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Distribution of *Chlamydia trachomatis* Serotypes in Clinical Urogenital Samples from North-Eastern Croatia

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Abstract The purpose of this study was to determine prevalence of *Chlamydia trachomatis* (Ct) urogenital infection and its serotype distribution from clinical samples in north-eastern Croatia. During a 3-year period, 2,379 urogenital samples were analyzed by real-time polymerase chain reaction (A group), while 4,846 genital swabs were analyzed by direct fluorescent antibody test (B group). 132 Ct positive specimens were genotyped by *omp1* gene sequencing. The prevalence rate of Ct was 3.2 % in A and 1 % in B group. The most prevalent chlamydial genotype was E (44 %), followed by F (33 %), K (11.5 %), G (8 %), J/UW (5.3 %), D-IC (4.4 %), D-B120 (1.8 %), and B/IU, J/IU, Ia/IU (0.9 % each) serotypes. Single-nucleotide polymorphisms (SNPs) of *omp1* gene were detected in E, K, and G serotypes. Some of these SNPs (C/T at position 272 and G/A at position 813 in E strain; C/T at position 884 in D strain) might represent novel *omp1* variants.

Keywords *Chlamydia trachomatis* · Prevalence · Serotype distribution · *Omp1* gene sequence analysis

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Introduction

Chlamydia trachomatis (Ct) is one of the leading causes of sexually transmitted diseases (STD) worldwide. This obligate intracellular Gram-negative bacterium causes a wide range of clinical infections: prostatitis and epididymitis in men, cervicitis and salpingitis in women and proctitis, lymphogranuloma venerum (LGV), trachoma, conjunctivitis and reactive arthritis in both sexes [31]. WHO data reported 90 million cases of infection with Ct in the world each year, including 4 million in the United States of America and 5.5 million in Europe. However, the documented cases encompass only a fraction of the infected individuals because 50 % of the infected males and 80 % of the infected females have asymptomatic infection [56]. Untreated asymptomatic infection can cause irreversible pelvic inflammatory disease (PID) leading to tubal infertility, ectopic pregnancy, chronic urethritis, and chronic pelvic pain [51]. Due to lack of efficient human vaccines, early detection and antibiotic treatment of Ct infection remain the main strategy for reduction of late complications.

Genetic variability of Ct could be one of the reasons for its wide tropism and difficulty in detection and eradication. There are at least 19 chlamydial serotypes found worldwide. Serotypes are defined by mutations in one of the four variable domains (VD1–4) of *omp1* gene. It codes for the highly abundant and immunogenic outer membrane protein MOMP, whose epitopes have been intensely studied in vaccine development [17, 20, 29, 30]. For example, serotypes A, B, and C have usually been associated with ocular trachoma, serotypes L1, L2, and L3 with LGV, while serotypes D to K have mostly been associated with urogenital and neonatorum infections [17, 20, 29, 30]. In addition, presence of ocular strains in the genital tract and vice versa was also

reported [13] suggesting broad Ct tropism. *Omp1* gene sequence also changes through single-nucleotide polymorphisms (SNPs) and through gene recombination. The latter was observed in populations with high rates of multiple infections with different Ct strains [14, 29, 34].

A large fraction of urogenital chlamydia infections, particularly those with E and K serotypes, fail to show any clinical symptoms and remain undetected for years [37]. For example, K serotype does not productively infect peripheral blood monocytes. However, K serotype chlamydia cells spread into the whole organism via monocytes in a Trojan-horse manner leading to long-term consequences such as arthritis [46]. Thus, the more is known about the Ct serotype distribution in local population, the easier it will be to develop strategies such as public awareness, routine screenings, vaccination, etc. to diminish chlamydia-related health issues.

Surprisingly, no research study has so far analyzed Ct serotype distribution in Croatia. Thus, objective of this work was to determine the prevalence of Ct serotypes and correlate them with gender and age. Initial Ct screening of urogenital clinical samples from the Osijek-Baranya County was performed using the real-time polymerase chain reaction (PCR)-based or direct fluorescent antibody (DFA)-based methods. Chlamydia positive samples were then genotyped by *omp1* gene-based sequencing and BLAST alignment.

Materials and Methods

Study Population

Study Group A

Between March 2007 and January 2010, 449 urines (349 male and 100 female) and 501 urethral swabs (from men) were collected at the Microbiology department of the Institution of Public Health Osijek-Baranya County, while 1,430 cervical swabs were provided by gynecologist's offices from the Osijek-Baranya County. Collection of specimens was performed according to the COBAS TaqMan real-time PCR instrument CT Test instructions (Roche Diagnostics, Germany). The mean age of 1,530 female and 850 male patients was 34 ± 11.2 years (range 18–78) and 39 ± 12.9 years (range 18–83), respectively.

Table 1 Study populations in relation to sex, initial diagnosis, and percentage of Ct infection detected by TaqMan (group A) or DFA (group B) method

	Study group A		Study group B	
Initial diagnosis	No. of cases (%)	Ct positive[a] (%)	No. of cases (%)	Ct positive[a] (%)
Women				
Cystitis	394 (25.8)	2 (0.5)	256 (7.6)	3 (1.1)
Salpingitis	332 (21.8)	5 (1.5)	81 (2.4)	2 (2.5)
STDs	212 (13.8)	17 (8.0)	740 (21.9)	6 (0.8)
Pregnancy	188 (12.3)	6 (3.2)	980 (29.0)	21 (2.1)
Arthritis	106 (7)	–	285 (8.5)	–
Cervical dysplasia	103 (6.7)	6 (5.8)	251 (7.4)	2 (0.8)
Infertility	99 (6.5)	2 (2.0)	358 (10.6)	–
Controls after treatment	58 (3.7)	–	345 (10.2)	–
Ovarian cysts	21 (1.3)	1 (4.8)	21 (0.6)	–
Genital skin infection	17 (1.1)	–	60 (1.8)	–
Total	1,530 (100)	39 (2.6)	3,377 (100)	34 (1.0)
Men				
Prostatitis	401 (47.1)	5 (1.2)	393 (26.8)	1 (0.3)
Cystitis	104 (12.3)	6 (5.7)	181 (12.3)	2 (1.1)
Arthritis	88 (10.3)	1 (1.1)	248 (16.9)	1 (0.4)
Urethritis	78 (9.3%)	14 (17.9)	201 (13.7)	7 (3.5)
Infertility	72 (8.4)	–	134 (9.1)	–
STDs	46 (5.5)	6 (13.0)	87 (5.9)	3 (3.4)
Epididymitis	28 (3.3)	2 (7.1)	59 (4)	–
Genital skin infection	19 (2.2)	2 (10.5)	–	–
Controls after treatment	12 (1.4)	–	158 (10.7)	–
Genital cancer	2 (0.2)	–	8 (0.6)	–
Total	850 (100)	36 (4.2%)	1,469 (100)	14 (0.95)

STDs sexually transmitted diseases

[a] Ct positive percentage calculated in relation to the total number of cases with identical initial diagnosis

Study Group B

Between January 2009 and January 2010, 3,377 cervical and 1,469 urethral swabs were collected according to the MicroTrak Direct Fluorescence Antigen (DFA) test instructions (MicroTrak, Trinity Biotech, USA). The mean age of 3,377 female and 1,469 male patients was 34 ± 11.2 years (range 18–80), and 40 ± 13.5 years (range 18–81), respectively.

All patients in this study had typically symptomatic urogenital infections and were referred to our department by their general practitioners, gynecologists, or other medical specialists. Table 1 shows study groups A and B in relation to sex, initial diagnosis, and Ct infection rate. Informed consent was obtained from all Ct processed patients and the Ethics Committee of the Institute of Public Health for Osijek-Baranya County approved the study. The patients who had Ct urogenital infection were treated with appropriate antibiotics. Once the Ct positive patients completed antibiotic treatment, they were requested to come for a check-up.

Ct Detection

DNA from the study group A patients was isolated using AMPLICOR CT/NG Specimen Preparation Kit and analyzed for Ct presence by COBAS TaqMan CT test (Roche Diagnostics, Germany) and COBAS TaqMan Analyzer. Ct positive urines and swab specimens were stored at −20 °C until DNA extraction for subsequent Ct genotyping.

Specimens from the study group B patients were analyzed by MicroTrak test (Trinity Biotech, USA) and positive DFA microscopic slides were stored at +4 °C until DNA extraction.

Ct Serotyping

DNA from the Ct positive study group A patients was isolated from 140 µl of urine using QIAamp Viral RNA Mini Kit (Qiagen) or from 200 µl M4RT (Remel, USA) urethral and cervical swabs using QIAamp DNA Mini Kit (Qiagen). All steps were performed in duplicates and 50 µl of eluted DNA was stored in aliquots at −20 °C for subsequent genotyping. DNA from the Ct positive study group B patients was prepared by scraping MicroTrack glass microscope slides for 15–20 times in 1.5 ml of ultra pure water and spinning at 11,500 rpm for 20 min at 25 °C. After resuspension of cell pellet in 600 µl 1× GIBCO™ phosphate-buffered saline buffer (PBS), pH 7.4 (Invitrogen, USA) and vigorous vortexing for 3 min, DNA was isolated using QIAamp DNA Mini kit (Qiagen, Germany) with following modifications: 2 µl polyadenylic acid (Amersham, Biosciences, USA) (conc. 1 µg/ml AE buffer) was used for each sample and DNA was eluted with 50 µl of the kit-provided elution buffer.

Initial PCR amplification of *omp1* gene (1,100 bp fragment, P1/OMP2 primers [20]) was followed by nested PCR (990 bp fragment, MOMP87/RVS1059 primers [48]). The 50 µl PCR mix contained 10 µl of DNA extracted from urine and swabs (or 3 µl from the initial PCR), 0.4 µM of primers, 2.0 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate and 1 unit of Platinum Taq DNA polymerase (Invitrogen, USA). PCR was performed in Applied Biosystems 9700 thermal cycler using following conditions: denaturation at 95 °C for 3 min; 40 cycles of 94 °C for 30 s, 55 °C (or 60 °C in nested PCR) for 30 s, 72 °C for 90 s; final elongation at 72 °C for 7 min. Amplification products were separated on 1.7 % agarose gels, stained with SYBRSafe DNA stain (Invitrogen, USA) and visualized with UV light. The MassRuler™ Low Range DNA Ladder (Fermentas GMBH, Germany) was included in each electrophoresis.

Both *Omp1* amplicons (1,100 and 990 bp) were purified using Qiaquick PCR Purification Kit (Qiagen, Germany) and sequenced using BigDye v3.1 terminator cycle sequencing kit (Applied Biosystems, USA) and Applied Biosystems 3130 Genetic Analyzer. Each PCR product was sequenced twice in both directions. Sequencing primers are shown in Table 2.

Obtained consensus sequences were aligned using the SeqScape v2.5 software (Applied Biosystems, USA) and screened against the library constructed from the reference strains of *Ct* using the BLAST search tool at the National Center for Biotechnology Information, GenBank http://www.ncbi.nlm.nih.gov/Genbank/ accession number in parentheses: A/Sa1 (M58938), B/TW-5 (M17342), B/IU-1226 (AF063208), C/TW3 (M17343), D/B-120 (X62918), D/IC-Cal8 (X62920), E/Bour (X52557), F/IC-Cal3 (X52080), G/UW57 (AF063199), H/Wash (= H/UW4) (X16007), I/UW-12 (AF063200), Ia/IU-4168 (AF063201), J/UW36

Table 2 PCR primers used for *omp1* sequence analysis

Primer	Nucleotide sequence	Accession number
P1	5′-ATG AAA AAA CTC TTG AAA TCG G-3′	X52557 (bp 1–22)
Omp2	5′-ACT GTA ACT GCG TAT TTG TCT G-3′	X52557 (bp 1,124–1,103)
MOMP87	5′-TGA ACC AAG CCT TAT GAT CGA CGG A-3′	X52557 (bp 87–111)
RVS1059	5′-GCA ATA CCG CAA GAT TTT CTA GAT TTC ATC-3′	X52557 (bp 1,079–1,050)
191S	5′-GTC YTS TGG GAR TGT GGR TGT GC-3′	X52557 (bp 598–620)

(AF063202), Ja/IU-A795 (AF063203), K/UW31 (AF 063204), L1/440 (M36533), L2/434 (M14738), and L3/404 (X55700).

Statistical Analysis

The prevalence of chlamydial urogenital infection and its relation to age and sex was calculated using Statistica 8.0 software (StatSoft) and differences between prevalence in relation to age and sex were analyzed using χ^2 test (Figs. 1,2). Difference was considered significant when $p < 0.05$. The Ct genotype distribution in relation to sex was analyzed with Microsoft Office Excel 2003 (Microsoft) (Fig. 3).

Results

Age- and Sex-Dependent Prevalence of Chlamydial Urogenital Infection in North-East Croatia

The gender-specific prevalences of chlamydial urogenital infection of patients from the study groups A and B are shown in Figs. 1 and 2. Both study groups showed similar trends in chlamydial prevalence: (i) men had higher Ct incidence then women; (ii) young male and female patients showed the highest Ct prevalence (3.7 and 2.6 %, respectively) while patients older than 41 had the lowest Ct prevalence.

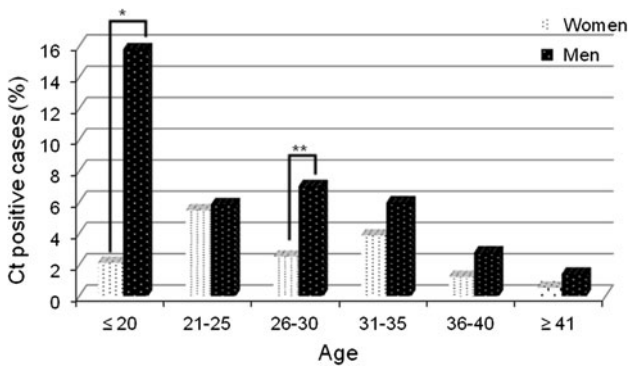

Fig. 1 Sex- and age-dependent prevalence of *Chlamydia trachomatis* (study group A). Urogenital samples were analyzed by COBAS TaqMan Ct test and presented as percentages (*$p < 0.01$, **$p < 0.05$)

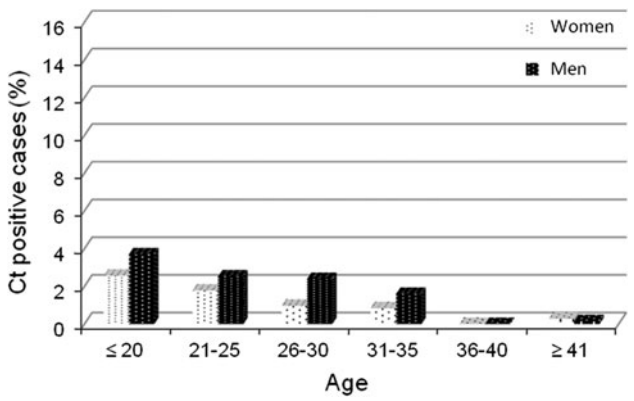

Fig. 2 Sex- and age-dependent prevalence of *Chlamydia trachomatis* (study group B). Urogenital samples were analyzed by Micro Track Ct test and presented as percentages

Statistical analysis of chlamydial infection based on the real-time PCR-based COBAS TaqMan Ct analysis of 449 urine, 501 urethral, and 1,430 cervical swab specimens (study group A) is shown in Fig. 1. Men showed almost two-fold higher Ct prevalence then women (4.1 vs. 2.6 %, respectively, $p = 0.044$). The most significant differences in Ct prevalence between men and women were found in age groups 26–30 years (7 vs. 2.5 %, $p = 0.017$) and under 20 years (15.8 vs. 2.1 %, $p = 0.008$). The highest incidence of Ct infection was detected in young subjects, that is, in men under 20 years (15.8 %) and in 21–25 years old women (5.5 %). The oldest category in our study, patients above 41 years, showed the lowest incidence of Ct infection both in men and women (1.4 vs. 0.5 %, respectively) (Fig. 1). Similar age- and gender-dependent trends in Ct prevalence were observed in 1,469 urethral and 3,377 cervical swabs (study group B) that were analyzed by the antibody-based MicroTrak DFA method (Fig. 2). Young male and female patients showed the highest Ct prevalence (3.7 and 2.6 %, respectively) while older than 41 patients had the lowest Ct prevalence although the difference in age- and gender-Ct prevalence was not statistically significant (Fig. 2).

Distribution of Chlamydia Serotypes

We successfully genotyped 93.3 % of specimens (i.e., 70 out of 75 from study group A) and 95.6 % (i.e., 43 out of 45 from study group B) that were found to be Ct positive by either MicroTrak DFA or COBAS TaqMan Ct method. Genotyping was performed by sequencing the 990 bp-long amplicon of the *omp1* gene that was synthesized either by one-step PCR (in 71.5 % of cases) or by nested PCR (in 17.1 % of cases) using DNA isolated from 48 males and 65 females. 6.7 % (study group A) and 4.4 % (study group B) of specimens failed to be amplified and genotyped. Sequencing and BLAST alignment analysis revealed that the most prevalent chlamydial genotypes corresponded to serotype E (44 %), followed by F (23 %), K (11.5 %), G (8 %), J/UW (5.3 %), D-IC (4.4 %), D-B120 (1.8 %) and B/IU, J/IU, Ia/IU (0.9 % each). In total, the genotypes E, F, and K accounted for 77.9 % of infections in both genders.

Distribution of Ct genotypes in relation to the sex of infected subjects is shown in Fig. 3. Genotype F was more found in men than in women, while the genotypes E and K were found more often in women than in men. Genotypes B/IU and D/B120 showed gender discrepancies.

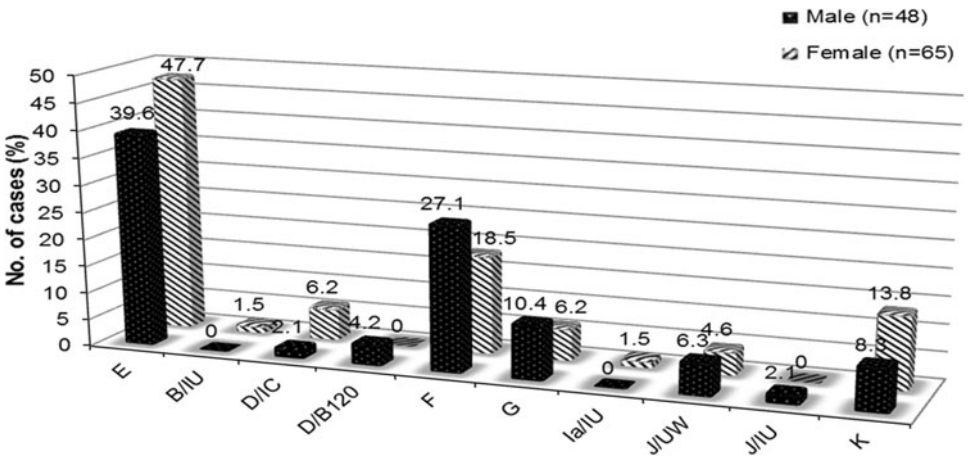

Fig. 3 Gender-specific distribution of *Chlamydia trachomatis* serotypes in clinical urogenital samples from the Osijek-Baranya County population (study groups A and B)

Omp1 Single-Nucleotide Polymorphisms

Omp1 gene sequences of 86 % of chlamydia positive clinical specimens (i.e., 97 out of 113) were identical to reference sequences for Ct serotypes/strains deposited in the NCBI database (Table 3). The remaining 14 % of chlamydia strains (i.e., 16 out of 113) showed SNPs in *omp1* gene. Interestingly, for some of the *omp1* gene variants we did not find corresponding GenBank sequences and thus they might represent novel genetic variants of *omp*1 gene (Table 3). Finally, we did not detect multiple chlamydial serotypes in the same clinical specimens neither any signs of *omp1* recombination.

We found a single C/T or G/A nucleotide substitution in *omp1* gene in 4 % of E strains (2 out of 50), causing a potential missense or nonsense mutation, respectively

Table 3 *Omp1* gene single-nucleotide polymorphisms in 113 Ct positive clinical samples (study groups A and B)

Chlamydia trachomatis serotype[a]	Tissue source (no. of total samples → no. of samples with SNPs)	SNP in *Omp1* gene (bp from the TSS)	Amino acid change	NCBI entries with the same SNP in *omp1* gene[b] (Acc. no.)
E	Urine (15)	No	No	
	Urethra (16→1)	C→T (272)	Thr→Ile	Not found
	Cervix (29→1)	G→A (813)	Trp→STOP	Not found
F	Urine (4)	No	No	
	Urethra (9)	No	No	
	Cervix (12)	No	No	
K	Urine (1)	No	No	
	Urethra (4→2)	C→A (1063)	Leu→Met	AM901164
	Cervix (8→2)	C→A (1063)	Leu→Met	AM901164
G	Urethra (5→5)	T→G (1003)	Ser→Ala	CP001930
	Cervix (4→3)	T→G (1003)	Ser→Ala	CP001930
	Cervix (4→1)	G→A (487)	Gly→Ser	AM901158, CP001888
D/IC	Urethra (1)	No	No	
	Cervix (4→1)	C→T (884)	Ala→Val	Not found
J/UW	Urine (3)	No	No	
	Cervix (3)	No	No	
D/B-120	Urethra (2)	No	No	
B/IU	Cervix (1)	No	No	
Ia/IU	Cervix (1)	No	No	
J-IU	Urethra (1)	No	No	

Acc. no. accession number, *SNP* single-nucleotide polymorphism, *bp* base pairs, *TSS* transcription start site

[a] Accession numbers of reference strains used for Ct serotype classification are in the "Materials and Methods" section

[b] Sequences showing the same SNP in *omp1* gene

(Table 3). For both of these SNPs no corresponding sequences were found in the NCBI database (September 2011). All F strains (25 out of 25) were identical to the NCBI reference sequence. 3 % of K genotypes (4 out of 13) displayed a C/A mutation, identical to AM901164 NCBI sequence. All G genotypes detected in our clinical samples contained SNPs in *omp1* gene. These SNPs corresponded either to a T/G (in 8 out of 9 cases) or G/A (in 1 out of 9 cases) substitution. While for the former we did not find corresponding sequence in the NCBI database, the latter was identical to two NCBI entries (AM901158 and CP001888). 2 % of D-IC genotypes (1 out of 5) showed C/T nucleotide substitution that was not found in the NCBI database.

Discussion

This study is the first to determine the prevalence of *Chlamydia trachomatis* in the Osijek-Baranya County, a north-eastern region of Croatia. When compared to previous studies in Croatia, the overall (both sexes, all age categories) Ct prevalence in our study was similar to that in Zagreb (3 vs. 2 %) [19]. In addition, Ct prevalence in young men in our study (7.3 %) was comparable to previous reports in Zagreb (~11 %) and across Croatia (5.9 %) [6, 19]. However, young women from Zagreb showed higher tendency to become infected then women from the Osijek-Baranya County (7 and 16.4 % vs. 2.1 % in women <20 as in [16, 19]; 11 vs. 5.5 % in 21–25 years old women as in [19]). This is probably due to earlier or more frequent sexual interaction in urban areas and not due to different detection methods since both Jarža-Danila et al. and our studies employed a PCR-based approach.

When compared to Europe, men from our study showed relatively low Ct prevalence. For example, Ct infection rate in Croatian men (4.1 %) was lower than in Norway (7.8–19.6 %), Scotland (9.8 %), Sweden (10 %), UK (9.5–13.3 %), the Netherlands (12.3 %), Bulgaria (25 %), Poland (40 %) and was similar to Switzerland (1.2–7.5 %) [32]. On the other hand, Ct prevalence of women from our study (2.1 %) was similar to that in Spain (1 %) [24], Belgium (1.4 %) [52, 54], Slovenia (1.6 %) [22], England (1.7 %) [24, 54], but was lower than in Poland (3.2 %) [12], Greece (3.5 %) [28], Italy (3.9 %) [24], Republic of Macedonia (~4 %) [47], Netherlands (4.4 %) [24], Turkey (4.9 %) [44], Hungary (5.1 %) [38], Bulgaria (6.1 %) [24], Lithuania (8.4 %) [24], and France (17 %) [54]. As most of these studies examined asymptomatic women whereas our study included only symptomatic ones, this further straightens our observation that women of the Osijek-Baranya County show generally lower Ct prevalence then women in other European regions.

We find that population younger than 25 is at the highest risk of Ct infection as noted previously [24, 26, 54]. Sexual activity (especially in early age), early age of menarche, new or several sexual partners, unmarried status and inconsistent use of condoms are additional risk factors [16, 24, 42].

Ct infection monitoring of pregnant women is not obligatory in Croatia. Since pregnant women younger than 20 years show higher prevalence of Ct infection than non-pregnant women [5], there is a high possibility of its transmission during delivery. Consequences such as neonatal conjunctivitis, nasopharyngeal infection, and chlamydial pneumonia have already been detected in Croatian newborns [2]. In order to prevent it and, ultimately, decrease the medical treatment expenses, we suggest that Ct monitoring becomes a compulsory test during pregnancy.

Ct serotype distribution in north-east Croatia is similar to trends in Europe and USA. Almost half of the chlamydia infected urogenital samples was E serotypic (44 %), followed by F, K, and G serotypes. Similar levels of E and F prevalence were detected in Swedish [7, 11, 20], American [15], and Dutch [35] studies. Asian population, to the contrary, is mainly F serotypic (25–29 %), followed by E (9–20 %) and D (14–23 %) serotypes [18, 34, 50]. This inconsistency may be caused by different Ct serotype distribution in symptomatic vs. asymptomatic population as well as the different laboratory methods used for Ct serotyping. Swedish men show low (1.4 %) [20], Croatian men show moderate (10.4 %, our study) and Greek and Italian men show more abundant G serotype prevalence (23 and 19 %, respectively) [10, 40]. Dutch men with symptomatic urogenital infection show more H serotype (2.4 vs. 0 %) but less K serotype (2.5 vs. 8.3 %) than men in our study [35]. Similarly to men, E serotype was also a predominant serotype in Croatian women. Moreover, levels of E and F serotypes in Croatian symptomatic women were similar to symptomatic women in Sweden [13] and Australia [27]. In summary, unlike more stable levels of E and F serotype, prevalence of G, H, and K serotypes appear to vary across Europe.

Whether there is a gender-specific distribution of Ct serotypes is still a controversial question. Some studies reported differences in the Ct serotype distribution between men and women [49, 55] while others found no difference [33].We indeed detected a disproportion of F, E, and K serotypes in relation to sex, similarly to the Seattle study [49].

Contrary to several studies [3, 8, 18, 34, 55], we did not detect any case of infection by mixed Ct serotypes. There might be several reasons to it: (i) our sequencing method was not enough sensitive due to, for example, large differences in proportions of each serotype [3], (ii) the source of the tested population—the majority of our specimens

were obtained from physicians and gynecologists and not from the STD clinics. Therefore, future studies should readdress this question through, for example, analysis of high-risk population such as adolescents and/or highly sexually active or promiscuous individuals.

Similarly to others, we also noticed that K and G chlamydia genotypes are the least stable and contain several SNPs in *omp1* gene [33]. We found one, previously unreported, mutation proximal to VD4 region of *omp*1 gene in K strain [17, 20, 30, 41]. We also detected two known SNPs in VD4 and VD2 region in G strain [20, 23, 30, 36]. To the contrary, E genotype was not only the most abundant but also the most stable genotype in our population [20, 43]. Only 4 % of E strains contained the *omp1* gene SNPs (in VD1 and VD4 regions) and one of them has not yet been reported [11, 17, 20, 29, 30]. To summarize, we detected three novel mutations in the *omp1* gene and further studies should be aimed to independently verify our observations.

The major limitation of our study (and other studies relying on clinical samples) is that asymptomatic infection remained largely undetected. Asymptomatic infection facilitates chlamydial spread, augments infection reservoir and may cause infertility and ectopic pregnancies. Moreover, asymptomatic individuals are more susceptible to human papillomavirus (HPV) [45] and human immunodeficiency virus (HIV) infections [25]. Consequences of asymptomatic and/or untreated Ct infections increase the costs of health care interventions [25]. Only in USA, the 1997 annual cost of Ct treatment exceeded 2.4 billion dollars [4] whereas in England, the 1998 annual cost exceeded 50 million pounds [53]. Therefore, routine screening of high-risk population would be a better approach for earlier and, ultimately, less expensive way in combating the chlamydia infection. Recent report in Croatia suggested the feasibility of routine chlamydial screenings through analysis of at-home collected urine and/or vaginal flush samples [6]. Moreover, at-home collected samples result in higher detection of chlamydial infection then samples obtained conventionally, at doctors' offices [1, 39].

Strikingly, almost one-third of infected individuals, especially those younger than 20 years, show recurrent chlamydial genital infections [21, 41]. In addition, infections with H, I, and J serotypes are often resistant to antibiotics and persist in the genital tract even 2–3 years after the treatment [9, 21]. In the case of individuals prone to recurrent infections, routine screenings could be combined with Ct serotype determination through *omp1* gene sequencing. This would help to understand whether those infections are caused by relapse, reinfection or new infection [41].

As a follow-up of the work presented here, a 2 years-long epidemiological study is necessary in order to determine the *true* (symptomatic and asymptomatic) prevalence of Ct infection in Croatia. Finally, we suggest a study predicting the costs of routine screenings of high-risk population and comparing them with the current treatment costs of Ct infection and its secondary consequences in Croatia.

In conclusion, this is the first study based on *omp1* gene sequence analysis showing the distribution of urogenital Ct serotypes in clinical specimens of north-eastern Croatia. Relatively low Ct prevalence, mainly restricted to E, F, and K serotypes, was the highest in men under 20 years and women in early twenties. However, the true incidence of chlamydial infection in Croatian population is, most probably, much higher due to asymptomatic nature of this disease and incomplete screening coverage and underreporting in our study.

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Conflict of interest The authors declare that they have no conflict of interests.

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