Genital and anal *Chlamydia trachomatis* bacterial load in concurrently infected women

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ORIGINAL ARTICLE

Genital and anal *Chlamydia trachomatis* bacterial load in concurrently infected women: a cross-sectional study

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ABSTRACT

Objectives Most international STI guidelines recommend *Chlamydia trachomatis* anorectal testing in women after self-reported sexual exposure or symptoms in women. However, it has been shown that the prevalence of anorectal *C. trachomatis* is as high (7%–17%) in women who do not report anorectal intercourse (AI) as in women who do. This study assessed the correlation between the genital and anorectal *C. trachomatis* load in concurrently infected women for increased microbiological insight.

Methods A convenience sample of 105 women with a concurrent (genital and anorectal) *C. trachomatis* infection was included from the STI clinic in South Limburg, the Netherlands. Women provided self-collected vaginal and anorectal swabs. The *C. trachomatis* load was quantified with qPCR and the human cell load was assessed to ensure sample adequacy. Genital and anorectal *C. trachomatis* loads were divided into tertiles for comparison. The χ^2 test and linear regression were used to compare genital and anorectal *C. trachomatis* loads and identify determinants associated with load.

Results The overall median *C. trachomatis* load was higher in genital (median 5.3 log₁₀ *C. trachomatis*/ml) than anorectal samples (median 3.4, $p \leq 0.001$), but both loads were within the same range. The genital and anal load categories were not related within one woman ($p = 0.99$), both in women with and without AI. The anorectal *C. trachomatis* load was in the same or higher load category than the genital load in 56% of women without AI, and 79% of women with AI.

Conclusions Although no cut-off for clinical relevance is known, an anorectal *C. trachomatis* load in the same or higher load category than the genital *C. trachomatis* load is likely to be clinically relevant. Other measurements should also be taken into account, such as leucocytes or bacterial viability to distinguish infection from contamination or exposure.

INTRODUCTION

With over 131 million prevalent cases in 2012, *Chlamydia trachomatis* is the most common bacterial STI worldwide.¹ *C. trachomatis* can infect the genital, anorectal and oropharyngeal sites. Although most of these infections are asymptomatic, up to 22% of genital *C. trachomatis* infections in women might have serious sequelae, such as

pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy and tubal factor infertility.²

Most international STI test guidelines recommend routine testing on the genital site and testing on indication of exposure or symptoms on the anorectal site for women.^{3–6} Recently, Dutch guidelines changed their anorectal testing recommendations for men who have sex with men (MSM) towards routine testing of all MSM.⁶ Studies have shown that anorectal *C. trachomatis* infections are as common in women (7%–17%)⁷ as in MSM (1%–18%). Notably, the prevalence of anorectal *C. trachomatis* (7%–17%) is as high in women who do not report anorectal intercourse (AI)⁸ as in women who report AI. When all women are tested for genital and anorectal *C. trachomatis*, 71%–95% of women with an anorectal *C. trachomatis* infection have a concurrent genital infection,^{7 9 10} and 33%–83% of women with a genital *C. trachomatis* infection have a concurrent anorectal *C. trachomatis* infection.^{7 9 11–14} Anorectal *C. trachomatis* positivity is equally high in women who report AI as those who do not report AI. Possible explanations of anorectal *C. trachomatis* in the absence of reported AI include under-reporting of AI, contamination in the laboratory, contamination during self-sampling (eg, accidentally touching the vagina or perineum before sampling anorectally), exposure of the rectum to *C. trachomatis* (no infection yet, but superficial presence of bacteria), and a ‘true’ infection through autoinoculation via seepage of infected vaginal secretions^{9 11} or via the digestive tract.^{15 16}

The bacterial *C. trachomatis* load has been implicated in affecting several aspects of the disease, such as duration of the infection, transmission, complications and symptoms.^{17–19} Previously,²⁰ we showed that the anorectal *C. trachomatis* load in MSM is equal to that of women who report AI, implicating similar clinical relevance. The *C. trachomatis* load in women that did not report AI was, however, lower than women who reported AI. Besides reported AI, other factors could influence the height of the *C. trachomatis* load. It is unknown whether the genital bacterial load is correlated with the anorectal *C. trachomatis* load in concurrently infected women. To obtain more microbiological insight in concurrent *C. trachomatis* infections in women, this study will investigate the relation between the genital *C.*

Figure 1 Chlamydia load in concurrently infected women, split per anatomical location. The median *Chlamydia trachomatis* load in genital samples was 5.3, and in anorectal samples 3.4 ($p < 0.05$). Dark grey indicates reported anorectal intercourse, while light grey dots represent women who did not report anorectal intercourse.

trachomatis load and the anorectal *C. trachomatis* load, both in women with and without AI.

METHODS

This study is a subanalysis of a previously described study of concurrently infected women from the STI clinic in South Limburg, the Netherlands.²⁰ Trained study nurses provided patients with a

Figure 2 *Chlamydia trachomatis* load in concurrently infected women. The anorectal and genital loads are depicted per woman in one dot. Dark grey indicates reported anorectal intercourse, while light grey dots represent women who did not report anorectal intercourse.

visual diagram and verbal instructions about how to take separate self-collected vaginal and anorectal swabs. For the vaginal swab, the patient was instructed to insert the swab 2.5 cm into the vagina, rotate it for 5 to 10 s, and then place it in a capped tube to avoid potential contamination. This procedure was repeated in the anus for the anorectal swab. A review of questionnaires completed by the patients provided data about age, number of partners in the preceding 6 months and self-reported symptoms. Genitourinary symptoms included vaginal discharge, intermenstrual bleeding, postcoital bleeding, abdominal pain and symptoms of a urinary tract infection (dysuria, urinary frequency and haematuria). Anorectal symptoms (proctitis) included rectal discharge, bleeding, pain, redness, burning sensation or itching. All data were registered in an electronic patient registry.

Since the retrospective data originated from standard care and were analysed anonymously, no further informed consent for data analysis was obtained.

C. trachomatis load quantification

Initial *C. trachomatis* screening was performed with either Roche Cobas Amplicor or Roche Cobas 4800 (Roche Diagnostics, Basel, Switzerland), which are both very sensitive commercial assays (lower detection limit 40 bacteria/ml²¹, sensitivity 91.2%–98%,^{21–23} specificity $\geq 98.8\%$).^{22, 23} The *C. trachomatis* load was quantified in positive samples by inhouse quantitative PCR (qPCR) targeting the chromosomal *OmpA*-gene.²⁴ The human cell load was also quantified using the human leucocyte antigen (HLA) gene²⁵ to ensure adequate sampling took place.

Cycle threshold values were entered into a master curve (calculated from over 10 dilution series), to calculate the *C. trachomatis* load ($\log_{10}$). The initial diagnostic *C. trachomatis* screening targets the chlamydial plasmid, which is present in abundance compared to the single-copy *OmpA* gene used in our load assessment. We assigned samples with a load below the quantification limit (30 *C. trachomatis* bacteria/ml), a load at half the lowest detection limit of our inhouse PCR. This is a generally accepted method to address values below the quantification limit.²⁶

C. trachomatis and human cell loads were significantly different in genital and anorectal samples. The anorectal and genital *C. trachomatis* loads were each divided into tertiles (low/middle/high) based on cycle threshold values to compare the anorectal and genital loads in different sample types. Anorectal swabs were divided into tertiles based on cycle threshold values of <33.0 , 33.1 – 38.3 and >38.4 , and genital swabs on cycle threshold values of <27.3 , 27.4 – 31.0 and >31.1 .

Three paired samples were excluded because the genital and anorectal swabs were not taken the same day. Four additional paired samples were excluded because HLA could not be detected in either sample. This resulted in the inclusion of the paired samples of 105 women with a concurrent genital and anorectal *C. trachomatis* for analyses.

Statistical analyses

At the group level, the median *C. trachomatis* load (continuous) was compared between the genital and the anorectal sites and compared between women who reported AI and women who did not report AI using Mann-Whitney U test. At the individual level, the distribution of the *C. trachomatis* load categories (low/medium/high) at the genital and anorectal sites were compared using Pearson's χ^2 test. Univariate linear regression analyses were performed to identify determinants associated with genital and anorectal load. The determinants

Table 1 The categorised genital and anorectal *Chlamydia trachomatis* loads in women

		All women			Women with AI			Women without AI		
		Genital <i>C. trachomatis</i> load			Genital <i>C. trachomatis</i> load			Genital <i>C. trachomatis</i> load		
		Low	Middle	High	Low	Middle	High	Low	Middle	High
Anorectal <i>C. trachomatis</i> load	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Low	N (%)	11 (31)	12 (34)	12 (34)	5 (26)	2 (13)	5 (38)	6 (38)	10 (53)	7 (32)
Middle	N (%)	12 (34)	12 (34)	11 (31)	6 (32)	5 (31)	3 (23)	6 (38)	7 (37)	8 (36)
High	N (%)	12 (34)	11 (31)	12 (34)	8 (42)	9 (56)	5 (38)	4 (25)	2 (11)	7 (32)

AI, anorectal intercourse.

tested were; age, number of sexual partners (1, 2–3, ≤ 4 , based on tertiles), AI and symptoms (genital and anorectal). The number of sexual partners, symptoms and AI were assessed for the past 6 months. No determinants were found to be associated with either the genital or anorectal *C. trachomatis* loads in women besides AI,²⁰ which is why all analyses are performed separately for women with and without AI. The results were considered statistically significant at $p \leq 0.05$. All statistical tests were performed using the IBM SPSS Statistics for Windows, V.24.0 (IBM Corp, Armonk, New York, USA).

RESULTS

Characteristics of the study population

The median age of the women was 22 years (range 16–63, IQR 20–28). In the past 6 months, 31% of women had one sexual partner, 38% had two to three partners, and 31% had four or more partners. AI was reported by 45.7% (48/105) of women. Proctitis symptoms were reported by 17% ($n=8$) of women who reported AI and by 5% ($n=2$) of women who did not report AI. Genital symptoms were reported by 64% ($n=67$) of the women included in the analyses.

Genital and anorectal chlamydial load in women

Overall, the median genital *C. trachomatis* load was higher than the median anorectal *C. trachomatis* load ($5.30 \log_{10}$ *C. trachomatis*/ml vs $3.42 \log_{10}$ *C. trachomatis*/ml, $p < 0.001$), both in women who did not report AI ($5.40 \log_{10}$ *C. trachomatis*/ml vs $2.65 \log_{10}$ *C. trachomatis*/ml, $p < 0.001$) and in women who reported AI ($5.06 \log_{10}$ *C. trachomatis*/ml vs $3.74 \log_{10}$ *C. trachomatis*/ml, $p = 0.004$) (figure 1). The genital load was similar in women who reported AI and women who did not ($5.06 \log_{10}$ *C. trachomatis*/ml vs $5.40 \log_{10}$ *C. trachomatis*/ml; $p = 0.1$).

Then we compared the distribution of the load (categories: high/medium/low) between both anatomical sites at the individual level. The categorical distribution of the genital load was similar to that of the anorectal load within the same woman ($p = 0.99$). Results were the same when analyses were stratified for self-reported AI ($p = 0.60$ for women with AI, $p = 0.52$ for women without AI, figure 2 and table 1).

Although no cut-off for clinical relevance is known, an anorectal *C. trachomatis* load equal to or higher than the genital *C. trachomatis* load is likely clinically relevant. As can be seen in table 1, this would mean that 32 (56%) of clinically relevant anorectal infections are found in women without AI, compared with 38 (79%) in women with AI.

This is the largest study to date to investigate the *C. trachomatis* load in concurrently infected women to assess the correlation between the genital and anorectal *C. trachomatis* loads within one woman. We demonstrated that the median genital load was

higher than the median anorectal *C. trachomatis* load at the group level, with a similar range. At the individual level, the categorical distribution of the genital load was similar to that of the rectal load within the same woman, both in women with and without AI. The anorectal *C. trachomatis* load was in the same or higher load category as the genital load category in the majority of women, including women that did not report AI, assumably with similar clinical relevance.

This study describes a unique data set, in that women are rarely sampled at the genital and anorectal sites simultaneously irrespective of symptoms or sexual behaviour, and the *C. trachomatis* load is quantified even less frequently. Only one other study by Dubbink *et al* has quantified the genital and anorectal *C. trachomatis* loads within one woman, but no direct comparison was made.¹⁴ In this study, the median genital load was higher than the median anorectal load in concurrently infected women, which was similar to the results found in the present study.

Direct comparison of the *C. trachomatis* load in genital and anorectal swabs within one women is problematic, as both the *C. trachomatis* and human cell loads are differently distributed,²⁷ which indicates different sample types, despite their sharing similar epithelium (data not shown). By dividing the *C. trachomatis* load into three load categories (low/middle/high), we were able to compare the *C. trachomatis* loads in the same woman while evading the problem of directly comparing the different sample types. Another way to overcome sample-type differences in load is normalisation of the *C. trachomatis* load for a common (human) marker, but our previous study showed that the currently used markers (eg, HLA or β -globin) are unsuitable because they cannot distinguish epithelial from inflammatory cells, and the quantity of human cells is further influenced by behavioural factors such as AI.²⁷ The use of lysis buffer in the storage solution necessitates the use of molecular markers for cell type analyses, as microscopy cannot be performed on these samples.

We are aware that our research is limited due to the use of a convenience sample for practical reasons, as too few women with an anorectal only *C. trachomatis* infection ($n=6$) were tested at the STI clinic in the same time period to be used as a control group. This limitation highlights the difficulty of collecting data in the absence of routine anorectal testing. Furthermore, we did not assess (all) possible sources of contamination, such as the order of sampling or hygiene practices. For example, patients were not instructed about the order of vaginal and anorectal sampling, whereas swabbing the anus before the vagina might change the results as contamination might be reduced when doing it in this order. This was assessed more in-depth in a subsequent study.²⁸ An additional possible source of error is the use of qPCR for screening and quantification, which is much more sensitive than culture, allowing the quantification of low loads, however, it does not distinguish between viable and non-viable

C. trachomatis bacteria. Congruently, it has been demonstrated that clinical determinants are better correlated to culture results than nucleic acid amplification test-based results.¹⁷

This study demonstrated that the genital and anorectal loads are highly variable within one woman (eg, high genital load with a low anorectal load, and vice versa). To obtain best insight into the data, we have chosen to present the data in two different ways: absolute data and in categories. This latter presentation was chosen due to the different sample types, in which we feel that the load cannot be compared directly. Although no cut-off for clinical relevance is known, we believe that an anorectal *C. trachomatis* load equal to or higher than the genital *C. trachomatis* load is unlikely to be due to contamination or exposure, and is therefore likely clinically relevant. This implies that the majority of women without AI (56%) have a clinically relevant anorectal infection (compared with 79% in women with AI). In addition to the *C. trachomatis* load, other measurements can be taken into account to determine clinical relevance, such as leucocytes or bacterial viability,²⁹ to distinguish infection from contamination or exposure.

In short, we found that in concurrently infected women the genital *C. trachomatis* load was on average higher than the anorectal *C. trachomatis* load in the majority of the women, but the *C. trachomatis* load range was similar. The categorical distribution of the genital load was similar to that of the rectal load within the same woman, notwithstanding self-reported AI. The anorectal *C. trachomatis* load appears to indicate a clinically relevant infection in the majority of women, including 56% of women without self-reported AI. Further studies are needed to establish a *C. trachomatis* load cut-off for clinical relevance, and to investigate the correlation to clinical disease and treatment necessity.

Key messages

- *Chlamydia trachomatis* bacterial load cannot be directly compared between genital and anorectal swabs because the samples are too different.
- At the group level, the median *C. trachomatis* load was higher in genital than anorectal samples, but both loads were within the same range.
- At the individual level, the categorical distribution of the genital and anorectal loads was similar to that of the rectal load within the same woman.

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