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CHLAMYDIA TRACHOMATIS UROGENITAL INFECTION AND ASSOCIATED RISK FACTORS AMONG UNIVERSITY STUDENTS IN CROATIA

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Objective – The study aimed to determine the prevalence of *Chlamydia trachomatis* infection among university students in Croatia and to determine risk factors associated with chlamydia infection. **Material and methods** – The study was conducted as an opportunistic screening of asymptomatic sexually active students attending routine health examinations during their first year of studying. The first void urine samples from males, cervical swab or first void urine samples from females and data regarding life style, sexual and other health-risk behaviour were collected and analysed. **Results** – Overall prevalence of chlamydia infection was 4.9%, with the testing response rate of 29.5%. The only identified risk factor was female sex. Female students preferred urine sample over cervical swab sample and avoided gynaecologist examination. **Conclusion** – Opportunistic screening is a feasible strategy to diagnose asymptomatic chlamydia infection among university students in Croatia. Results may contribute to decision making on the form of chlamydia control policy, including choice of screening settings and specimens suitable for target population.

Introduction

Chlamydia trachomatis (CT) is an obligate intracellular bacterium estimated to infect, by sexual transmission, over 100 million people each year worldwide (1). The majority of urogenital *Chlamydia trachomatis* infections (CTI) are asymptomatic (2). Untreated CTI can lead to serious outcomes in women, e.g. pelvic inflammatory disease (PID) that can result as infertility, ectopic pregnancy and chronic pelvic pain (3). A minority of infected men develop complications (e.g. epididymitis, orchitis) and they rarely affect reproductive health (4). However, CT has been associated with male infertility as a result of deterioration of sperm (2,5). CTI also increases the risk of cervical cancer (6), and transmission of human immunodeficiency virus (7).

CTI is the most common bacterial sexually transmitted infection (STI) and a major public health concern globally (8). In 2014, 396128 cases of CTI were reported in 26 EU/EEA countries, with the overall notification rate of 187 per 100 000 persons (9). In the same year, 386 cases of CTI were reported in Croatia, with the overall notification rate of 9 per 100 000 persons (9). There is a substantial variation across the EU/EEA

in reported chlamydia cases, with rates ranging from below 1 to more than 500 reported cases per 100 000 population. The true incidence and prevalence of chlamydia are likely to be higher than suggested by rates of reported infection (10).

Appropriate testing of symptomatic and asymptomatic sexually active individuals is recommended to identify and treat the CTI (3). Guidelines in many countries recommend annual CT screening for all sexually active young (<25 years of age) women and extend to young men in some countries (3). More than half of European EU/EEA countries recommend opportunistic testing to a certain group of asymptomatic individuals. The most common target groups are pregnant women and young adults, followed by other groups such as men having sex with men, commercial sex workers, migrants, women undergoing abortion (11). Eastern European EU countries either have CT guidelines for opportunistic screening, but infrequently implement them in practice, such as Bulgaria, Hungary, Czech Republic and Romania, or have no guidelines for opportunistic screening at all, such as Slovakia and Slovenia (11). National polices on opportunistic testing should be based on reliable information about chlamydia prevalence and the characteristics of those at risk (12).

Croatia does not have CT testing guidelines or a screening programme due to the lack of epidemiological data regarding prevalence in different groups and associated risk factors. University students in Croatia are the population of late adolescents and young adults integrated in the preventive healthcare system specifically organised for school children and adolescents in Croatia, and therefore as a population they are easily approachable and available for offering screening and counselling.

The study aimed to determine the prevalence of CTI among university students in Croatia and to determine risk factors associated with CTI, information that would be useful for planning future prevention interventions and decision making on the form of CT control policy.

Materials and methods

The study protocol received ethical approval from the Ethics Committee of the Institute of Public Health for the Osijek-Baranja County, Osijek, Croatia. The study population was recruited from university in Osijek, in Eastern Croatia. The study was conducted between 2011 and 2013, as a pilot opportunistic screening of asymptomatic sexually active students attending routine health examination during their first year of studying at the Department of School Medicine of the Institute of Public Health for the Osijek-Baranja County. Routine health examination of the first-year university students in Croatia includes obtaining data regarding life-style, sexual and other health-risk behaviour. All asymptomatic sexually active students attending routine health examination were offered by a school doctor to test for *Chlamydia trachomatis*. Students willing to participate signed an informed consent and the significance of positive CT test and its subsequent management were explained to them.

First void urine samples from males and cervical swab or first void urine samples from females were collected. Detection of C. trachomatis DNA was performed using a real-time PCR assay COBAS® TaqMan® CT Test, v2.0 (Roche Diagnostics Ltd, Germany) at the Department of Microbiology, Institute of Public Health for the Osijek-Baranja County. Following manual DNA extraction, real-time PCR was preformed using COBAS® TaqMan® 48 Analyser (Roche Diagnostics Ltd, Germany), as recommended by the manufacturer. This real-time PCR assay employs two sets of probes and primers for amplification of two

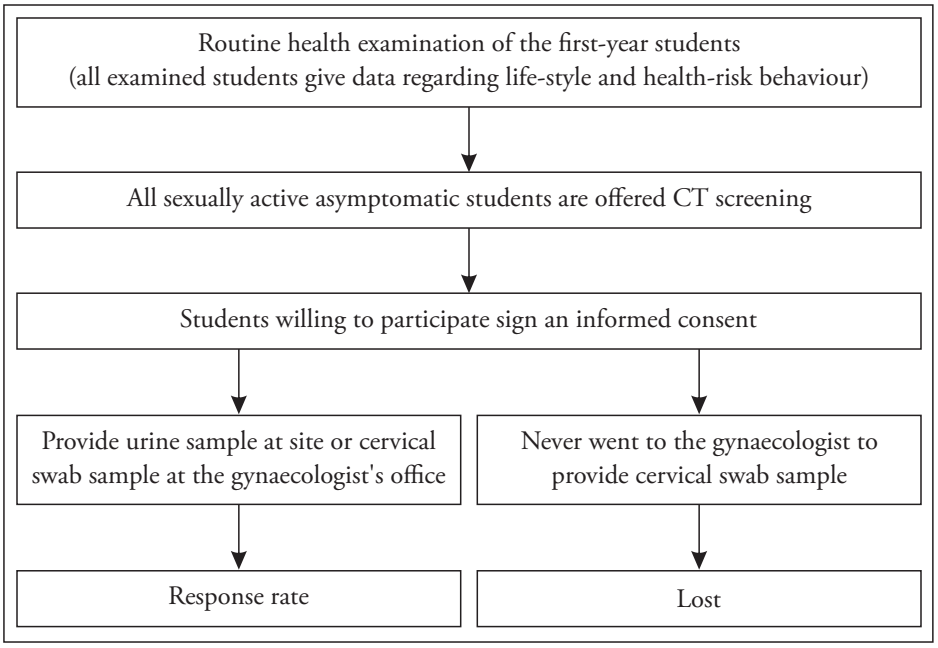

Fig. 1 *Flowchart of the participant recruitment process.*

separate target sequences, one for the C. trachomatis cryptic plasmid and other for the ompA gene of the C. trachomatis chromosome, which ensures the detection of all C trachomatis strains (including the new Swedish variant).

Students who tested positive for CT were treated according to treatment guidelines, counselled and advised to inform their sexual partners, and finally retested at least 30 days after treatment at the Department of School Medicine at the Institute of Public Health for the Osijek-Baranja County. Data regarding life style, sexual and other health-risk behaviours of the participants were collected from medical documentation obtained at the routine health examination, before the results of the CT test were available (Fig. 1).

Statistical analysis

The obtained data was analysed using descriptive statistics, chi-square test and Fisher's exact test in statistical programme Statistica 12.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Prevalence rates were calculated with 95% confidence intervals. P values less than 0.05 were described as significant.

Results

Population characteristics

A total of 667 sexually active first-year university students were tested for *Chlamydia trachomatis*, 315 (47.2%) males and 352 (52.8%) female students (Table 1). Median age of participants was 20 years, ranging from 18 to 35 years. 87.0% of participants were aged 18-20 years. The collected samples were: 315 urines from males, 303 urines from females and 47 cervical swabs.

The response rate for CT testing was 29.5%. An especially low response rate was observed among female students who were offered to preform sampling at a gynaecologist's office using cervical swab sample. The results showed that 46.0% of sexually active female students had never visited a gynaecologist and there was a significant proportion of female students who signed an informed consent to get tested, but never went to the

Table 1 Description of participants

Variables	Details	Participants n (%)
Age	Mean 20 years	
	Median 20 years	
	Range 18-35 years	
	18-20 years	580 (87.0)
	>20 years	87 (13.0)
Sex	Female	352 (52.8)
	Male	315 (47.2)
Living in a place of studying	With parents	223 (33.4)
	Own or lend apartment	401 (60.1)
	Student dormitory	43 (6.4)
Faculty	Medicine	4 (0.6)
	Agriculture	25 (3.7)
	Food technology	82 (12.3)
	Nursing	6 (0.9)
	Economy	279 (41.8)
	Electro-technics	56 (8.4)
	Mathematics	52 (7.8)
	Physics	13 (1.9)
	Arts	9 (1.3)
	Biology	23 (3.4)
	Philosophy	22 (3.3)
	Construction	25 (3.7)
	Law	7 (1.0)
	Chemistry	13 (1.9)
	Teaching	51 (7.6)
Self-esteem	Low	12 (1.8)
	Normal	448 (67.2)
	High	207 (31.0)
Samples taken	Urine-males	315 (47.2)
	Urine-females	303 (45.4)
	Cervical swab-females	49 (7.3)
CT infection	Positive	33 (4.9)
	Negative	634 (95.1)
Age of the first sexual intercourse	<15	18 (2.7)
	15-16	200 (30.0)
	17-18	404 (60.6)
	>18	45 (6.7)
Number of sex partners in the past 12 months	0-1	407 (61.0)
	2-3	204 (30.6)
	4-5	41 (6.1)
	>5	15 (2.2)
Condom use	Consistent	352 (52.8)
	Occasional/Rare	252 (37.8)
	No	63 (9.4)

Continuation of Table 1

Variables	Details	Participants n (%)
Oral contraceptives	Consistent	74 (11.1)
	Occasional/Rare	34 (5.1)
	No	559 (83.8)
Coitus interruptus	Consistent	139 (20.8)
	Occasional/Rare	176 (26.4)
	No	352 (52.8)
Safe days	Consistent	57 (8.5)
	Occasional/Rare	137 (20.5)
	No	473 (70.9)
Smoking experience	No	267 (40.0)
	Yes	400 (60.0)
Smoking in the past month	No	402 (60.3)
	Yes	265 (39.7)
Psychoactive substance experience	No	574 (86.1)
	Yes	93 (13.9)
Alcohol in the past month	0-2 times	514 (77.1)
	3-5 times	103 (15.4)
	>5 times	50 (7.5)
Getting drunk	Never	50 (7.5))
	1-2 times	209 (31.3
	3-9 times	214 (32.1)
	>9 times	194 (29.1)
Recreational sports	No	113 (16.9)
	Yes	554 (83.1)
Walking activity	No	145 (21.7)
	Yes	522 (78.3)
Gynaecologist/Urologist visit	No	448 (67.2)
	Yes	219 (32.8)
Pregnancy (females)	No	348 (98.9)
	Yes	4 (1.1)

gynaecologist to perform the test. After we started offering first void urine as a sample taken immediately at the Department for school medicine, as with the male students, the number of tested female students increased 6-fold (Table 1).

Life-style and health-risk behaviour of students is presented in Table 1. The mean age of the first sexual intercourse was 16.9 years (range 12-22, median 17 years) and the mean number of sexual partners in the past 12 months was 1.8 (range 0-30, median 1). Among tested students, 60.6% reported having the first sexual intercourse at the age 17-18 years, 61.0% reported 0-1 sexual partner in the past 12 months, 52.8% reported using condoms consistently, 11.1% reported using oral contraceptives consistently, 20.8% reported using coitus interruptus consistently and 8.5% reported using calendar-based contraceptive method consistently. Smoking experience reported 60.0% of students and 39.7% reported smoking in the past month. Psychoactive substance experience

Table 2 Demographic and behavioural differences of university students according to gender

Variables	Males n (%)	Females n (%)	Level of significance
CT infection			
Yes	10 (3.2)	23 (6.5)	p<0.05[a]
No	305 (96.8)	329 (93.5)	
Living in a place of studying			
With parents	117 (37.1)	106 (30.1)	p=0.07[a]
Own or lend apartment	183 (58.1)	218 (61.9)	
Student dormitory	15 (4.8)	28 (8.0)	
Self-esteem			
Low	4 (1.3)	8 (2.3)	p<0.01[b]
Normal	195 (61.9)	253 (71.9)	
High	116 (36.8)	91 (25.8)	
Age of the first sexual intercourse			
<15	17 (5.4)	1 (0.3)	p<0.01[b]
15-16	121 (38.4)	79 (22.4)	
17-18	165 (52.4)	239 (67.9)	
>18	12 (3.8)	33 (9.4)	
Number of sexual partners in the past 12 months			
0-1	124 (39.3)	283 (80.4)	p<0.01[b]
2-3	136 (43.2)	68 (19.3)	
4-5	40 (12.7)	1 (0.3)	
>5	15 (4.8)	0 (0)	
Condom use			
Consistent	175 (55.6)	177 (50.3)	p=0.06[a]
Occasional/Rare	119 (37.8)	133 (37.8)	
No	21 (6.6)	42 (11.9)	
Condom use			
Yes	294 (93.3)	310 (88.1)	p<0.05[a]
No	21 (6.7)	42 (11.9)	
Oral contraceptives use			
Consistent	15 (4.8)	59 (16.8)	p<0.01[a]
Occasional/Rare	13 (4.1)	21 (6.0)	
No	287 (91.1)	272 (77.2)	
Coitus interruptus			
Consistent	33 (10.5)	106 (30.1)	p<0.01[a]
Occasional/Rare	81 (25.7)	95 (27.0)	
No	201 (63.8)	151 (42.9)	
Safe days			
Consistent	20 (6.4)	37 (10.5)	p=0.14[a]
Occasional/Rare	64 (20.3)	73 (20.7)	
No	231 (73.3)	242 (68.8)	
Gynaecologist/urologist visit			
No	286 (90.8)	162 (46.0)	p<0.01[a]
Yes	29 (9.2)	190 (54.0)	

Continuation of Table 2

Variables	Males n (%)	Females n (%)	Level of significance
Smoking experience			
No	122 (38.7)	145 (41.2)	p=0.52[a]
Yes	193 (61.3)	207 (58.8)	
Smoking in the past month			
No	184 (58.4)	218 (61.9)	p=0.35[a]
Yes	131 (41.6)	134 (38.1)	
Psychoactive substance experience			
No	259 (82.2)	315 (89.5)	p<0.01[a]
Yes	56 (17.8)	37 (10.5)	
Alcohol in the past month			
0-2 times	211 (67.0)	303 (86.1)	p<0.01[a]
3-5 times	61 (19.4)	42 (11.9)	
>5 times	43 (13.6)	7 (2.0)	
Getting drunk			
Never	18 (5.7)	32 (9.0)	p<0.01[a]
1-2 times	56 (17.8)	153 (43.5)	
3-9 times	92 (29.2)	122 (34.7)	
>9 times	149 (47.3)	45 (12.8)	
Recreational sports			
No	27 (8.6)	86 (24.4)	p<0.01[a]
Yes	288 (91.4)	266 (75.6)	
Walking activity			
No	79 (24.1)	66 (18.8)	p<0.05[a]
Yes	236 (74.9)	286 (81.2)	

[a]Chi square; [b]Fisher's exact test.

reported 13.9% of students, 29.1% reported getting drunk more than 9 times in their life and 77.1% reported drinking alcohol in the past month 0-2 times. Practicing recreational sports reported 83.1% of students and 78.3% of students reported practicing walking activity.

Health-risk behaviour and lifestyle of first-year university students was significantly different between male and female students (Table 2). Male students reported higher self-esteem, more frequent engagement in recreational sports, more frequent experience with psychoactive substances, more frequent alcohol use in their life and in the past month, higher number of sexual partners in the past 12 months and earlier age of the first sexual intercourse. On the other hand, female students reported more frequent walking activity, more frequent use of oral contraceptives and coitus interruptus and also more frequently reported not using a condom.

Prevalence and risk factors

Prevalence of *C. trachomatis* infection was 4.9% (95 CI 3.26% - 6.54%), 3.2% (95 CI 1.26% - 5.14%) in male students and 6.5% (95 CI 3.92% - 9.03%) in female students, the difference was significant (p<0.05). Of all investigated demographic and behavioural variables presented in Table 3, female sex was significantly associated with CT infection. There were no significant differences between CT

Table 3 Prevalence of *C. trachomatis* infection by demographic and behavioural variables

Variables	CT infected n (%)	Tested students (n)	Level of significance
Gender			
Male	10 (3.2)	315	p< 0.05[a]
Female	23 (6.5)	352	
Age			
≤20 years	27 (4.7)	580	p=0.37[a]
>20 years	6 (6.9)	87	
Living in a place of studying			
With parents	7 (3.1)	223	
Own or lend apartment	22 (5.5)	401	p=0.14[b]
Student dormitory	4 (9.3)	43	
Self-esteem			
Low	0 (0)	12	
Normal	22 (4.9)	448	p=0.92[b]
High	11 (5.3)	207	
Age of the first sexual intercourse			
<15	1 (5.6)	18	
15-16	9 (4.5)	200	p=0.95[b]
17-18	21 (5.2)	404	
>18	2 (4.4)	45	
Number of sex partners in the past 12 months			
0-1	20 (4.9)	407	
2-3	10 (4.9)	204	p=0.40[b]
4-5	1 (2.4)	41	
>5	2 (13.3)	15	
Condom use			
Consistent	17 (4.8)	352	
Occasional/Rare	11 (4.4)	252	p=0.50[a]
No	5 (7.9)	63	
Condom use			
Yes	28 (4.6)	604	p=0.25[a]
No	5 (7.9)	63	
Oral contraceptives			
Consistent	3 (4.1)	74	
Occasional/Rare	1 (2.9)	34	p=1[b]
No	29 (5.2)	559	
Coitus interruptus			
Consistent	5 (3.6)	139	
Occasional/Rare	12 (6.8)	176	p=0.37[a]
No	16 (4.5)	352	
Safe days			
Consistent	2 (3.5)	57	
Occasional/Rare	6 (4.4)	137	p=0.87[b]
No	25 (5.3)	473	

Continuation of Table 3

Variables	CT infected n (%)	Tested students (n)	Level of significance
Smoking experience			
No	12 (4.5)	267	p=0.65[a]
Yes	21 (5.3)	400	
Smoking in the past month			
No	19 (4.7)	402	p=0.75[a]
Yes	14 (5.3)	265	
Psychoactive substance experience			
No	29 (5.1)	574	p = 1[b]
Yes	4 (4.3)	93	
Alcohol in the past month			
0-2 times	26 (5.1)	514	
3-5 times	5 (4.9)	103	p=1[b]
>5 times	2 (4.0)	50	
Getting drunk			
Never	2 (4.0)	50	
1-2 times	12 (5.7)	209	p=0.97[b]
3-9 times	10 (4.7)	214	
>9 times	9 (4.6)	194	
Recreational sports			
No	3 (2.7)	113	p=0.34[b]
Yes	30 (5.4)	554	
Walking activity			
No	9 (6.2)	145	p=0.43[a]
Yes	24 (4.6)	522	

[a]Chi square; [b]Fisher's exact test.

positive and negative females, nor between CT positive and negative male students. Significant risk factors for CTI were not determined for female students, nor for the male students.

Discussion

There is no epidemiological data regarding CTI prevalence among asymptomatic university students in Croatia and studies regarding CTI prevalence in general are scarce in Croatia. A recent cross-sectional study of CTI among asymptomatic young people aged 18-25 years in Croatia, conducted on a nationally representative, multi-stage stratified probability sample, found prevalence of 6.2% (13), which was similar to CTI prevalence among university students found in this study. This may suggest that the results obtained for the university students might be representative for young adults. The previously mentioned study had a response rate of 32.5% and a small sample size (n=274), which might be the reason for finding no significant difference between men and women.

Obtained CTI prevalence in this study is comparable to other studies that used opportunistic screening in asymptomatic student population in Europe and worldwide with prevalence rates ranging from 2.7% to 9.7% (14-21). Although behavioural risk factors, such as inconsistent condom use and new or multiple partners per year are the main risk factors associated to CTI beside young age (22), this study found no health-risk behaviours associated to CTI among university

students. A similar study conducted among Croatian young adults aged 18-25 years found no significant risk factors for CTI (13). The only significant risk factor for CTI determined in this study was female sex. Although significant differences in health-risk behaviour of female and male students were determined, it is difficult to argue that these are the only explanations for the increased risk for CTI in females. Significantly more female students reported not using a condom, but on the other hand, male students reported a significantly higher number of sexual partners in the past 12 months. Another explanation of higher risk for CTI among female students might be the anatomic characteristics of cervix of younger women that makes them more susceptible to CTI (2, 22, 23), since 90.1% of tested female students were aged ≤20 years.

The response rate for CT testing was low in this study, especially among female students who were offered to preform sampling at gynaecologist's office using cervical swab sample. 2015 European guideline on the management of CTI recommends first choice specimen for diagnosis of urogenital CTI to be first-void urine sample for men and vulvo-vaginal swab for women since it is at least as sensitive as a cervical specimen (3). Due to suboptimal sensitivity, first-void urine samples for women should only be used if other specimens are not available (3). Although vulvo-vaginal swabs are the first choice specimens recommended for women, several studies found that young women prefer urine samples over self-collected or clinician taken swabs (24, 25), and this study also showed female students prefer urine samples over cervical swabs.

These results suggest that decision making on the possible policy of chlamydia control in the form of opportunistic screening should take into account settings and sampling techniques that are acceptable to the target population of each country. This study showed that a gynaecologist's office is not an appropriate setting since female students avoid going there and that the cervical swab is not an acceptable sample for this population. There are studies suggesting school-based health centres and education-based settings are preferred by students and young adults (26, 27).

Obtained results of prevalence may support a screening strategy for university students, but higher participation rates need to be achieved for population level impact on CT transmission (28). Mathematical modelling suggests that screening 38% of women aged 15 to 24 years annually would significantly reduce CT transmission and that screening both men and women up to 29 years may affect CT transmission or sequelae (29). Randomised control trials suggest that CT screening reduces the incidence of PID in comparison to control groups (10). Assumptions about model structure and the probability of complications of CT in several studies tend to favour screening (22).

Although sexual health education is organized in Croatia and performed by the school doctors during high-school education, results showed that a significant proportion of university students practice high-risk sexual behaviours. Inconsistent condom use, coitus interruptus use, early sexual initiation, a high number of sexual partners and avoiding gynaecologists were identified as targets for sexual health education strategies needed to prevent health-risk behaviour among university students.

There are several limitations of this study. Because the participants were self-selected, the results may not be representative of all university students. Most of the samples collected from females were urine samples. This may have missed some infections in women since vulvovaginal and cervical swabs demonstrated slightly higher sensitivity (22, 30). Also, only urogenital infections were identi-

fied in this study, whereas CTI can also occur in the rectum and oropharynx.

Conclusion

Opportunistic screening is a feasible strategy to diagnose asymptomatic chlamydia infection among university students in Croatia. The study's results may contribute to decision making on the form of chlamydia control policy in Croatia, including the choice of screening settings and specimens suitable for target population. Prevention of health-risk behaviours of students should be a part of CT control policy.

Authors' contribution: Conception and design: JB, ZB and VB; Acquisition, analysis and interpretation of data: JB, ZB, MP, VB and VBK; Drafting the article: JB; Revising it critically for important intellectual content: JB and ZB; Approved final version of the manuscript: JB, ZB, MP, VB and VBK.

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