

Molecular Epidemiology of Genital *Chlamydia Trachomatis* Infection in Asymptomatic Adolescent-Young People

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Abstract Infections by *Chlamydia trachomatis* are usually asymptomatic or minimally symptomatic and for this reason, diagnosis is often delayed. The aim of this study was to investigate the genotype of genital *Chlamydia trachomatis* infections among asymptomatic adolescent and young people. Detection and genotyping of *Chlamydia trachomatis* were performed with polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis directly on crude cells of first-void urine. Crude samples (n=427; 222 men/205 women) from young people with different social and economic conditions were screened for *C. trachomatis*. The prevalence of chlamydial infection was 13.7% among women and 4.1% among men. Socioeconomically disadvantaged women had a significantly higher prevalence compared with the rest of the women 27.4% vs. 6.1% (p=0.0006). Genotype E was the most frequently found (N=27 73%) among both genders. Screening programs focussed on sexually active adolescents and young people attending health clinics are important for detection of *Chlamydia trachomatis* infections.

Keywords *Chlamydia Trachomatis*, Asymptomatic, Young People

1. Introduction

Infections by *Chlamydia trachomatis* are the most prevalent sexually transmitted bacterial infections recognized throughout the world. The World Health Organization[1] estimated 92 million new cases worldwide in 1999 and indicates that the incidence of the infection has continued to rise every year in both industrialized and developing countries.

The original Wang and Grayston classification[2] that defined 15 *C. trachomatis* serovars was based on antigenic differences of the major outer membrane protein (momp). According to the reclassification of the order *Chlamydiales* of 1999, the family *Chlamydiaceae* is now divided in two genera, *Chlamydia* and *Chlamydophila*[3]. The genus *Chlamydia* comprises the species *C. trachomatis*, *C. suis* and *C. muridarum*.

C. trachomatis is the causative agent of a variety of

diseases and syndromes, including trachoma (genotypes A, B, Ba, C), urogenital infections, chlamydial conjunctivitis, infant pneumonia (genotypes D-K), and lymphogranuloma venereum (genotypes L1, L2, L2a, L3)[4,5]. Lymphogranuloma venereum (LGV) is endemic in several areas of developing countries and represents a major risk factor for HIV acquisition[6,7].

More than 70% of genital infections in women and 50% in men are asymptomatic[8,9]. The factors that determine whether infections are symptomatic or asymptomatic are still unknown. From the public health perspective, this leads to the persistence of undetected *C. trachomatis* reservoirs with significant perpetuation of transmission. Thus, an efficient and rapid method for detection and typing of *C. trachomatis* genotypes, particularly a highly sensitive method that allows the finding and analysis of low number of copies of *C. trachomatis* DNA is needed, mainly for the study of asymptomatic populations. Compared to other techniques, PCR provides higher sensitivity and specificity. Reports have shown that plasmid-targeted PCR is more sensitive than ompA gene-targeted PCR[10]; however the last one allows detection of strains that lack plasmids[11].

Furthermore, PCR has been successfully applied to

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different Chlamydia genotypes by means of restriction fragment length polymorphism (RFLP) analysis and direct sequencing of amplified ompL DNA [12,13]. Genotypic characterization of *C. trachomatis* isolates can not only provide valuable insights into circulating *C. trachomatis* serovars within a given community, but can also improve understanding of their epidemiology, which may assist in developing strategies to increase the control of sexually transmitted disease (STD).

The aim of this study was to genotype *C. trachomatis* samples recovered from non-symptomatic adolescent-young people with different socio economic conditions from several areas of Cordoba city.

2. Material and Methods

Urine samples from 427 individuals (205 females/222 males) were collected in two-year periods. The inclusion criteria were: sexually active asymptomatic individuals aged 18 to 24 years who had not received antimicrobial therapy during the 15 days prior to the study; females should have a negative pregnancy test. Written informed consent was obtained and the project was approved by the Ethics Council of the Catholic University of Córdoba. A survey was used to collect the data about socio economic conditions. The sample included 272 individuals with satisfied basic necessities (SBN) and 155 individuals under socio economically disadvantaged conditions (SDC). These conditions included precarious housing, parents with null or basic educational level, overcrowded accommodations (three or more persons per room) or lack of toilet.

Data Processing: The data were processed and tabulated and the variables were quantified; the distribution and trend measures were determined and the analysis of independent variables was performed with statistics tests. Data were compared by univariate analysis with Pearson test and odds ratio.

Univariate comparison between patients with Chlamydial infection and control group was performed using χ^2 test. Variables with significance level lower than 0.1 on univariate analysis were analyzed with a multivariate logistic regression model using a forward conditional (likelihood ratio) method.

Urine: Subjects were instructed not to urinate within the previous hour and females should not clean the perineum before urinating. The first catch of 10 to 20 ml of urine was collected in a clean collection cup and refrigerated immediately at 2-8°C for no more than 4 days for PCR.

Plasmid PCR and ompA PCR: In order to determine the sensitivity of plasmid PCR and ompL PCR, a dilution assay was performed with genotype L₂/434/Bu. This assay showed that the plasmid PCR was capable to detect 0.1 inclusion-forming units. OmpL PCR was about 100 times less sensitive than plasmid PCR; for this reason, when making the Hemi-nested PCR for MOMP, sensitivity values were equalled.

PCR pre-treatment methods: Two hundred micro liters of the samples were centrifuged at 16,000 x g for 30 min. The pellet was resuspended in 200 μ l of 10 mM Tris-HCl (pH 7.5), stored at -80°C and thawed before use. This freeze-thawed suspension was used for PCR. The subsequent pre-treatment method was digestion with proteinase K (0.5 mg/ml) in the presence of non-ionic detergent Tween 20 (0.45%). The solution used for pre-treatment contained 100 mM KCl, 3.0 mM MgCl₂, and 20 mM Tris-HCl (pH 8.3). All pre-treated samples were boiled for 10 minutes before PCR.

Detection of *C. trachomatis* by plasmid PCR: The primers used for generating a 201-bp fragment of the *C. trachomatis* cryptic plasmid were CTP1 (plus strand; 5'-TAGTAACTGCCACITCATCA-3') and CTP2 (minus strand; 5'-TTCCCCTTGTAATTCGTTGC-3') [13].

C. trachomatis ompA PCR and genotyping of *C. trachomatis* were performed as previously described [13]. Briefly, the primers used for generating an approximately 1.1-kb fragment of the ompL gene were SERO1A (plus strand; 5'-ATGAAAAAACTCTTGAAATCGG-3') and SERO2A (minus strand; 5'-TTTCTAGAT/CTTCATT/CTTGTT-3').

PCR products were analyzed by 1.5% agarose gel electrophoresis. Briefly, 2 μ l of the primary ompA PCR product was pipetted with an aerosol-tipped pipette into a PCR mixture containing the SERO2A primer and one nested primer, pCTM3 (5'-TCCTTGCAAGCTCTGCCTGTGGG AATCCT-3') located 55 bp downstream of SERO1A. The amplification conditions of the nested PCR were the same as those of the primary ompA PCR.

RFLP analysis of ompA PCR- and nested PCR-positive samples was carried out as described by Lan et al. [13] with minor modifications. Briefly, 10 μ l of positive PCR product were digested with 2.5 U of AluI (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. If necessary, samples were analyzed with a second enzyme (HinfI, CfoI, or a combination of EcoRI and DdeI). The samples were then analyzed by 3% agarose gel electrophoresis, stained with 10 μ g of ethidium bromide per ml during 45 min and photographed under UV light. The patterns observed for each genotype were in accordance with those reported by Lan et al [13]. The samples were amplified with primers for β -globin in order to detect the inhibitor's presence.

3. Results

Of the 427 individual screened, 8.7% (N=37) yielded positive results with plasmid PCR; 11 samples that were negative by ompA PCR were positive by plasmid PCR; all of these were clearly positive after Hemi nested ompA PCR. Plasmid free *C. trachomatis* strains not were detected in this study.

The prevalence of *C. trachomatis* in the female groups was 13.7% (28/205) and 4.1% in males (9/222). The difference

was statistically significant, $p < 0.0004$, OR 3.74 (1.66-9.24, IC 95%).

The prevalence was 5.1% (14/272) in the SBN group and 14.8% (23/155) in the SDC group. Socioeconomically disadvantaged women had a significantly higher prevalence compared with the rest of the women 27.4% vs. 6.1% ($p = 0.0006$). The difference was statistically significant, $p < 0.0006$, OR 3.21 (1.52-6.97, IC 95%). Genotyping of *C. trachomatis* in urine samples was carried out in 37 ompA PCR-positive samples. Genotyping of these samples showed that they were identified directly after AluI digestion.

Table 1. Distribution of *C. trachomatis* genotypes according to gender and socio economic conditions

Genotype	Women (13.7%) N=28/205		Men (4.1%) N=9/222	
	SBN ¹	SDC ²	SBN	SDC
Total=37/427 (8.7%) SBN (5.1%) N=14/272 SDC (14.8%) N=23/155	(6.1%) N=8/132	(27.4%) N=20/73	(4.3%) N=6/140	(3.7%) N=3/82
E (73%) N=27/37	N=5	N=16	N=4	N=2
D (16.2%) N=6/37	N=1	N=4		N=1
F (5.4%) N=2/37	N=1		N=1	
G (5.4%) N=2/37	N=1		N=1	

¹SBN: satisfied basic necessities. ²SDC: socio economically disadvantaged conditions

The overall genotype distribution of the 37 typed samples is summarized in Table 1; genotype E was the most frequently found (73%), genotype D was found in 6 samples (16.2%) and genotypes F and G were found in 2 (5.4%).

4. Discussion

The nucleic acid amplification tests worked significantly better than alternative methods like culture isolation or ELISA [14] for the detection of *C. trachomatis* both for urine samples and cervical swabs [15].

The existence of a stable plasmid-free *C. trachomatis* may cause undesirable problems when using PCR for diagnosis, although it is 10 times more sensitive than ompA PCR. In our study, both PCRs demonstrated to have no differences regarding sensitivity, possibly due to the fact that nested ompA Hemi nested PCR was performed.

Studies among clinically healthy populations have shown a prevalence rate equal or major than values detected in our population of asymptomatic young people (8.7%). Two reports have shown a lower prevalence rate of 0.9% in the US [16] and Germany [17] in a population of military and adolescent students; however, higher prevalence rates (8.8%) have been reported for Chinese students [18] and young people of England (8.3%) [19]. The rate for a population who

attended health care centers in Brazil showed an average prevalence rate of 11.7%, ranging from 4 to 25.7% [20,21]

Infection with *C. trachomatis* can be asymptomatic in up to 80% of the females [22], this fact can difficult its diagnosis and detection. The asymptomatic nature of chlamydial infection makes screening essential for the control of this infection. *C. trachomatis* has its highest prevalence among young people. More than 13.5% of the women younger than 25 years old present infection of the lower genital tract; however, this rate decreases to less than 4.9% in women over 25 years old [23].

We found large variations of the incidence of *C. trachomatis* among different groups of subjects within Córdoba, a large city of Argentina. We correlated the dependent contribution of socio economic conditions to the risk of acquiring *C. trachomatis* infection. We detected extremely high rates of *C. trachomatis* infection among asymptomatic young women with socio-economical disadvantages. Genotype E was the most frequently found genotype among women of the SDC group. A high prevalence of genotype E (27 of 37, 73%) and the lack of associated clinical symptoms may suggest that this genotype is more successful than other less-prevalent genotypes in our studied population. Indeed, a successful genotype would be the one that remains undetected for longer periods of time, enhancing dissemination. Other studies have demonstrated frequencies of genotype E similar to ours in asymptomatic subjects [24-25]. Even among High-Risk Women in China, serovar E was associated with asymptomatic infection [26]. In Buenos Aires city, Argentina, Gallo Vaullet et al have confirmed an increased frequency of genotype E in neonatal ocular samples and genital adult samples [27].

This technique has provided a valuable and sensitive resource for molecular epidemiological analysis to identify high-risk groups. To the best of our knowledge, this is the first study of genotype distribution of *C. trachomatis* in asymptomatic young people in Córdoba Argentina.

In conclusion, we detected a high prevalence of *C. trachomatis* genital infection among healthy young people in Córdoba, Argentina, by using a highly sensitive diagnostic PCR.

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REFERENCES

- [1] World Health Organization. Global prevalence and incidence of selected curable sexually transmissible disease: overview and estimates. Geneva. WHO, 2001.
- [2] Wang SP, Grayston JT; 1970, Immunologic relationship between genital TRIC, lymphogranuloma venereum and

- related organisms in a new microtiter indirect immunofluorescence test. *Am J Ophthalmol*; 70:367-374.
- [3] Everett, K. D. E., R. M. Bush, and A. A. Andersen; 1999, Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *Int. J. Syst. Bacteriol*; 49:415-440.
- [4] Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, Klausner JD; 2003, Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California. *Clin Infect Dis*; 41(1):67-74.
- [5] Sethi G, Allason-Jones E, Richens J, Annan NT, Hawkins D, Ekbote A, Alexander S, White J; 2009, Lymphogranuloma venereum presenting as genital ulceration and inguinal syndrome in men who have sex with men in London, UK. *Sex Transm Infect*; 85(3):165-70.
- [6] Blank S, Schillinger JA, Harbatkin D; 2005, Lymphogranuloma venereum in the industrialised world. *Lancet*; 365(9471):1607-8.
- [7] Cai L, Kong F, Toi C, van Hal S, Gilbert GL; 2010, Differentiation of *Chlamydia trachomatis* lymphogranuloma venereum-related serovars from other serovars using multiplex allele-specific polymerase chain reaction and high-resolution melting analysis. *Int J STD AIDS*; 21(2):101-4.
- [8] Hay, P. E., and Ghaem-Maghami S.; 1997, *Chlamydia* and non-gonococcal urethritis. *Curr. Opin. Infect. Dis*; 10:44-49.
- [9] Zimmerman HL, Potterat JJ, Dukes RL, Muth JB, Zimmerman HP, Fogle JS, Pratts CI; 1990, Epidemiologic differences between chlamydia and gonorrhea. *Am J Public Health*; 80(11):1338-42.
- [10] Mahony JB, Luinstra KE, Sellors JW, Jang D, Chernesky MA; 1992, Confirmatory polymerase chain reaction testing for *Chlamydia trachomatis* in first-void urine from asymptomatic and symptomatic men. *J Clin Microbiol*; 30(9):2241-5.
- [11] Matsumoto A, Izutsu H, Miyashita N, Ohuchi M; 1998, Plaque formation by and plaque cloning of *Chlamydia trachomatis* biovar trachoma. *J Clin Microbiol*; 36(10):3013-9.
- [12] Black CM; 1997, Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev*; 10:160-84.
- [13] Lan J, Walboomers MM, Roosendaal R, van Doornum G, Maclaren DM, Meijer CJ, van den Brule AJC; 1993, Direct Detection and Genotyping of *Chlamydia trachomatis* in Cervical Scrapes by Using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis. *J Clin Microbiol*; 31: 1060-1065.
- [14] Hollblad-Fadiman K, Goldman SM. 2003. American College of Preventive Medicine Practice Policy Statement Screening for *Chlamydia trachomatis* *Am J Prev Med*; 24(3)
- [15] Watson EJ, Templeton A, Russell I, Paavonen J, Mardh PA, Stry A, Pederson BS; 2002, The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol*; 51(12):1021-31.
- [16] Jordan NN, Lee SE, Nowak G, Johns NM, Gaydos JC; 2011, *Chlamydia trachomatis* reported among U.S. active duty service members, 2000-2008. *Mil Med*; 176(3):312-9.
- [17] Desai S, Meyer T, Thamm M, Hamouda O, Bremer V; 2011, Prevalence of *Chlamydia trachomatis* among young German adolescents, 2005-06. *Sex Health*; 8(1):120-2.
- [18] Hsieh YH, Shih TY, Lin HW, Hsieh TC, Kuo MJ, Lin CW, Gaydos CA; 2010, High-risk sexual behaviours and genital chlamydial infections in high school students in Southern Taiwan. *Int J STD AIDS*; 21(4):253-9.
- [19] Skidmore S, Copley S, Cordwell D, Donaldson D, Ritchie D, Spraggon M; 2011, Positive nucleic acid amplification tests for *Neisseria gonorrhoeae* in young people tested as part of the National Chlamydia Screening Programme. *Int J STD AIDS*; 22(7):398-9.
- [20] Rodrigues, M.; Fernandes, P.; Haddad, J.; Paiva, M.; Souza, Mdo.; Andrade, T.; Fernandes A; 2011, Frequency of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma* species in cervical samples. *J Obstet Gynecol*; 31: 237-241.
- [21] Ramos, B.; Poletini, J.; Marcolino, L.; Vieira, E.; Marques, M.; Tristão, A.; Nunes, H.; Rudge, M.; Silva, M; 2011. Prevalence and risk factors of *Chlamydia trachomatis* cervicitis in pregnant women at the genital tract infection in obstetrics unit care at Botucatu Medical School, São Paulo State University-UNESP, Brazil. *J Low Genital Tract Disease*; 15: 20-24.
- [22] Gaydos CA, Howell MR, Pare B. et al; 1998, *Chlamydia trachomatis* infections in female military recruits. *N Engl J Med*; 339: 739-744.
- [23] Svensson LO, Mares I, Olsson SE, Nordstrom ML; 1991, Screening for *Chlamydia trachomatis* infection in women and aspects of the laboratory diagnostics. *Acta Obstet Gynecol Scand*; 70: 587-590
- [24] Pineiro L, Montes M, Gil-Setas A, Camino X, Echeverria MJ and Cilla G. 2009. Genotipado de *Chlamydia trachomatis* en un area del norte de Espana. *Enferm Infecc Microbiol Clin*; 27(8):462-464
- [25] Morré SA, Rozendaal L, van Valkengoed IG, Boeke AJ, van Voorst Vader PC, Schirm J, de Blok S, Hoek van Den JA, van Doornum GJ, Meijer CJ, Brule van Den AJ. 2000. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection: an association with clinical manifestations?. *J Clin Microbiol*; 38(6):2292-6.
- [26] Gao X, Chen XS, Yin YP, Zhong MY, Shi MQ, Wei WH, Chen O, Peeling RW and Mabey D. 2007. Distribution Study of *Chlamydia trachomatis* Serovars among High-Risk Women in China Performed Using PCR-Restriction Fragment Length Polymorphism Genotyping. *J Clin Microbiol*. 1185-1189
- [27] Gallo Vaulet L, Entrocassi C, Corominas AI, Rodríguez Fermepin M. 2010. Distribution study of *Chlamydia trachomatis* genotypes in symptomatic patients in Buenos Aires, Argentina: association between genotype E and neonatal conjunctivitis *BMC Research Notes*. 3:34.