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 the current study were to determine the incidence of concurrent infections on a serovar level, to determine the incidence of multiple anatomical infected sites on detection and genotyping level, and analysis of site specific serovar distribution, to identify tissue tropism in urogenital vs. rectal specimens.

Methods: *Chlamydia trachomatis* (CT) infected patients in two populations were analysed in this study: 75 patients visiting the outpatient department of Obstetrics and Gynaecology of the MC Haaglanden and 358 patients visiting the outpatient STD clinic, The Hague, the Netherlands. The PACE 2 assay (Gen-Probe) was used for detection of CT from urethral, cervical, vaginal, oropharyngeal, and anorectal swabs. CT genotyping was determined on all CT positive samples, using the CT-DT genotyping assay.

Results: Samples of 433 patients (256 female and 177 male) with confirmed CT 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 considerable percentage of women tested on the cervical/vaginal and rectal site were found positive on both sites (36.1%, 22 out of 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 relative low. Significant differences in serovar distribution are found in rectal specimens of men with serovar G/Ga as the most prominent, suggesting tissue tropism.

Introduction

*Chlamydia trachomatis* (CT) is the most prevalent bacterial sexually transmitted disease (STD) worldwide. Many CT infections are asymptomatic [1] and, if undiagnosed and untreated, they 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. Depending on sexual behaviour rectal and pharyngeal swabs can also be taken.

Nineteen CT serovars have been identified causing different types of infections; A-C cause ocular infections, D-K anogenital infections, and the serovars L1-L3 cause the disease lymphogranuloma venereum (LGV).[2-4] Based on similarities on the major outer membrane protein (MOMP), the serovars can be divided into three serogroups, namely the B-group (serovars B, Ba, D, Da, E, L1, L2, and L2a), the intermediate group (serovars F, G, and Ga), and the C-group (serovars I, Ia, J, K, C, A, H, and L3). The most prevalent CT strains worldwide are serovars D, E, and F, accounting for approximately 70% of the typed urogenital serovars.[4-8] Serovar identification is clinically important since, for example, the LGV serovars needs different treatment than the other ano-urogenital serovars D-K.[9-11]

Most of the previous studies on CT serovar distribution focused on one anatomical site, usually the urogenital tract. However, when there is a preference of specific serovars for specific sample sites, i.e. urogenital vs. rectal, serovar distributions might differ. Studies have reported 2-15% multiple serovar infections in one anatomical site and widespread percentages of 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 infects more common the rectum, while others are more common in the cervix/vagina.[16-18] Since there is limited information on this subject, the current study has three aims: to determine the number of concurrent infections on a serovar level, to determine the percentage of multiple anatomical infected sites on detection and genotyping level, and analysis of site specific serovar distribution, to identify tissue tropism in urogenital vs. rectal specimens.
Methods

Specimen collection
From January - October 2008, 433 CT infected patients in two populations were analysed in this study: 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.
i) OPD Obstetrics and Gynaecology, MC Haaglanden, The Hague;
MC Haaglanden is an inner city hospital with patients from various ethnic origins. Patients visited the OPD O&G for various reasons, for example pregnancy, discharge, menstrual disorders, subfertility, contraception etc. If required, cervical and urethral swabs were taken.
ii) STD clinic, The Hague;
Patients could be visiting because of complaints, because they were warned by an infected partner, or for general check-up. In women cervical or vaginal swabs taken, and in some women urethral swabs or first-void urine (FVU). In men, urethral swabs or FVU was collected. In men-who-have-sex-with-men (MSM) (n=46) anorectal and oropharyngeal swabs were taken as well. In women these swabs were taken when oral or anal sex was reported.
In both clinics information was collected concerning age, gender (STD clinic only, OPD all female), age and ethnicity. All patient and sample data were anonymised by each center and analysed according to local ethical regulations.

CT detection
For the detection of CT we used a probe hybridisation assay on urethral, cervical, vaginal, pharyngeal, and rectal swabs (PACE 2 assay, Gen-Probe). Swabs were analysed within 24 hours according to Gen-Probe’s packet insert instructions. For urine analysis we used amplification of CT-rRNA by transcription-mediated amplification (TMA) in urine samples with the Gen-Probe AMP CT 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 previous determined CT positive samples according to the manufacturer’s instructions (Labo Biomedical Products BV, Voorburg, The Netherlands). The CT-DT genotyping assay is a reverse hybridization probe line blot (RHA) with a probe for the detection of the endogenous plasmid, and probes to detect the 3 different CT 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’s Exact statistics. A p-value < 0.05 was considered as statistically significant.

Results
During the study period samples of 433 patients (256 female and 177 male) were collected sequentially and were used for CT 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
In 2.6% of the patients (11/430) concurrent serovar infections in one sample site were found (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 eleven patients were infected with the serovars E, F, or serovar 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.

Multiple sample site infections on a CT detection and serovar level
For the analysis of multiple site infections the 11 patients with concurrent serovar infections were excluded, as well as one patients with different serovars on different sites. The DNA probe (PACE2) 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 patients were positive on the cervical sampling site only, 38 were positive on both sites, and six patients were positive on the urethral sampling site only.
In the STD clinic population several patients were only tested on one sample site.
In the male STD population (n=170, of which 44 MSM), 146 were positive on the urethral sampling site or in urine analysis. Of the remaining 24 patients, 20 are positive on the rectal site, two on the pharyngeal site, and two on both rectal and pharyngeal site. 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 all DNA probe positive patients. Also 13 CT positive rectal samples were not available for serovar determination.

In the female STD clinic population (n=177), 168 patients were positive on the cervical or vaginal sampling sites. Of the remaining nine patients, five were positive on the rectal site, three on the pharyngeal site, and one in 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 considerable percentage of women tested on the cervical/vaginal and rectal site were found positive on both sites (36.1%, 22 out of 61).

Serovars could not be determined in all DNA probe positive patients. Also 13 CT positive rectal samples were not available for serovar determination.

In the remaining 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 patients were positive on the cervix/vagina and urethra, ten on the cervix/vagina and

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**Table 1** The distribution of concurrent CT serovars per sampling site.

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Patients (n=11)</th>
<th>Cervix (n=87)</th>
<th>Vagina (n=142)</th>
<th>Urethra (n=86)</th>
<th>Rectum (n=87)</th>
<th>Urine (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD male</td>
<td>M</td>
<td>l-group (F)</td>
<td>l-group (G/Ga)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>B-group (E)</td>
<td>C-group (K)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>B-group (E)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD female</td>
<td>F</td>
<td>l-group (F)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C-group (E)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C-group (H)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>B-group (E)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPD O&amp;G</td>
<td>F</td>
<td>l-group (G/Ga)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C-group (H)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>B-group (E)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>B-group (D/ Da)</td>
<td>C-group (J)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M=male, F=female; Serovars presented as Serogroup with Serovar; ?=unknown/untypeable serovar.

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**Table 2** Multiple site infections (DNA probe (PACE2) positive) in male and female patients tested at multiple sample sites.

<table>
<thead>
<tr>
<th>Sample sites</th>
<th>Female* Tested (n)</th>
<th>Positive (n)</th>
<th>%</th>
<th>Male† Tested (n)</th>
<th>Positive (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix/Vagina + Urethra</td>
<td>75</td>
<td>43</td>
<td>57,3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cervix/Vagina + Rectum</td>
<td>29</td>
<td>19</td>
<td>65,5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cervix/Vagina + Pharynx</td>
<td>139</td>
<td>7</td>
<td>5,0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cervix/Vagina + Rectum + Pharynx</td>
<td>31</td>
<td>2</td>
<td>6,5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cervix/Vagina + Urethra + Rectum</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urethra/Urine + Rectum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39</td>
<td>5</td>
<td>12,8</td>
</tr>
<tr>
<td>Urethra/Urine + Pharynx</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41</td>
<td>1</td>
<td>2,4</td>
</tr>
<tr>
<td>Rectum + Pharynx</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
<td>2</td>
<td>5,3</td>
</tr>
<tr>
<td>Urethra/Urine + Rectum + Pharynx</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39</td>
<td>1</td>
<td>2,6</td>
</tr>
</tbody>
</table>

* 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. 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. i.e. female cx/vag + rc n= 32 tested, but 31 were also pharynx tested and 1 urethra.
rectum, eight on the cervix/vagina and pharynx, and one patient on the vagina, rectum, and pharynx. In the male patient group three patients were positive on the rectum and pharynx, and one patient on the urethra and pharynx. In all but one patient (male, rectum and pharynx) the same serovars were observed.

**Single serovar and anatomical sites**

Overall, the 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 (134 vaginal samples: E 34.5% (n=49), F 23.9% (n=34), and G/Ga 16.2% (n=23); 105 urine samples: E 41.9% (n=44), F 21.9% (n=23), and G/Ga 12.4% (n=13); 17 oropharyngal samples: D/Da 29.4% (n=5), E 41.2% (n=7), and G/Ga 11.8% (n=2); and 47 rectal samples: D/Da 27.7% (n=13), E 21.3% (n=10), and G/Ga 34.0% (n=16)). In all sample sites (except 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 due to the low sample size of rectal infections (p=0.0592).

In men, significant differences were found for the serovars D/Da, E, and G/Ga between 25 rectal and 140 urogenital (urethra and urine) specimens. In 28% (n=7) of the rectal specimens serovar D/Da was identified versus 79% (n=11) in urogenital specimens (p=0.0081; OR 4.6, 95% CI 1.6-13.3). 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 1.8-35). In the male rectal specimens 10 contained serovar G/Ga (40%), while 19 urogenital specimens (13.6%) contained serovar G/Ga identical to the percentage found in women (p=0.0033; OR 4.2, 95% CI 1.7-11).

All 25 men of which rectal samples were taken were men-who-have-sex-with-men (MSM). Serovar G/Ga was significantly more prevalent in this group, followed by serovar D/Da. Fourteen out of the 140 males were MSM in the group from which urogenital specimens were obtained. 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 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 prostitute), and one (0.9%) of 109 rectal swabs of MSM attending a STD clinic.[20] In the current study none of the women with multiple serovars were engaged in prostitution. One of the three men with multiple serovars was bisexual, 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 current study).

Multiple sample site infections
In 72 out of 213 (33.8%) of the female patients in which multiple samples were taken, DNA probe (PACE 2) results were positive on more than one site. In nine out of 43 (20.9%) of the male patients in which multiple samples were taken, DNA probe (PACE 2) results were positive on more than one site. All male patients were MSM. Kent et al describe multiple site infections in 48 (10.5%) patients in a population of MSM who have 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 CT prevalence (7.1% in MSM tested at urethra and pharynx vs. 13.3% in MSM tested at 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, serovars analysis at one sample site seems to justifiable, although CT detection remains necessary for all sample sites. In female patients, cervix or vagina sampling revealed 94.9% of the CT infected patients. Sexual behaviour questionnaires help to reveal the other sample sites (rectum and oropharynx). In male patients, urethral site sampling or urine revealed 85.9% of the CT infected patients. In MSM rectal samples are taken as well, to detect more patients, since the proctum can be a reservoir of CT infections. The pharyngeal sampling site does not seem to contribute much to the detection of CT 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, in 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 as the third most prevalent serovar in young women visiting an OPD of Obstetrics and Gynaecology.[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%).

In men, serovars D/Da and G/Ga were significantly more prevalent in rectal than in urogenital swabs (28% vs. 7.9% and 40% vs. 13.6%). In women a similar tendency was observed for these serovars, although 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%), while in women it was approximately the same (35.3% vs. 44.4%), and in normal range as compared to other Dutch studies. The serovars B/Ba, H, I/Ia, and K were not detected in the rectal swabs in both men and women. In women serovar J and F were not found in rectal swabs.

In female rectal specimens serovar E was most frequently detected. Similar results were described by Barnes et al. The rectal swabs were obtained from MSM. They also tested the rectal swabs of 32 women and found two serovars B, one I/Ia, and one K. In our study those serovars were not identified in men or women. It is suggested that these serovars are less viable in the rectum. The permeability to toxic substances could be influenced by the porin activity of the major outer membrane protein (MOMP), therefore serotype might reflect organism permeability.[18, 23]

Two explanations for the prevalence differences between rectal and urogenital specimens, not mutually exclusive are present: 1) serovars G/Ga and D/Da have a higher affinity to epithelial cells of the rectum compared to urogenital epithelial cells, potentially partially mediated by the environment, suggesting tissue tropism, still based on unknown virulence factors, and 2) the high incidence of serovars G/Ga and D/Da in rectal specimens of MSM can find its origin in differences in sexual behaviour and group dynamics compared to heterosexuals. However, since in the heterosexual women included in our study the same trend was found, serovar distribution linked to core groups is
less likely as an explanation. Other studies find 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% resp.).[17, 24] In San Francisco, rectal specimens of MSM were tested in two populations.[16] The prevalence of CT infections 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. describe significant higher prevalences of serovar G/Ga in cervical isolates of heterosexual women and rectal isolates of MSM.[18] The prevalence of serovar G/Ga (13%) in the rectal isolates is 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 to discover 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 individual is relatively low. Therefore serovar analysis could be performed on one positive sample site. Significant differences in serovar prevalences are found between rectal and urogenital specimens in men. The serovar distribution in rectal specimens of MSM showed significant differences, with serovar G/Ga as the most prominent.

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References