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Published in:
Sexually Transmitted Infections

DOI:
10.1136/sti.2010.048173

Citation for published version (APA):

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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 asymptomatic1 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 Ia, J, K, C, A, H and L5). 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 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 serovar infections 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 C/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, e.g., 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/1a, J, K, L1, L2/L2a and L3).19

Statistical analysis

Serogroup and serovar distributions were compared using χ² 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>
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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).

Sex Transm Infect 2011; 87:503—507. doi:10.1136/sti.2010.048173
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, 58 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 (56.1%, 22/41).

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=57), and 25.3% (n=22), respectively) and 86 urethral samples (11.6% (n=10), 41.9% (n=56) and 22.1% (n=19), respectively). In the other sites, serovar G/Ga was the third most prevalent serovar (142 vaginal samples: E, 54.5% (n=49); F, 29.3% (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 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 15.3). 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 (15.6%) contained serovar G/Ga, identical with the percentage found in women (p=0.0058; 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 F (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
Infectious diseases in multiple sample sites

Infections in multiple sample sites

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

The prevalence of urogenital serovar G/Ga for men and women was much lower (16% vs 11%, respectively). In San Francisco, rectal specimens from MSM were tested in two populations. 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. 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 Chlamydia that are associated with clinical presentation.

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.

The aims of this work are in part line with the European EpiGenChlamydia Consortium which is supported by the European Commission within the Sixth Framework Program through contract No LSHC-CT-2007-037637. See http://www.EpiGenChlamydia.eu for more details about this consortium.

Contributors CJB, wrote manuscript, collected data and samples at the obstetrics and gynaecology department; KDO, wrote manuscript, developed serovar typing technique; RPWF, collected data and samples at STD clinic, read manuscript; SO, built and gynaecology department; KDQ, wrote manuscript, developed serovar typing technique; RPHP, collected data and samples at STD clinic, read manuscript; SO, built and gynaecology department; KDQ, wrote manuscript, developed serovar typing technique; JBT, read manuscript; CJ, supervised sample collection, read manuscript; SAA, supervised study, collected data, read manuscript (guarantor of study). All authors had access to all data in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

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*Sex Transm Infect* 2011 87: 503-507 originally published online August 19, 2011
doi: 10.1136/sti.2010.048173

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