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Treatment Effectiveness of Azithromycin and Doxycycline in Uncomplicated Rectal and Vaginal *Chlamydia trachomatis* Infections in Women: A Multicenter Observational Study (FemCure)

Nicole H. T. M. Dukers-Muijrers,[1,2] Petra F. G. Wolffs,[2] Henry de Vries,[3,4,5] Hannelore M. Götz,[5,6,7] Titia Heijman,[3] Sylvia Bruisten,[3,4] Lisanne Eppings,[1] Arjan Hogewoning,[3,4] Mieke Steenbakkers,[1] Mayk Lucchesi,[2] Maarten F. Schim van der Loeff,[3,4] and Christian J. P. A. Hoebe[1,2]

[1]Department of Sexual Health, Infectious Diseases, and Environmental Health, Heerlen, South Limburg Public Health Service, [2]Department of Medical Microbiology, Care and Public Health Research Institute, Maastricht University Medical Center, [3]Department of Infectious Diseases, Public Health Service of Amsterdam, [4]Amsterdam Infection and Immunity Institute, Amsterdam University Medical Center, [5]National Institute of Public Health and the Environment, Epidemiology and Surveillance Unit, Centre for Infectious Disease Control, Bilthoven, [6]Department Infectious Disease Control, Municipal Public Health Service Rotterdam-Rijnmond, and [7]Department of Public Health, Erasmus Medical Center–University Medical Center Rotterdam, The Netherlands

Background. Rectal infections with *Chlamydia trachomatis* (CT) are prevalent in women visiting a sexually transmitted infection outpatient clinic, but it remains unclear what the most effective treatment is. We assessed the effectiveness of doxycycline and azithromycin for the treatment of rectal and vaginal chlamydia in women.

Methods. This study is part of a prospective multicenter cohort study (FemCure). Treatment consisted of doxycycline (100 mg twice daily for 7 days) in rectal CT–positive women, and of azithromycin (1 g single dose) in vaginally positive women who were rectally untested or rectally negative. Participants self-collected rectal and vaginal samples at enrollment (treatment time-point) and during 4 weeks of follow-up. The endpoint was microbiological cure by a negative nucleic acid amplification test at 4 weeks. Differences between cure proportions and 95% confidence intervals (CIs) were calculated.

Results. We analyzed 416 patients, of whom 319 had both rectal and vaginal chlamydia at enrollment, 22 had rectal chlamydia only, and 75 had vaginal chlamydia only. In 341 rectal infections, microbiological cure in azithromycin-treated women was 78.5% (95% CI, 72.6%–83.7%; n = 164/209) and 95.5% (95% CI, 91.0%–98.2%; n = 126/132) in doxycycline-treated women (difference, 17.0% [95% CI, 9.6%–24.7%]; $P < .001$). In 394 vaginal infections, cure was 93.5% (95% CI, 90.1%–96.1%; n = 246/263) in azithromycin-treated women and 95.4% (95% CI, 90.9%–98.2%; n = 125/131) in doxycycline-treated women (difference, 1.9% [95% CI, –3.6% to 6.7%]; $P = .504$).

Conclusions. The effectiveness of doxycycline is high and exceeds that of azithromycin for the treatment of rectal CT infections in women.

Clinical Trials Registration. NCT02694497.

Keywords. women; *Chlamydia trachomatis*; rectal; treatment effectiveness.

There is an ongoing debate regarding the widespread use of a single dose of azithromycin for uncomplicated *Chlamydia trachomatis* (CT) infections [1], and especially for CT infections at the rectal site [2]. Rectal infections are commonly found in women [3–6]. In a meta-analysis, the summary prevalence of rectal CT in women attending sexual health services was 6% (95% confidence interval [CI], 3%–9%), and among those who tested positive for vaginal chlamydia, it was 68% (95% CI, 57%–80%) [7]. In women, rectal CT may indirectly pose a risk for adverse reproductive outcomes if rectal CT, by autoinoculation, infects the genital area [8, 9].

A 1-g single dose of azithromycin was found to be slightly less effective than 7 days of doxycycline (100 mg twice per day) for urogenital chlamydia (94% vs 97%) in randomized controlled clinical trials (RCTs) [10, 11]. Azithromycin was found substantially less effective than doxycycline for rectal chlamydia (83% vs 99%) in a meta-analysis of observational studies [12]. Nevertheless, in these rectal CT treatment studies, women are largely underrepresented and no rectal RCT data are available [12–15].

We assessed rectal and vaginal CT infections by nucleic acid amplification test (NAAT) in a large prospective multicenter

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Correspondence: N. H. T. M. Dukers-Muijrers, Department of Sexual Health, Infectious Diseases and Environmental Health, PO Box 33, 6400 AA Heerlen, The Netherlands (nicole.dukers@ggdzl.nl).

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cohort (FemCure) of female patients treated with azithromycin or doxycycline. Treatment effectiveness by microbiological cure at week 4 was evaluated for both anatomical sites, adjusting for confounders. In addition, follow-up measurements, proxies for bacterial load, and culture were included to explore clinically relevant variations in the cure estimates. Findings contribute to CT management in women in the context of current clinical care.

METHODS

Study Design

This was a prospective cohort study (Figure 1) as part of the FemCure study, 2016–2017 [16].

Regular Sexually Transmitted Infection Clinic Care

Patients were recruited from regular sexually transmitted infection (STI) clinic care at 3 public health STI clinics in the Netherlands [17]. At the regular care STI clinic testing consultation (T_{-1}) women were vaginally tested by NAAT, and women reporting unprotected anal sex (in the past 6 months) or current anal symptoms were also rectally tested by NAAT. At enrollment (T_0), treatment was provided, based on the test result at T_{-1} [17]. Women who were rectally CT positive were treated with a 7-day course of oral doxycycline 100 mg twice daily. Vaginal CT–positive women who were rectally untested or rectally negative received a 1-g single oral dose of azithromycin; some patients with vaginal CT received doxycycline because of a contraindication to azithromycin. Azithromycin and the first doxycycline dose were directly observed.

Enrollment at Regular Care Treatment

Women were enrolled at T_0, the treatment visit. Eligible were nonpregnant adult women (aged ≥18 years) who had a vaginal or rectal CT infection; were not infected with human immunodeficiency, syphilis, or *Neisseria gonorrhoeae*; and who had not used antibiotics since T_{-1}. Participation started after written informed consent.

Study Sample Collection and Laboratory Analyses

Women collected rectal and vaginal swabs at enrollment (T_0) immediately prior to treatment, and after 4 weeks (T_1) at the STI clinic; women also collected samples at week 1 and week 2 at home [16]. Individual test results were not available to clinic staff and participants. Samples were tested using commercial NAAT platforms according to manufacturers' instructions (COBAS 4800; Roche Diagnostics, Basel, Switzerland). The NAAT quantitation cycle (Cq) values were taken as a proxy for bacterial load [18]. Positive week 0 and 4 samples were cultured [19]. Week 4 NAAT-positive samples with a low Cq value (<31) were genotyped by multilocus sequence typing; the accompanying week 0 sample was also genotyped [20].

Demographic, Clinical, and Sexual Behavior Data

Data were collected [16] using structured online patient-administered questionnaires (weeks 0, 2, and 4) regarding antibiotic use, symptoms, and sexual practices in the 2 weeks preceding enrollment. At week 1, vomiting and doxycycline pill intake were assessed using an online questionnaire; participants were encouraged to notify the study nurse in case they had vomited or forgotten to take the doxycycline treatment. Clinical data were extracted from the patient registries.

Primary Outcome

The primary outcome was microbiological cure (ie, a negative NAAT at week 4) [12, 13].

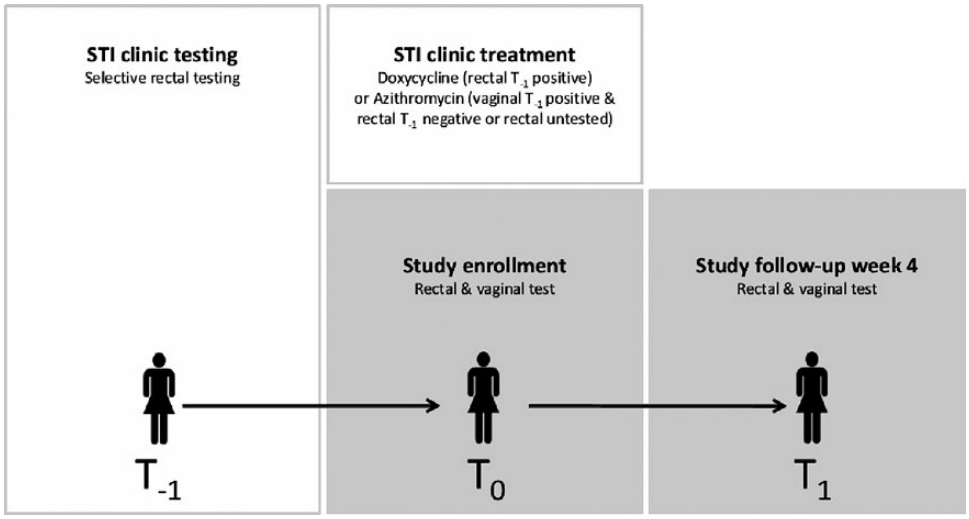

Figure 1. Study design. Abbreviations: STI, sexually transmitted infection; T_{-1}, STI clinic testing consultation, T_0, enrollment; T_1, follow-up (week 4).

Statistical Analyses

Aim

To assess and compare the proportion of microbiological cure after doxycycline and azithromycin treatment, separately for rectal and vaginal chlamydia.

Infections Studied (Main Population)

The rectal and vaginal infections studied were based on the T_0 sample test results. Numbers of patients by their T_{-1} and T_0 results and treatment are presented in Supplementary Table 1.

Proportion of Microbiological Cure and Difference Between Treatments

The proportions of women with rectal chlamydia reaching microbiological cure were assessed for azithromycin and doxycycline. Proportions were compared between the 2 treatment groups, using 2-sided exact tests. Similar analyses were done for women with vaginal chlamydia. We present the difference in proportions with exact 95% CIs.

Potential Confounders for Treatment Effect

Treatment allocation was based on regular clinic care test results (at T_{-1}), possibly leading to differences in enrollment characteristics between the treatment groups. To test for such differences, we compared treatment groups using the Mann-Whitney *U* test for continuous variables (age and Cq value) and a χ^2 test for other variables. Also, using logistic regression analyses, the association between enrollment characteristics and the outcome ("not reaching microbiological cure") was tested. Characteristics that were associated ($P < .10$) with treatment or with the outcome were considered possible confounders for the treatment effect.

Treatment Effect

Univariate logistic regression analyses were used to assess the association between treatment type and the inverse of the outcome (ie, "not reaching microbiological cure") defined by a week 4 positive NAAT. In multivariate models, we adjusted for the identified potential confounders. Subgroup heterogeneity of associations was tested by including interaction terms (between age, education, migration background, and treatment type), but all interaction terms were not statistically significant.

Analyses in a Restricted Subset of the Main Population

To minimize potential bias. In some women, a positive NAAT at week 4 may indicate a reinfection after having unprotected sex or due to suboptimal antibiotic treatment intake. Conversely, additional antibiotic treatment during the study could have contributed to a negative NAAT at week 4. To minimize such potential bias, the main population was restricted to a subset that included only those women who received the treatment according to the regular care protocol [16, 17]—that is, patients had to report at least a compliance of 10 doxycycline pills without direct (<3 hours) vomiting [11] or no vomiting after azithromycin intake. Furthermore, the subset did not include women who reported unprotected vaginal or anal sex, had missing questionnaire data, or received additional antibiotics after enrollment. In the subset, we evaluated microbiological cure as described above.

To assess and compare secondary outcomes in sensitivity analyses. We assessed secondary outcomes to explore clinically relevant variations in the estimates. A positive NAAT at week 4 possibly only indicates the presence of remnant CT DNA originating from dead chlamydia bacteria [21], and we hypothesized that a positive week 4 NAAT may in that case reflect an actual "true cure." To approach this hypothetical situation, we redefined microbiological cure in 3 separate secondary outcomes:

1. Week 4 NAAT negative or week 4 NAAT positive with week 1 or week 2 NAAT negative [22, 23].
2. Week 4 NAAT negative or week 4 NAAT positive with a high Cq value (>36) as a proxy for low bacterial load [18].
3. Week 4 NAAT negative or week 4 NAAT positive with a negative culture, appreciating that this does not prove absence of viable CT, due to the low sensitivity of culture [19, 21].

All 3 secondary outcomes were taken as imperfect proxies for "true cure" and were used to compare proportions of microbiological cure.

Analyses were performed using SPSS package version 21 (IBM Corporation, Armonk, New York) and Stata version 13.1 software (StataCorp, College Station, Texas).

Ethical Considerations

All participants provided written informed consent. This study was approved by the Medical Ethical Review Committee from the Maastricht University Medical Centre, the Netherlands (NL51358.068.15/METC153020, 20-01-2016). This study was monitored by the Clinical Trial Centre Maastricht (Maastricht University).

RESULTS

Main Population

In total, 1763 women were invited to participate and 560 (31.8%) were enrolled. Of the participants, 36 were excluded from further analyses as they were NAAT negative at enrollment (some due to spontaneous clearance or perhaps initial remnant CT DNA detection). Of the 524 remaining women, 102 patients did not provide follow-up samples, and 6 patients had a missing sample at week 1, 2, or 4. After excluding these 108 women (20.6%), 416 women remained for analyses. Women included and excluded did not differ regarding care diagnosis, treatment type, reported anal or vaginal sex, and symptoms. However, in excluded women the proportion of

women with a medium or low education (87.0%) and with non-Western migration background (12%) was higher than in included women (69.7% [$P < .001$] for education and 6.0% [$P = .042$] for migration background). Excluded women were younger (median age, 21 years [interquartile range {IQR}, 20–23 years]) than included women (median, 23 years [IQR, 20–24 years]) ($P < .001$).

Of the 416 patients included in analyses, the median number of days between T_0 and T_{-1} was 8 (IQR, 7–12 days). Of the 416 patients, all sampled within 6 days of the planned 4-week time point (at 28 days posttreatment), and 407 (98%) patients sampled within 3 days. Of the 416 patients, 319 (76.7%) had a rectal and vaginal CT, 22 (5.3%) had a single rectal CT, and 75 (18.0%) had a single vaginal CT. In total, the women contributed 341 rectal infections and 394 vaginal infections (Figure 2). Table 1 and Supplementary Table 2 present characteristics at enrollment.

Microbiological Cure in the Main Population

Microbiological cure was 78.5% (95% CI, 72.6%–83.7%; n = 164/209) in azithromycin-treated rectal infections and 95.5% (95% CI, 91.0%–98.2%; n = 126/132) in doxycycline-treated rectal infections. The difference was 17.0% (95% CI, 9.6%–24.7%; $P < .001$) (Table 2).

Microbiological cure was 93.5% (95% CI, 90.1%–96.1%; n = 246/263) in azithromycin-treated vaginal infections and 95.4% (95% CI, 90.9%–98.2%; n = 125/131) of the doxycycline-treated vaginal infections (Table 2). The difference was 1.9% (95% CI, −3.6% to 6.7%; $P = .504$).

Supplementary Figure 1 shows the percentages of microbiological cure at the patient level.

Treatment Effect Adjusted for Confounders in the Main Population

In rectal CT, in the univariate logistic regression analyses, the odds for not reaching microbiological cure were 5.8 (95% CI, 2.4–13.9; $P < .001$) times higher for those treated with azithromycin than for those treated with doxycycline (Table 2). Adjusting for potential confounders (see next section) in the multivariate analyses, the odds ratio (OR) increased (OR, 9.4 [95% CI, 3.2–27.2]; $P < .001$). In vaginal CT, treatment type showed no association in univariate analyses (OR, 1.4 [95% CI, .6–3.7]; $P = .455$) or in multivariate analyses (OR, 1.4 [95% CI, .5–4.0]; $P = .528$) (Table 2).

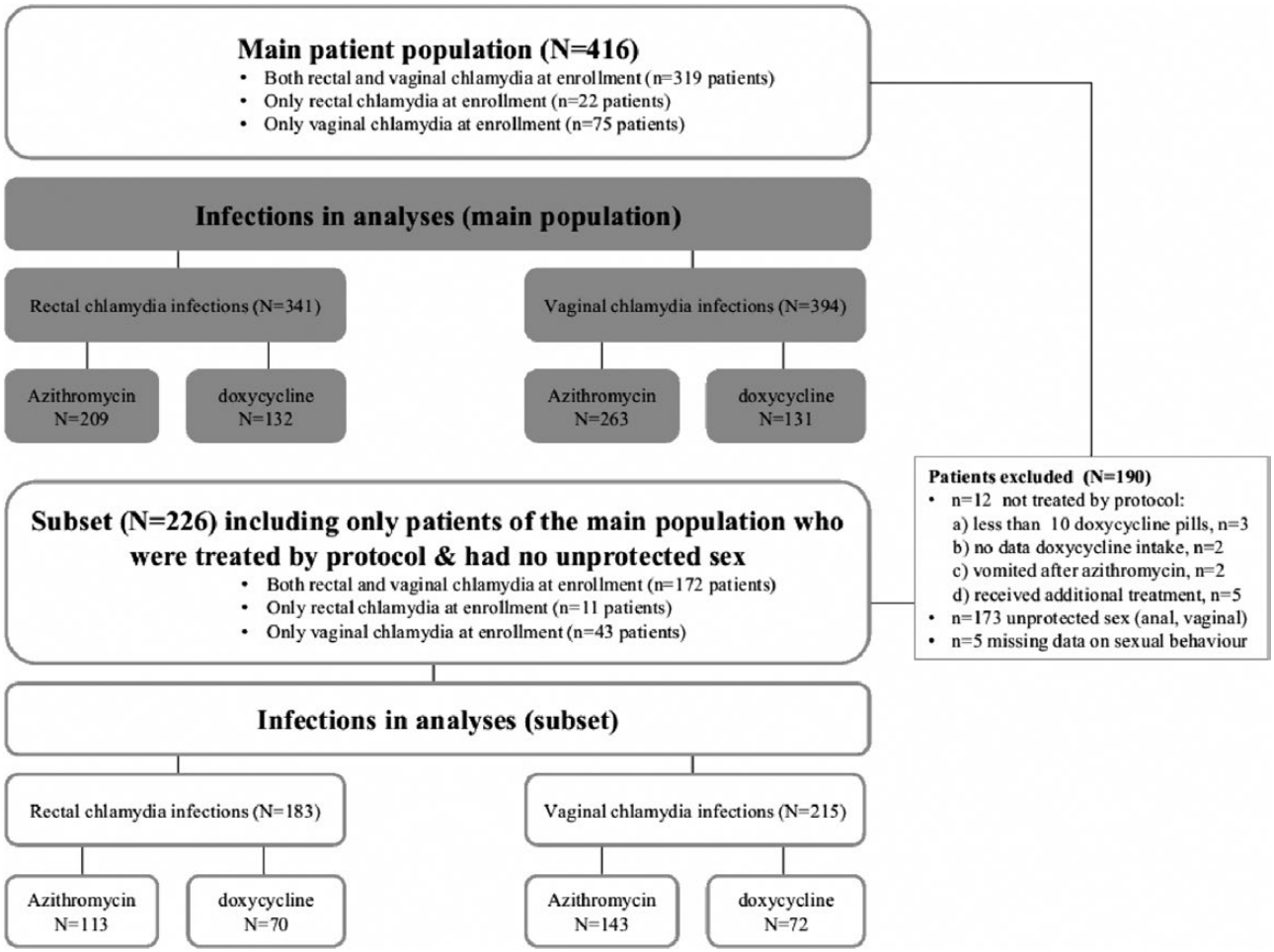

Figure 2. Flowchart of chlamydia infections in the analyses.

Table 1. Characteristics of the Main Population at Enrollment by *Chlamydia trachomatis* Infection Site and by Treatment Regimen

Characteristic at Enrollment	Rectal Infection at Enrollment			Vaginal Infection at Enrollment		
	Azithromycin (n = 209)	Doxycycline (n = 132)	*P* Value[a]	Azithromycin (n = 263)	Doxycycline (n = 131)	*P* Value[a]
Study site			< .001			< .001
Clinic 1: South Limburg	91 (43.5)	41 (31.1)		111 (42.2)	34 (26.0)	
Clinic 2: Rotterdam	65 (31.1)	23 (17.4)		84 (31.9)	24 (18.3)	
Clinic 3: Amsterdam	53 (25.4)	68 (51.5)		68 (25.9)	73 (55.7)	
Age, y, median (IQR)	22 (20–24)	23 (21–25)	< .001	22 (20–24)	23 (21–25)	.005
Migration background			.244			.365
Western	193 (92.3)	126 (95.5)		245 (93.2)	125 (95.4)	
Non-Western	16 (7.7)	6 (4.5)		18 (6.8)	6 (4.6)	
Educational level			.001			.003
Low or medium	158 (75.6)	78 (59.1)		197 (74.9)	79 (60.3)	
High	51 (24.4)	54 (40.9)		66 (25.1)	52 (39.7)	
Number of sex partners preceding 3 mo			.099			.341
0–1	78 (37.3)	44 (33.8)		95 (36.1)	45 (35.2)	
2–3	102 (48.8)	56 (43.1)		128 (48.7)	56 (43.8)	
>3	29 (13.9)	30 (23.1)		40 (15.2)	27 (21.1)	
Previous episode of chlamydia reported			.822			.888
No	159 (76.1)	99 (75.0)		193 (73.4)	97 (74.0)	
Yes	50 (23.9)	33 (25.0)		70 (26.6)	34 (26.0)	
Vaginal sex 2 wk preceding enrollment			.978			.922
No	100 (47.8)	62 (47.7)		126 (47.9)	62 (48.4)	
Yes	109 (52.2)	68 (52.3)		137 (52.1)	66 (51.6)	
Anal sex 2 wk preceding enrollment			.003			.015
No	207 (99.0)	121 (93.1)		260 (98.9)	121 (94.5)	
Yes	2 (1.0)	9 (6.9)		3 (1.1)	7 (5.5)	
Vaginal chlamydia at enrollment			< .001			…
No	1 (0.5)	21 (15.9)		NA	…	
Yes	208 (99.5)	111 (84.1)		…	…	
Rectal chlamydia at enrollment						.172
No	NA	…	…	55 (20.9)	20 (15.3)	
Yes	…	…		208 (79.1)	111 (84.7)	
Genital symptoms at enrollment[b]						.976
No	NA	…	…	110 (41.8)	55 (42.0)	
Yes	…	…		153 (58.2)	76 (58.0)	
Anal symptoms at enrollment[b]			< .001			
No	193 (92.3)	90 (68.2)		NA	…	…
Yes	16 (7.7)	42 (31.8)		…	…	
Vaginal CT NAAT Cq value at enrollment, median (IQR)	NA	…	…	30 (28–33)	30 (28–33)	.211
Rectal CT NAAT Cq value at enrollment, median (IQR)	35 (32–38)	34 (30–37)	.034	NA	…	…
Vaginal CT culture positive at enrollment	…	…	…	…	…	.679
No	NA	…	…	204 (77.6)	104 (79.4)	
Yes	…	…	…	59 (22.4)	27 (20.6)	
Rectal CT culture positive at enrollment			.182			…
No	152 (72.7)	87 (65.9)		NA	…	
Yes	57 (27.3)	45 (34.1)		…	…	
Included in subset[c]			.852			.912
No	96 (45.9)	62 (47.0)		120 (45.7)	59 (45.0)	
Yes	113 (54.1)	70 (53.0)		143 (54.4)	72 (55.0)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: Cq, quantitation cycle; CT, *Chlamydia trachomatis*; IQR, interquartile range; NA, not assessed; NAAT, nucleic acid amplification test.

[a] *P* values for differences between the 2 treatment groups were determined using the Mann-Whitney *U* test for continuous variables (age and Cq value) and χ^2 test for all other characteristics.

[b] Symptoms for vaginal CT: dysuria, irregular menstruation, lower abdominal pain, pain during intercourse, vaginal discharge; symptoms for rectal CT: anal discharge, anal blood loss during or after intercourse, pain during or after intercourse.

[c] Took azithromycin without direct vomiting (<3 hours) or took at least 10 doxycycline pills without direct (<3 hours) vomiting and did not receive additional treatment. They also had no unprotected anal sex, no unprotected vaginal sex, and no missing data on sexual practices during the 4 weeks after treatment.

Table 2. Proportions and Differences of Microbiological Cure for Azithromycin- or Doxycycline-treated Rectal and Vaginal *Chlamydia trachomatis* Infections, and the Treatment Effect (ie, the Odds of Azithromycin Compared to Doxycycline in Not Reaching Microbiological Cure) in the Main Population and the Restricted Subset

	Proportion Cured							
	Azithromycin		Doxycycline		Difference in % Cured		Treatment Effect	
Patients	no./No.	% (95% CI)	no./No.	% (95% CI)	% (95% CI)	*P* Value[a]	OR (95% CI)	aOR (95% CI)
Rectal chlamydia								
All patients	164/209	78.5 (72.6–83.7)	126/132	95.5 (91.0–98.2)	17.0 (9.6–24.7)	< .001	5.76 (2.38–13.93)	9.38 (3.24–27.17)
Subset[b]	89/113	78.8 (70.6–85.6)	68/70	97.1 (91.4–99.5)	18.4 (8.7–27.5)	< .001	9.17 (2.09–40.14)	18.51 (3.20–106.96)
Vaginal chlamydia								
All patients	246/263	93.5 (90.1–96.1)	125/131	95.4 (90.9–98.2)	1.9 (–3.6 to 6.7)	.504	1.44 (.55–3.74)	1.40 (.49–3.96)
Subset[b]	134/143	93.7 (88.9–96.9)	69/72	95.8 (89.5–98.3)	2.1 (–6.1 to 9.1)	.755	1.55 (.41–5.89)	0.89 (.19–4.11)

For rectal chlamydia, the OR was adjusted for study site, age, education, number of sexual partners, anal sex, vaginal *Chlamydia trachomatis* at enrollment, anal symptoms, prior chlamydia reported, quantitation cycle (Cq) value of the rectal nucleic acid amplification test (NAAT), and culture result of the rectal sample. For vaginal chlamydia, the OR was adjusted for study site, age, education, anal sex, number of sexual partners, Cq value of the vaginal NAAT test, and culture result of the vaginal sample.

Abbreviations: aOR, odds ratio adjusted for characteristics measured at enrollment; CI, confidence interval; no., number of patients with microbiological cure; No., total number of patients; OR, unadjusted odds ratio.

[a]Exact *P* values.

[b]The subset included only participants who were treated according to the protocol and who reported no sex or safe sex only. This means that the subset (compared to all participants in analyses) did not include those who directly vomited after azithromycin, who did not take at least 10 doxycycline pills without direct vomiting, who received additional treatment, who reported unprotected anal sex, who reported unprotected vaginal sex, and those who had missing data on sexual behavior.

Confounders Adjusted for in Multivariate Analyses

Treatment groups differed regarding study site, age, education, number of sex partners, anal sex, single rectal infection, anal symptoms, and Cq NAAT value for rectal CT and regarding study site, age, education, and anal sex for vaginal CT (Table 1). Characteristics associated with "not reaching microbiological cure" were for rectal CT: no history of chlamydia, a lower Cq value, and a positive culture; for vaginal CT, these were a low or medium educational level, more sex partners, a lower Cq value, and a positive culture (Table 3).

Analyses in a Restricted Subset of the Main Population

Fourteen patients with CT at week 4 could be evaluated by sequence typing (13 rectal infections and 2 vaginal infections). All evaluated patients had the same genotype at week 4 compared with their week 0 sample (Supplementary Table 3). Excluding 190 patients from the main study population who were not treated according to the protocol or who reported unprotected sex, 226 patients remained in the subset analyses who contributed 183 rectal CT infections and 215 vaginal CT infections (Figure 2). Proportions of microbiological cure and differences between treatments were similar as described before for the main study population.

The week 1 and 2 NAAT results, the week 4 NAAT Cq values, and the week 4 culture results (Supplementary Table 3) were used to construct 3 secondary outcomes. Evaluating secondary outcomes (Supplementary Table 4), cure proportions for azithromycin-treated cases were 82%–91% for rectal CT and 94%–99% for vaginal CT. Cure proportions for doxycycline-treated cases were 97%–100% for rectal CT and 96%–100% for vaginal CT. The difference between treatments was 9%–15% for rectal CT and 1%–2% for vaginal CT.

DISCUSSION

This prospective observational multicenter study in outpatient STI clinic women assessed the effectiveness of doxycycline and azithromycin for the treatment of rectal and vaginal chlamydia. We observed high proportions of microbiological cure for doxycycline in rectal and vaginal CT and for azithromycin in vaginal CT. The proportion of rectal CT infections reaching microbiological cure was substantially and significantly lower for women treated with azithromycin.

Strengths of this study include the study population (women) for whom scarce rectal treatment data are available, the rigorous and detailed data collection conducted by assessing both rectal and vaginal CT, the comparison of 2 treatments within one study, and adjustment for putative confounders. With analyses in a subset, we aimed to further reduce bias due to suboptimal treatment compliance and possible CT reexposure. A unique feature of this study was the genotyping, the multiple time sequential sampling, Cq value assessment, and culture of week 4 samples. This allowed us to evaluate secondary outcomes that perhaps approximate more clinically relevant definitions of "true cure" as NAAT tests are highly sensitive and may detect remnant CT DNA from dead chlamydia. Doing so, the cure proportions remained substantially lower for azithromycin compared with doxycycline in rectal CT. Several posttreatment samples, especially in the azithromycin group, had consistent preceding positive samples, higher loads, or culture-positive results, which suggested or proved presence of viable posttreatment CT. Moreover, viable infections may have been missed by culture due to its low sensitivity. Enhanced viability testing may provide more clues on these issues, as planned in our future research [16, 19, 21].

Table 3. Associations Between Enrollment Characteristics and Not Reaching Microbiological Cure at 4 Weeks After Treatment for Rectal or Vaginal *Chlamydia trachomatis* Adjusted for Treatment Type in Logistic Regression Analyses

	Rectal Chlamydia		Vaginal Chlamydia	
Enrollment Characteristic	Odds Ratio[a]	*P* Value[a]	Odds Ratio[a]	*P* Value[a]
Study site (compared to clinic 1: South Limburg)		.227		.207
Clinic 2: Rotterdam	1.80 (.85–3.81)		1.23 (.48–3.13)	
Clinic 3: Amsterdam	1.80 (.82–3.80)		0.41 (.12–1.37)	
Age, per year	1.02 (.95–1.09)	.645	0.88 (.75–1.03)	.104
Migration background non-Western (compared to Western)	1.11 (.35–3.51)	.865	NA	…
Educational level high (compared to low or medium)	1.21 (.61–2.40)	.578	0.35 (.10–1.20)	.094
No. of sex partners past 3 mo (compared to 0 or 1)		.505		.082
2–3	1.25 (.62–2.50)		1.14 (.40–3.29)	
>3	1.17 (.70–4.18)		3.03 (1.00–9.17)	
Previous chlamydia reported: no (compared to yes)	2.78 (1.12–6.88)	.027	1.76 (.58–5.30)	.316
Vaginal symptoms[b]: yes (compared to no)	NA	…	1.70 (.68–4.22)	.256
Anal symptoms[b]: yes (compared to no)	0.29 (.07–1.28)	.102	NA	…
Vaginal sex preceding 2 wk: yes (compared to no)	NA	…	0.85 (.37–1.98)	.706
Anal sex preceding 2 wk: yes (compared to no)	3.23 (.57–18.28)	.186	NA	…
Vaginal chlamydia: yes (compared to no)	1.21 (.14–10.48)	.861	NA	…
Rectal chlamydia: yes (compared to no)	NA	…	1.65 (.48–5.73)	.427
Vaginal chlamydia: Cq value, per unit decrease	NA	…	1.14 (1.00–1.31)	.054
Rectal chlamydia: Cq value, per unit decrease	1.25 (1.14–1.37)	< .001	NA	…
Vaginal chlamydia culture positive (compared to negative)	NA	…	2.44 (1.02–5.85)	.046
Rectal chlamydia culture positive (compared to negative)	4.02 (2.11–7.66)	< .001	NA	…

Numbers in brackets represent the 95% confidence interval. For migration background, the odds ratio (OR) was not assessed due to low numbers or patients with non-Western migration background and zero patients with the outcome in the doxycycline group; for the other characteristics, the OR was not assessed as characteristics related to the alternate anatomic site infection.

Abbreviations: Cq, quantitation cycle value of nucleic acid amplification test; NA, not applicable (odds ratio was not assessed).

[a]OR and *P* value were adjusted for treatment type, using the Wald test. Adjustment for treatment was applied as characteristics differed between treatment groups and unadjusted analyses may merely reflect clinic practice (ie, provision of doxycycline in rectally tested women) rather than the independent association between characteristics and outcome.

[b]Symptoms for vaginal chlamydia: dysuria, irregular menstruation, lower abdominal pain, pain during intercourse, vaginal discharge; symptoms for rectal chlamydia: anal discharge, anal blood loss during or after intercourse, pain during or after intercourse.

We also recognize limitations. First, treatment was neither randomized nor blinded and confounding cannot be ruled out [12, 13]. Participants originated from STI clinic regular care practice [17], and this practice reflected in the study population (eg, doxycycline users more often reported anal sex and symptoms). We aimed to minimize confounding by performing analyses that adjusted for various potentially important determinants. Doing so, the treatment effect increased even more (rectal CT) or remained similar (vaginal CT).

Second, under- or overreporting of behaviors may have affected the findings. If doxycycline pill intake by participants was overreported, the actual proportion "cured" in doxycycline-treated cases may even be higher. Differential underreporting of anal sex in rectally positive azithromycin-treated women is possible but has not led to a major bias as (1) women who practice anal sex usually also practice vaginal sex [24], and we excluded women who had unprotected vaginal sex in the subset; (2) when restricting analyses to the 141 women who reported no anal sex and no vaginal sex, the results remained similar (data not shown); and (3) strain typing, although incomplete, revealed the same types at enrollment and week 4.

Third, as our NAAT did not have an internal human control, we could not rule out the possibility that negative NAAT results were due to inadequate self-sampling. However, a previous study showed that the majority (98.4%) of CT-negative samples contained human DNA and thus were considered as being adequately sampled [22].

Fourth, it is unknown whether our findings are generalizable to other STI clinic or non-STI clinic settings. Young women, women with a low educational level, and those with a non-Western migration background were less represented in the study population. This did, however, not affect the internal validity of the study as there was no subgroup heterogeneity of associations.

In both treatment groups and both anatomic sites there were cases with CT DNA detection after treatment. For azithromycin and doxycycline, it is yet unknown whether antibiotic concentrations are sufficiently high to cure high pretreatment bacterial loads. Heterotypic resistance has been previously associated with a higher bacterial load [25], in agreement with the observed association with the pretreatment Cq value in our study and in other studies [21, 26]. Still, the reasons for a lower cure proportion in azithromycin-treated rectal CT are unknown. In vitro,

CT strains showed higher minimum inhibitory concentration values of macrolides in colorectal compared with endocervical cells; this was not the case for doxycycline [27]. Others have suggested that a longer duration of azithromycin treatment may be the key to better target the specific CT life cycle [28].

The observed proportions of microbiological cure correspond with previous findings [10–12, 14, 15]. For azithromycin-treated rectal chlamydia, proportions are below the World Health Organization threshold of 95% recommended for a first-line treatment [29]. Rectal chlamydia is widely treated with azithromycin in the United States, but also in Europe and Australia where doxycycline is recommended [17]. This is because selective rectal testing policies result in many untested rectal infections that are coincidentally azithromycin-treated for vaginal CT [3, 6, 7]. Rectal testing is highly acceptable [4–6], and universal rectal testing has been found to be cost-effective in the STI clinic context [30]. Pooling of samples may contribute to reduced costs. When selective rectal testing of women remains the practice of choice, doxycycline use for vaginal CT may be considered as this would treat most rectal CT infections. Universal rectal testing as well as universal doxycycline use would provide a comprehensive (ie, vaginal and rectal) CT clinical management [4]. However, doxycycline use has limitations. It is contraindicated in pregnant women, which hampers its use as a simple treatment for all women. Furthermore, photosensitivity may occur with doxycycline use, although the occurrence of main side effects (minor gastrointestinal upset) was found to be similar between azithromycin (24%) and doxycycline (23%) [10]. Even though studies show consistently high doxycycline microbiological cure rates [10, 11, 14, 15] in the context of possible incomplete doxycycline pill intake, in routine clinical settings, compliance is a major issue in antibiotic regimens that require twice-daily administration for multiple days [31, 32]. We may need to seek practical care strategies to improve adherence, such as the use of text message reminders or online applications.

In conclusion, the results of this observational study indicate that the microbiological cure proportion of doxycycline exceeds that of azithromycin for the treatment of rectal CT infections in women.

Supplementary Data

Supplementary materials are available at *Clinical 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

Author contributions. N. D. drafted the report and performed the statistical analyses. N. D., M. S., C. H., H. G., and H. V. designed the statistical analysis. P. W., M. L., and S. B. set up and performed the laboratory analyses. All authors reviewed the results; provided guidance on the method; and drafted, reviewed, and provided critical feedback on the report.

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