MAJOR ARTICLE

Rapid and Profound Shifts in the Vaginal Microbiota Following Antibiotic Treatment for Bacterial Vaginosis

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Background. Bacterial vaginosis (BV) is a common polymicrobial disease associated with numerous negative reproductive health outcomes, including an increased risk of human immunodeficiency virus acquisition. BV is treatable with antibiotics, but relapse is common. A more detailed understanding of bacterial dynamics during antibiotic therapy for BV could identify conditions that favor establishment, maintenance, and eradication of BV-associated bacterial species, thereby improving treatment outcomes.

Methods. We used mathematical models to analyze daily quantitative measurements of 11 key bacterial species during metronidazole treatment for 15 cases of BV.

Results. We identified complete reorganization of vaginal bacterial composition within a day of initiating therapy. Although baseline bacterial levels predicted a longer time to clearance, all anaerobic species were eliminated rapidly within a median of 3 days. However, reemergence of BV-associated species was common following treatment cessation. *Gardnerella vaginalis*, a facultative anaerobe, was cleared more slowly than anaerobic BV-associated species, and levels of *G. vaginalis* often rebounded during treatment. We observed gradual *Lactobacillus* species growth, indicating that untargeted microbes fill the transient vacuum formed during treatment.

Conclusions. Under antibiotic pressure, the human microbiome can undergo rapid shifts on a scale of hours. When treatment is stopped, BV-associated bacteria quickly reemerge, suggesting a possible role for intermittent prophylactic treatment.

Keywords. bacterial vaginosis; vaginal microbiota; mathematical modeling; metronidazole; *Gardnerella vaginalis*; *Lactobacillus*; qPCR.

The human microbiome plays a key role in human health. Diverse microbial metabolic activity maintains ecosystems in anatomic niches throughout the body [1], and deviations in microbial composition may result in a dysbiotic disease state [2]. While therapeutic antibiotics or probiotics recalibrate bacterial communities, the effects of these treatments at the individual species level are less well understood. A clearer understanding of polymicrobial dynamics is needed to optimize microbiome-targeted treatments.

Bacterial vaginosis (BV) is a highly prevalent dysbiotic condition in women [3] that is associated with increased risk for preterm birth [4, 5], pelvic inflammatory disease (PID) [6–8], herpes simplex virus shedding [9], and acquisition of sexually transmitted infections [10–12], including human immunodeficiency virus (HIV) infection [12, 13]. BV is characterized by a depletion of specific *Lactobacillus* species and increased quantities of numerous anaerobic species, many of which have been described only recently, using molecular techniques [14].

Successful treatment of BV may depend on both elimination of BV-associated anaerobes and stabilization of species associated with a healthy vaginal

Received 16 October 2014; accepted 4 February 2015; electronically published 12 February 2015.

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The Journal of Infectious Diseases® 2015;212:793–802

DOI: 10.1093/infdis/jiv079

microbiota [15]. However, the composition of the vaginal microbiota is dynamic [16–19], and temporal analyses are necessary to characterize a healthy state. Previous studies observed compositional changes over weeks [16–18], but individual species' turnover rates over shorter time frames remain unexplored. This information may provide important insights for designing BV treatment strategies.

Here, we develop mathematical models to quantify changes in bacteria concentrations during antibiotic treatment for BV. Similar models have been used to better understand the pathogenesis and treatment of chronic viral infections, such as those due to HIV [20–23], hepatitis C virus [24], and herpes simplex virus [25, 26]. Our models demonstrate rapid changes in most prevalent anaerobic bacteria species. Metronidazole treatment results in a transient microbial vacuum that is often filled by growth of *Lactobacillus iners*. When treatment is stopped, BV-associated bacteria quickly reemerge, suggesting a possible role for intermittent prophylactic treatment. Our findings highlight that the vaginal microbiota can undergo dramatic changes over extremely narrow intervals.

METHODS

Ethics Statement

Vaginal samples were collected using protocol 1789, which was approved by the institutional review board (IRB) at the Fred Hutchinson Cancer Research Center (approval no.: 5485). All participants provided written informed consent prior to study enrollment. Consent forms were approved by the IRB as part of protocol 1789.

Study Population

The study population comprised 45 women enrolled in a longitudinal study of BV at the Public Health–Seattle and King County Sexually Transmitted Diseases Clinic between March 2007 and March 2010. At enrollment, participants were evaluated for BV by means of the Amsel criteria [27]. They then returned for follow-up evaluation at 1 month. In the intervening period, women obtained vaginal swab specimens daily for 7 days and then on days 14, 21, and 28 (protocol 1), or they performed vaginal swabs daily for 30 days (protocol 2). Diagnosis, sample collection, storage, and processing of swabs are described elsewhere [19].

Among the study population, 12 women were diagnosed with BV by means of the Amsel criteria. Eleven received metronidazole treatment and were therefore included in this study. Among these women, 7 followed protocol 1 and 4 followed protocol 2. In protocol 1, 1 woman was enrolled 3 times and 2 were enrolled twice. We analyzed each episode independently, for a total of 15 enrollments. Because including repeated enrollments might introduce bias, we also performed analyses without repeat enrollments.

Thirteen episodes were treated with topical metronidazole gel (5 g with 37.5 mg of metronidazole) nightly for 5 days [28]. Two participants were treated with oral metronidazole (500 mg) twice daily for 7 days [28]; both were included because sensitivity analysis indicated similar clearance between oral treatment and topical treatment.

DNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

Concentrations of bacterial DNA were measured using qPCR assays targeting 11 key vaginal bacteria: *Atopobium vaginae*, BV-associated bacterium 1 (BVAB1), BVAB2, BVAB3, *Gardnerella vaginalis, Lactobacillus crispatus, Lactobacillus jensenii, L. iners, Leptotrichia/Sneathia* species, *Megasphaera* species, and *Mobiluncus* species [19, 29]. Hereafter, we classify the non-*Lactobacillus* groups as "BVAB" for this article because all are significantly associated with BV [29].

Bacterial Suppression and Reemergence

We defined bacterial suppression when the treatment resulted in a decrease of a particular bacteria species below the threshold of qPCR detection during treatment. On the basis of laboratory protocol and bacterial qPCR assay, 4 threshold values were used to establish eradication, depending on the species: counts of 375, 750, 1500, and 3000 16S ribosomal RNA (rRNA) gene copies per swab. We defined reemergence as at least 2 positive qPCR-based detections or 1 high qPCR concentration (>6 log measurement) of a given bacterium any time after suppression occurred (including the remaining treatment period and the 3 observed weeks following treatment cessation).

To determine potential predictors of reemergence, we used logistic regression with reemergence of the specific BV-associated bacteria as the outcome. Only species that were present at baseline and suppressed were included in the analysis. To control for within subject correlation, we used a generalized linear mixed model with random intercepts.

Mathematical and Statistical Modeling of Bacterial Dynamics

Analyses were conducted in R (R Foundation for Statistical Computing, Vienna, Austria) using the intraclass correlation coefficient (ICC), lme4, lmerTest, and plyr add-on packages. Figures were made using the ggplot2 add-on package.

To calculate the effect of metronidazole on BV-associated bacteria, we modeled bacterial DNA concentrations across the treatment period. For each enrollment, we used a monophasic exponential model to describe clearance of BV-associated bacteria DNA over time. To estimate clearance rates, we conducted a linear regression of the natural log of the number of bacterial 16S rRNA gene copies per swab on treatment days. For *Lactobacillus* species, which exhibited nonlinear patterns of change over treatment time, we fit loess splines to establish general trends.

Total clearance time was defined on the basis of when the BV-associated bacteria were suppressed. If suppression was not reached, the clearance window ended the day after the final day of treatment (day 6 for topical treatment or day 8 for oral treatment) or when a large bacterial rebound occurred (>1 log; see the Supplementary Materials for a discussion on selection of clearance end points). Regression was not performed when suppression occurred within 2 days, owing to the inability to establish a trend.

For each fitted exponential model, the estimated clearance rate was converted into its corresponding $\log_{10}$ estimate and half-life for interpretability. We assessed model fit by calculating r^2.

To calculate correlation between clearance rate and initial values, we used the Pearson correlation coefficient to measure overall correlation and the Spearman rank correlation coefficient to measure species-specific correlations.

We compared women who experienced emergence of *L. crispatus* or *L. jensenii* to women who did not across several continuous predictors, using the Kolmogorov–Smirnov 2-sample test. We classified emergence as a positive swab sample obtained any time after baseline. Episodes positive for a species at baseline (2 women with *L. crispatus* and 1 woman with both) were excluded.

Analysis of Clearance Rates

To assess the difference in clearance rates among bacterial groups, we regressed our subject-specific clearance rates on bacterial group, using a linear mixed model with a random intercept to control for within subject variability (see the Supplementary Materials for more details). To evaluate clustering by subjects and bacteria, we calculated an ICC, which describes the proportion of the total variance of an outcome attributable to the between-group (within-variable) variance.

Summed Bacteria Calculation

As a crude estimate of total bacteria, we summed the total bacterial DNA concentrations for *Lactobacillus* species and BVAB levels for each participant on each treatment day.

RESULTS

Study Participants

We documented bacterial dynamics during metronidazole treatment for 15 treatment episodes in 11 women with BV diagnosed by means of clinical criteria [27] and confirmed by means of the Nugent score [30]. A separate analysis that excluded repeated enrollments is presented in the Supplementary Materials and revealed similar findings overall. Topical metronidazole was used for 13 treatment episodes, and oral metronidazole was used for 2 episodes.

Pretreatment Microbial Characteristics Differ Among Species

Atopobium vaginae, G. vaginalis, and *L. iners* were the most prevalent species in women before treatment and were present at the onset of each treatment course (Figure 1). The least common bacteria at baseline were *L. crispatus* and *L. jensenii*. A mean of 6 BVAB (range, 2–8 BVAB) were present in each episode.

Mean pretreatment concentrations of individual bacteria ranged from 10^3 to 10^9 16S rRNA gene copies/swab (Figure 1). Within a given bacterium, baseline value ranges were narrower, generally ranging over 1–2 log units. BVAB1, when present, had the highest mean baseline value. *Gardnerella vaginalis* levels were uniformly high. *L. crispatus* had the lowest overall mean and maximum level, while *L. iners* had high pretreatment levels (Figure 1).

BVAB Suppression Was Rapid and Associated With Baseline Bacterial Levels

With the exception of *Leptotrichia/Sneathia* species and *G. vaginalis*, BVAB DNA levels were uniformly driven to levels below the qPCR detection threshold during treatment (Table 1).

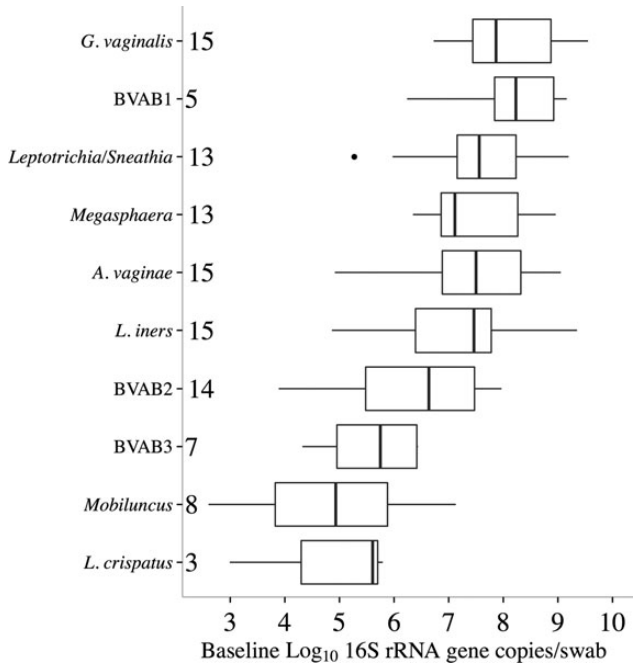

Figure 1. Pretreatment ranges for bacterial concentration during bacterial vaginosis. Distributions of pretreatment $\log_{10}$16S ribosomal RNA (rRNA) gene copies/swab for each measured bacterium, ordered by increasing mean. Boxes represent the interquartile range (IQR) of the data, the whiskers extend to cover all data within 1.5 times the IQR of the first or third quartile, and solid dots represent outliers. Sample sizes at baseline are given for each species (out of 15 total episodes). *Lactobacillus jensenii* was removed because of small sample size (n = 1 with a baseline $\log_{10}$ 16S rRNA count of 6.40 gene copies/swab). Abbreviations: *A. vaginae, Atopobium vaginae*; BVAB, BV-associated bacterium; *G. vaginalis, Gardnerella vaginalis*.

Table 1. Effect of Metronidazole Therapy on Bacterial Vaginosis–Associated Bacteria (BVAB) During and After Treatment

Bacterial Group, Species, or Genus	Clearance		Reemergence,[a] Treatments, No. (%)	
	Total Treatments, No. (%)	Time, d, Mean	During Treatment[b]	Overall[c]
A. vaginae	15 (100)	3.47	3 (20)	9 (60)
BVAB1	5 (100)	4.00	0 (0)	1 (20)
BVAB2	14 (100)	2.93	1 (7.14)	6 (42.86)
BVAB3	7 (100)	1.57	0 (0)	0 (0)
G. vaginalis	3 (20.00)	4.00	1 (33.33)	3 (100)
Leptotrichia/Sneathia	11 (84.62)	3.36	1 (9.09)	5 (45.45)
Megasphaera	13 (100)	3.15	0 (0)	5 (23.08)
Mobiluncus	8 (100)	2.12	1 (12.5)	2 (25)

Abbreviations: *A. vaginae, Atopobium vaginae*; *G. vaginalis*, *Gardnerella vaginalis*.

[a] At least 2 positive results of quantitative polymerase chain reaction (qPCR) or 1 high qPCR-based concentration (>6 log) after suppression occurred.

[b] Reemergence criteria met before 5-day treatment ended (or, for 2 episodes, before 7-day oral metronidazole treatment ended).

[c] Reemergence during 4 weeks of observation including treatment.

Leptotrichia/Sneathia species were not eliminated in 2 of 13 treatments; however, in 1 episode, *Leptotrichia/Sneathia* species were undetectable 2 days after treatment cessation. *Gardnerella vaginalis* levels reached the threshold of PCR detection during only 3 of 15 treatment sessions.

Time until suppression was similar across species (Table 1 and Figure 2*A*), with the exception of BVAB3. Most suppression occurred rapidly (median time to suppression, ≤4 days). Each species had cases that were cleared in ≤2 days (Figure 2*A*). However, responses were variable, and some times to suppression exceeded 5 days. Longer times to suppression were associated with higher baseline bacterial loads (Figure 2*B*). Each log increase in initial bacterial load increased mean clearance time by 0.50 days (95% confidence interval [CI], .19–.81).

Reemergence of BVAB Was Common Following Treatment

After treatment cessation, we observed examples of reemergence (detection after suppression within the 28 day observation period) in all species but BVAB3 (Table 1). While reemergence sometimes occurred prior to treatment cessation, it was more frequent after treatment ended. We observed reemergence throughout the 3-week observation period after treatment but most commonly during the first week (Supplementary Figure 1). *Gardnerella vaginalis* and *A. vaginae* had the highest reemergence rates. Although *G. vaginalis* elimination was rare (3 of 15 episodes), all reemerged. *Atopobium vaginae* was present and eliminated in all 15 episodes, only to reemerge during or after treatment in 10 instances. Excluding *G. vaginalis*, we observed 5 episodes in which all measured BV-associated bacteria were cleared with

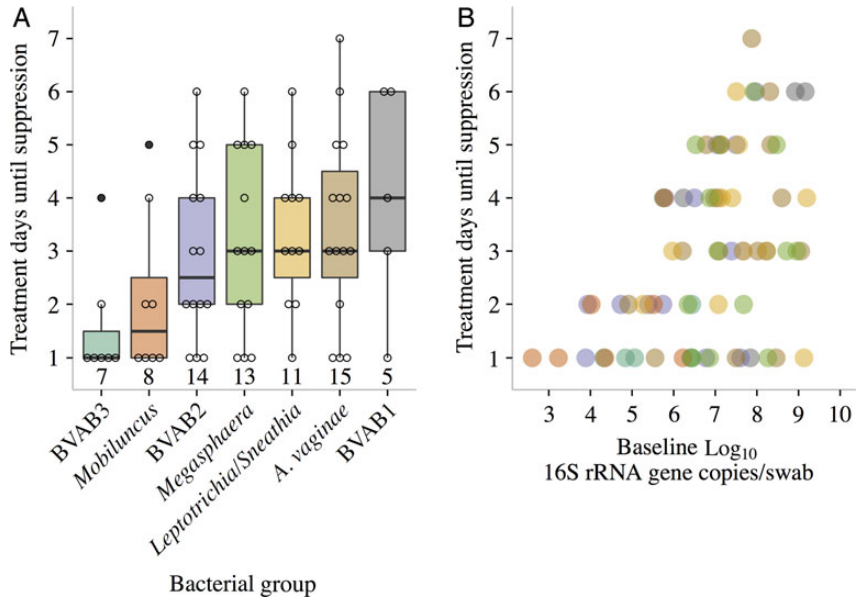

Figure 2. Time until suppression for bacterial vaginosis (BV)–associated bacteria during metronidazole therapy according to pretreatment bacterial concentration. *A*, Distributions of total treatment days until suppression for each BV-associated bacterium (BVAB). Bacteria are listed in order of increasing pretherapy mean. Boxes represent the interquartile range (IQR) of the data, the whiskers extend to cover all data within 1.5 times the IQR of the first or third quartile, and circles represent raw data. Sample sizes are given below the box plots. *B*, Scatterplot of $\log_{10}$ baseline counts versus treatment days until bacterial suppression. *Gardnerella vaginalis* is not included because of low numbers of participants with complete suppression (3 total on days 1, 3, and 8). Colors of dots correspond to colors of box plots in panel *A*. Abbreviations: *A. vaginae, Atopobium vaginae*; rRNA, ribosomal RNA.

Table 2. Vaginal Bacteria Dynamics During Metronidazole Treatment

Bacterial Group, Species, or Genus	Treatments, No.	Clearance Rate,[a] Mean (Range)	R^2, Mean (Range)	Half-life, h, Mean
A. vaginae	11	1.43 (0.67–2.37)	0.88 (0.67–0.99)	5.94
Megasphaera	8	1.23 (0.76–1.85)	0.88 (0.65–1)	6.57
Leptotrichia/Sneathia	10	1.17 (0.63–2.21)	0.87 (0.64–1)	7.13
BVAB1	4	1.1 (0.91–1.52)	0.8 (0.62–0.99)	6.85
BVAB3	1	0.92	0.97	7.86
BVAB2	7	1.01 (0.72–2)	0.93 (0.78–1)	7.94
Mobiluncus	2	0.81 (0.8–0.83)	0.91 (0.84–0.97)	8.87
G. vaginalis	13	0.78 (0.18–1.67)	0.77 (0.38–0.99)	13.41

Abbreviations: *A. vaginae, Atopobium vaginae*; BVAB, bacterial vaginosis–associated bacterium; *G. vaginalis*, *Gardnerella vaginalis*.

[a] Log_{10} 16S ribosomal RNA gene copies/swab/day.

no reemergence within 21 days of treatment cessation. Time to suppression, bacterial clearance rates, and presence of *Lactobacillus* species were not predictive of protection against reemergence (see Supplementary Material).

Exponential Clearance of BVAB Other Than *G. vaginalis* During Therapy

Using an exponential clearance model, we estimated each species' bacterial DNA clearance rate during antibiotic treatment. The mathematical model provided a good fit for all BV-associated bacteria (R^2 range, 0.62–0.99; mean R^2, >0.80) except *G. vaginalis* (R^2 range, 0.38–0.99; mean R^2, 0.77; Table 2). Most BVAB underwent rapid and predictable monotonic clearance (Figure 3*A*), with mean clearance half-lives ranging from 6 to 14 hours (Table 2). Baseline bacterial levels did not correlate with the clearance rates (Pearson correlation, −0.12; 95% CI, −.37 to .15). Bacterial DNA levels also did not correlate with clearance rates at the single species level. Although at least a

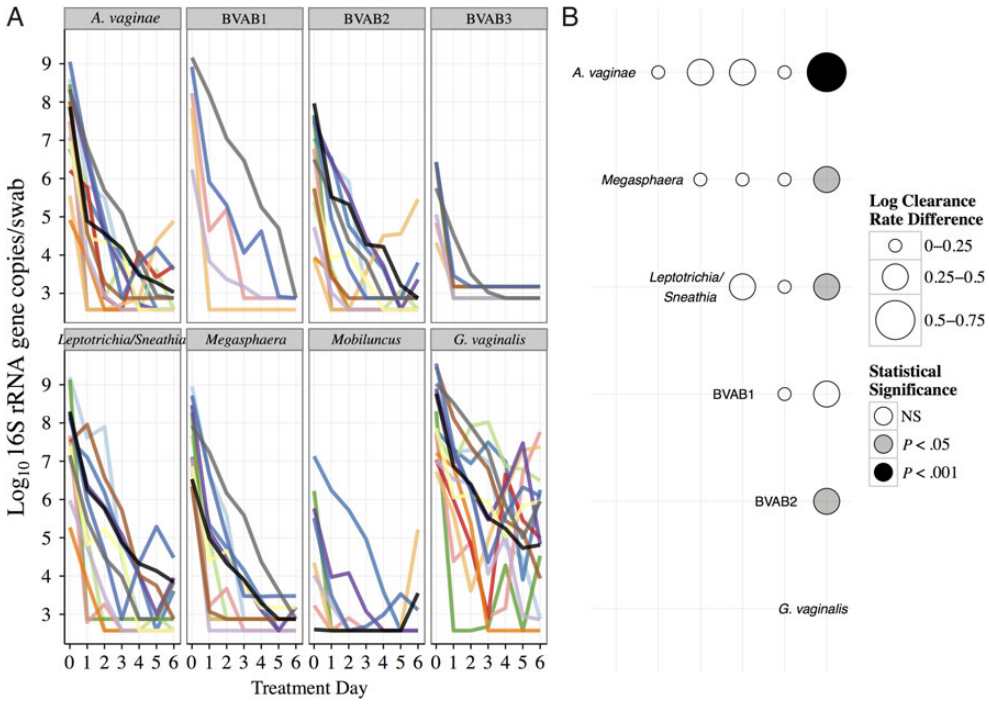

Figure 3. Individual clearance curves and clearance rate comparisons for bacterial vaginosis (BV)–associated bacteria (BVAB) during metronidazole treatment. *A*, Each color denotes a different participant. Only *Gardnerella vaginalis* is notable for frequent bacterial rebound. *B*, Each bubble represents the mean differences in clearance rates (log_{10} 16S ribosomal RNA [rRNA] gene copies/swab/day) between the bacterial group listed to the left and the bacterial group listed below. Rate differences and *P* values were estimated using a linear mixed model. *Atopobium vaginae* had the fastest mean clearance rate and *G. vaginalis* the slowest mean clearance rate, compared with the other bacteria. Abbreviation: NS, not significant.

100-fold reduction in *G. vaginalis* was observed in each episode, responses were more variable than for other species (Figure 3*A*).

Clearance Rates Were Similar Across BVAB Species

To further investigate differences among bacterial clearance rates, we fit a mixed model of estimated clearance rates by bacterial group. *Atopobium vaginae* was cleared the most rapidly among BVAB, and the *G. vaginalis* clearance rate was slower than that for all other BVAB (Figure 3*B*). Oral metronidazole treatment did not appear to have a different effect than topical therapy (Figure 4).

Variability of BVAB Clearance Rates Depend More on Subject-Specific Than Bacteria-Specific Factors

The range of median half-lives was lower among the BVAB (5.13–9.64 hours; Figure 4*A*) than among the episodes (3.96–10.73 hours; Figure 4*B*). We calculated ICCs to determine the extent to which clearance rates from the same groups tended to be similar. The ICC is the proportion of overall variance attributable to between-group variance. The ICC for clearance rates was higher across subjects than across species (0.45 vs 0.12), implying that clearance rates cluster more strongly by host. Therefore, although bacterial species explains some variance in clearance, other unmeasured host-specific factors must also be considered.

Five different episodes were associated with clearance of at least 1 species in a single day despite a baseline level of >6 log genomic copies. Because we could not use regression to estimate clearance rates when clearance occurred in 1 or 2 days, we examined the last detectable DNA concentration before suppression as an estimate for clearance rate. We reasoned that if the last positive values for bacteria cleared within 1–2 days are the same as bacteria cleared in >2 days, then differences in clearance times are attributable to lower baseline values. However, the last detectable DNA concentrations were considerably higher for single-day eradications than multiple-day eradications (Supplementary Figure 2), indicating an extremely rapid treatment effect during a subset of treatment episodes. Two women experienced suppression of all BVAB (one with and one without *G. vaginalis*) within

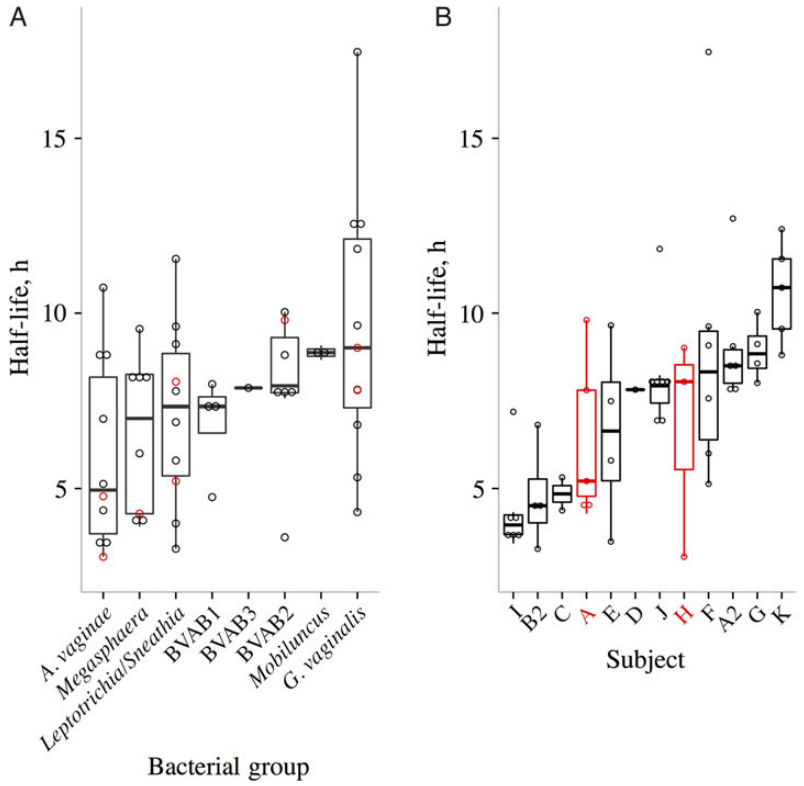

Figure 4. Higher clearance half-life variability within bacterial vaginosis (BV)–associated bacterial (BVAB) species as compared to within study participants. *A*, Distribution of estimated bacterial clearance half-lives (days) for each BV-associated bacterial group, sorted by increasing median. *B*, Distributions of half-lives (days) for each participant, arranged by increasing median. Participants who received oral metronidazole treatment are denoted in red. Repeat episodes in the same subject share the same letter. Boxes represent the interquartile range (IQR) of the data, and the whiskers extend to cover all data within 1.5 times the IQR of the first or third quartile. Dots represent each individual clearance half-lives: black denotes topical metronidazole use and red denotes oral metronidazole use. Two *Gardnerella vaginalis* outliers are not shown (bacterial half-lives of 28 hours for episode G and 41 hours for episode A3). One episode (A3) was removed from panel *B* because it had only 2 data points, and 2 other episodes (B and C2) were not included owing to a lack of estimated clearance half-lives. Abbreviation: *A. vaginae, Atopobium vaginae*.

a single day. Both had concurrent growth of *L. crispatus* and *L. jensenii* during treatment but did not have distinct initial bacterial profiles. Rapid clearance did not predict long-term suppression: 100% reemergence (7 of 7 species) was observed in the woman who rapidly cleared *G. vaginalis,* while 50% reemergence (3 of 6 species) was noted in the other woman.

Lactobacillus Species Expand During Treatment

Metronidazole therapy resulted in complete reorganization of the vaginal microbiota in the majority of treated women, with a massive decrease in BVAB quantities (Figure 3*A*) and emergence of *Lactobacillus* species (Figure 5). At baseline, only 3 episodes were positive for either *L. crispatus* or *L. jensenii*; however, we observed swabs positive for either species in 7 episodes by the end of the treatment period and in 11 episodes by the end of the observation period (28 days; Supplementary Figure 3). By fitting a spline through the data during treatment, we found that average quantities of *L. crispatus* changed very little during treatment, whereas levels of *L. jensenii* increased by several log units among the 6 women with this species (Figure 5*A*). BVAB clearance rate, time until suppression, baseline *L. iners* levels, baseline BVAB levels, and total BVAB present at baseline were not predictive of *Lactobacillus* species emergence (Supplementary Materials).

Lactobacillus iners was detected at high levels in all women at baseline (Figure 1) and was present in all episodes throughout the entire observation period. During treatment, population levels of *L. iners* increased by about a log, but individual dynamics were highly variable (Figure 5*A*). Although we observed nonmonotonic growth patterns in most women, a spline indicated that *L. iners* often expanded slowly after 2 days, filling the vacuum created by antibiotic treatment.

To assess the extent of microbiota transition, we measured a sum of all available qPCR levels for each woman, grouped separately by *Lactobacillus* species and BV-associated species. Owing to variable amplification efficiency among BV-associated species and exclusion of potential key BV-associated species from our qPCR measurements, the summed measures of BVAB are likely underestimates of true values. Therefore, this analysis establishes general trends only. While BVAB dominated at baseline (90% of the total bacteria level), levels dropped below that of *Lactobacillus* species after 1 day of treatment (Figure 5*B*). By the end of treatment, BVAB represented only a small proportion of the summed total, compared with *Lactobacillus* species.

DISCUSSION

Using mathematical models, we demonstrate that the human microbiome is dynamic within a single anatomic niche and

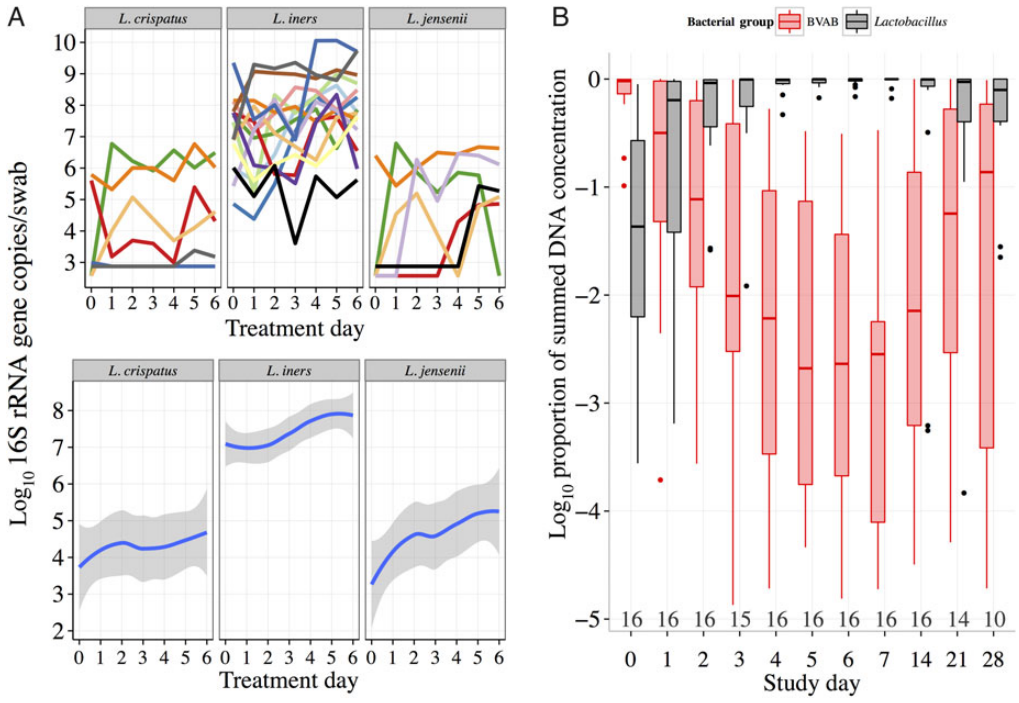

Figure 5. *Lactobacillus* species growth during antibiotic therapy. *A*, Time series plots (top half) for each *Lactobacillus* species during antibiotic treatment (each color denotes a different participant). Loess splines (bottom half) correspond to the data in the top half of figure. *B*, Daily distribution of the vaginal microbiota composition (log_{10} proportion of total bacteria), comparing bacterial vaginosis (BV)–associated bacteria (BVAB; red box plots) to *Lactobacillus* species (black box plots) during the observation period (sample sizes are depicted below box plots). Boxes represent the interquartile range (IQR) of the data, the whiskers extend to cover all data within 1.5 times the IQR of the first or third quartile, and solid dots represent outliers. Abbreviation: rRNA, ribosomal RNA.

can undergo complete reorganization in less than a day during antibiotic treatment. Within 24 hours of metronidazole treatment initiation, most BVAB undergo rapid exponential depletion. Levels of *Lactobacillus* species, particularly *L. iners,* often surge to fill the transient microbial vacuum. The microbiome remains unstable when antibiotic therapy is stopped; reemergence of anaerobic species is common, often within a week.

Many studies correlate cross-sectional profiles of diversity with disease risk, based on the assumption that the microbiome is a rather stable entity. However, we demonstrate that cross-sectional analyses may be insufficient to capture the dynamic landscape of polymicrobial composition. Other human microbiota niches, such as the gut and mouth, may also undergo frequent rapid shifts akin to those in the vagina. Permanent or transient alterations to bacterial composition can occur both during and after antibiotic use [31–33]. Future studies of the microbiome should incorporate serial, high-frequency quantitative measures of multiple bacterial species.

While the presence of BVAB does not always result in BV, an understanding of bacterial reemergence may be useful to prevent BV recurrence. If reemergence depends on seeding from sources outside of the vagina, such as the gastrointestinal tract or the genital tract of sex partners, then preventive interventions may include risk-modification counseling. Alternatively, levels of metabolically dormant BVAB may persist in anatomic drug sanctuaries or in *G. vaginalis* biofilms [34–37]. Under these conditions, intermittent suppressive regimens may prevent BVAB from reemerging and causing disease [38].

We demonstrate that metronidazole therapy is not an effective treatment against *G. vaginalis*. Among the measured BVAB in our study, *G. vaginalis* had markedly lower clearance rates. The exponential model fit poorly to *G. vaginalis* levels during treatment, owing to frequent bacterial rebound. The lack of efficacy of metronidazole against *G. vaginalis* is only partially understood [39, 40]. Rebound may indicate emergent drug resistance or persistence within biofilms [34–37, 41].

For sustained treatment effect, the drivers of successful *Lactobacillus* expansion and persistence may be of central importance. *Lactobacillus* reemergence could result from reduction in spatial constraints on growth or diminished competition with BVAB for limited nutritional resources. Thus, metronidazole may indirectly but positively affect *Lactobacillus* growth. In our study, emergence of either *L. crispatus* or *L. jensenii* occurred in 8 of the 12 episodes that had negative tests for these species at baseline. Given that *L. crispatus* is inversely associated with BVAB [17, 19, 29, 41–47], strategies to ensure its sustained presence in the vaginal microbiota could be beneficial. *Lactobacillus iners* was the most dominant *Lactobacillus* species in these women, although its role in protection against BV is uncertain [19, 41, 43–48].

While clearance rates are similar within BV-associated anaerobic species, host-specific features play an even more important role in determining clearance rates. Factors that determine more-rapid clearance rates during certain treatment sessions warrant further study. Aside from species, possible determinants of the bacterial clearance rate include individual variability related to pharmacodynamics, microbiota profile at baseline, treatment adherence, and timing of treatment during the menstrual cycle [17, 19, 47].

Our interpretation of clearance rates is limited because estimates encompass several possible ongoing processes, including, (1) natural bacterial death rate, (2) bactericidal effects of metronidazole, and (3) bacterial fission during treatment. If metronidazole's bactericidal effects [49, 50] predominate in vivo, then our measured clearance rate may exceed the natural death rate of bacteria. Yet, if bacterial fission continues to occur at a meaningful rate during treatment, then our measured clearance rate may be lower than the natural death rate. The estimated clearance rates would approximate natural bacterial death rates if metronidazole is bacteriostatic in vivo and the natural birth and death rates of BVAB exceed the killing rate of metronidazole. The subtle differences in clearance rates between species likely include some differential combination of these processes.

Some limitations arise when estimating bacterial levels by using qPCR. Bacteria may, in theory, die at a faster rate than their DNA degrades. Our estimated daily clearance rates of BVAB DNA exhibited a clear log-linear relationship, and this trend should be confirmed using colony counts of cultivatable bacteria. We were also not able to measure levels of all species in the vaginal niche. It is therefore not possible to estimate a true total bacterial count: our estimates of BVAB levels relative to *Lactobacillus* levels are imprecise. Nevertheless, there is a clear trend toward complete turnover in microbial composition within a very short time of antibiotic initiation.

In summary, we documented extremely rapid turnover of resident vaginal bacteria during and after antibiotic treatment. The vaginal microbiota can be highly dynamic, particularly in response to antibiotics, and future work should investigate the impact of antibiotic and other perturbations on microbial community structure and function.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. S. S., J. M. M., and D. N. F. conceived and designed the experiments. S. S. and T. L. F. performed the experiments. B. T. M. and J. T. S. analyzed the data and wrote the manuscript.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grants R01 AI061628 [to D. N. F.], K23 AI087206 [to J. T. S.], and U19 AI113173).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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