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Analyses of multiple-site and concurrent *Chlamydia trachomatis* serovar infections, and serovar tissue tropism for urogenital versus rectal specimens in male and female patients

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ABSTRACT

Objectives The aims of this study were: to determine the incidence of concurrent infections on a serovar level; to determine the incidence of multiple anatomical infected sites on a detection and genotyping level and analyse site-specific serovar distribution; to identify tissue tropism in urogenital versus rectal specimens.

Methods *Chlamydia trachomatis*-infected patients in two populations were analysed: 75 visiting the outpatient department of obstetrics and gynaecology of the MC Haaglanden, and 358 visiting the outpatient sexually transmitted disease clinic, The Hague, The Netherlands. The PACE 2 assay (Gen-Probe) was used to detect *C trachomatis* from urethral, cervical, vaginal, oropharyngeal and anorectal swabs. *C trachomatis* genotyping was performed on all *C trachomatis* positive samples, using the CT-DT genotyping assay.

Results Samples from 433 patients (256 female and 177 male) with confirmed *C trachomatis* infection were analysed. In 11 patients (2.6%), concurrent serovars in one anatomical sample site were present. In 62 (34.1%) female and four (9.3%) male patients, multiple sample site infections were found. A substantial percentage of women tested at the cervical/vaginal and rectal site were found to be positive at both sites (36.1%, 22/61). In men, D/Da and G/Ga serovars were more prevalent in rectal than urogenital specimens ($p=0.0081$ and $p=0.0033$, respectively), while serovar E was more prevalent in urogenital specimens ($p=0.0012$).

Conclusions The prevalence of multiple serovar infections is relatively low. Significant differences in serovar distribution are found in rectal specimens from men, with serovar G/Ga being the most prominent, suggesting tissue tropism.

INTRODUCTION

Chlamydia trachomatis is the most prevalent bacterial sexually transmitted disease (STD) worldwide. Many *C trachomatis* infections are asymptomatic[1] and, if undiagnosed and untreated, provide a reservoir for the disease and long-term complications. The most common sample types are cervical, urethral and vaginal swabs, and first-void urine (FVU). Depending on sexual behaviour, rectal and pharyngeal swabs can also be taken.

Nineteen *C trachomatis* serovars have been identified causing different types of infection: A–C cause ocular infections, D–K anogenital infections, and the serovars L1–L3 cause the disease lymphogranuloma venereum.[2–4] On the basis of similarities in the major outer membrane protein, the serovars can be divided into three serogroups: the B group (serovars B, Ba, D, Da, E, L1, L2 and L2a); the intermediate (I) group (serovars F, G and Ga); and the C group (serovars I, Ia, J, K, C, A, H and L3). The most prevalent *C trachomatis* strains worldwide are serovars D, E and F, accounting for ~70% of the typed urogenital serovars.[4–8] Serovar identification is clinically important, because, for example, the lymphogranuloma venereum serovars need different treatment from the other ano-urogenital serovars D–K.[9–11]

Most of the previous studies on *C trachomatis* serovar distribution focused on one anatomical site, usually the urogenital tract. However, when there is a preference of specific serovars for specific sample sites—that is, urogenital versus rectal—serovar distributions may differ. Studies have reported 2–15% of multiple serovar infections in one anatomical site and widespread concurrent anatomical site infection.[5–7 12–14] Lan *et al* found 5/37 women with a single identical serovar infection in multiple sample sites and 2/37 women with different serovars in multiple sample sites. No double infections were found in men.[15] It has been suggested that the prevalence of infection varies by anatomical site, and that serovar G/Ga more commonly infects the rectum, whereas others are more common in the cervix/vagina.[16–18] As there is limited information on this subject, the present study had three aims: to determine the number of concurrent infections on a serovar level; to determine the percentage of multiple anatomical infected sites on a detection and genotyping level and analyse site-specific serovar distribution; to identify tissue tropism in urogenital versus rectal specimens.

METHODS

Specimen collection

From January to October 2008, 433 *C trachomatis*-infected patients in two populations were analysed: 75 (female) patients visiting the outpatient department of obstetrics and gynaecology (OPD O&G) of the MC Haaglanden, and 358 patients (177 male and 181 female) visiting the outpatient STD clinic in the centre of The Hague, The Netherlands.

1. OPD O&G, MC Haaglanden, The Hague. MC Haaglanden is an inner city hospital with patients of various ethnic origin. Patients visit the OPD O&G for various reasons, eg, pregnancy, discharge, menstrual disorders, subfertility, contraception. If required, cervical and urethral swabs are taken.
2. STD clinic, The Hague. Patients could be attending because of symptoms, because they were warned by an infected partner, or for a general check-up. In women, cervical or vaginal swabs are taken, and in some women urethral swabs or FVU. In men, urethral swabs or FVU are collected. In men who have sex with men (MSM) (n=46), anorectal and oropharyngeal swabs are taken as well. In women, these swabs are taken when oral or anal sex is reported.

In both clinics, information was collected on age, gender (STD clinic only, as OPD O&G all female), age and ethnicity. All patient and sample data were anonymised by each centre and analysed according to local ethics regulations.

Detection of *C trachomatis*

For the detection of *C trachomatis*, we used a probe hybridisation assay on urethral, cervical, vaginal, pharyngeal and rectal swabs (PACE 2 assay; Gen-Probe, San Diego, USA). Swabs were analysed within 24 h according to Gen-Probe's packet insert instructions. For urine analysis, we used amplification of *C trachomatis* rRNA by transcription-mediated amplification in urine samples with the Gen-Probe AMP *C trachomatis* assay. Urine specimens were collected before swab specimens were gathered and stored at +4°C. The urine was analysed on a weekly basis according to Gen-Probe procedures.

Amplification, detection and genotyping using the CT-DT assay

The CT-DT amplification and genotyping assay was performed on all previously determined *C trachomatis* positive samples according to the manufacturer's instructions (Labo Biomedical Products BV, Voorburg, The Netherlands). The CT-DT genotyping assay is a reverse hybridisation probe line blot with a probe for detecting the endogenous plasmid and probes to detect the three different *C trachomatis* serogroups (B, C and intermediate) and the 14 major serovars (A, B/Ba, C, D/Da, E, F, G/Ga, H, I/Ia, J, K, L1, L2/L2a and L3).[19]

Statistical analysis

Serogroup and serovar distributions were compared using χ^2 and Fisher exact statistics. $p<0.05$ was considered significant.

RESULTS

During the study period, samples from 433 patients (256 female and 177 male) were collected sequentially and used for *C trachomatis* serovar and typing. Three male patients were excluded because of gender and sample site mismatch. For analysis, we used 430 patients (75 OPD O&G, 181 STD female and 174 STD male).

There were no significant differences in age between patients visiting the OPD O&G or the STD clinic (OPD O&G: median 25, range 15–47; STD female: median 24, range 15–72; STD male: median 26, range 17–72).

Concurrent serovar infections per sampling site

Concurrent serovar infections at one sample site were found in 2.6% of the patients (11/430) (table 1). In the OPD O&G, the prevalence was 5.3% (4/75), and in the STD clinic it was 2.0% (2.2% (4/181) in female and 1.7% (3/174) in male patients). Eight of these 11 patients were infected with serovars E, F or G/Ga. Nine patients had serovars from different serogroups. Three patients had different serovars from the same serogroup. In four patients, it was only possible to identify the serogroup, but not the serovar.

Table 1 Distribution of concurrent *Chlamydia trachomatis* serovars per sampling site

Patients (n=11)	Cervix	Vagina	Urethra	Rectum	Urine
OPD O&G					
Female	I group (G/Ga)				
	C group (?)				
Female	C group (H)				
	C group (K)				
Female	B group (E)				
	C group (?)				
Female	B group (D/Da)				
	C group (?)				
STD clinic					
Female		I group (F)			
		C group (?)			
Female		B group (E)			
		C group (J)			
Female		C group (H)			
		C group (K)			
Female		B group (E)			
		C group (J)			
Male				I group (F)	
				I group (G/Ga)	
Male			B group (E)		
			C group (K)		
Male					B group (E)
					I group (F)

Serovars presented as serogroup with serovar.
OPD O&G, outpatient department of obstetrics and gynaecology; STD, sexually transmitted disease; ?, unknown/untypeable serovar (possible explanations are given in the discussion).

Table 2 Multiple-site infections (DNA probe (PACE 2) positive) in male and female patients tested at multiple sample sites

Sample site	Female, tested (n)*	All sites positive (n)	%	Male, tested (n)†	All sites positive (n)	%
Cervix/vagina + urethra	75	43	57.3	–	–	–
Cervix/vagina + rectum	29	19	65.5	–	–	–
Cervix/vagina + pharynx	139	7	5.0	–	–	–
Cervix/vagina + rectum + pharynx	31	2	6.5	–	–	–
Cervix/vagina + urethra + rectum	1	1	100	–	–	–
Urethra/urine + rectum	–	–	–	39	5	12.8
Urethra/urine + pharynx	–	–	–	41	1	2.4
Rectum + pharynx	–	–	–	38	2	5.3
Urethra/urine + rectum + pharynx	–	–	–	39	1	2.6

This table shows combinations of sample sites. Some patients were tested at more sites than the two mentioned, but the third site was then always negative; eg, female cervix/vagina + rectum, n=32 tested, but 31 were also pharynx tested and one urethra.
*All but one female patient (tested urine only) were tested on the cervical/vaginal sample site.
†All but one male patient (tested at pharyngeal site only) were tested at the urethral site/urine.

Multiple sample site infections on a *C trachomatis* detection and serovar level

For the analysis of multiple site infections, the 11 patients with concurrent serovar infections were excluded, as well as one patient with different serovars at different sites. The DNA probe (PACE 2) results of tested sample sites are shown in table 2. In our OPD O&G population, all patients (n=71) were tested at both the cervical and urethral sampling sites. Twenty-seven were positive at the cervical sampling site only, 38 were positive at both sites, and six were positive at the urethral sampling site only.

In the STD clinic population, several patients were only tested at one sample site.

In the female STD clinic population (n=177), 168 patients were positive at the cervical or vaginal sampling sites (in combination with zero, one or two other sample sites). The remaining nine patients had a single-site infection: five were positive at the rectal site, three at the pharyngeal site, and one in the urine analysis. In 19.8% (n=35) of the patients, only one sample was taken (27 vagina only, seven cervix only, and one urine only). A substantial percentage of women tested at the cervical/vaginal and rectal site were found positive at both sites (36.1%, 22/61).

In the male STD population (n=170, of which 44 were MSM), 146 were positive at the urethral sampling site or in urine analysis (in combination with zero, one or two other sample sites). The remaining 24 patients had a single-site infection; 20 were positive at the rectal site, two at the pharyngeal site, and two at both rectal and pharyngeal sites. In 74.7% (n=127) of the patients, only one sample was taken (98 urine only, 28 urethra only, and one pharynx only).

Serovars could not be determined in 19 DNA probe-positive patients (in six patients only the serogroup was available, and 13 *C trachomatis*-positive rectal samples were not available for serovar determination).

In the remaining 484 samples used for serovar determination, multiple sample site infections were observed in 34.1% (62/182) of female (38 from the OPD O&G) and 9.3% (4/43) of male patients. In the female patient group, 43 were positive at the cervix/vagina and urethra, 10 at the cervix/vagina and rectum, eight at the cervix/vagina and pharynx, and one at the vagina, rectum and pharynx. In the male patient group, three patients were positive at the rectum and pharynx, and one at the urethra and pharynx. In all but one patient (male, rectum and pharynx), the same serovars were observed.

Single serovar and anatomical sites

Overall, serovars D, E and F were the most prevalent in 87 cervical samples (12.6% (n=11), 42.5% (n=37), and 25.3% (n=22), respectively) and 86 urethral samples (11.6% (n=10), 41.9% (n=36) and 22.1% (n=19), respectively). In the other sites, serovar G/Ga was the third most prevalent serovar (142 vaginal samples: E, 34.5% (n=49); F, 23.9% (n=34); G/Ga, 16.2% (n=23). 105 urine samples: E, 41.9% (n=44); F, 21.9% (n=23); G/Ga, 12.4% (n=13). 17 oropharyngeal samples: D/Da, 29.4% (n=5); E, 41.2% (n=7); G/Ga, 11.8% (n=2). 47 rectal samples: D/Da, 27.7% (n=13); E, 21.3% (n=10); G/Ga, 34.0% (n=16)). In all sample sites (except the rectum), serovar E was the most prevalent.

When the serovar distribution between rectal and urogenital specimens was compared, no differences for women were observed (figure 1).

In women, 16 serovars D/Da were identified among 167 urogenital (cervix and vagina) specimens (9.6%), while, in rectal specimens, in three out of nine (33.3%) serovars D/Da were observed. Although in rectal specimens, the percentage of serovars D/Da was three times the percentage in urogenital specimens, this difference was only borderline significant, possibly because of the low sample size of rectal specimens (p=0.0592).

In men, significant differences were found for serovars D/Da, E and G/Ga between 25 rectal and 140 urogenital (urethra and urine) specimens. Serovar D/Da was identified in 28% (n=7) of the rectal specimens versus 7.9% (n=11) of the urogenital specimens (p=0.0081; OR 4.6, 95% CI 1.6 to 13.3). For serovar E, we found 40.7% (n=57) in urogenital specimens and 8% (n=2) in rectal specimens (p=0.0012; OR 7.8, 95% CI 1.8 to 35). Of the male rectal specimens 10 contained serovar G/Ga (40%), whereas 19 urogenital specimens (13.6%) contained serovar G/Ga, identical with the percentage found in women (p=0.0033; OR 4.2, 95% CI 1.7 to 11).

All 25 men from whom rectal samples were taken were MSM. Serovar G/Ga was significantly more prevalent in this group, followed by serovar D/Da. Fourteen of the 140 men in the group from which urogenital specimens were obtained were MSM. In these 14 men, serovar D/Da was most prevalent (n=6, 42.9%) followed by serovar J (n=4, 28.6%), serovar G/Ga (n=3, 21.4%) and serovar F (n=1, 7.1%).

DISCUSSION

Overall, the prevalence of multiple serovar infections is relatively low. Significant differences in serovar distribution (D/Da, E and G/Ga) are found when comparing anatomical sites (rectal versus urogenital) in men, with the same trend observed in women for serovar D/Da, suggesting tissue tropism.

Concurrent serovar infections per sampling site

A prevalence of concurrent serovar infections at the same sample site (2.6%) in the same (low) range as detected in other studies

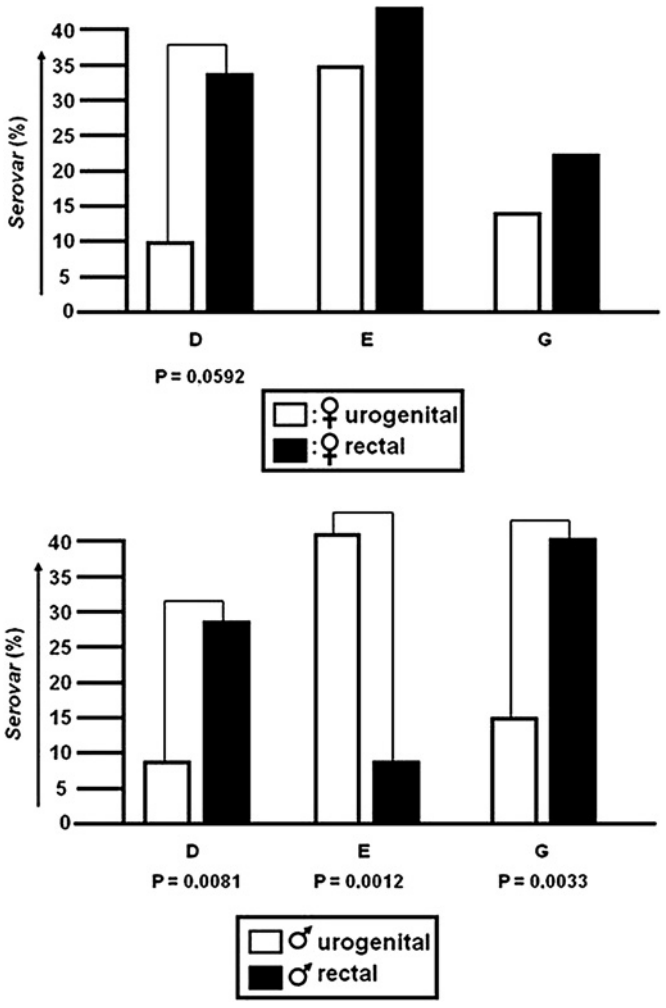

Figure 1 Distribution of serovars D/Da, E and G/Ga in urogenital versus rectal specimens from female and male patients.

was found.[5–7 12–14] Barnes *et al* describe seven (2%) multiple serovars: three (1.4%) of 213 cervical swabs of women visiting a STD clinic, three (10%) of cervical swabs of jailed women (mostly prostitutes), and one (0.9%) of 109 rectal swabs of MSM attending a STD clinic.[20] In the present study, none of the women with multiple serovars were engaged in prostitution. One of the three men with multiple serovars was bisexual, and the remaining two were heterosexual.

In six patients, it was only possible to identify the serogroup (C), but not the serovar. This is probably due to a new genovariant of the serovar which does not respond to the specific serovar probe, but does respond to the more conservative serogroup probe. Also, an infection with multiple serovars within the same serogroup can be caused by a new genovariant. For example, a new genovariant of serovar K was revealed by sequencing potential multiple infections of serovar H&K (both serogroup C) in women visiting a women's health clinic in Uganda.[19] This leads to the recommendation to sequence multiple infections belonging to the same serogroup, to exclude new variants (not performed in the present study).

Infections in multiple sample sites

In 72 of 213 (33.8%) female patients from whom multiple samples were taken, DNA probe (PACE 2) results were positive at more than one site. In nine of 43 (20.9%) male patients from whom multiple samples were taken, DNA probe (PACE 2) results were positive at more than one site. All male patients were MSM. Kent *et al* reported multiple site infections in 48 (10.5%) patients in a population of MSM who had been tested at three sites (rectum, urethra and pharynx). In MSM who had only been tested at the urethral and pharyngeal site, only two (1.6%) were found positive at two sites, possibly caused by a lower *C trachomatis* prevalence (7.1% in MSM tested at the urethra and pharynx versus 13.3% in MSM tested at the rectum as well).[16]

In 66 patients, serovars at multiple sample sites were positive. Since in all but one patient the same serovar was found, serovar analysis at one sample site seems justifiable, although *C trachomatis* detection remains necessary for all sample sites.

In female patients, cervix or vagina sampling revealed 94.9% of the *C trachomatis*-infected patients. Sexual behaviour questionnaires helped to reveal the other sample sites (rectum and oropharynx). In male patients, urethral site sampling or urine revealed 85.9% of the *C trachomatis*-infected patients. In MSM, rectal samples were taken as well, to detect more patients, since the proctum can be a reservoir of *C trachomatis* infections. The pharyngeal sampling site does not seem to contribute much to the detection of *C trachomatis*-infected patients.

Single serovar and anatomical sites

In our study, we found serovar G/Ga to be the third most prevalent serovar after D and E, at most sampling sites. The prevalence of serovar G/Ga was the lowest (5.7%) at the cervical sampling site in our study. Similar to our results, Lan *et al* found serovar G to be the third most prevalent serovar in young women visiting an OPD O&G.[19 21] In some Asian countries, higher prevalences of serovar G are observed (7–15%).[22] These prevalences are observed mostly in STD clinic populations, but also in obstetrical and gynaecological patients (14.9%).

In men, serovars D/Da and G/Ga were significantly more prevalent in rectal than urogenital swabs (28% vs 7.9% and 40% vs 13.6%). In women, a similar tendency was observed for these serovars, although the differences were not significant (33.3% vs 9.6% and 22.2% vs 13.8%). In men, serovar E was more prevalent in the urogenital swabs than in the rectal swabs (40.7% vs 8%), whereas in women it was approximately the same (35.3% vs 44.4%), and in the normal range compared with other Dutch studies. Serovars B/Ba, H, I/Ia and K were not detected in rectal swabs from either men or women. In women, serovars J and F were not found in rectal swabs.

In rectal specimens from female patients, serovar E was most commonly detected.

Similar results were described by Barnes *et al*.[18] The rectal swabs were obtained from MSM. They also tested rectal swabs from 32 women and found two B serovars, one I/Ia and one K. In our study, these serovars were not identified in men or women. It suggests that these serovars are less viable in the rectum. Permeability to toxic substances may be influenced by the porin activity of the major outer membrane protein, therefore the serotype may reflect organism permeability.[18 23]

We have two explanations for the prevalence differences between rectal and urogenital specimens, which are not mutually exclusive: (1) serovars G/Ga and D/Da have a higher affinity for the epithelial cells of the rectum than for urogenital epithelial cells, potentially partially mediated by the environment, suggesting tissue tropism, still based on unknown virulence factors; (2) the high incidence of serovars G/Ga and D/Da in rectal specimens from MSM may originate from differences in MSM sexual behaviour and group dynamics compared with heterosexuals. However, as the same trend was found in the

Key messages

- The prevalence of concurrent or multiple serovar infections is low.
- Significant differences in serovar distribution are found between rectal and urogenital specimens in male patients, suggesting tissue tropism.
- Serovar analysis can be performed on one positive sample site.

heterosexual women included in our study, serovar distribution linked to core groups is less likely as an explanation. Other studies have found similar results; most rectal chlamydia infections were caused by serovar G/Ga (47.9%) in MSM, while in the same population the prevalence of urogenital serovar G/Ga for men and women was much lower (16% vs 11%, respectively).[17 24] In San Francisco, rectal specimens from MSM were tested in two populations.[16] The prevalence of *C trachomatis* infection was 8.8% and 5.7% in patients visiting a STD clinic and a gay men's health centre, respectively. Unfortunately, no serovar analysis was performed. Barnes *et al* report significantly higher prevalences of serovar G/Ga in cervical isolates from heterosexual women and rectal isolates from MSM.[18] The prevalence of serovar G/Ga (13%) in the rectal isolates was, however, significantly lower than in our study (40%) (p=0.0026).

Recently, Jeffrey *et al* demonstrated that polymorphisms in open reading frame sequences have a correlation with different tissue tropisms of serovars. Genome sequence analysis is an effective approach for discovering variable loci in *Chlamydiae* that are associated with clinical presentation.[25]

In conclusion, the prevalence of multiple serovar infections at different sites of the same patient is relatively low. Therefore serovar analysis could be performed on one positive sample site. Significant differences in serovar prevalence are found between rectal and urogenital specimens in men. The serovar distribution in rectal specimens from MSM showed significant differences, with serovar G/Ga being the most prominent.

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Competing interests None.

Contributors CJB, wrote manuscript, collected data and samples at the obstetrics and gynaecology department; KDQ, wrote manuscript, developed serovar typing technique; RPHP, collected data and samples at STD clinic, read manuscript; SO, built database, performed statistical analysis, read manuscript; PMO, supervised data and sample collection, read manuscript; JAEMM, supervised sample collection/storage; PJD, read manuscript; SS, organised sample storage; CJ, supervised sample collection/storage; APvL, supervised data and sample collection at STD clinic; WGVQ, developed serovar typing technique; JBT, read manuscript; CJLMM, read manuscript; SAM, supervised study, collected data, read manuscript (guarantor of study). All authors had access to all data in the study.

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