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Nonoptimal Vaginal Microbiota After Azithromycin Treatment for *Chlamydia trachomatis* Infection

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We characterized the composition and structure of the vaginal microbiota in a cohort of 149 women with genital *Chlamydia trachomatis* infection at baseline who were followed quarterly for 9 months after antibiotic treatment. At time of diagnosis, the vaginal microbiota was dominated by *Lactobacillus iners* or a diverse array of bacterial vaginosis-associated bacteria including *Gardnerella vaginalis*. Interestingly, *L. iners*-dominated communities were most common after azithromycin treatment (1 g monodose), consistent with the observed relative resistance of *L. iners* to azithromycin. *Lactobacillus iners*-dominated communities have been associated with increased risk of *C. trachomatis* infection, suggesting that the impact of antibiotic treatment on the vaginal microbiota could favor reinfections. These results provide support for the dual need to account for the potential perturbing effect(s) of antibiotic treatment on the vaginal microbiota, and to develop strategies to protect and restore optimal vaginal microbiota.

Keywords. vaginal microbiome; sexually transmitted infection; *Chlamydia trachomatis*; antibiotics; 16S rRNA gene sequencing; longitudinal.

In the United States, >1.7 million *Chlamydia trachomatis* (CT) genital infections were reported in 2017 (528.8 cases per 100 000), representing a 6.9% increase since 2016 [1]. However, this rate is considered an underestimate, as most CT-positive cases are asymptomatic [2]. CT infection is particularly prevalent in females between the ages of 15 and 24 years, with a reported infection rate 4 times higher than the general population [3]. Without appropriate treatment, approximately 10%–20% of infected women develop pelvic inflammatory disease [4, 5], which is associated with tubal infertility and ectopic pregnancy [6].

The vaginal microbiota provides the first line of defense against sexually transmitted infections (STIs). *Lactobacillus*

spp produce lactic acid and other antimicrobial compounds that maintain a protective environment [7]. The absence of *Lactobacillus* spp, as in the clinical diagnosis of bacterial vaginosis (BV), is associated with increased risk of STIs [8–12]. CT transmission rates after exposure to an infected partner are estimated at between 25% and 40% [13–15], indicating that not all exposures result in successful infection and that other factors, such as the vaginal microbiota, could be important cofactors in susceptibility to infection. Recent large-scale molecular surveys of the vaginal microbiota have revealed 5 broad vaginal bacterial community-state types (CSTs) [16, 17]. Four CSTs are dominated by *Lactobacillus* spp, while a fifth is deficient in *Lactobacillus* but comprised of a diverse set of strict and facultative anaerobes as often seen in BV. It has been hypothesized that different CSTs respond differently to disturbance events such as menstruation and medication [16–20] and display different resilience, that is, the ability to respond to, withstand, and recover from disturbance [21]. We sought to evaluate if the vaginal microbiota returns to a more optimal state following antibiotic treatment for genital CT infection. If the vaginal microbiota is not fully restored in the months and years following CT treatment, it may help to explain observed high rates of CT reinfection [22, 23]. We aimed to characterize the vaginal microbiota composition and structure of a cohort of 149 women with genital CT infection who were followed quarterly for 9 months after azithromycin treatment.

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METHODS

Study Design

Adolescents and young adults with positive tests for CT infection were screened at clinic point-of-care centers and community-based outreach sites by clinical staff at the University of Maryland School of Medicine's Adolescent and Young Adult Center. Participants in the Chlamydia Adolescent/Adult Reproductive Management (CHARM) research study were invited upon notice of positive CT infection. The study was approved by the institutional review board of the University of Maryland, Baltimore (protocol number HP-00042350). Included in this report are eligible females who were 12–40 years old and self-reported a history of sexual activity. The CHARM cohort is described in detail elsewhere [24]. In addition, 99 CT-negative African American women, 19–44 years old, enrolled in the Vaginal Microbiota 400 Women Study (VM400) [17], a cross-sectional study at the University of Maryland School of Medicine, served as controls.

Study Procedure

At each CHARM enrollment visit (enrollment, 3 months, 6 months, and 9 months), an audio computer-assisted self-interview was administered. Clinicians conducted a physical examination and specimen collection, and provided treatment for CT using azithromycin 1 g orally in a single dose. When women were CT positive at visit 2, 3, or 4, azithromycin treatment was prescribed as needed and women remained in the study. Vaginal specimens for microbiota analysis (ESwabs, Copan) were collected from the mid-vaginal wall prior to antibiotic treatment, stored in 1 mL liquid Amies, and archived at –80°C. CT was determined by BD ProbeTec on urine specimens.

Sample Processing, 16S Ribosomal RNA Gene Amplification, and Sequencing

Whole genomic DNA was extracted from 300-μL aliquots of Amies solution as previously reported [17]. High-throughput amplification and sequencing of the V3–V4 hypervariable regions of the 16S ribosomal RNA gene were performed using a validated and improved dual-indexing approach [25]. Further bioinformatic processing followed the DADA2 Workflow for Big Data and *dada2* (version 1.5.2) (<https://benjjneb.github.io/dada2/bigdata.html> [26]) as previously reported [27]. Taxonomy was assigned to each amplicon sequence variant generated by *dada2* using SpeciateIt (version 1.0, <http://ravel-lab.org/SpeciateIt>). Read counts for amplicon sequence variants assigned to the same taxonomy were summed for each sample. Data analyses include hierarchical clustering of each community profiles using Euclidian distance and assignment to one of the CSTs described previously [16, 17], and community diversity estimation using the Shannon diversity index [28].

Statistical Analyses

Analyses were carried out on samples with a total read count of at least 1000, and on phylotypes present in at least 2 samples.

Baseline characteristics between CHARM participants who were lost to follow-up after the first visit and those who did not were compared using Fisher tests. To compare vaginal microbiota composition and structure among different groups, we conducted analyses at the CST and at the phylotype levels. At the phylotype level, we fitted negative binomial regression models for each phylotype present in at least 20% of all samples, using the DESeq2 package in R [29]. At the CST level, we fitted logistic regression models to compare vaginal microbiota of CHARM visit 1 (CT positive) or CHARM visit 2 to those of the VM400 controls. We applied a mixed-effect logistic regression model to compare CHARM visit 1 and visit 2 taking into account intrawoman correlation between samples, using the lme4 package in R. To describe vaginal microbiota at all visits and in the VM400 cohort controls, we used the Shannon diversity index, which accounts for both the number of different taxa and their evenness. Values of the Shannon diversity index were compared across CHARM visits and with the VM400 controls using the Wilcoxon matched-pairs signed-rank test and the Wilcoxon–Mann–Whitney rank-sum test, respectively. For all these analyses, CT-positive samples at visits 2, 3, and 4 were excluded because of the difficulty to distinguish between reinfections and treatment failures (see Results).

RESULTS

We enrolled 149 women with confirmed CT infection, mostly African American (86%) and 13–33 years old who provided 141 baseline samples (visit 1), prior to treatment with 1 g single-dose azithromycin. Additional samples were collected at each subsequent visit and tested for CT (92, 85, and 77 samples, respectively; [Supplementary Figure 1](#)). CT positivity was 18% ($n = 17$), 14% ($n = 12$), and 18% ($n = 14$), respectively.

Participants' demographic, behavioral, and medical history is summarized in [Supplementary Table 1](#). Women lost to follow-up after visit 1 ($n = 49$) were less likely to have pelvic inflammatory disease at baseline (data not shown); no other baseline characteristics were significantly associated with cohort retention ($n = 100$), including age, race, marital status, education, sexual orientation, number of partners (lifetime and in the last 3 months), smoking status, Nugent score, hormonal contraception, or CST.

For the CHARM cohort, we obtained 7 396 180 high-quality sequences with an average of 18 725 (standard deviation [SD], 18 325) sequences per sample. For the VM400 controls, we generated 3 349 907 sequences with an average of 33 837 (SD, 17 024) sequences per sample. A total of 319 phylotypes was identified in the combined CHARM and VM400 datasets. The relative abundance of each phylotype is illustrated in [Figure 1](#) (data available at https://github.com/ravel-lab/charm_longitudinal).

Vaginal Microbiota Composition Association With Prevalent CT Infection

To lower the dimensionality of the dataset, samples were assigned to CSTs. CST I and III are often dominated by *Lactobacillus*

crispatus and *Lactobacillus iners*, respectively, whereas CST IV lacks *Lactobacillus* spp and includes a diverse array of facultative and strict anaerobes. Further refinement revealed subtypes CST III-A/B and IV-A/B (Figure 1). Broadly, in CST III-A, *L. iners* is dominating the vaginal community at a relative abundance higher than approximately 80%, whereas in CST III-B, *L. iners* remains the dominant species but at lower abundance while anaerobes are also present. Within CST IV, CST IV-B is represented by a higher abundance of *Gardnerella vaginalis*.

Using a logistic regression model, we found that the frequencies of each CST in CHARM visit 1 and VM400 cohort control samples were significantly different (Supplementary Table 2). We observed that CST III-A, CST III-B, CST IV-A, and CST IV-B were significantly overrepresented in CHARM samples compared to controls (odds ratios [ORs], 4.51 [95% confidence interval {CI}, 1.41–16.42], 5.20 [95% CI, 1.66–18.72], 8.98 [95% CI, 3.38–28.59], and 26.52 [95% CI, 8.84–94.91], respectively;

P values .015, .007, < .001, and < .001, respectively) (Figure 2). The majority of CST IV samples (91%) were confirmed to have Nugent scores >7, which is indicative of BV [31].

We fitted a negative binomial regression model to evaluate specific phylotypes associated with CT infection (visit 1) compared to the control group. We compared the relative abundance of 40 phylotypes present in at least 20% of controls and CHARM visit 1 samples (240 observations). Phylotypes significantly associated with either CHARM visit 1 or VM400 cohort controls are listed in Supplementary Table 3. Overall, 25 phylotypes had relative abundance that significantly differed between the 2 groups (Supplementary Figure 2), among which 11 phylotypes were overrepresented in CT-positive vaginal microbiota, including *G. vaginalis*, *Atopobium vaginae*, bacterial vaginosis-associated bacterium (BVAB) 2, and *Mobiluncus curtisii*, whereas *Lactobacillus* spp were overrepresented in VM400 CT-negative samples.

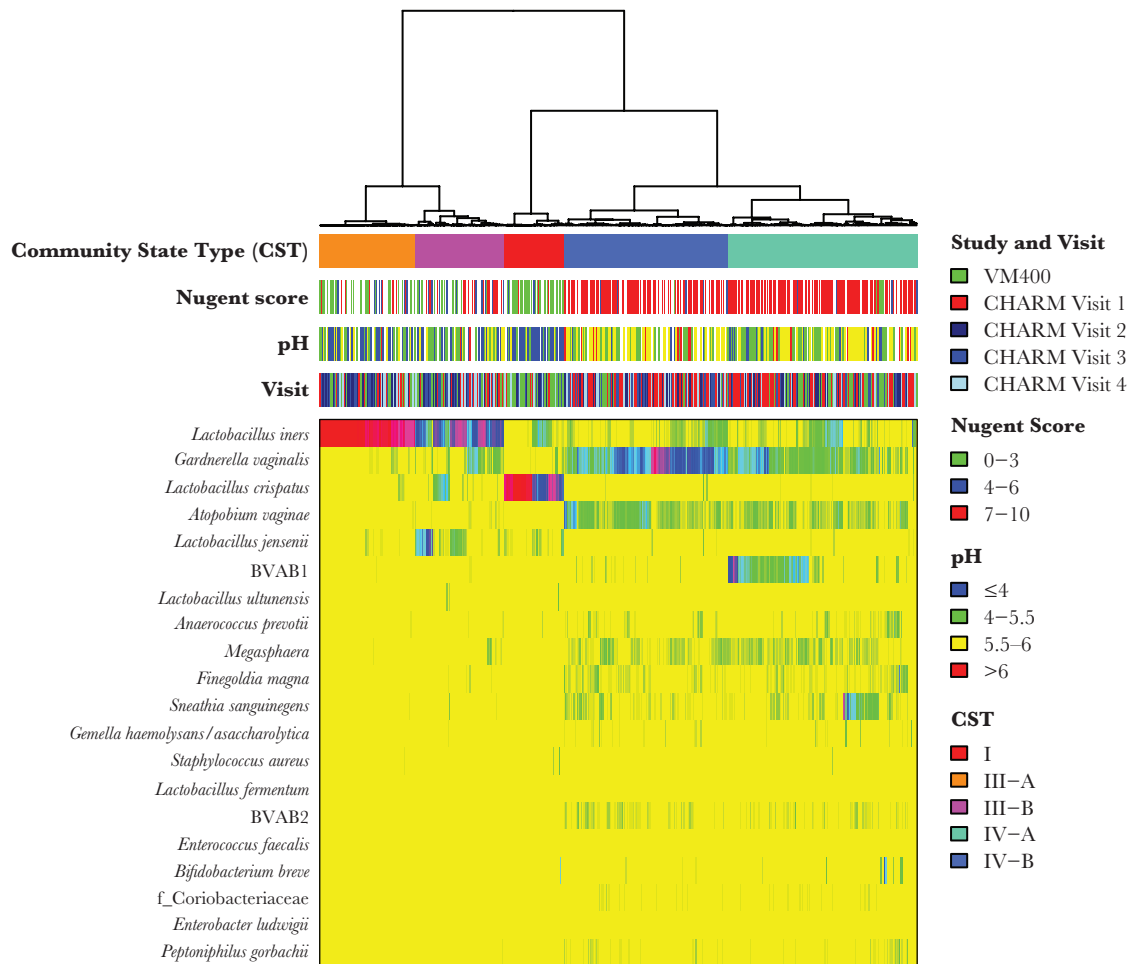


Figure 1. Heatmap representing the relative abundance of the 20 most abundant phylotypes found in the vaginal microbiota of 395 samples collected every 3 months for 9 months after azithromycin treatment for *Chlamydia trachomatis* by 149 young females in the Chlamydia Adolescent/Adult Reproductive Management prospective cohort, Baltimore, Maryland, and 99 samples from 99 women enrolled in the VM400 cross-sectional control study, Baltimore, Maryland [30]. Ward linkage clustering was used to cluster samples based on their Euclidian distance calculated in the “vegan” package in R. The 4 bars on top indicate community state types, according to the previous naming convention [30], Nugent Score, vaginal pH, and visit number. Abbreviations: BVAB, bacterial vaginosis-associated bacteria; CHARM, Chlamydia Adolescent/Adult Reproductive Management; CST, community state types.

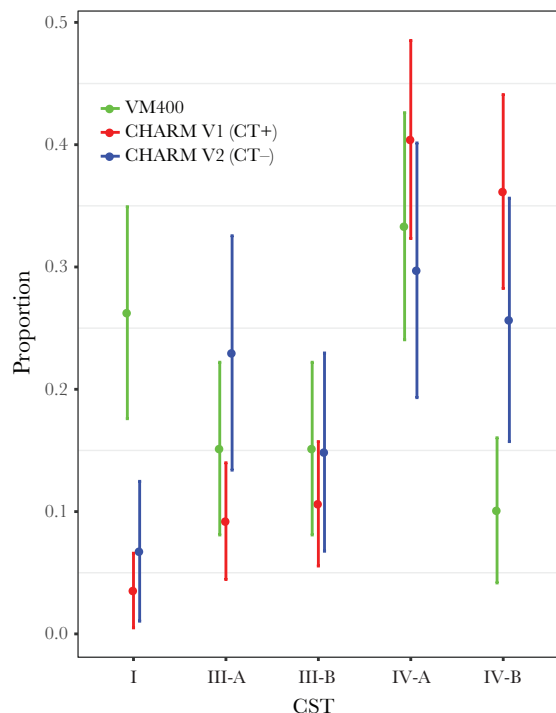


Figure 2. Proportions of community state types (CSTs) in Chlamydia Adolescent/Adult Reproductive Management (CHARM) samples from visit 1 (*Chlamydia trachomatis* [CT] positive) and visit 2 (CT negative, 3 months posttreatment) and in VM400 controls.

CT Infection and Antibiotic Treatment Footprint on the Vaginal Microbiota

All women from the CHARM study were treated with azithromycin after a CT-positive diagnosis at baseline. Interestingly, 18% ($n = 17$) of women were CT positive at visit 2 (either because of treatment failure, reinfection with the same untreated partner, or reinfection with another partner). The frequency of each CST at visit 2 in CT-negative women ($n = 74$) were compared to that of the VM400 cohort (cleared infection vs noninfected). We observed that CST III-A, CST III-B, CST IV-A, and CST IV-B were again overrepresented in CHARM CT-negative samples (ORs, 5.89 [95% CI, 1.91–21.01], 3.81 [95% CI, 1.16–14.13], 3.47 [95% CI, 1.23–11.47], and 9.88 [95% CI, 3.09–36.88]), respectively; P values .003, .033, .027, and < .001, respectively) (Supplementary Table 4 and Figure 2). At the phylotype level, among 49 phylotypes present in at least 20% of CHARM visit 2 CT-negative and VM400 control samples (173 observations), 24 were statistically significant with 6 overrepresented in CHARM CT-negative samples compared to controls, including *A. vaginae*, *Gemella haemolysans*, or *Peptoniphilus gorbachii*. However, *G. vaginalis*, BVAB1, BVAB2, and, interestingly, *L. iners*, were not statistically significant but trended toward overrepresentation in CHARM CT-negative samples (Supplementary Table 5 and Supplementary Figure 3).

Additionally, the frequency of CSTs in CHARM visit 2 CT-negative samples was compared to that of CHARM visit 1

(CT-positive). CST III-A was overrepresented in CT-negative samples compared to CT-positive samples (Supplementary Table 6 and Figure 2) (OR, 3.51 [95% CI, 1.45–8.75], using CST IV-B as reference). In addition, among 33 phylotypes present in at least 20% of CHARM visit 1 and visit 2 CT-negative samples (215 observations), only *L. iners* appeared to be significantly overrepresented in CT-negative samples at visit 2 compared to visit 1 samples (negative \log_2 fold change), this after correcting for multiple testing (Supplementary Table 7 and Supplementary Figure 4).

Long-Lasting Effect of CT Infection and Azithromycin Treatment on the Vaginal Microbiota

The composition and structure of the vaginal microbiota in CT-negative samples at visit 3 and visit 4 (6 months and 9 months after azithromycin treatment: $n = 73$ and $n = 63$, respectively) were analyzed to evaluate whether CT infection and azithromycin treatment had a long-term effect on the vaginal microbiota. While a major shift in CST was observed between visit 1 and visit 2 (probably due to CT infection and azithromycin treatment as suggested above), the proportions of CST III and CST IV remained stable from visit 2 to visit 4 while women remained CT negative (Figure 3). Interestingly, *L. iners* relative abundance increased greatly after azithromycin treatment (visit 1 to visit 2; Figure 4A) whereas *Sneathia sanguinegens* decreased, but the relative abundance of no phylotype was statistically significantly different between visit 2 and visit 4 (Figure 4B and 4C). Shannon diversity was significantly higher in CHARM visit 1 samples compared to other CHARM samples and VM400 samples, and no difference was found between visits 2, 3, and 4 (Supplementary Table 8 and Supplementary Figure 5).

L. iners and *G. vaginalis* Strain Resistance to Azithromycin

To evaluate whether the patterns we observed were associated with antibiotic resistance of phylotypes associated with CST IV (*G. vaginalis*) and CST III (*L. iners*), we performed antimicrobial susceptibility tests of 2 front-line antibiotic treatments for CT genital infection, azithromycin and doxycycline, on several strains of these 2 species (Figure 5 and Supplementary Table 9). We observed low resistance to azithromycin (0.094–1.5 $\mu\text{g/mL}$) for *L. crispatus*, *Lactobacillus jensenii*, and *Lactobacillus gasseri*; however, some *L. iners* (5/10) and *G. vaginalis* (1/8) strains were resistant to the highest concentration of azithromycin tested (256 $\mu\text{g/mL}$). In contrast, doxycycline sensitivity was similar for all inspected vaginal species (0.016–12 $\mu\text{g/mL}$), and no doxycycline resistance was observed.

Community-State Types Transition Patterns From One Visit to Another According to CT Infection Status

For each CHARM participant with follow-up samples ($n = 100$), we generated CST transition patterns over the course of the

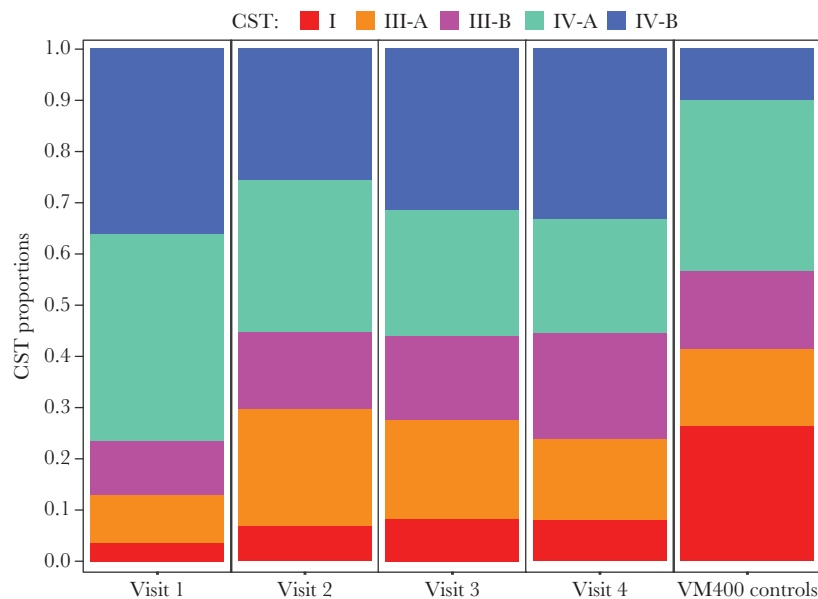


Figure 3. Community state type (CST) proportions in Chlamydia Adolescent/Adult Reproductive Management (CHARM) samples from visit 1 (*Chlamydia trachomatis* [CT] positive, pretreatment), visit 2 (CT negative only, 3 months posttreatment), visit 3 (CT negative only, 6 months posttreatment), and visit 4 (CT negative only, 9 months posttreatment) and in VM400 controls.

study (Figure 6A). Analysis of individual trajectories confirmed our findings that women commonly transitioned to *L. iners*-dominated CST III-A after azithromycin treatment. However, over time, women who were CST IV-A and CST IV-B before treatment and transitioned to CST III at visit 2 then transitioned back to CST IV or CST III-B, which both contain significant levels of strict and facultative anaerobes. We further stratified these CST transition patterns by restricting the analysis to (1) participants who shifted from CT positive at visit 1, 2, or 3 to CT negative at the following visit (Figure 6B); (2) participants

who remained CT negative for 2 consecutive visits (Figure 6C); and (3) participants who tested CT negative at visit 2 or 3 but CT positive at the following visit (Figure 6D).

Transitions from CT positive to CT negative (Figure 6B) at any time during the study period (after azithromycin treatment) were mostly associated with a transition to CST III-A (+75%), supporting the findings obtained when comparing CHARM visit 1 (CT positive) to CHARM visit 2 (CT negative). These transitions were observed in women who were either CST III-B or CST IV-A/B when CT positive. Surprisingly, CST I was observed after

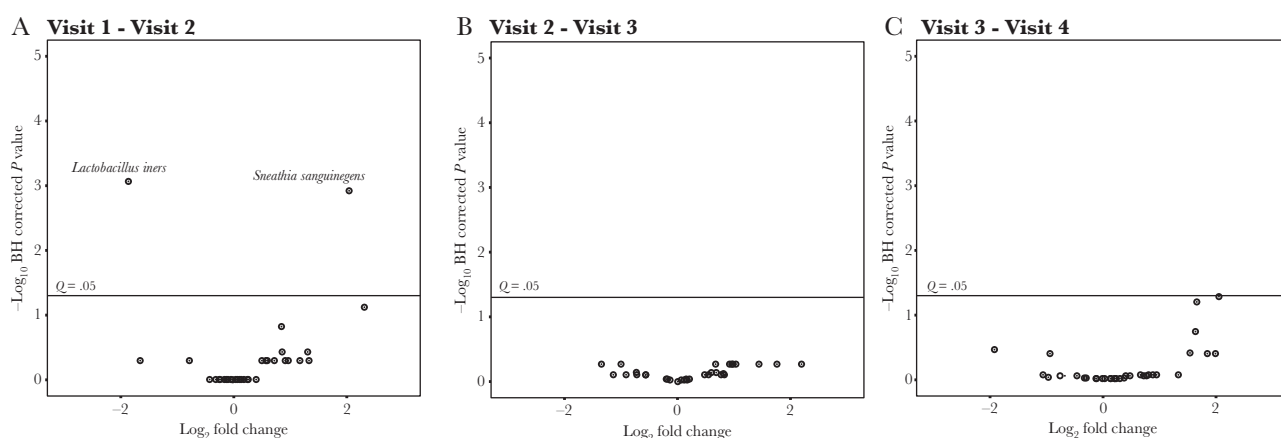


Figure 4. Volcano plots displaying results of negative binomial regression model using the DESeq2 package on R [29], comparing phylotypes differentially expressed between Chlamydia Adolescent/Adult Reproductive Management (CHARM) samples: visit 1 (*Chlamydia trachomatis* [CT] positive) to visit 2 (CT negative) (A), visit 2 (CT negative) to visit 3 (CT negative) (B), visit 3 (CT negative) to visit 4 (CT negative) (C), for phylotypes present in at least 20% of samples for each analysis (33, 32, and 34 phylotypes, respectively). The \log_2 fold change is plotted against the $-\log_{10}$ of the Q value, which is the P value corrected for multiple testing using Benjamini–Hochberg correction (BH). Positive values of the \log_2 fold change indicate phylotypes overrepresented in the first visit of the 2 visits considered, whereas negative values indicate phylotypes overrepresented in the second visit.

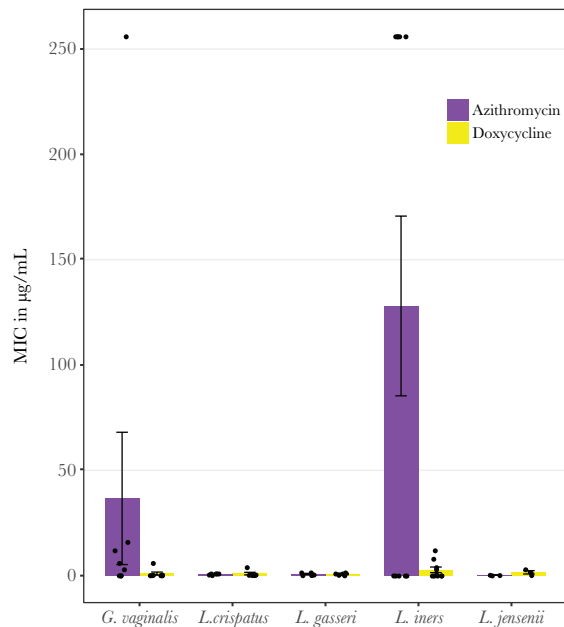


Figure 5. Antimicrobial susceptibility test of 5 major vaginal bacterial species for azithromycin and doxycycline. Minimum inhibitory concentration (MIC) was determined by broth microdilution protocol [32] with concentrations ranging from 0.016 µg/mL to 256 µg/mL. Number of strains tested: *Gardnerella vaginalis*: 8; *Lactobacillus crispatus*: 6; *Lactobacillus gasseri*: 5; *Lactobacillus iners*: 10; *Lactobacillus jensenii*: 3.

azithromycin treatment, despite the fact that in vitro *L. crispatus* was shown to be sensitive to azithromycin. As expected, in the absence of azithromycin treatment (CT negative at 2 consecutive

visits; Figure 6C), we observed limited CST transitions that did not affect the overall CST proportions (Figure 3). Transitions between CST III-B and CST IV-A were rare, whereas subgroup transitions within CST IV and within CST III were more frequent. We also observed very few transitions between CST I and CST IV-B ($n = 1$ of 61 transitions overall) or CST IV-A ($n = 1$ of 61) (Supplementary Figure 6 and Supplementary Table 10). In cases of transitions from CT negative to CT positive, there was an increase of CST III-B (+150%) and CST IV-B (+33%), though numbers were too small to draw any conclusions. As expected, no transition to CST I was observed (Figure 6D).

DISCUSSION

By evaluating the vaginal microbiota composition and structure at time of CT infection and every 3 months for 9 months after azithromycin treatment, our study identified specific characteristics of the vaginal microbiota associated with CT infection and resolution. Unsurprisingly, the vaginal microbiota of women infected with CT in CHARM encompassed bacterial taxa commonly associated with BV or CST IV, including *G. vaginalis*, *A. vaginae*, or *M. curtisii* [33–37]. It is important to note that studies of the vaginal microbiota during prevalent CT infection do not resolve whether the observed microbiota is causal to the increased risk of CT infection, or if it is a consequence of CT infection. To potentially establish causality, prospective longitudinal studies must be performed and focused on incident cases of infection. Such a study undertaken in the Netherlands has indicated that women presenting an *L. iners*-dominated CST

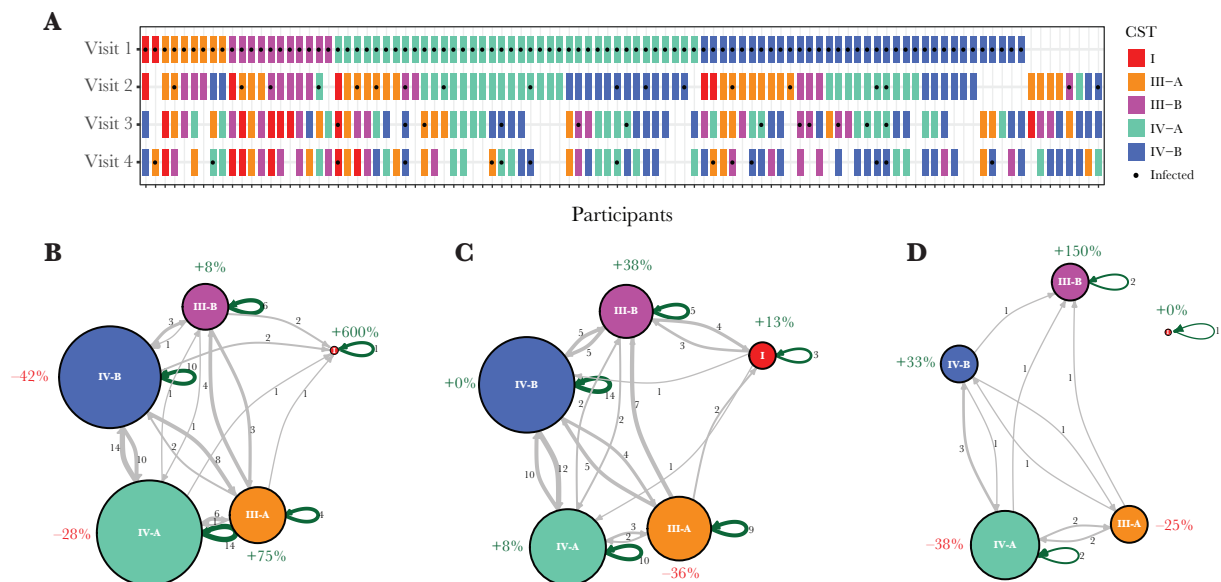


Figure 6. Transitions between community state types (CSTs) from one visit to another in Chlamydia Adolescent/Adult Reproductive Management (CHARM) samples. A, Individual trajectories of women included in the study. B, CST transitions from *Chlamydia trachomatis* (CT)-positive samples pretreatment to CT-negative samples posttreatment at 2 consecutive visits. C, CST transitions among CT-negative samples at 2 consecutive visits. D, CST transitions from CT-negative samples to CT-positive samples at 2 consecutive visits. The number next to a line represents the number of women transitioning from one CST to another. Looped arrows represent the number of women staying in the same CST between 2 visits and are colored green. In B, C, and D, circle size is proportional to total frequency of CSTs.

III were at increased risk of CT infection compared to women with *L. crispatus*-dominated CST I [38]. While *L. iners* often dominates CST III, it can share the ecological niche with other bacterial taxa, such as *G. vaginalis*, *A. vaginae*, and other strict and facultative anaerobes, whose presence could limit the potential benefit of having a *Lactobacillus* spp.-dominated vaginal microbiota. When that is the case, CST III is thought to transition more frequently to CST IV, as previously observed in longitudinal studies of the vaginal microbiota [16, 18], particularly following antibiotic treatment for BV [20]. Importantly, it is well established that CST IV is associated with an increased risk for CT infection [12, 39].

The longitudinal study design of CHARM gave us the unique opportunity to observationally study the vaginal microbiota following azithromycin treatment. Interestingly, we found that 3 months after azithromycin treatment for CT infection, most women had vaginal microbiota that were either CST IV or CST III-A. The relative frequency of each CST in CT-negative women at visit 2 was significantly different from that observed in our control CT-negative cohort drawn from the same clinic. Modeling the transitions from visit 1 to visit 2 demonstrated that after azithromycin treatment, *L. iners* relative abundance (and CST III) increases substantially. Because of the observational and interval censored study design, we cannot differentiate azithromycin's direct effects (observed 3 months later) vs the community changes resulting from CT clearance. However, we hypothesize that the observed microbiota could be explained by either the effect of antibiotic exposure or by a return to a preinfection vaginal microbiota or a combination of both. We observed that strains of *L. iners* and *G. vaginalis* displayed a higher level of resistance to azithromycin, and thus could be selected posttreatment, whereas sensitive *Lactobacillus* spp were diminished. Nonetheless, because *L. iners* is a potential risk factor for CT infection [40], this finding is important as it suggests that after antibiotic treatment, a woman's risk of STI is not reduced. This result could contribute significantly to the high rate of reinfections observed in CHARM and in other studies (20–30 cases per 100 person-years [22, 23]). In our cohort, 74.2% of women reported at baseline having been CT positive in the last 3 months. Though this could be due to (re-)exposure to infected partners, it is likely that they received antibiotic treatment, thus maintaining their susceptibility to reinfection. Another study evaluating the effect of metronidazole treatment for BV showed that *L. iners* was often increased after antibiotic treatment, sometimes replacing *G. vaginalis* [20]. Thus metronidazole treatment can also result in nonoptimal vaginal microbiota. More importantly, we provide evidence that a posttreatment vaginal microbiota remains stable for up to 9 months with high relative abundances of *L. iners* and *G. vaginalis*, resulting in persistently increased CT infection risk.

Interestingly, doxycycline, another recommended antibiotic for CT infection, efficiently killed all strains tested in

our minimum inhibitory concentration studies. Though both antibiotics are reported to be 95% effective to treat CT infection, a meta-analysis reported doxycycline as more effective than azithromycin [41]. If, in vivo, azithromycin eliminates *L. crispatus*, *L. jensenii*, and *L. gasseri*, which are considered beneficial [42–47], the treatment would favor the proliferation of *L. iners* or *G. vaginalis*, thus increasing the risk of CT infection. This result is novel since only one previous study reported no effect of azithromycin on 4 strains of *G. vaginalis* and 2 of *L. iners* [48]. This finding supports the use of live biotherapeutic products to restore a protective vaginal microbiota after antibiotic treatment for CT and potentially for other health conditions treated by antibiotics. Unfortunately, very little is known about the effect of frequently used antibiotics on the vaginal microbiota.

Our study presents some limitations. Owing to our inclusion criteria focusing on CT-positive women at baseline, we are unable to determine whether bacterial phylotypes and CSTs overrepresented in CHARM visit 1 CT-positive samples were present before infection. Similarly, we are unable to distinguish between CT clearance or antibiotic treatment as the causal determinant in microbiota composition. However, we found that some strains of *L. iners* and *G. vaginalis* are resistant to azithromycin, which could explain the observed patterns. Unfortunately, the study was not sufficiently powered to detect a statistically significant impact of reinfection at visits 3 or 4. Finally, we used a cohort of 99 healthy African American women from the same geographical area and clinic for recruitment as a control population in our analyses. However, the CHARM cohort is not entirely African American (87.4%), and we cannot exclude that they differ for other characteristics.

This study has potentially important consequences for the management and control of CT infections. It confirms the association between CT infection and not only non-*Lactobacillus* spp microbiota, but also microbiota dominated by *L. iners*. Furthermore, the study shows that high risk for infection may be maintained in part by antibiotic treatment. Our results stress the importance of taking into account the potential perturbing effects of antibiotic treatment on the vaginal microbiota, whether it is for the treatment of CT infections or other indications. Studies of the effect of antibiotic therapy on the composition of the vaginal microbiota are urgently needed. Such studies will provide the necessary guidance in the development of strategies to protect and restore optimal vaginal microbiota composition prior to and after antibiotic treatment.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. J. R. is co-founder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutics drugs for women's health. All other authors report no potential conflicts of interest.

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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