Urogenital Tract Infection with *Chlamydia trachomatis* among Women Attended at Different Units in a Referral Hospital in Spain

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Authors PM, PSL and JAB participated in the planning of the study, analysis and interpretation of the data, laboratory studies and wrote the first draft of the manuscript, revised it critically and approved the final version.

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**ABSTRACT**

**Aims:** The objective of this study was to estimate the prevalence and characteristics of *Chlamydia trachomatis* infections in a group of women visiting different Units in a referral Hospital from Spain. **Study Design:** This was a hospital retrospective and descriptive study for the presence of *C. trachomatis* in endocervical, vaginal and urine swabs obtained from consecutive sexually active women attendees at different Units. Also their medical records were reviewed. Retrospective ethical approval was granted by the Ethical Committee of Clinical Investigation of Principality of Asturias. **Place and Duration of Study:** Units of Gynecology, Obstetrics and Infertility of Hospital Universitario Central de Asturias, between January 2007 to December 2011. **Methodology:** We included 1997 symptomatic and asymptomatic unselected women (mean age 29.1 range 18 to 45 years) who were evaluated for urogenital chlamydial infection. **Results:** The overall prevalence of *C. trachomatis* was 6.3%. The *C. trachomatis* infection had the highest prevalence among the age group below 25 years of age (n=30, 7.5%). Genotypes E, G
and D constitute 89.4% of the genotyped strains. Infections with genotypes G and F were more often (n=31, 42%) associated with clinical manifestations that suggest cervical infection and genotype E was observed more frequently (n=17, 85%) in asymptomatic women. 

Conclusions: In our study, similar prevalence rates between both symptomatic and asymptomatic women, under 25 years, were found. Self-collected vaginal swabs are an appropriate alternative for routine diagnosis of C. trachomatis infection. The findings of this work highlighted the need for a possible Chlamydia screening program, offered especially in younger women. Delays in seeking a diagnosis and treatment among asymptomatic females can result in increased transmission of this bacterium and its serious consequences for women reproductive health.

Keywords: C. trachomatis; prevalence; sexual transmitted infections; infertility; genotypes.

1. INTRODUCTION

C. trachomatis is currently, the major cause of bacterial sexually transmitted infections (STIs) in Europe as well as many other countries around the world [1]. An important characteristic of these infections is their ability to cause long-term sequels in the upper genital tract. In women, chlamydial urogenital infections, having a clinical course varying for asymptomatic to clinical manifestations, which include urethritis, cervicitis and pelvic inflammatory disease (PID) may lead to serious sequelae such as ectopic pregnancy, infertility and chronic lower abdomen pain [2,3].

Due to their frequent asymptomatic nature and the prevalence among young women, this infection is a public health problem to resolve [4]. Despite major advances in the diagnosis and management of chlamydial infections, in Spain there is no a general cost-effective plan of screening programs that would guarantee a decrease of the aftermath caused by untreated infections. Undiagnosed and untreated chlamydial infections are, thus, not only creating major health problems and consequences for individuals, but also result in major social and economical problems. The aim of this study was to determine the prevalence and characteristics of Chlamydia trachomatis infections in a group of women attended to at different Units from a referral Hospital in Spain.

2. MATERIALS AND METHODS

2.1 Patients and Clinical Samples

A total of 2255 biological samples (1381 vaginal, 763 endocervical dracon swabs and 111 urine specimens) were tested, for Chlamydia trachomatis by Cobas TaqMan CT system according to manufactures’ instructions (Roche Diagnostic Systems, Branchburg, NJ). These specimens were collected between January 2009 to December 2011, from 1997 (1469 symptomatic and 528 asymptomatic) consecutive sexually active women (mean age 29.4, range 18 to 45 years), attending Gynecology, Obstetrics and Infertility Units of the Hospital Central de Asturias, Spain. Retrospective ethical approval (nº128/2012) was granted by the Ethical Committee of Clinical Investigation of Principality of Asturias.

Symptomatic women were defined as those suffering from one genitourinary clinical symptom (abnormal vaginal discharge, cervicitis, PID, lower abdominal pain, dyspareunia, dysuria or vulvar erythema). Asymptomatic women were defined as those who contacted a clinician for pregnancy control, routine Pap smear annual examination or infertility, and they did not have symptoms or signs of infection at the time the specimen was taken.

On the other hand, STI microbiologic testing: culture of bacteria, VHS-2, Candida spp, serological testing (HBV, syphilis and HIV) or NAAT for VPH were routinely performed considering clinical signs or symptoms of patients.

To compare vaginal samples and endocervical samples as better detection specimen, 129 vaginal and endocervical swabs belonging to 129 women were acquired simultaneously.

Furthermore, a possible association between particular genotypes and the occurrence of clinical manifestations was studied in 85 C. trachomatis single infected-women.
2.2 Chlamydia trachomatis Detection

*C. trachomatis* DNA was extracted and detected using AMPLICOR CT/NG Specimen Preparation Kit (Roche Diagnosis System) and COBAS TaqMan CT Test v2.0 (Roche Diagnosis) respectively. Positive *C. trachomatis* specimens were kept at -70°C in 2SP medium until retrospective ompA genotyping.

2.3 Genotyping

*C. trachomatis* DNA was extracted using the Nuclisens Easy Mag system (bioMerièux, Marcy l’Etoile France). To genotype *C. trachomatis* specimens, a 990pb fragment of the ompA gene was amplified according to a nested-PCR methodology previously described [5]. PCR amplified ompA gene fragments were purified from agarose gels using Montage DNA Gel Extraction Kit (Millipore, Bedford, MA) and sequenced with BigDye Terminator Sequencing Kit (Applied Biosystems, Foster City, CA) using inner primers (MOMP87-RSV1059).

The amplicon sequences were aligned to analogous sequences from reference strains of each genotype by using Clustal-W2 program. The strains were A/Sa1 (accession number 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-Cal9 (X52080), G/UW57 (AF063199), H/UW4 (X16007), I/UW-12 (AF063200), Ia/IU-4168 (AF063201), J/UW36 (AF063202), Ja/IU-A795 (AF063203), K/UW31 (AF063204), L1/440 (M36533), L2/434 (M14738), L2b/144276 (DQ217607), L3/404 (X55700) and *Chlamydia muridarum* MoPn (M64171), a murine variant of *C. trachomatis*. The alignments were used to obtain phylogenetic trees with 1.6.6 Tree-view program.

2.4 Statistical Analyses

Statistical analyses of the data were performed using the Χ² or Fisher’s exact test. A p- value < .05 was considered to be statistically significant. Absolute and relative frequencies and 95% accuracy were given. Also, the kappa index was used to measure the correlation in the diagnostic yield of different samples.

3. RESULTS

Out of 2255 samples studied, a total of 134 specimens [88 (6.4%) vaginal, 43 (5.6%) endocervical and 3 (2.7%) urine] belonging to 125 women, were positive for *C. trachomatis* infection. The overall prevalence of *C. trachomatis* was 6.3% (6.9% in symptomatic and 4.5% in asymptomatic females) (p= .06). In women with an age between 25 and 40 years, the highest prevalence (7.1%) was found in those with some genitourinary clinical symptom. We observed similar prevalence rates (7.7% and 7%) among the age group younger than 25 years in both symptomatic and asymptomatic women, respectively (Table 1).

Prevalence rates observed in women attending a Gynecology Unit, were higher (6.9%) in patients with clinical manifestations than in those visiting the Unit for an annual routine Pap smear examination (3.8%) (p= .04). Prevalence rates found in evaluated pregnant women and in asymptomatic women with infertility were 5% and 10%, respectively (Table 1).

Table 1. Prevalence of *C. trachomatis* infection in relation to age and the units of precedence in symptomatic and asymptomatic tested women

| Age range (years) | Total | Symptomatic | Asymptomatic |
|-------------------|-------|-------------|--------------|
| <25               | 1997  | 125 (6.3)   | 1469 (101)   |
| 25-40             | 1272  | 81 (6.4)    | 940 (67)     |
| >40               | 326   | 14 (4.3)    | 230 (11)     |
| Units             |       |             |              |
| Gynecology        | 1837  | 115 (6.2)   | 1469 (101)   |
| Obstetric         | 120   | 5 (0)       | 0 (0)        |
| Infertility       | 40    | 4 (10)      | 0 (0)        |

CT: *C. trachomatis* infected women
The most often clinical symptom reported was abnormal vaginal discharge (n= 591; 40.2%). C. trachomatis was found as a single infection in 46 (90.2%) out of 51 positive women. Nevertheless, C. trachomatis was the only microorganism detected in all of the cases with PID or lower abdominal pain. The study of the relationship among C. trachomatis infection and the presence of concomitant STIs, showed that 16 (15.8%) women had concurrent pathogens such as HPV (9, 47.4% of cervicitis cases), Candida spp (5, 9.8% of cases with abnormal vaginal discharge) and HSV-2 (2, 100% of vulvar erythema cases) (Table 2).

C. trachomatis was detected in both swabs (vaginal and endocervical) in 35 (27.1%) patients, in vagina in 3 (2.3%) women and in endocervix in 1 (0.8%) female Kappa index (0.924) (Table 3).

The phylogenetic analysis of the ompA gene from 94 positive specimens showed that the most frequent genotypes were G (n=35, 37.2%) followed by D (n=26, 27.7%), E (n=23, 24.5%), F (n=8, 8.5%) and K (n=2, 2.1%). In the 74 positive specimens belonging to symptomatic women infected only with C. trachomatis, genotype G (47.3%) was the most prevalent, followed by D (31%), F (10.8%), E (8.1%) and K (2.7%). On the other hand, 20 asymptomatic females presented E (85%) and D (15%) genotypes. The most relevant difference between symptomatic and asymptomatic women was the detection of genotype E in 8.1% of the symptomatic patients vs 85% in the asymptomatic females (p< .001). Infections with genotypes G and F were more often (n=31, 42%) associated with clinical manifestations that suggest cervical infection (cervicitis, lower abdominal pain and PID). Genotype G was found only in symptomatically infected patients (p< .001) and genotype D was associated with abnormal vaginal discharge (p<0.0001) (Fig. 4).

### 4. DISCUSSION

Infections caused by C. trachomatis, have showed a progressive increase in the past decade in Europe and others parts of the developed world [1].

In our research, a clear age dependency was observed. The highest C. trachomatis prevalence rate was found among women younger 25 years of age (7.5%), in both symptomatic (7.7%) and asymptomatic patients (7%). The C. trachomatis prevalence rate decreased significantly in women older than 40 years. This is in accordance with previous publications reporting higher percentage of patients with asymptomatic infections [4]. It is therefore of importance to not forget the silent nature of this infection. The microbiology laboratory, by using sensitive and specific techniques, plays a significant role allowing a rapid confirmation of the diagnosis.

Young age is the factor that is most strongly associated with the infection (relative risk among women younger than 25 years as compared with older women, 2.0 to 3.5) [6]. This association is largely attributed to the higher level of sexual activity among younger women. Also, in younger women, the squamocolumnar junction of the cervix often lies well out on the ectocervix, forming a bright red central zone of ectopic columnar epithelium called ectropion [7].

### Table 2. Clinical manifestations in symptomatic and positive women tested for C. trachomatis infection and relation with concurrent STIs

| Symptoms                  | Tested  | Positive | Single infection | Dual infection |
|---------------------------|---------|----------|------------------|---------------|
| Abnormal vaginal discharge| 591 (40.2) | 51 (8.6) | 46 (90.2) | 50 (8.8) |
| Cervicitis                | 444 (30.2) | 19 (4.27) | 10 (52.6) | 9 (47.4) |
| PID                       | 263 (17.9) | 16 (6)   | 16 (100) | - |
| Lower abdominal pain      | 100 (6.8) | 9 (9)    | 9 (100) | - |
| Dysuria                   | 28 (1.9)  | 2 (7.1)  | 2 (100) | - |
| Vulvar erythema           | 23 (1.5)  | 2 (8.7)  | - | 2 (100) |
| Dyspareunia               | 20 (1.3)  | 2 (10)   | 2 (100) | - |
| Total                     | 1469 (100) | 101 (6.8) | 85 (84) | 16 (15.8) |

*a with respect to total tested women; b with respect to number of tested women with each symptom; c with respect to number of positives women with each symptom.

dCandida spp, eHPV, fHSV-2
Table 3. *C. trachomatis* detection in vaginal and endocervical samples recovered simultaneously, in 129 symptomatic and asymptomatic women

| Vaginal samples | PCR (+) | PCR (-) | Total |
|-----------------|---------|---------|-------|
| PCR (+)         | 35 (27.1%) | 3 (2.3%) | 38 (27.9%) |
| PCR (-)         | 1 (0.8%) | 90 (69.8%) | 91 (72.1%) |
| Total           | 36 (20.7%) | 93 (79.2%) | 129 (100%) |

Fig. 4. Association between *C. trachomatis* genotypes and symptoms

Reviewing data from different consultations, the prevalence of *C. trachomatis* infection is higher (6.9%) in females who attended the Gynecologic Units showing clinical manifestations, compared to the 3.8% asymptomatic women attending the Gynecology Unit for a routine gynecological checkup. This data are in agreement with previously published reports showing similar prevalence rates among sexually active adolescent women and also confirm the prevalence observed in asymptomatic females. In Spain, among females under 25 years from the community, Benitez et al. [8] found similar prevalence (4%). Corbeto et al. [9] studied women attending in sexual health clinic observed higher prevalence (5.8%) than in our non-selected group.

We observed a prevalence of *C. trachomatis* higher than expected in pregnant women (10%) and in asymptomatic patients suffering from infertility (5%) [10]. There is increasingly more evidence that *C. trachomatis* infection can cause a number of complications during pregnancy: early and late abortion, intrauterine fetal and neonatal infection, premature labor, premature rupture of membranes, chorioamnionitis and endometritis post-partum. Studies in populations of pregnant women show that they are at increased risk of preterm birth if infected by *C. trachomatis* before 32 weeks of gestation [11,12]. Also, the risk of infertility after *C. trachomatis* infection is not known but, between 64% and 90% of women who are infertile because of tubal occlusion have antibodies to *C. trachomatis* [13]. This is 2 to 8 times greater than the findings in women who are infertile from other causes [14]. An estimated two thirds of all cases of infertility due to tubal factor and one-third of all cases of ectopic pregnancy may be
due to silent and undiagnosed infection with *C. trachomatis* [15-17]. For this reason, screening during antenatal care, could reduce the rate of adverse outcomes of pregnancy [18-22] and should be part of routine investigations for infertility. In our hospital, pregnant women are routinely tested for syphilis, hepatitis B and HIV, but not for *C. trachomatis* and there are limited data regarding prevalence of this infection among infertile women.

Furthermore, it has been shown that screening is cost effective at prevalence of 3.1-10% and cost saving (over testing symptomatic women) at prevalence as low as 1.1%, if age was chosen as a selection factor and DNA based test were used. These studies reveal a significant reduction in the number of cases of PID and ectopic pregnancies, after the introduction of *C. trachomatis* screening [23-28]. However, due to the low number of pregnant and infertile women analyzed as well as to the unavailability of demographic data relating to them, further studies and strategies for suitable control are needed in our hospital.

There are several studies that defend the increased sensitivity of endocervical specimens [29,30]. Other authors, point out that vaginal samples have better sensitivity because collecting more DNA from the two potential sites for *C. trachomatis* infection in women, the urethra and endocervix [31,33]. This work, showed that both vaginal and endocervical swabs were optimal at detecting *C. trachomatis* infection, in women. We showed that self-collection of vaginal specimens is an appropriate alternative for *C. trachomatis* infection screening.

On the other hand, we observed that the genotypes E, G and D constitute 89.4% of the genotyped strains. Sequence analysis showed that the most prevalent *ompA* sequence corresponded to genotype G. The genotypes D, E and F are the most common genotypes in Europe [34,35] as well as in Spain [36], where the most frequent genotypes are also E, D, G and F. Genotype E is the most prevalent genotype in both men and women.

The E genotype was observed more frequently in samples from women without clinical symptoms or signs of infection (85%) compared to females with clinical manifestations, which are a reservoir able to transmit the infection. In symptomatic females (the majority of tested women), infections with genotypes G and F were more often (41.9%) associated with clinical manifestations that suggest cervical infection (cervicitis, lower abdominal pain and PID). These results are in agreement with two previously published studies in which an association was found between genotype G and developing cervical cancer and genotype F and lower abdominal pain and dyspareunia [37,38]. Perhaps, the highest number of women with cervical pathology explains the high frequency of G genotype in our study but, it is also likely that patient characteristics are a key component to understanding the genotype distribution. Unfortunately data such us sexual behaviors, history of previous *Chlamydia trachomatis* infection or prior history of gynecological pathology were not made available to the present study.

Considering all the data presented in this research and knowing that many infections are asymptomatic, it is essential to investigate in detail the infection and carefully design the most appropriate methods for detection. Thus, with the implementation of cost-effective screening we could increase our knowledge about the epidemiology and transmission of infections caused by this bacterium.

5. CONCLUSION

*Chlamydia trachomatis* infections are an important public health problem due to their frequent asymptomatic nature, the prevalence among people younger than 25 years and their severe reproductive complications. Thus, interrupting their route of transmission by identifying and treating patients with *Chlamydia trachomatis* is essential in the prevention of this STI. In Spain, there is a need for effective strategies for an early detection of *Chlamydia trachomatis* infection especially in asymptomatic women of childbearing age.

ETHICAL APPROVAL

Retrospective ethical approval was granted by the Ethical Committee of Clinical Investigation of Principality of Asturias (registration number 128/2012).

CONSENT

It is not applicable.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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