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Original article

Effects of azithromycin and doxycycline on the vaginal microbiota of women with urogenital *Chlamydia trachomatis* infection: a substudy of the Chlazidoxy randomized controlled trial

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ABSTRACT

Objectives: Dysbiotic bacterial communities within the vagina are associated with *Chlamydia trachomatis* infection. We compared the effect of treatment with azithromycin and doxycycline on the vaginal microbiota in a cohort of women with a urogenital *C. trachomatis* infection randomly assigned to one of these treatments (Chlazidoxy trial).

Methods: We analysed vaginal samples from 284 women (135 in the azithromycin group and 149 in the doxycycline group) collected at baseline and 6 weeks after treatment initiation. The vaginal microbiota was characterized using 16S rRNA gene sequencing and classified into community state types (CSTs).

Results: At baseline, 75% (212/284) of the women had a high-risk microbiota (CST-III or CST-IV). A cross-sectional comparison 6 weeks after treatment showed that 15 phylotypes were differentially abundant, but this difference was not reflected at the CST (p 0.772) or diversity level (p 0.339). Between baseline and the 6-week visit, α-diversity (p 0.140) and transition probabilities between CSTs were not significantly different between the groups, and no phylotype was differentially abundant.

Discussion: In women with urogenital *C. trachomatis* infection, the vaginal microbiota does not seem to be affected by azithromycin or doxycycline 6 weeks after treatment. Because the vaginal microbiota remains susceptible to *C. trachomatis* infection (with CST-III or CST-IV) after antibiotic treatment, women remain at risk of reinfection, which could originate from unprotected sexual intercourse or untreated anorectal *C. trachomatis* infection. This last consideration advocates for the use of doxycycline instead of azithromycin because of its higher anorectal microbiological cure rate. **Jeanne Tamarelle, Clin Microbiol Infect 2023;29:1056**

Introduction

The vaginal microbiota is considered to be the cornerstone of vaginal health. Large microbiological molecular surveys have revealed five broad vaginal community state types (CSTs) in reproductive-age women [1]. Four CSTs are dominated by *Lactobacillus* spp., whereas the fifth CST is deficient in *Lactobacillus* spp. and comprises a diverse set of strict and facultative anaerobes. Dysbiotic vaginal bacterial communities and communities dominated by *Lactobacillus iners* are associated with prevalent urogenital *Chlamydia trachomatis* infection [2,3]. Van der Veer et al. [3] showed that in a population of women notified by sex partners infected with *C. trachomatis*, those who tested positive for *C. trachomatis* were more likely to have a vaginal microbiota dominated by *L. iners* or diverse anaerobic bacteria than those negative for *C. trachomatis*. More interestingly, Van Houdt et al. [4]

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showed that *L. iners*-dominated communities are associated with incident urogenital *C. trachomatis* infection, even after adjustment on sexual activity, meaning that this type of bacterial community predisposes to acquiring *C. trachomatis*. Although widely prescribed, antibiotic use is likely to disturb the fine-tuned equilibrium of the vagina. If antibiotic treatment for urogenital *C. trachomatis* infection had a detrimental effect on the vaginal bacterial communities, it would place women at risk of *C. trachomatis* reinfection or other genital morbidity. A single dose of azithromycin (1 g) and a 7-day course of doxycycline (100 mg twice daily) both showed >95% efficacy for the treatment of urogenital *C. trachomatis* infection [5]. However, little is known about the effect of these antibiotics on the vaginal microbiota. In a cohort of 52 women with urogenital *C. trachomatis* or *Mycoplasma genitalium* infection, the vaginal bacterial communities were not affected by azithromycin or tetracycline treatment [6]. In a previous work [2], we showed that 9 months after azithromycin treatment for urogenital *C. trachomatis* infection, most women had a vaginal microbiota that was either CST-IV or CST III-A, thus maintaining their susceptibility to reinfection.

We sought to evaluate whether the vaginal microbiota returns to a more optimal state after doxycycline treatment for urogenital *C. trachomatis* infection compared with treatment with azithromycin. We aimed to characterize the vaginal microbiota composition of women with urogenital *C. trachomatis* infection included in the Chlazidoxy trial before and after their random assignment to either azithromycin or doxycycline.

Methods

Study population and sample collection

Women included in this study were participants of the Chlazidoxy randomized controlled trial, recruited at four sexually transmitted disease (STI) screening centres and three pregnancy termination centres in France [7]. Inclusion and exclusion criteria have already been described [7]. In short, eligible women with a urogenital *C. trachomatis* infection provided a self-collected anal swab and were randomly assigned (1:1) to receive orally either a 1-g single dose of azithromycin or 100 mg of doxycycline twice daily for 7 days. If the anal swab at baseline was *C. trachomatis* positive, women provided new self-collected vaginal and anal swabs for *C. trachomatis* detection 6 weeks after the treatment was initiated. Demographic, clinical, biological data, and sexual behaviour were collected. The women provided written informed consent to participate in the trial. The study received ethics approval (number 2017-74-2) and was authorized by the French regulatory authority (IDRCB 2017-002595-15) [7]. For this substudy, the study population consisted of participants with vaginal swabs available both at baseline and at the 6-week visit.

Biological analyses

DNA extraction was done using the MagNA Pure 96 DNA and viral NA small-volume kit on the MagNA Pure 96 instrument (Roche Diagnostics). The DNA was diluted to 10 ng/μL, and amplified using the Kappa HiFi HotStart ReadyMix (Roche, Switzerland) with the tailed primers V3_341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and V4_805R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) for bacterial identification. Amplicon libraries were prepared according to the Illumina metagenomic sequencing library construction workflow. Final libraries were quantified by fluorescence using a Synergy H1 device (BioTek, USA), standardized and combined into two pools (of 288 and 312 samples, respectively). The two pools were purified using homemade solid-phase reversible immobilization beads at a 1× ratio. The size of each pool was evaluated on a TapeStation 4200 device (Agilent Technologies, USA) using High Sensitivity D1000 ScreenTapes. The pools were sequenced on a MiSeq device (Illumina, USA) using V2 chemistry and 2 × 250-bp reads.

Bioinformatics analysis

Cutadapt (v.2.10 [8]) was used to exclude reads that did not contain primers from the sample used for analysis. Then, the data were processed using dada2 (v.1.16 [9]) with the pseudopooling algorithm up until the chimera filtering step. Researchers at the Ravel laboratory of the University of Maryland generously performed taxonomic assignment from the sequence table without chimera using their speciateIT tool (http://ravel-lab.org/speciateit). The assigned phylotypes were then filtered, and all phylotypes with abundances $<10^{-4}$ of the total number of reads were removed.

Statistical analysis

Participants' baseline characteristics were compared using Fisher's exact test. The vaginal microbiota was analysed using three approaches. First, individual phylotypes were analysed cross-sectionally in relation to the intervention group using negative binomial regression models implemented in the *DESeq2* package in R [10]. A corrected p value < 0.05 was considered indicative of statistical significance. Second, the level of α-diversity (within-sample diversity) was assessed using the Shannon diversity index (in the *vegan* package in R [11]). In cross-sectional analyses, the means of this index per treatment group were compared using the non-parametric Wilcoxon test. For both phylotypes and α-diversity, an analysis of change was performed (longitudinal analyses). Differences in abundance of each phylotype or in α-diversity between baseline and 6 weeks after treatment followed normal distributions; therefore, we implemented linear regression models, with the intervention group serving as the independent variable and controlling for baseline abundance or baseline α-diversity, as recommended [12]. Third, we used a data reduction approach to describe the vaginal microbiota in terms of CSTs [13]. A CST was assigned to each observation (i.e. sample) using the algorithm VALENCIA [14]; the output is illustrated in Figs S1 and S2. We used seven CST classes: CST-I-A and CST-I-B (*Lactobacillus crispatus*—dominated communities, high and low diversity, respectively), CST-II/V (*Lactobacillus gasseri*— and *Lactobacillus jensenii*—dominated communities), CST-III-A and CST-III-B (*Lactobacillus iners*-dominated communities, high and low diversity, respectively), CST-IV-A/B (diverse communities with either *CaLachnocurva vaginae* [formerly BVAB1], *Gardnerella vaginalis*, or *Atopobium vaginae* prominently represented), and CST-IV-C (diverse communities with opportunistic pathogens such as *Streptococcus*, *Enterococcus* or *Staphylococcus*, and *Bifidobacterium* or *Prevotella*). In cross-sectional analyses, the distributions of CSTs per treatment group were compared using the Fisher test. For longitudinal analyses, we used a multistate Markov model implemented in the *msm* package in R [15], with the number of CSTs reduced to five (CST I-A, CST I-B/II/V, CST III-A, CST III-B, and CST-IV-AB/C) based on similarity in α-diversity (Fig. S2) because of model convergence issues.

Results

In total, 284 women out of 357 with available vaginal swabs both at baseline and at 6 weeks after treatment were included in this substudy, 135 in the azithromycin group and 149 in the doxycycline group (Fig. S3). The comparison of characteristics

between participants included and those excluded (lost to follow-up, missing samples, uninterpretable results) from this longitudinal analysis is presented in Table S1.

The demographic characteristics and baseline exposures of the participants are presented in Table 1. The women were aged 18–48 years (median 22 years, IQR 20–24), 89% (254/284) of them were born in France (metropolitan and overseas departments). Seventy-eight per cent (222/284) of them had no known STI history. Women in both groups did not differ regarding demographic characteristics, history of STIs or sexual behaviour, except for urogenital symptoms that were more frequent in the azithromycin group (59% (79/135) vs. 46% (68/149) in the doxycycline group, p 0.033).

16S rRNA sequencing of vaginal swabs yielded 11,586,329 sequences, with an average of 20,398 sequences per sample (standard deviation [SD]: 8662; azithromycin group 20,158 [SD: 8567], doxycycline group 20,617 [SD: 8756]). In total, 109 phylotypes were identified in the dataset after filtering.

Table 1
Baseline characteristics of participants

Variables	Total ($n = 284$)		Azithromycin group ($n = 135$)		Doxycycline group ($n = 149$)		
	n	%	*n*	%	*n*	%	p[a]
Age (y)							0.798
Median [IQR]	22	[20–24]	22	[20–24]	22	[20–24]	
Recruiting centre							0.492
Pregnancy termination centre	70	(25)	36	(27)	34	(23)	
STI screening centre	214	(75)	99	(73)	115	(77)	
Country of birth							0.248
France (with overseas)	254	(89)	124	(92)	130	(87)	
Other	30	(11)	11	(8)	19	(13)	
Marital status							0.126
Single	194	(68)	86	(64)	108	(72)	
In a relationship	90	(32)	49	(36)	41	(28)	
Level of education							0.404
Low	68	(24)	29	(21)	39	(26)	
High[b]	216	(76)	106	(79)	110	(74)	
Professional status							0.460
Working	101	(36)	45	(33)	56	(38)	
Not working	183	(64)	90	(67)	93	(62)	
STI history							0.765
Unknown	8	(3)	3	(2)	5	(3)	
No	222	(78)	108	(80)	114	(77)	
Yes	54	(19)	24	(18)	30	(20)	
Number of pregnancies							0.672
None	220	(77)	103	(76)	117	(79)	
≥1	64	(23)	32	(24)	32	(21)	
Genital symptoms							0.033
No symptom	137	(48)	56	(41)	81	(54)	
≥1 symptom	147	(52)	79	(59)	68	(46)	
Coinfection							
HIV	0	(0)	0	(0)	0	(0)	
Syphilis	0	(0)	0	(0)	0	(0)	
Neisseria gonorrhoeae	14	(5)	4	(3)	10	(7)	
HBV	2	(1)	2	(1)	0	(0)	
Age at first intercourse (y)							0.970
10–14	15	(5)	7	(5)	8	(5)	
15–19	236	(83)	113	(84)	123	(83)	
≥20	33	(12)	15	(11)	18	(12)	
Sexual partner in the last 12 mo							0.245
None	8	(3)	3	(2)	5	(3)	
Only regular	75	(26)	41	(30)	34	(23)	
Only occasional	88	(31)	35	(26)	53	(36)	
Occasional and regular	113	(40)	56	(41)	57	(38)	
Condom use with occasional partner							0.913
Never	15	(7)	6	(7)	9	(8)	
Sometimes/often	147	(73)	68	(75)	79	(72)	
Always	39	(19)	17	(19)	22	(20)	
Number of sexual partners in lifetime							0.619
1–5	122	(43)	58	(43)	64	(43)	
6–10	74	(26)	32	(24)	42	(28)	
≥10	88	(31)	45	(33)	43	(29)	
Anal sex in lifetime							0.331
No	173	(61)	78	(58)	95	(64)	
Yes	111	(39)	57	(42)	54	(36)	
Oral sex in lifetime							0.858
No	35	(12)	16	(12)	19	(13)	
Yes	249	(88)	119	(88)	130	(87)	

HBV, hepatitis B virus; STI, sexually transmitted disease.
[a] The p value was computed using Fisher's exact test for categorical variables and using the Wilcoxon rank sum test for continuous variable (age).
[b] Postbachelor.

Baseline vaginal microbiota

The phylotypes did not differ between the two groups, except that *Escherichia coli* was significantly more frequent or abundant in the azithromycin group (Fig. 1(A), Table S2). The level of vaginal microbiota α-diversity, as measured by the Shannon index (based on abundance and evenness of bacterial species), was slightly higher in the doxycycline group, but the difference was not statistically significant (p 0.284; Fig. 2(A)). In terms of CST, 75% (212/284) of women had a CST-III or CST-IV. There was no significant difference in the CST distribution between the groups (p 0.904), although the doxycycline group had a larger proportion of CST-III-B (26% [38/149] vs. 21% [29/135]) and smaller proportions of CST-I-A (9% [13/149] vs. 12% [16/135]) and CST-I-B (11% [16/149] vs. 14% [19/135]) than the azithromycin group (Fig. 3(A), Table S3). Of note, women recruited in pregnancy termination centres did not significantly differ from women recruited in STI screening centres in terms of vaginal microbiota composition (Table S4).

Vaginal microbiota 6 weeks after treatment

Overall, two phylotypes, *Streptococcus oralis* and *Bacteroides coagulans*, were significantly more frequent or abundant in women treated with doxycycline, whereas 13 phylotypes were more frequent or abundant in those who received azithromycin (Fig. 1(B), Table S5); these taxa included *CaLachnocurva vaginae* (formerly BVAB1), *Atopobium vaginae*, *Raoultella planticola*, *Fusobacterium nucleatum*, and species of the genera *Prevotella*, *Dialister*, *Anaerococcus*, *Gemella*, and *CaSaccharibacteria*. Diversity did not differ significantly between the two groups at 6 weeks after treatment (p 0.339), although it was higher in the azithromycin group (Fig. 2(B)). The doxycycline group had a higher proportion of CST-III-A after treatment than the azithromycin group (Fig. 3(B), Table S3), but the CST distribution did not differ significantly between groups (p 0.772). Seventy-six per cent (215/284) of women still harboured CST-III or CST-IV after treatment.

Interestingly, 11.1% (15/135) of women in the azithromycin group and 10.7% (16/149) in the doxycycline group were *C. trachomatis* positive at the 6-week follow-up visit (either because of treatment failure, reinfection with the same untreated partner, or reinfection with another partner).

Vaginal *C. trachomatis* positivity was not associated with the CST at week 6 (p 0.325). In addition, no exposure occurring between baseline and 6 weeks after treatment was significantly associated with either the treatment group (Table S6) or vaginal microbiota composition (Table S7).

Evolution of the vaginal microbiota between baseline and 6 weeks

We modelled the evolution of the vaginal microbiota between baseline and the 6-week visit in terms of phylotypes and diversity. Over time, the prevalence or abundance of *Dialister*_KQ960846 and *Megasphaera*_ADGP decreased in the doxycycline group relative to that in the azithromycin group, whereas the prevalence or abundance of *Lactobacillus fermentum* and *Bacteroides coagulans* relatively increased in the doxycycline group (uncorrected $p < 0.05$) (Fig. 1(C) and S4, Table S8). However, these differences were not significant after correction for multiple testing. The change of α-diversity between baseline and the 6-week visit did not significantly differ in the trajectory between groups (p 0.140, although $\beta = -0.120$, suggesting a relative decrease of diversity in the doxycycline group). The type of treatment received did not differentially affect the probabilities of transitioning from one CST to another between visits (Table S9).

Discussion

Azithromycin and doxycycline are the two widely used treatments for urogenital *C. trachomatis* infection, with similar effectiveness in eradicating *C. trachomatis* at the urogenital site [5]. The effects of these antibiotics on the vaginal microbiota likely depend on the spectrum of activity (narrow vs. broad spectrum), pharmacokinetics, and pharmacodynamics, as well as the dose and duration of administration. All women included in this study had a urogenital *C. trachomatis* infection and were randomly assigned to azithromycin or doxycycline treatment. This gave us a unique opportunity to compare the vaginal microbiota before and after these treatments. Interestingly, we found that 6 weeks after treatment with azithromycin or doxycycline, most women still had a vaginal microbiota that was either CST-IV or CST-III-A.

The CST-III and CST-IV were the most prevalent vaginal microbiota types found in our population. In a study in young *C. trachomatis*-positive French women attending an STI clinic, the five CSTs were found at frequencies of 19.1%, 4.8%, 52.4%, and 23.8% for CST-I, CST-II/V, CST-III, and CST-IV, respectively [16]. This is in accordance with our findings, although we found a smaller proportion of CST-I or CST-II/V. In two other studies involving *C. trachomatis*-positive women, the proportions of CST-I were even smaller (11.5% and <5%, respectively), and the majority of women had a vaginal microbiota composed of a diverse array of anaerobic bacteria (48.1% and >75%, respectively) [2,3]. CST-III and CST-IV encompass indole-producing bacterial communities that may provide sufficient tryptophan precursors to help *C. trachomatis* escape clearance by IFN-γ-mediated tryptophan depletion [17].

The cross-sectional analysis of week 6 data showed that the vaginal microbiota of women treated with azithromycin or doxycycline was not radically different. In terms of phylotypes, several Gram-negative anaerobic bacteria were more common in the azithromycin group, possibly because of the different antibiotic spectrum of these antibiotics [18,19]. However, this difference was not reflected at the CST or diversity level, probably because these bacteria often have low read counts. The longitudinal analysis confirmed these results.

Our results showed that the use of azithromycin and doxycycline does not result in a significant difference in the composition of the vaginal microbiota. This can be interpreted because there is no effect of these two antibiotics on the vaginal microbiota, in accordance with the findings of Ahrens et al. [6], who showed that azithromycin and tetracycline had no effect on the vaginal microbiota of women followed over one menstrual cycle. However, we cannot exclude that an effect might have occurred without being observable 6 weeks after treatment. Indeed, in the context of bacterial vaginosis treatment with metronidazole, vaginal lactobacilli increase and anaerobes decrease after treatment, but post-treatment relapse often quickly occur [6,20,21]. Our findings suggest that after antibiotic treatment, women are still at risk of Chlamydia infection. This could explain the high observed rates (20%–30%) of *C. trachomatis* reinfection within 3–6 months of treatment [22].

Strengths of this multi-centric study include the random assignment of women to the treatment groups and the large number of samples for analysis. This work also has some limitations. First, we did not have access to information on menses and to the types of contraceptives used by participants recruited in STI centres, which are two factors known to influence the vaginal microbiota [23–25]. Second, the 6-week follow-up period, corresponding to the recommended time for the test of cure, may have missed the observation of short-term changes in the vaginal microbiota. Many factors can be involved in changes in the microbiota, and the optimal duration of vaginal microbiota monitoring after infection treatment has not been established.

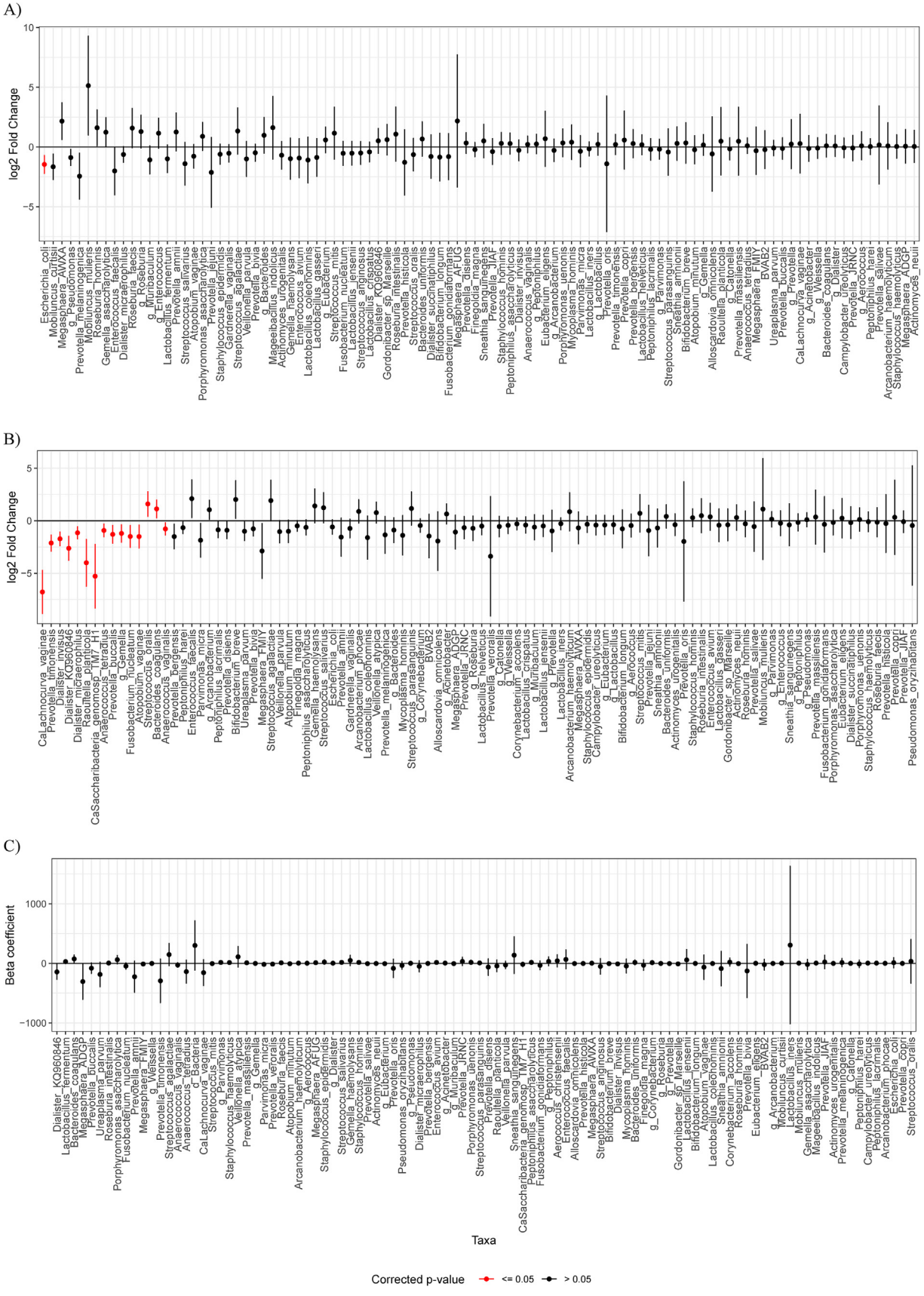

Fig. 1. Differentially abundant phylotypes in the azithromycin and the doxycycline groups. Data are presented (A) at baseline (cross-sectional analysis), (B) 6 weeks after treatment (cross-sectional analysis), and (C) in terms of relative change between baseline and 6 weeks (longitudinal analysis). Positive values of the $\log_2$ fold change indicate phylotypes over-represented in the doxycycline group, whereas negative values indicate phylotypes over-represented in the azithromycin group. Positive values of the β-coefficient indicate phylotypes which abundancy increases in the doxycycline group, relatively to the azithromycin group, whereas negative values indicate phylotypes which abundancy decreases in the doxycycline group, relatively to the azithromycin group. P values were corrected for multiple testing using Benjamini–Hochberg correction.

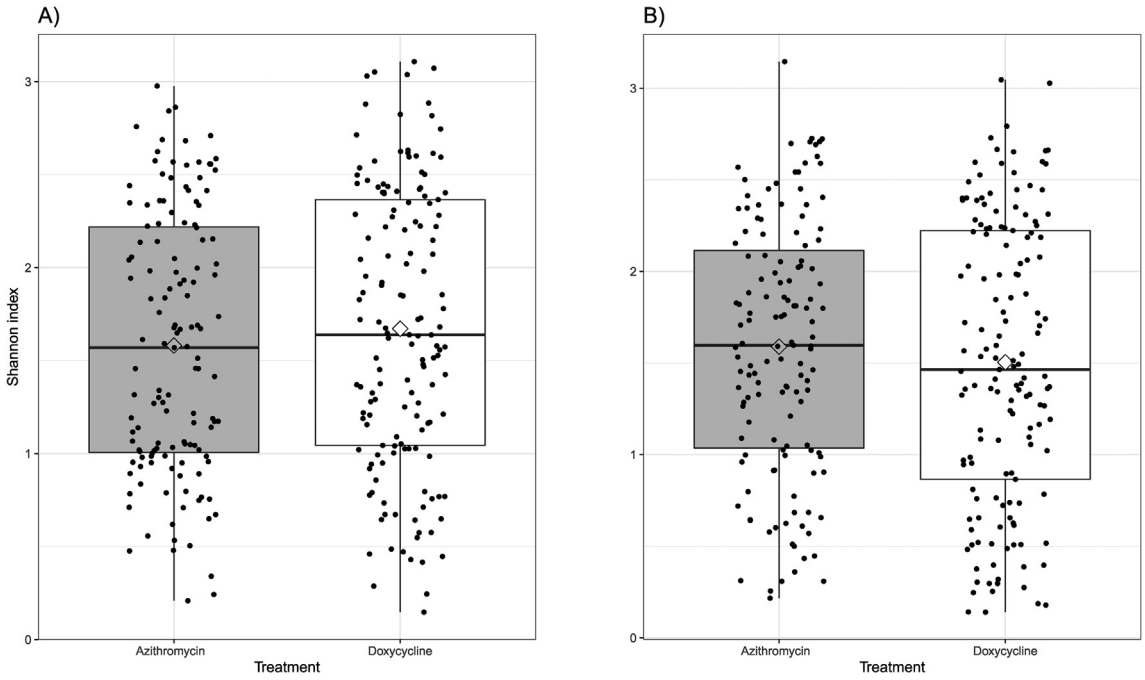

Fig. 2. Distribution of the Shannon diversity index (A) at baseline and (B) 6 weeks after treatment, in the azithromycin and in the doxycycline groups.

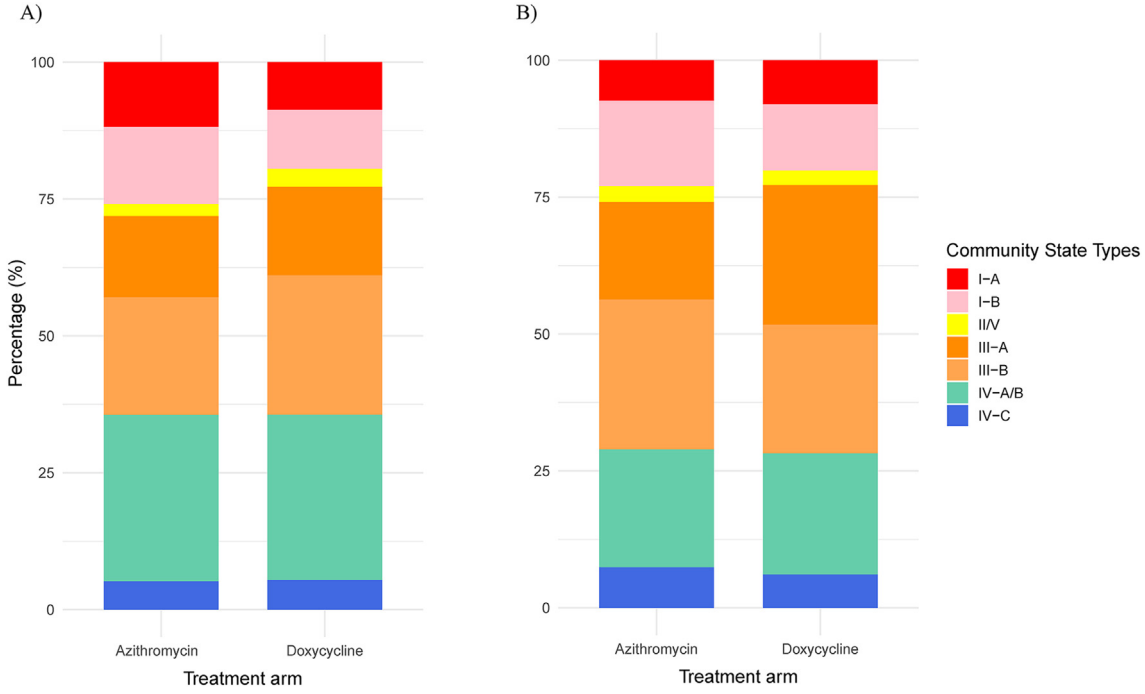

Fig. 3. Community state types proportions (A) at baseline and (B) 6 weeks after treatment, in the azithromycin and in the doxycycline groups.

Finally, the vaginal microbiota before chlamydial infection was unknown, and around 20% of women reported at baseline previous STI, suggesting they already had a vaginal microbiota at risk of such infections.

In conclusion, the vaginal microbiota composition of *C. trachomatis*-positive women did not substantially change before and 6 weeks after treatment by azithromycin or doxycycline. *C. trachomatis*-positive women treated with these drugs kept a vaginal microbiota associated with a higher risk of *C. trachomatis* reinfection.

Author contributions

JT and OP were major contributors to the writing of the paper. BT and EG amplified and sequenced the DNA from the vaginal samples. BP carried out the bioinformatics analyses and JT the statistical analyses. OP and BdB were the main contributors to the conception and implementation of the protocol and the definition of research hypotheses and objectives. All authors have read and approved the final manuscript.

Supporting information

Trial registration numbers: EudraCT number: 2017-002595-15.

ClinicalTrials.gov. Identifier: NCT03532464. Date of registration: 31 May 2018.

World Health Organisation International Clinical Trials Registry: NTC03532464. Secondary ID: CHUBX 2016/26. Date of registration: 9 May 2018.

The full trial protocol has been published under the reference: Peuchant O, Lhomme E, Krêt M, Ghezzoul B, Roussillon C, Bébéar C et al. Randomized, open-label, multi-centre study of azithromycin compared with doxycycline for treating anorectal *Chlamydia trachomatis* infection concomitant to a vaginal infection (CHLAZIDOXY study). Medicine (Baltimore). 2019; 98:e14572.

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Data availability

Methodologists involved in this trial and the coordinating investigator have full access to the data and are the guarantor of the data.

Transparency declaration

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.04.020.

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