkins-Chalgren (WC) media or tryptic soy (TS) broth (for E. coli). Data show average ± SEM of six replicate experiments for P. amnii, P. bivia, and M. mulieris and four replicates for E. coli, compared with five replicate abiotic controls. P values are calculated by Mann-Whitney U test. ns, not significant.

(P = 0.0077). A subanalysis showed that genital tenofovir concentrations negatively correlated with G. vaginalis protein abundance (correlation coefficient $r = -0.19$, $P = 0.0014$) and other anaerobic bacteria (Prevotella, $r = -0.14$, $P = 0.023$), suggesting a relationship between BV-associated bacteria and tenofovir levels.

**Metabolism by G. vaginalis and BV-associated bacteria leads to tenofovir depletion**

Given the decreased levels of mucosal tenofovir in non-LD-dominant women with G. vaginalis and other BV-associated bacteria versus LD women, we aimed to determine whether interactions between microbes and tenofovir may underlie altered drug levels. We used an in vitro culture system to assess potential biodegradation of tenofovir by the major bacterial species present in this cohort. We found that tenofovir concentrations in culture with G. vaginalis decreased rapidly by 50.6% compared with marginal changes in either L. iners ($P = 0.0037$), L. crispatus ($P = 0.0019$), or abiotic (same NY3II media without bacteria) control ($P < 0.0001$) at 4 hours (Fig. 3A). The differential decline continued; by 24 hours, tenofovir concentrations in the culture medium had dropped 67.4% with G. vaginalis but only...
critical component is whether *G. vaginalis* can metabolize tenofovir more rapidly than target
active tenofovir diphosphate. To assess this, we measured tenofovir diphosphate in cell pellets
and found that although tenofovir diphosphate was made at equal levels in Jurkat cells alone
and the presence of *Lactobacillus ssp.*, it was signifi-
cantly decreased in the presence of *G. vaginalis*
($P = 0.0002$, $\beta = 0.0022$, and $P = 0.0238$ relative to
tenofovir + Jurkat alone, tenofovir + Jurkat +
*L. iners*, or tenofovir + Jurkat + *L. crispatus*, re-
spectively) (Fig. 4C). Total drug recovery mea-
surements (tenofovir + tenofovir diphosphate +
adenine) demonstrated that all components of
tenofovir were fully recovered (Fig. 4D). These
data demonstrate that *G. vaginalis* is capable of
decreasing pharmacologically active tenofovir di-
phosphate by metabolizing tenofovir before drug
uptake by target cells.

**Implications for antiretroviral-based HIV prevention**

The efficacy of tenofovir-containing topical micro-
bicide to prevent HIV infection varied more than
threefold depending on vaginal bacterial profiles;
tenofovir gel reduced HIV incidence by 61% in LD
women but only by 18% in non-LD women. These
efficacy differences between LD and non-LD women
were consistently present in the most gel-
aderent women (78% versus 26%), as well as
in the least adherent women (17% versus 4%).
In vitro studies demonstrated that metabolism of
tenofovir occurred by *G. vaginalis*, *P. bivia*, *P. amnii*,
and *M. mulieris*, and slightly by *E. coli*, but not
by *L. iners* or *L. crispatus*, indicating a putative
mechanism for the observed differences in topi-
cal microbicide efficacy. The modifying effect of
*Lactobacillus* dominance on tenofovir gel efficacy
underscores the importance of both high adherence
and LD vaginal bacterial communities for women
to benefit maximally from topical microbicides for
HIV prevention.

In searching for an underlying cause for the
disordance between applicator adherence and
detectable vaginal tenofovir concentrations be-
tween the LD and non-LD groups, we found that
non-*Lactobacillus*-dominant bacteria associated with
BV rapidly metabolized tenofovir, thereby
likely reducing extracellular drug availability. The
rapid loss of tenofovir by *G. vaginalis* and other
bacteria may also affect gel adherence estimates
based on vaginal drug levels in non-LD women,
particularly considering that *Gardnerella* metab-
olizes tenofovir more rapidly than target cells
uptake and convert the drug to active form. Thus,
although adherence is important for efficacy
(23, 24), the lack of *Lactobacillus* dominance
may also be a contributing factor. These data
demonstrate that women with BV may have to be
more rigorous in their adherence to tenofovir
gel administration to be protected against HIV
infection, due to the rapid metabolism of tenofovir
by dyshoric bacteria. Because of the potential in-
terplay between vaginal bacteria and adherence
in topical microbicide efficacy, a useful next step
could be to evaluate the proportion of non-LD
women in other topical microbicide trials of
tenofovir conducted in Africa such as VOICE and
FACTS (23, 24). These findings may have broader
implications for other topical antiretroviral deliv-
ery strategies, such as vaginal rings, for tenofovir-
based HIV prevention.

The prevalence of *Lactobacillus* dominance in
women in CAPRISA 004 (59%) was comparable
to that observed in self-described black women
from a North American study (58%) (12) but con-
siderably lower than that reported in Caucasian
women (90%). However, the extent of *Lactobacillus*
dominance may differ even within the same
country or region. The *Lactobacillus* species and
overall CSTs in our study participants were simi-
lar to those described in a previous South African
study (13, 25) but somewhat different from those
reported recently by the FRESH (Females Rising
through Education, Support, and Health) co-
hort in KwaZulu-Natal in South Africa (13).
The reasons for these differences in the prevalence
of *Lactobacillus* dominance are not known. Some
notable differences were that the CAPRISA 004
women were slightly older (24 versus 21 years) and
had higher hormonal contraceptive usage (97% versus
54% on DMPA, norethisterone oenan-
thate, or combined oral contraceptive pill), both
of which have previously been associated with
higher *Lactobacillus* (26, 27).

Although *Lactobacillus* has been associated
with lower HIV incidence in previous observa-
tional studies, we did not find a strong relation-
ship between LD status and HIV protection in the
placebo arm. This may be due to the predomi-
nance of *L. iners* in the women in our study,
rather than *L. crispatus*, which has been associ-
ated with lower HIV incidence (19, 28) (whereas
*L. iners* has been associated with increased HIV
risk (29)), or simply being underpowered with
few *L. crispatus*-dominant women. Nevertheless,
we did observe a nonsignificant trend of reduced
HIV incidence with *L. crispatus*-dominant women
compared with women who had non-*Lactobacillus-
dominant profiles, regardless of tenofovir or placebo
gel assignment.

The ecological diversity in the vaginal micro-
bione has been previously linked to changes in
mucosal immunity in the female genital tract,
including elevated cytokine levels, increased
HIV target cells (CD4+CCR5+ T cells), as well as
epithelial barrier disruption (20, 30, 31). Thus,
the presence of BV-associated inflammatory bac-
teria could have influences at the vaginal mucosa
that may add collectively to a multifactorial mech-
anism affecting microbicide efficacy. The relative
contributions of drug depletion and these poten-
tial host modulatory effects on microbicide
efficacy would be an important avenue of future
investigation.

Some limitations need to be taken into ac-
count when interpreting these data. The lack of
clinical BV measurements, such as Nugent score
or Amstel’s criteria, precludes our ability to asso-
ciate these findings with clinical BV criteria, an
important factor in vaginal health. Instead, these
data are limited to associations with metaprome-
tic and metagenomic characterization of
BV. Another caveat of this study is the assumption

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**Table 1. Effect of adherence on the HIV prevention efficacy of 1% tenofovir gel in women participating in the CAPRISA 004 trial, stratified by *Lactobacillus* dominance in the female genital tract.** One woman did not produce adherence data. *n*, number of women.

<table>
<thead>
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<th>Gel adherence</th>
<th>No. of HIV infections/women-years</th>
<th>HIV incidence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tenofovir</td>
<td>Placebo</td>
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<tr>
<td>All participants</td>
<td>407</td>
<td>407</td>
</tr>
<tr>
<td>Lactobacillus-dominant</td>
<td>281</td>
<td>281</td>
</tr>
<tr>
<td>Non-Lactobacillus-dominant</td>
<td>416</td>
<td>416</td>
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<tr>
<td>Greater than 50% adherence</td>
<td>271</td>
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<tr>
<td>Less than 50% adherence</td>
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<td>Lactobacillus-dominant</td>
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<td>244</td>
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<tr>
<td>Greater than 50% adherence</td>
<td>172</td>
<td>172</td>
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<tr>
<td>Less than 50% adherence</td>
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<td>15/176</td>
<td>15/176</td>
</tr>
<tr>
<td>Greater than 50% adherence</td>
<td>163</td>
<td>163</td>
</tr>
<tr>
<td>Less than 50% adherence</td>
<td>108</td>
<td>108</td>
</tr>
</tbody>
</table>

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...
that a single random sample represents the vaginal microbiome for the duration of the trial. Previous longitudinal evaluations of women demonstrated that some bacterial communities shift substantially in short periods of time, whereas others, including those dominated by *Lactobacillus* species, demonstrated considerably more stability, where outgrowth of anaerobic bacteria was less frequent (~10%) over time (32). In a sensitivity analysis to address this question, unrealistically high levels of misclassification would be required to double the HIV incidence rate observed in this study. In randomly selected LD women (n = 21), 76% maintained LD status at previous visits (median days = 301), and a 24% misclassification had a minimal effect on the overall incidence rate. Further, as neither the trial participants nor investigators were aware of LD status at randomization, overall random allocation in the CAPRISA 004 trial is expected to lead to comparable groups assigned to study arms of the LD and non-LD strata.

Although it is a limitation of the data that the metabolism of tenofovir was only demonstrated in vitro here, the decreased tenofovir levels in women with vaginal dysbiosis support this as a potential in vivo mechanism. Further, the in vitro studies were performed with a range from 1 to 10 mg/ml, representing predicted in vivo tenofovir concentrations after gel use (33), and we observed similar kinetics of tenofovir metabolism despite tenofovir concentration. Because multiple BV-associated bacteria could biodegrade tenofovir in vitro, future studies assessing the effects of mixed bacteria cultures on tenofovir metabolism would be an important next step. Though we have not yet assessed whether *Pseudomonas* species metabolize tenofovir, they (group Ile) represent a small fraction of women (3.5%), and removal of this group in LD versus non-LD comparisons did not affect the reported outcomes. Other BV-associated bacteria not evaluated in this study may also metabolize tenofovir and would be important to explore. We demonstrated that clinical isolates of *G. vaginalis* are also capable of degrading tenofovir, albeit at reduced rates as compared with type strains, possibly due to decreased growth rates in the clinical isolates. In addition, this mechanism is supported by several studies showing in vivo metabolism of drugs by microbiota, just never in the context of HIV infection and antiretroviral metabolism (34). Furthermore, the kinetics of bacterial metabolism of tenofovir relative to host cellular uptake and conversion of tenofovir to the pharmacologically active phosphorylated form (tenofovir diphosphate) demonstrated that *G. vaginalis* can actually metabolize tenofovir more rapidly that target cells can convert it to tenofovir diphosphate. Previous studies in CAPRISA 004 demonstrated that low levels of tenofovir diphosphate were negatively correlated with *G. vaginalis* levels in the vagina, as well as markers of BV, demonstrating the clinical relevance of our results (35).

This study used mass spectrometry–based proteomics, an emerging technology for the study of mucosal systems, to characterize vaginal bacteria. Both mass spectrometry and standard 16S rRNA sequencing had high concurrence in the classification of *Lactobacillus*-dominant profiles. There were some differences in the proportion of detected taxa. Particularly, in mass spectrometry analysis, the predominant genera—such as *Lactobacillus, Gardnerella*, and *Prevotella*—more frequently dominate the bacterial proteome load as compared with 16S results, similar to previous observations (20). This could be due to sensitivity differences as the proteome spans a larger dynamic range, posttranscriptional regulatory mechanisms that modulate protein translation from
genes, a reflection of greater diversity of metabolic states, or divergence of functionality between the same taxa in different individuals or CSTs. Future studies of the bacterial metaproteome may provide further insights into in vivo microbial systems.

We assessed whether LD and non-LD women differed with respect to clinical symptoms associated with STIs and found that reported vaginal symptoms did not differ between LD and non-LD women. This finding is concordant with other descriptions suggesting that the presence of *G. vaginalis* is often asymptomatic (17, 12), making it unlikely that *G. vaginalis* led to behavioral changes affecting gel use. Whereas previous results showed that tenofovir is protective against HSV-2 (37), our subanalysis did not find an effect of *Lactobacillus* dominance on efficacy of tenofovir against HSV-2 acquisition. However, given the many differences between HIV and HSV-2, including the target cells in the genital tract and the unknown levels required for protection in vivo, it is challenging to compare these two infections in this context. Future studies aimed at assessing tenofovir in decreased HSV-2 infection relative to vaginal microbiota will be very important.

Because *Lactobacillus* dominance corresponds with a relatively low vaginal pH, typically below 4.5 (12), vaginal pH testing may be a pragmatic approach to identify women most likely to benefit from topical tenofovir-containing microbicides and potentially other prevention strategies. However, current treatment strategies for BV may not be sufficiently efficacious (38), and better strategies that can both deplete anaerobic bacteria and support recolonization with *Lactobacillus* may be required. If validated in other trials, this could be a compelling reason for integrating topical microbicide implementation with sexual and reproductive health services so that vaginal health becomes an integral component of HIV prevention approaches.

**Conclusions**

Vaginal microbiota modulated the efficacy of the topical tenofovir microbicide in CAPRISA 004. The rapid depletion of tenofovir by *G. vaginalis* and other BV-associated anaerobic bacteria by metabolism provides a biological mechanism likely contributing to a multifactorial process, including increased vaginal inflammation and adherence, leading to varying levels of HIV prevention efficacy observed across topical microbicide trials. This suggests that women with non-*Lactobacillus*-dominant vaginal bacteria may be more sensitive to the timing of gel application and adherence, whereas bacterial communities with *Lactobacillus* dominance may facilitate an environment more conducive to topical microbicide efficacy. Thus, vaginal pH and/or microbiota screening are interventions that can potentially be added to topical microbicide initiation to guide and enhance prevention of HIV in women. These findings provide evidence about the importance of vaginal microbial communities on prevention efficacy, which could help improve this HIV-specific prevention strategy for women.

**REFERENCES AND NOTES**


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**SUPPLEMENTARY MATERIALS**

www.sciencemag.org/content/356/6341/938/suppl/DC1 Materials and Methods Figs. S1 to S9 Tables S1 to S3 References (39–47) Data S1 16 September 2016; resubmitted 25 January 2017 Accepted 13 April 2017 10.1126/science.aar9383
Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women


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Vaginal microbiome influences HIV acquisition
Tenofovir is a preexposure drug used to prevent HIV infection. In clinical trials, tenofovir was effective for men, but not women. Klatt et al. now show that tenofovir efficacy in women depends on the composition of the vaginal microbiome (see the Perspective by Tuddenham and Ghanem). In a clinical trial of 688 women, tenofovir was three times as effective among those with a Lactobacillus-dominant vaginal microbiome as it was among other women. Gardnerella vaginalis tended to predominate in the women for whom tenofovir was less effective, and the authors found that the organism could rapidly metabolize and thereby inactivate the drug.

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