Viable Bacterial Load Is Key to Azithromycin Treatment Failure in Rectally Chlamydia trachomatis Infected Women (FemCure)

Viable Bacterial Load Is Key to Azithromycin Treatment Failure in Rectally *Chlamydia trachomatis* Infected Women (FemCure)

To the Editor—With great interest we read the article by Khosropour et al [1], in which the health implications of rectal *Chlamydia trachomatis* in women was studied by daily sampling of rectal and vaginal specimens. Undetected or inappropriately treated rectal *C. trachomatis* could have consequences both for the patient and sexual partners via ongoing transmission. Khosropour et al [1] conclude that rectal *C. trachomatis* infections are common in women, and anticipate that azithromycin treatment might fail in the case of rectal *C. trachomatis*. As acknowledged, their sample size (n = 13) was too small to observe rectal *C. trachomatis* treatment failure.

We recently described treatment effectiveness of azithromycin and doxycycline in women with rectal or vaginal *C. trachomatis* infections using nucleic acid amplification testing (NAAT) at week 4 [2]. As rightfully noted by Khosropour et al [1], NAAT cannot distinguish DNA derived from viable *C. trachomatis* or nonviable *C. trachomatis* remnants. Frequently observed posttreatment blips, that is *C. trachomatis* positivity after initial clearance, are also notable in this respect with unknown meaning for viability. In the current postculture era, where *C. trachomatis* is routinely diagnosed using NAAT, we developed a viability polymerase chain reaction assay (V-PCR) to assess viability of *C. trachomatis* with higher sensitivity than culture [3].

In our FemCure study, we applied viability testing to assess treatment failure in 112 women who were rectal and vaginal NAAT-*C. trachomatis*–positive, and who, in the 4 weeks posttreatment, did not use antibiotics, nor vomited, and denied rectal and vaginal unprotected sex. Just prior to treatment and at 4 weeks after azithromycin treatment (1 g single dose), participants self-collected vaginal and rectal samples, and tested for *C. trachomatis* DNA (NAAT) and viable *C. trachomatis* (V-PCR). Based on the week 4 samples, we evaluated 2 endpoints: (1) failure per *C. trachomatis* DNA and (2) failure per viable *C. trachomatis*.

Regarding failure per *C. trachomatis* DNA at week 4, 19 (17.0%) of the 112 women had *C. trachomatis* DNA at the rectal site only, 3 (2.7%) had *C. trachomatis* DNA at the vaginal site only, and 5 (4.5%) had *C. trachomatis* DNA at both anatomic sites (Table 1). Regarding failure per viable *C. trachomatis*, 17 (15.2%) had viable *C. trachomatis* at the rectal site only, 2 (1.8%) had viable *C. trachomatis* at the vaginal site only, and 1 (0.9%) had viable *C. trachomatis* at both the rectal and vaginal sites.

A few notable observations arise when we also consider the presence (or absence) of viable *C. trachomatis* just prior to treatment.

1. Just prior to treatment, almost all participants had viable *C. trachomatis* at the vaginal site (96%, 108/112) and a substantial number (58%, 65/112) had viable *C. trachomatis* at the rectal site.
2. As we observed previously for *C. trachomatis* DNA [2], viable *C. trachomatis* at week 4 was more frequently observed at the rectal site (16.1%) than at the vaginal site (2.7%).
3. Pretreatment viable *C. trachomatis* at the rectal site (or the absence thereof) affected the later occurrence of failure per viable *C. trachomatis*; failure was 25.4% in women with pretreatment viable *C. trachomatis* and 4.4% in women without pretreatment viable *C. trachomatis* (Table 1). We could not evaluate the impact of pretreatment viable *C. trachomatis* at the vaginal site as only 4 women did not have viable *C. trachomatis* at the vaginal site.

We also explored the impact of "blips" by assessing *C. trachomatis* DNA at week 1 and 2. We considered women with *C. trachomatis* DNA at the rectal site at week 4 (n = 24) as failures with blips when women did not have *C. trachomatis* DNA at week 1 or week 2 (n = 10). Of the failures with blips, 8 (57.1%) had viable *C. trachomatis* at the rectal site at week 4; of the failures (n = 14) without blips, all had viable *C. trachomatis* at week 4. As Khosropour et al [1] concluded, the cause of rectal *C. trachomatis* blips is unclear, but may be a result of persistent infection, reinfection via autoinoculation, or persistence of nonviable organisms. We add that possible clinical relevance of blips should not be ruled out, as in our study over half of the failures with blips showed viable *C. trachomatis*.

Given the frequent cooccurrence of rectal *C. trachomatis* with vaginal *C. trachomatis*, it is possible that providing azithromycin to women with vaginal *C. trachomatis* and unnoticed rectal *C. trachomatis* infections, may result in rectal *C. trachomatis* treatment failure. The current observations suggest that rectal treatment failure may reflect viable *C. trachomatis*, likely depending on the presence of viable *C. trachomatis* prior to treatment.

We hope that our presented results emphasize the importance of rectal *C. trachomatis* infections in women. Appropriate diagnostics and treatment are of importance both for the patient to prevent late sequalae and for society to prevent ongoing transmission.

Notes

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

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Table 1. Proportions of Treatment Failure

Test Result Prior to Treatment	Rectal *Chlamydia* at Week 4, n (%)		Vaginal *Chlamydia* at Week 4, n (%)	
	NAAT Positive	V-PCR Positive	NAAT Positive	V-PCR Positive
Chlamydia DNA at vaginal and rectal site, n = 112	24 (21.4)	18 (16.1)	8 (7.1)	3 (2.7)
Viable *Chlamydia*				
No viable *Chlamydia* at vaginal and rectal site, n = 2	0	0	0	0
No viable *Chlamydia* at vaginal site, viable *Chlamydia* at rectal site, n = 2	0	0	0	0
Viable *Chlamydia* at vaginal site, no viable *Chlamydia* at rectal site, n = 45	5 (11.1)	2 (4.4)	2 (4.4)	1 (2.2)
Viable *Chlamydia* at vaginal and rectal site, n = 63	19 (30.2)*	16 (25.4)*	6 (9.5)	2 (3.2)

Treatment failure was defined as NAAT positive at week 4 or V-PCR positive at week 4 in rectal and vaginal infections, in 112 women who were vaginal and rectal NAAT positive just prior to treatment, FemCure, the Netherlands 2016–2017.

NAAT positive indicates presence of *Chlamydia* DNA; V-PCR positive indicates presence of viable *Chlamydia*.

*$P < .05$ X^2 test.

Abbreviations: NAAT, nucleic acid amplification testing; V-PCR, viability polymerase chain reaction.

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Reply to Dukers-Muijrers et al

To the Editor—Dukers-Muijrers et al provide new data that are complementary to those from our study [1]. Namely, they found that over one quarter of women who had viable *Chlamydia trachomatis* detected in the rectum before receiving azithromycin treatment also had viable *C. trachomatis* detected 4 weeks after treatment, suggesting possible treatment failures. They also note that over half of women with intermittent *C. trachomatis*–positive results (or "blips") of rectal specimen tests showed evidence of viable *C. trachomatis*.

Together, the data from our study and those from Dukers-Muijrers et al add key pieces to the puzzle of the clinical significance of rectal *C. trachomatis* among women. We now have data to demonstrate that, among women, (1) rectal *C. trachomatis* is prevalent, commonly cooccurs with vaginal *C. trachomatis*, and is not associated with reporting anal sex [2]; (2) detection of rectal *C. trachomatis* via nucleic acid amplification testing likely represents the presence of viable bacteria in the rectum; and (3) failure of azithromycin treatment for rectal *C. trachomatis* occurs somewhat frequently. Together, these findings suggest that limiting rectal screening to women who only report anal sex will miss a substantial number of cases but that providing azithromycin treatment to women with vaginal *C. trachomatis* without regard to whether they have concurrent rectal *C. trachomatis* could result in persistent rectal *C. trachomatis*.

Thus, the following question remains—what additional evidence is needed to recommend routine screening for rectal infections among women? Arguably, the strongest evidence would demonstrate that these infections lead to adverse health outcomes. As we have previously noted [1], data from animal models suggest that autoinfection from the rectum to the vagina occurs [3–5], which could lead to ongoing transmission and reproductive tract morbidity. But the extent to which autoinfection occurs in humans is still unclear, and the most appropriate