Determinants associated with viable genital or rectal Chlamydia trachomatis bacterial load (FemCure)

Original research

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Received 8 April 2020
Revised 7 December 2020
Accepted 22 December 2020
Published Online First 13 January 2021

To cite: Janssen KJH, Wolffs PFG, Hoebe CJPA, *et al*. *Sex Transm Infect* 2022;**98**:17–22.

ABSTRACT

Background *Chlamydia trachomatis* (CT) is routinely diagnosed by nucleic acid amplification tests (NAATs), which are unable to distinguish between nucleic acids from viable and non-viable CT organisms.

Objectives We applied our recently developed sensitive PCR (viability PCR) technique to measure viable bacterial CT load and explore associated determinants in 524 women attending Dutch sexual health centres (STI clinics), and who had genital or rectal CT.

Methods We included women participating in the FemCure study (Netherlands, 2016–2017). At the enrolment visit (pre-treatment), 524 were NAAT positive (n=411 had genital and rectal CT, n=88 had genital CT only and n=25 had rectal CT only). We assessed viable rectal and viable genital load using V-PCR. We presented mean load (range 0 (non-viable) to 6.5 $\log_{10}$ CT/mL) and explored potential associations with urogenital symptoms (coital lower abdominal pain, coital blood loss, intermenstrual bleeding, altered vaginal discharge, painful or frequent micturition), rectal symptoms (discharge, pain, blood loss), other anatomical site infection and sociodemographics using multivariable regression analyses.

Results In genital (n=499) CT NAAT-positive women, the mean viable load was 3.5 $\log_{10}$ CT/mL (SD 1.6). Genital viable load was independently associated with urogenital symptoms—especially altered vaginal discharge (Beta=0.35, p=0.012) and with concurrent rectal CT (aBeta=1.79; p<0.001). Urogenital symptoms were reported by 50.3% of women; their mean genital viable load was 3.6 $\log_{10}$ CT/mL (vs 3.3 in women without symptoms). Of 436 rectal CT NAAT-positive women, the mean rectal viable load was 2.2 $\log_{10}$ CT/mL (SD 2.0); rectal symptoms were reported by 2.5% (n=11) and not associated with rectal viable load.

Conclusion Among women diagnosed with CT in an outpatient clinical setting, viable genital CT load was higher in those reporting urogenital symptoms, but the difference was small. Viable genital load was substantially higher when women also had a concurrent rectal CT.

Trial registration number ClinicalTrials.gov NCT02694497.

INTRODUCTION

For various STIs, such as HIV and *Neisseria gonorrhoeae*, organism load has been associated with clinical complications and onward transmission.[1–6] For example, rectal *N. gonorrhoeae* load was found higher in men with symptoms of proctitis compared with men with asymptomatic rectal infections (5.4 log vs 4.1 log).[3] More recently, urethral *N. gonorrhoeae* load was found higher among men with purulent urethral discharge (3.7×10^6 copies/swab; IQR 2.5×10^6–4.7×10^6) compared with asymptomatic men(2.0×10^5 copies/swab; IQR 2.7×10^4–4.5×10^5).[6] For *Chlamydia trachomatis* (CT), the association between organism load and clinical course of disease, that is, clinical presentation and complications over time, remains unclear.[7 8] We recently validated a PCR technique to quantify viable CT in clinical samples, also known as viability PCR (V-PCR).[9] In V-PCR, samples are treated with a membrane-impermeable DNA binding dye (eg, propidium monoazide; PMAxx) prior to DNA isolation, eliminating extra-bacterial DNA and DNA from membrane-comprised bacteria, to ensure the amplification of DNA from viable bacteria only.[10 11] The aim of the current study was to assess the viable CT load in genital and rectal swab samples and evaluate the association between viable CT load and symptoms and other potential determinants in CT-positive women attending Dutch sexual health centres (STI clinics).

METHODS

Study design

We conducted a cross-sectional study, as part of the FemCure study, 2016–2017.[12]

Study population and sample collection

Patients were recruited at the STI clinics of the Public Health Services in South Limburg, Rotterdam-Rijnmond, and Amsterdam, the Netherlands, as described previously.[12] In short, all women were tested for CT according to European guidelines, that is, standard genital testing; rectal testing in women who reported unprotected anal sex, anal symptoms or belonging to a high-risk group.[13] Eligible for inclusion were heterosexual adult (18 years or older) women diagnosed with a genital or rectal CT infection (symptomatic or asymptomatic). Women were not eligible for inclusion when they had a co-infection with HIV, syphilis or *N. gonorrhoeae* at time of clinic diagnosis, or when they were pregnant, or reported recent (<1 month) use

of antibiotics at enrolment.[12] Women (n=560) were enrolled in the study when they returned to the clinic for treatment. Participants were instructed by a study nurse to self-collect rectal and genital swabs, that is, genital swabs: insert the swab 5–10 cm, rotate it for 30 s and place the swab in a capped tube; rectal swabs: insert the swab 2.5 cm into the anus, rotate it for 5 to 10 s, and place the swab in a capped tube). Swabs were taken just prior to treatment. From each anatomical site, two swab samples were collected, as laboratory procedures for NAAT testing and V-PCR testing demand different handling and storage conditions. The first swab was placed in 4 mL 2SP buffer (Smith, 1977) and used to evaluate the viable CT load by V-PCR.[12] The data describe the number of chlamydia per millilitre of sample of 2SP buffer in which either a rectal or vaginal swab was resuspended. The second swab was used to test for CT positivity by a commercial NAAT platform according to the manufacturers' guidelines (COBAS 4800; Roche Diagnostics, Basel, Switzerland). The swab for viability testing was taken first. The viability test is less sensitive than the NAAT, which is conducted on the second swab taken. Thereby, we believe that any loss of signal due to multiple swabbing is negligible. Data were further collected using structured online questionnaires (participant and study nurse self-administered). The study population in analyses were all participants who tested genital or rectal CT positive by NAAT at study enrolment (n=524).

DNA extraction

Total nucleic acids from 200 µL sample were isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) platforms according to the manufacturers' guidelines, and eluted in 120 µL elution buffer. DNA was stored at −20°C and thawed once for quantification by using V-PCR.

Viability testing by V-PCR

Two different V-PCR assays were used to evaluate CT viability. The first was used as a quantitative V-PCR assay, targeting the single-copy *ompA* gene coding for the major outer membrane protein (MOMP).[9 14] A second assay was used as a qualitative V-PCR assay, targeting the CT plasmid (PLA). As previously demonstrated, the number of plasmids is variable and generally ranges from 1 to 10 plasmid copies per CT, resulting in an increased sensitivity as compared with the single-copy MOMP target.[14] For the qualitative V-PCR assay, we designed a new set of primers and probe to amplify a 180 bp region of the CT plasmid. The forward primer sequence used was *CTPLAv-F 5′*-TTGG TACGAGAAGGTGATTCTAAG-3′, the reverse primer sequence was *CTPLAv-R 5′*-GCCATTAGAAAGGGCATTAACC-3′ and the *6FAM-CTPLAv probe 5′*-ACACCGCTTTCTAAACCGCC TACACGTAA-3′. V-PCR was conducted as previously described with minor adjustments.[9 15] V-PCR reactions were carried out in a total volume of 50 µL per reaction. Furthermore, PMA was substituted with PMAxx and photoactivation was completed by using the PMA-Lite LED Photolysis Device (Biotium, Hayward, CA) with the exposure time set to 10 min.

Quantification of viable load

When the NAAT test was positive, samples were evaluated for viable CT load by using both the quantitative and the qualitative V-PCR assay (figure 1). The cycle of quantification (Cq) value of the quantitative V-PCR assay was entered into the master curve (calculated from a 10 dilution series) to calculate viable load (presented as $\log_{10}$ CT/mL).[7] Samples testing positive only for the qualitative V-PCR assay, due to a low total CT load, were assigned a viable CT load at half of the lower detection limit of the quantitative V-PCR assay (0.75 $\log_{10}$ CT/mL). This is a generally accepted method for evaluation of organism load below the detection limit.[7 16] NAAT-positive samples testing negative for both the quantitative and qualitative V-PCR assay were considered to contain remnant CT DNA only (ie, 0 $\log_{10}$ CT/mL; no evidence of viable CT detected).

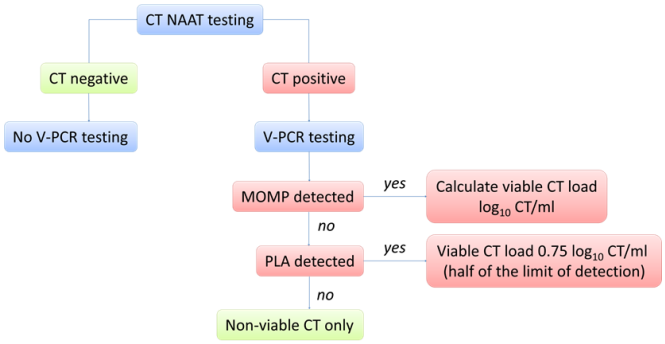

Figure 1 Algorithm for interpretation of V-PCR assay results. CT, *Chlamydia trachomatis*; MOMP, major outer membrane protein; NAAT, nucleic acid amplification testing; V-PCR, viability PCR.

Statistical analyses

We presented the mean load (range 0 (no viable CT detected) to 6.5 $\log_{10}$ CT/mL). We also tested associations with a range of determinants, information on which was collected in the questionnaires (see next section). Analyses were performed separately for genital and rectal viable CT load. Evaluated determinants were age, study site, ethnicity, educational level, CT at the other (rectal or genital) anatomical site, number of sex partners (past 3 months), genital or rectal intercourse (<2 weeks), urogenital symptoms (ie, lower abdominal pain, blood loss, intermenstrual bleeding, altered vaginal discharge, painful micturition or frequent micturition) or rectal symptoms (ie, anal discharge, pain, blood loss). We used univariable linear regression, and multivariable linear regression, by entering all determinants that were statistically significantly associated in univariable analyses. We calculated betas (B) indicating the Δ viable $\log_{10}$ load with 95% CIs. Statistical tests with a p value <0.05 was considered statistically significant. IBM SPSS Statistics V.24 was used for statistical analyses.

RESULTS

Study population

Of the 524 women, median age was 22 years (IQR 20–24) (table 1). Measured by NAAT, 78.4% (411/524) had concurrent genital and rectal CT, 16.8% (88/524) had genital-only CT and 4.8% (25/524) had rectal-only CT. Of women with genital CT (n=499), 50.3% (251/499) reported urogenital symptoms. Of women with rectal CT (n=436), 2.5% (11/436) reported rectal symptoms.

Viable load in women with genital CT

The viable genital CT load was quantifiable in genital swabs of 84.6% (422/499) of women by using the quantitative V-PCR assay. In another 9.4% (47/499) of women, viable CT in genital swabs was detected by the more sensitive qualitative V-PCR testing. In the genital swabs of the remaining 6.0% (30/499), no evidence of viable CT was detected (ie, absence of PCR signal in both the qualitative and quantitative V-PCR assay). The mean viable genital CT load was 3.46 $\log_{10}$ CT/mL (SD 1.58).

Table 1 Characteristics of the study population of women with *Chlamydia trachomatis* at study enrolment, FemCure

	Total n=524, n (%)	Genital CT by NAAT n=499, n (%)	Rectal CT by NAAT n=436, n (%)
Study site (sexual health centre—STI clinic)			
South Limburg	193 (36.8)	182 (36.5)	164 (37.6)
Amsterdam	145 (27.7)	143 (28.7)	119 (27.3)
Rotterdam-Rijnmond	186 (35.5)	174 (34.9)	153 (35.1)
Age (years)			
18–20	155 (29.6)	152 (30.5)	134 (30.7)
21–23	194 (37.0)	187 (37.5)	157 (36.0)
≥24	175 (33.4)	160 (32.1)	145 (33.3)
Ethnicity			
Western	486 (92.7)	462 (92.6)	403 (92.4)
Non-western	38 (7.3)	37 (7.4)	33 (7.6)
Educational level			
Low	187 (35.7)	179 (35.9)	153 (35.1)
Middle	196 (37.4)	188 (37.7)	165 (37.8)
High	140 (26.7)	131 (26.3)	118 (27.1)
Unknown	1 (0.2)	1 (0.2)	–
Genital or rectal CT			
Genital CT (rectal negative)	88 (16.8)	88 (17.6)	
Genital and rectal CT	411 (78.4)	411 (82.4)	411 (94.3)
Rectal CT (genital negative)	25 (4.8)		25 (5.7)
No of sex partners (past 3 months)			
0 or 1	180 (34.4)	171 (34.3)	151 (34.6)
2 or 3	249 (47.5)	241 (48.3)	208 (47.7)
≥4	92 (17.6)	84 (16.8)	75 (17.2)
Unknown	3 (0.6)	3 (0.6)	2 (0.5)
Vaginal sex (past 2 weeks)			
No	254 (48.5)	240 (48.1)	
Yes	270 (51.5)	259 (51.9)	
Anal sex (past 2 weeks)			
No	507 (96.8)		421 (96.6)
Yes	17 (3.2)		15 (3.4)
Urogenital symptoms*			
Any	258 (49.2)	251 (50.3)	
Lower abdominal pain during intercourse	43 (8.2)	42 (8.4)	
Blood loss during intercourse	57 (10.9)	55 (11.0)	
Inter menstrual bleeding	64 (12.2)	62 (12.4)	
Altered vaginal discharge	169 (32.3)	165 (33.1)	
Painful micturition (dysuria)	107 (20.4)	105 (21.0)	
Frequent micturition (pollakisuria)	69 (13.2)	67 (13.4)	
Rectal symptoms*			
Any	12 (2.3)		11 (2.5)
Anal discharge	10 (1.9)		9 (2.1)
Blood loss during intercourse	1 (0.2)		1 (0.2)
Pain during intercourse	1 (0.2)		1 (0.2)

The educational level was measured as current education or highest educational level completed and was categorised into three categories: lower educated (pre-vocational secondary education, secondary vocational education), medium educated (senior general secondary education, pre-university education) and higher educated (higher professional education, university education.
*Symptoms for genital CT: lower abdominal pain during intercourse, blood loss during intercourse, intermenstrual bleeding, altered vaginal discharge, painful micturition (dysuria), frequent micturition (pollakisuria); symptoms for rectal CT: anal discharge, anal blood loss during or after intercourse, pain during or after intercourse.
CT, *Chlamydia trachomatis*; NAAT, nucleic acid amplification testing.

Determinants associated with viable genital CT load

Urogenital symptoms and concurrent rectal CT were associated with genital viable CT load in univariable analyses. Women reporting urogenital symptoms had a higher load compared with asymptomatic women (3.65 vs 3.27 $\log_{10}$ CT/mL, p=0.007) (table 2). Women with altered vaginal discharge had a higher load than women without altered discharge (3.78 vs 3.30 $\log_{10}$ CT/mL, p=0.001).

Women with a concurrent rectal CT had a higher mean viable genital load than women who were negative for rectal CT (3.78 vs 1.96 $\log_{10}$ CT/mL) (table 2). In multivariable analysis, altered vaginal discharge (aBeta=0.33; p=0.015) and concurrent rectal CT (aBeta=1.79; p<0.001) remained independently associated with viable genital CT load. Table 3 shows the proportion of women reporting symptoms and having concurrent rectal CT, by their viable vaginal load.

Viable load in women with rectal CT

In 52.8% (230/436) of the women, the viable rectal CT load was quantifiable; in 13.8% (60/436) of the women, the viable rectal CT was detected by qualitative V-PCR testing. In the remaining women (33.5%; 146/436), no viable rectal CT was detected. The mean viable rectal CT load was 2.20 $\log_{10}$ CT/mL (SD 2.0).

Determinants associated with viable rectal CT load

Viable CT load was associated with study site (clinic) (higher in Rotterdam-Rijnmond compared with South Limburg), but not with any other studied determinant (table 2).

DISCUSSION

Using V-PCR to asses viable CT load in a large cohort of women attending three Dutch sexual health centres (STI clinics), we demonstrated that the viable CT load in self-collected genital swabs was only slightly associated with urogenital symptoms, especially with altered vaginal discharge. Viable genital CT load was strongly associated with the presence of concurrent rectal CT. The mean viable genital CT load was 3.46 $\log_{10}$ CT/mL and the rectal load was 2.20 $\log_{10}$ CT/mL.

Previous studies used either culture-based methods or NAAT-based methods to assess associations between organism load and symptoms. Culture based studies, detecting viable CT, observed that mucopurulent cervical and vaginal discharge were associated with cervical CT infection.[17 18] In a primary care population of women, no such association was found using culture.[19] Likewise, no association between reported symptoms and total CT load was found using quantitative PCR (in women attending STI clinics or participating in population-based screening).[7] A greater burden of viable CT at the cervix may induce a greater inflammatory response, leading to a more severe clinical course of disease and subsequently might lead to an increased shedding of viable CT from the cervical site to the vagina.[20] Recently, the total CT load at the cervical site was found to be positively correlated with cytokine levels detected in cervical secretions.[21] Using V-PCR to assess viable load, in women attending STI clinics, we here demonstrated an association with urogenital symptoms, mainly altered vaginal discharge, and viable genital CT load. However, the mean viable load differed only slightly between symptomatic and asymptomatic women, and a substantial proportion of women in the highest quartile of viable genital load did not report urogenital symptoms.

Rectal CT is prevalent in women who have genital CT, with the summary estimate in women visiting an STI clinic 68.1% (95% CI 56.6% to 79.6%), and is not associated with history of

Table 2 Viable load and univariable associations with participant characteristics in genital and in rectal *Chlamydia trachomatis* (CT), FemCure

	Genital			Anal		
	Mean viable log_{10} load (SD)	Δ log_{10} load (95% CI)*	P value	Mean viable log_{10} load (SD)	Δ log_{10} load (95% CI)*	P value
Age (years)			0.138			0.247
18–20	3.65 (1.30)	ref		2.06 (1.94)	ref	
21–23	3.30 (1.73)	−0.34 (−0.68 to −0.01)		2.09 (2.07)	0.03 (−0.44 to 0.50)	
>24	3.47 (1.61)	−0.18 (−0.53 to 0.17)		2.42 (2.08)	0.36 (−0.12 to 0.84)	
Study site			0.451			**0.005**
South Limburg	3.51 (1.52)	ref		1.93 (1.92)	ref	
Amsterdam	3.54 (1.46)	0.03 (−0.32 to 0.38)		2.00 (1.93)	0.07 (−0.41 to 0.55)	
Rotterdam-Rijnmond	3.34 (1.72)	−0.17 (−0.50 to 0.16)		2.62 (2.18)	0.69 (0.25 to 1.14)	
Ethnicity			0.387			0.854
Western	3.48 (1.56)	ref		2.19 (2.03)	ref	
Non-western	3.24 (1.75)	−0.23 (−0.76 to 0.30)		2.26 (2.17)	0.07 (−0.66 to 0.79)	
Educational level			0.495			0.083
Low	3.38 (1.55)	*ref*		2.03 (1.99)	ref	
Middle	3.47 (1.57)	0.10 (−0.22 to 0.43)		2.10 (2.00)	0.07 (−0.38 to 0.52)	
High	3.58 (1.60)	0.21 (−0.141 to 0.57)		2.55 (2.13)	0.52 (0.03 to 1.01)	
Genital or rectal CT			**<0.001**			0.873
Genital CT (rectal negative)	1.96 (1.45)	ref				
Genital and rectal CT	3.78 (1.41)	1.83 (1.50 to 2.15)		2.19 (2.05)	−0.07 (−0.89 to 0.76)	
Rectal CT (genital negative)				2.26 (1.92)	ref	
No of sex partners			0.605			0.539
0 or 1	3.53 (1.48)	ref		2.13 (1.95)	ref	
2 or 3	3.46 (1.61)	−0.07 (−0.37 to 0.24)		2.17 (2.10)	0.03 (−0.40 to 0.46)	
≥4	3.32 (1.69)	−0.21 (−0.62 to 0.20)		2.43 (2.05)	0.31 (−0.26 to 0.87)	
Vaginal sex			0.212			
No	3.37 (1.59)	ref				
Yes	3.55 (1.56)	0.18 (−0.10 to 0.45)				
Anal sex						0.908
No				2.20 (2.05)	ref	
Yes				2.13 (1.87)	−0.06 (−1.12 to 0.99)	
Urogenital symptoms						
Any†			**0.007**			
No	3.27 (1.63)	ref				
Yes	3.65 (1.50)	0.38 (0.10 to 0.65)				
Lower abdominal pain			0.968			
No	3.46 (1.57)	ref				
Yes	3.45 (1.71)	−0.01 (−0.51 to 0.49)				
Blood loss at intercourse			0.050			
No	3.41 (1.58)	ref				
Yes	3.85 (1.52)	0.44 (−0.00 to 0.88)				
Intermenstrual bleeding			0.382			
No	3.44 (1.56)	ref				
Yes	3.62 (1.67)	0.19 (−0.23 to 0.61)				
Altered vaginal discharge			**0.001**			
No	3.30 (1.62)	ref				
Yes	3.78 (1.44)	0.48 (0.18 to 0.767)				
Painful micturition			0.297			
No	3.42 (1.62)	ref				
Yes	3.60 (1.42)	0.18 (−0.16 to 0.52)				
Frequent micturition			0.862			
No	3.46 (1.59)	ref				
Yes	3.49 (1.48)	0.04 (−0.37 to 0.44)				
Any rectal symptoms†‡						0.754
No				2.19 (2.04)	ref	
Yes				2.38 (2.07)	0.20 (−1.03 to 1.42)	

*The regression coefficient is presented as Δ viable load.
†Only symptoms associated with CT were included. Symptoms for genital CT: lower abdominal pain during intercourse, blood loss during intercourse, intermenstrual bleeding, altered vaginal discharge, painful micturition (dysuria), frequent micturition (pollakisuria); symptoms for rectal CT: anal discharge, anal blood loss during or after intercourse, pain during or after intercourse.
‡Specific rectal symptoms not shown due to low number of cases.

Table 3 Proportion of women reporting urogenital symptoms (any) and proportion of women with concurrent rectal *Chlamydia trachomatis* (CT), by categories of genital viable CT load, FemCure

	By urogenital symptoms		By concurrent rectal CT	
	Symptoms	No symptoms	Concurrent	No concurrent
	%	%	%	%
All women with genital CT	50.3	49.7	82.4	17.6
By viable genital load categories				
Women with viable load <2.6 $\log_{10}$ CT/mL (n=109)	40.4	59.6	53.2	46.8
Women with viable load ≥2.6 and <3.5 $\log_{10}$ CT/mL (n=112)	48.2	51.8	76.8	23.2
Women with viable load <4.5 $\log_{10}$ CT/mL (n=136)	53.7	46.3	93.4	6.6
Women with viable load 4.5 $\log_{10}$ CT/mL (n=142)	56.3	43.7	98.6	1.4

Symptoms for genital CT: lower abdominal pain during intercourse, blood loss during intercourse, intermenstrual bleeding, altered vaginal discharge, painful micturition (dysuria), frequent micturition (pollakisuria).
CT, *Chlamydia trachomatis*.

anal sex or rectal symptoms.[22–24] In our study, viable rectal CT load was not associated with anal sex or with rectal symptoms. Several possible reasons for the detection of rectal CT in women have been suggested, including autoinoculation via genital secretions. Autoinoculation would require infected cervical secretions to migrate from the vagina to the rectum via the perineum, during which the bacterium *C. trachomatis* might lose some of its infectivity. Likelihood of such migration might be enhanced by higher viable genital loads, suboptimal hygiene or sexual practices. In women diagnosed with concurrent rectal CT, we demonstrated higher viable genital CT load than in women with CT only at the genital site. This was in line with previous studies that examined total genital CT loads.[25 26] Recently, we reported that of untreated female patients with genital CT who also had rectal CT, only a small proportion spontaneously cleared CT, and in fact many had rectal viable CT at their follow-up visit (median 9 days). Among single anatomical site–infected women, clearance was much higher.[27] With this study, we add that the load of viable genital CT increases with the presence of rectal CT, suggesting that in clinical practice, the presence of rectal CT may be taken as a proxy for higher viable genital CT loads.

Study site of enrolment was the only determinant associated with the load of viable rectal CT, an observation for which we do not have an explanation.

This study also has limitations. (1) By using self-taken swabs (as is routine care), we do not know whether the viable CT load observed in our samples perfectly reflects the viable load at the site of infection (more upward in the cervix or in the columnar cells of the rectal canal). However, V-PCR results do likely represent the amount of viable CT in genital secretions. (2) We did not perform genotyping, thus in theory it is possible that the rectal and the genital CT have a different genotype (which would rule out autoinoculation). (3) We did not standardise by a human cell marker, so we could not rule out an effect of sampling variability when comparing rectal and vaginal test results. (4) Findings may not be fully representative for all female STI clinic attendees, as only a subgroup of female STI clinic attendees were eligible for participation (based on predefined exclusion criteria such as very young age (<18 years), recent use of antibiotics (<1 month), and co-infection with other STI).[28] (6) Findings may not be generalisable to other healthcare settings (eg, general practitioners). (7) Sample contamination cannot be entirely ruled out, as all samples were patient-collected swabs. However, patients were well instructed (including both visual and verbal instructions) by trained study nurses.[12] Previous studies have shown that patient-collected swabs yield the same accuracy as clinician-collected swabs in routine CT testing.[29 30] (8) Bacterial cell death in the time interval between sampling and laboratory analysis may lead to an underestimation of CT viability. However, we expect this effect to be minimal, as samples were immediately stored at −80°C and transported to the laboratory by cooled transport using dry ice (−80°C).[12]

To conclude, women diagnosed with genital CT who report symptoms (altered vaginal discharge) have a slightly higher genital viable load than women who do not report symptoms. Yet, findings do not provide sufficient evidence to optimise testing and treatment guidelines, as the difference was small and viable load is found in many asymptomatic women. Notably, women had a substantially higher genital viable load when they also had a rectal CT (compared with women with a single genital CT). Rectal CT detection in genital CT diagnosed women may be an indicator for viable genital CT load in women.

Key messages

- Altered vaginal discharge and concurrent rectal *Chlamydia trachomatis* (CT) were independently associated with viable genital CT load.
- Viable genital CT load was higher than viable rectal CT load in women.
- Viable rectal CT was detected in women, irrespective of reported anal intercourse or rectal symptoms.
- The biological mechanisms of potential transmission and clinical implications of rectal CT in women warrant further study.

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Acknowledgements We are grateful to the staff at the Public Health Service (GGD) South Limburg, Lisanne Eppings, Dr Ronald van Hoorn, Maria Mergelsberg, Mandy Sanders, Emily Suijlen, Bianca Penders, Helen Sijstermans and Ine de Bock, the staff at GGD Rotterdam-Rijnmond, Astrid Wielemaker, Angie Martina, Roselyne Uwimana, Mieke Illidge, Klaas de Ridder and Bram (A) Meima for data management, and the staff at GGD Amsterdam, Dieke Martini, Myra van Leeuwen, Claudia Owusu, Jacqueline Woutersen, Princella Felipa, Mayam Amezian, Arjdal Khadija and Iris Deen, who are involved in the logistics and inclusion, Martijn van Rooijen for data management, and Anders Boyd for statistical advice. We also thank the staff at the laboratories of Medical Microbiology of the Maastricht University Medical Center, especially Judith Veugen, Laura Saelmans and Mayk Luchessi. Also, we thank the staff of the microbiological laboratory of the GGD Amsterdam, Esther Heuser and Michelle Himschoot.

Contributors KJHJ and NHTMD-M drafted the paper. NHTMD-M coordinated the study, performed the statistical analyses, wrote the final draft and supervised the study. NHTMD-M, CJPAH and PFGW designed the study. PFGW and SMB coordinated laboratory data collection and testing (study). All authors critically edited the manuscript and approved the final manuscript.

Funding This study is funded by a governmental organisation grant from the Netherlands: Organization for Health Research and Development (ZonMW Netherlands, registration numbers 50-53000-98-109 and 52-2008-002).

Disclaimer The funding sources had no role in the writing or publication of this paper.

Competing interests None declared.

Patient consent for publication Not required.

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

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data from the FemCure study are available on reasonable request.

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