

CHLAMYDIA TRACHOMATIS SEROVAR DISTRIBUTIONS IN RUSSIAN MEN AND WOMEN: A COMPARISON WITH DUTCH SEROVAR DISTRIBUTIONS

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SUMMARY

The data on serovar distributions of *Chlamydia trachomatis* – the most diagnosed sexually transmitted infection (STI) worldwide – are important for epidemiologic purposes and transmission studies but are completely lacking in Russia. The aim of the current study is to determine the serogroup and serovar distributions in Russian men and women and compare these data with Dutch serogroup and serovar distributions. In Russian men and women, serogroup B was the most prevalent (46%), followed by the intermediate serogroup (I group; 33%) and serogroup C (21%). The distribution was comparable between men and women. The serogroup distribution was similar to the previously published distribution in Dutch cohorts. However, on a serovar level statistically very significant differences were observed, reaching up to $P < 0.0001$. The serovars B and G/Ga had higher prevalences compared with the reported Dutch prevalences, while serovars F, H, I/la, J and K had lower prevalences compared with the Dutch studies. In conclusion, this is the first report of Russian *C. trachomatis* serovar/serogroup distributions. Serogroup B is the most prevalent, followed by serogroup I and serogroup C with no statistical differences on the serogroup level. However, significant differences between Russia and the Netherlands were observed in the distribution of *C. trachomatis* serovars.

INTRODUCTION

In Russia, reliable figures regarding incidence and prevalence of the sexually transmitted bacterium *Chlamydia trachomatis* infections remain highly limited. This is primarily the result of suboptimal diagnostics, case reporting and surveillance systems. However, in recent years, several DNA and RNA amplification systems (NAATs) have been developed and widely used in Russia in *C. trachomatis* diagnostics (1-3). Recently, the first prevalence figures have been published: 12.6% (56 of 446) in youth center attendees (4).

Dividing *C. trachomatis* into separate strains is a valuable tool for epidemiologic purposes and transmission studies. The *C. trachomatis* species is currently classified into 19 serovars: A, B, Ba, C, D, Da, E, F, G, Ga, H, I, Ia, J, K, L1, L2, L2a and L3. This classification is based on immunopeptide analysis of the major outer membrane protein (MOMP) with polyclonal and monoclonal antibodies (MABs). The MOMP is the immunodominant antigen of *C. trachomatis* and contains four variable domains (VDs) that are flanked and interspaced by five constant domains (CDs). Three of the variable domains (VD1, VD2

and VD4) are surface exposed and contain antigenic epitopes which are known at the nucleotide level (5).

In order to study the epidemiology and transmission of *C. trachomatis* infections, laboratory techniques for differentiating *C. trachomatis* serovars have been developed and include standard MOMP serotyping, restriction fragment length polymorphism (RFLP) analysis of the polymerase chain reaction (PCR)-amplified *omp1* gene (encoding the MOMP protein) (6-8), and nucleotide sequencing of the *omp1* gene. Recently we have developed a novel *C. trachomatis* amplification, detection and genotyping method (CT-DT assay) (9). The CT-DT detection step involves a DNA enzyme immunoassay (DEIA) using probes for the three *Chlamydia* serogroups (group B, C and Intermediate) and the cryptic plasmid, permitting sensitive detection of all 19 serovars. *C. trachomatis*-positive samples are analyzed by a nitrocellulose-based reverse hybridization assay (RHA) containing probes for the 19 different serovars, including probes for the three *Chlamydia* serogroups.

With this study we are the first to determine the serogroup and serovar distribution in Russian women and men. In addition, the obtained serogroups and serovars are compared with two independent large distributions assessed in the Netherlands.

PATIENTS AND METHODS

Russian cohort

Samples were collected consecutively from January 2006 to January 2008, during which time 83 *C. trachomatis*-positive urethral samples were collected from men in St. Petersburg, Russia (34 from the D.O. Ott Research Institute of Obstetrics and Gynecology and 49 from St. Petersburg State University Outpatient Clinic). Ninety-eight cervical samples from women were included (88 from the D.O. Ott Research Institute of Obstetrics and Gynecology and 10 from St. Petersburg State University Outpatient Clinic).

C. trachomatis positivity was assessed by either culture or inhouse NAATs test used in St. Petersburg, as described elsewhere (4) and *C. trachomatis* positivity was confirmed by plasmid-based PCR in Amsterdam (10).

Amplification, detection and genotyping using the CT-DT assay

C. trachomatis genotyping was determined in the Netherlands for all samples positive for *C. trachomatis*

with an inhouse PCR or cell culture, using the CT-DT detection and genotyping assay (9). The CT-DT amplification, detection and genotyping steps were performed exactly according to the manufacturer's instructions (Labo Biomedical Products BV, Rijswijk, the Netherlands).

Briefly, first the CT-DT amplification step was performed on extracted DNA to amplify all serovars available in GeneBank. This multiplex primer set generates an 89 bp amplicon of the cryptic plasmid and a 160/157 bp amplicon of the variable region 2 of the *omp1* gene.

Secondly, the *C. trachomatis* detection step was done to confirm the results detected with the inhouse PCR or cell culture. Reverse primers containing a biotin label at the 5' end enabled capture of the reverse strand onto streptavidin-coated plates. Captured amplimers were denatured by alkaline treatment, and detected by a defined cocktail of digoxigenin-labeled probes. Finally, all PCR products that proved positive with the *C. trachomatis* detection step were further analyzed with the CT-DT genotyping assay. The CT-DT genotyping assay is a reverse hybridization probe line blot (RHA) with a probe for the detection of the cryptic plasmid, and probes to detect the three different *C. trachomatis* serogroups (B, C and Intermediate) and 14 serovars (A, B/Ba, C, D/Da, E, F, G/Ga, H, I/Ia, J, K, L1, L2/L2a and L3). The different serovars are divided into serogroups based on phylogenetic mapping (Table I).

Each genotyping run contained a positive (serovar L2) and a negative control; 10% of the samples were retyped by the conventional PCR-based RFLP assay (6-8).

Comparison with Dutch serovar distribution studies

The obtained serovar and serogroup distribution in the Russian population studied were compared with two independent serovar distribution studies obtained in the Netherlands: A) 440 determined serovars published in 2000 (11), including 13 serovariants which were excluded for the comparison, leaving 108 serovar samples in men and 317 serovar samples in women for the comparison; B) 407 serovars determined in 2004 in a population with sexually transmitted disease (STD) at the Municipal Health Service in Amsterdam (12).

Statistical analyses

Serogroup and serovar distributions were compared between Russian men and women and between the Russian and Dutch cohorts, using chi-square statistics. $P < 0.05$ was considered statistically significant.

Table I. Serovar distribution into serogroups.

Serogroup	Serovar
B	B, Ba, D, Da, E, L1, L2, L2a
C	A, C, H, I, Ia, J, K, L3
I (Intermediate)	F, G, Ga

RESULTS

Russian serogroups and serovars

Of the Russian cohort in seven samples (one female, six males), only plasmid DNA was detected, and these were excluded from the comparison analyses. Eight Russian samples (seven females, one male) contained double infections (three with E'/G/Ga'; one with D'/G/Ga'; one with E/undefined [serogroup I]; two with E/K; and one with F/K) and were excluded from the comparison analyses. This resulted in 90 women and 76 men for the final serovar distribution analyses. The distribution of the serogroups in Russian patients is shown in Table II.

In the Russian population studied, serogroup B was the most prevalent (46%), followed by serogroup I (33%) and serogroup C (21%). The serovar distribution within the serogroups was comparable between men and women. A slight difference was observed in the distribution of serovars H (4 vs. 1%) and K (6 vs. 11%) (serogroup C) between men and women, respectively, although this did not reach statistical significance.

Comparison of the Russian and Dutch serogroups and serovars

The distribution of the serogroups in Russian patients was similar to the data on distribution in Dutch populations published in literature (Table II) (11, 12). When the serovar distributions were compared between the Russian cohort and the published Dutch cohorts, significant differences could be observed (Table II). In serogroup B, a significantly higher prevalence of serovar B was observed in the Russian cohort (5%) compared with the Dutch cohorts (1%) ($P < 0.0001$; OR: 12.06, 95% CI: 3.5-41.1). In serogroup I, serovar F had a decreased prevalence (8 vs. 23%), while serovar G/Ga (25 vs. 9%) had an increased prevalence compared to the Dutch cohorts ($P < 0.0001$, OR: 7.4, 95% CI: 3.8-14.5).

In serogroup C, serovar H had a significantly lower prevalence compared with the study by Spaargaren et

Table II. Serovar distribution in Russian patients divided into serovars and serogroups and by gender. Previously published results in Dutch cohorts (11, 12) are given for comparison.

Serovar	This study			Spaargaren et al. 2004 (12)	Morré et al. 2000 (11)		
	Women n (%)	Men n (%)	Total		Women n (%)	Men n (%)	Total
B serogroup							
B	4 (4.4)	4 (5.3)	8 (4.8)	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
D/D-	9 (10.0)	5 (6.6)	14 (8.4)	50 (12.3)	41 (12.9)	12 (10.8)	53 (12.4)
E	31 (34.4)	24 (31.6)	55 (33.1)	136 (33.4)	130 (41.0)	47 (42.3)	177 (41.4)
Subtotal	44 (48.9)	33 (43.4)	77 (46.4)	190 (46.7)	171 (53.9)	59 (53.2)	230 (53.7)
Intermediate serogroup							
F	7 (7.8)	7 (9.2)	14 (8.4)	95 (23.3)	72 (22.7)	21 (18.9)	93 (21.7)
G/Ga	23 (25.6)	18 (23.7)	41 (24.7)	38 (9.3)	24 (7.6)	12 (10.8)	36 (8.4)
Subtotal	30 (33.3)	25 (32.9)	55 (33.1)	133 (32.7)	96 (30.3)	33 (29.7)	129 (30.1)
C serogroup							
H	4 (4.4)	1 (1.3)	5 (3.0)	34 (8.4)	9 (3.4)	7 (6.3)	16 (3.7)
I/Ia	0 (0.0)	0 (0.0)	0 (0.0)	30 (7.4)	11 (3.5)	6 (5.4)	17 (4.0)
J	7 (7.8)	9 (11.8)	16 (9.6)	12 (2.9)	17 (5.4)	2 (1.8)	19 (4.4)
K	5 (5.5)	8 (10.5)	13 (7.8)	8 (2.0)	13 (4.1)	4 (3.6)	17 (4.0)
Subtotal	16 (17.8)	18 (23.7)	34 (20.5)	84 (20.6)	50 (15.8)	19 (17.1)	69 (16.1)
Total	90	76	166	407	317	111	428

al. (12) ($P = 0.0089$, OR: 0.25, 95% CI: 0.09–0.72), while it was comparable to the study by Morré et al. (11) (Table II). The prevalence of serovar I/Ia was lower than in the Dutch cohorts ($P < 0.0001$, OR: 0.03, 95% CI: 0.002–0.54). Prevalence of the serovars J and K were decreased in the Russian cohort compared with the study of Spaargaren et al. (12) ($P = 0.0003$, OR: 5.3, 95% CI: 2.2–13.3; $P = 0.0008$, OR: 5.9, 95% CI: 2.2–16.1, respectively), while they do not differ significantly from the study by Morré et al. (11).

DISCUSSION

This is the first study to describe the *C. trachomatis* serovar distribution in a Russian population. The serogroup distribution in this cohort was as follows: B: 46%, I: 33% and C: 21%, with comparable distributions between males and females. The serogroup distribution was also comparable to that found worldwide (13–17), with serogroup B as the most prevalent serogroup. A slightly higher *C. trachomatis* prevalence was observed in St. Petersburg, Russia, compared with

that reported in the Netherlands (6 vs. 2%) (18). Although serogroup distribution was identical to reported distributions in Dutch populations, significant differences were observed in the serovar level distributions between these cohorts. A higher prevalence of serovars B and G/Ga was observed in this study compared with the published prevalences in Dutch cohorts (11, 12), while a lower prevalence of serovars F and I/Ia was observed. Serovars H, J and K had a lower prevalence when compared with the study by Spaargaren et al. (12), but were comparable to that of Morré et al. (11). Similar serovar distributions have been observed worldwide, with small differences depending on the studied region and study size (13–17).

The samples found to be positive in Russia were retested in the Netherlands and all were confirmed, showing the reliability of the *C. trachomatis* detection setting in St. Petersburg, Russia. When the *C. trachomatis*-positive samples were used for serovar typing, seven samples (3.9%) were plasmid-positive only, but negative on the *omp1* level. This can be explained by the fact that the

omp1 target is present only once per cell, while the plasmid target used to detect *C. trachomatis* is 10 times more present per cell. This makes the detection more sensitive compared with typing. Most likely, these seven samples were either low bacterial load samples and/or the DNA was partially degraded, making it impossible to type the serovars. Although the numbers were small, most of these plasmid-only infections were obtained from men (6/7). The new typing technique used (9) is more sensitive compared with the PCR-based RFLP typing (6-8), which amplifies the complete *omp1* gene of 1.1 kb, while the new technique only amplifies a small fragment, variable segment 2, ensuring high typing efficiency.

In the current study we found eight (4.4%) double infections. These all included at least one serovar from the two most prevalent serogroups. This percentage of double infections is in the same range, although slightly higher as compared with previously reported double infections in the Netherlands (2%) (11). In general, double infections were detected in 1–10% of the infections, based on the population studied (high risk vs. low risk) and the sensitivity of the typing technique used (19–21). Remarkably, seven out of the eight double infections in this study were detected in women.

When looking into detail to the Russian Intermediate serogroup containing the serovars F and G/Ga, and comparing them with the frequencies of these serovars in the Dutch Intermediate serogroup, one notices that they are exactly reverse: 7–9% vs. 21–24%. This could potentially be a typing artefact. However, we retyped 10% of the samples by PCR-based RFLP (data not shown), and all F and G/Ga results between these two techniques were identical, ensuring reliable serovar allocation of the samples. This statistically significant difference has not been reported before, when comparing serovar distributions from countries.

Future studies are needed to extend the number of isolates in these studies and cohorts with different risk profiles for infections. In addition, these epidemiologic studies on the spread of different serovars can be used in transmission studies and identifying specific sexual networks.

CONCLUSIONS

This is the first report of Russian *C. trachomatis* serovar/serogroup distributions. Serogroup B was found to be the most prevalent, followed by serogroup I and serogroup C. Significant differences were observed in the

distribution of *C. trachomatis* serovars between Russia and the Netherlands.

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DISCLOSURE

The authors have nothing to disclose.

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