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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

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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 suffereing 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 -70ºC 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 Clustall-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-Cal3 (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^T (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 X^2 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

Total	Total			Symptomatic			Asymptomatic		
	N	CT	%	N	CT	%	N	CT	%
	1997	125	6.3	1469	101	6.9	528	24	4.5
Age range (years)									
<25	399	30	7.5	299	23	7.7	100	7	7
25-40	1272	81	6.4	940	67	7.1	332	14	4.2
>40	326	14	4.3	230	11	4.8	96	3	3.1
Units									
Gynecology	1837	115	6.2	1469	101	6.9	368	14	3.81
Obstetric	120	6	5	0	0	0	120	6	5
Infertility	40	4	10	0	0	0	40	4	10

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. DISCUSION

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
	N (%[a])	N (%[b])	N (%[c])	N (%[c])
Abnormal vaginal discharge	591 (40.2)	51 (8.6)	46 (90.2)	5[d] (9.8)
Cervicitis	444 (30.2)	19 (4.27)	10 (52.6)	9[e] (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[f] (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.
[d]Candida spp, [e]HPV, [f]HSV-2

Table *3. C. trachomatis* detection in vaginal and endocervical samples recovered simultaneously, in 129 symptomatic and asymptomatic women

Vaginal samples	Endocervical 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%)

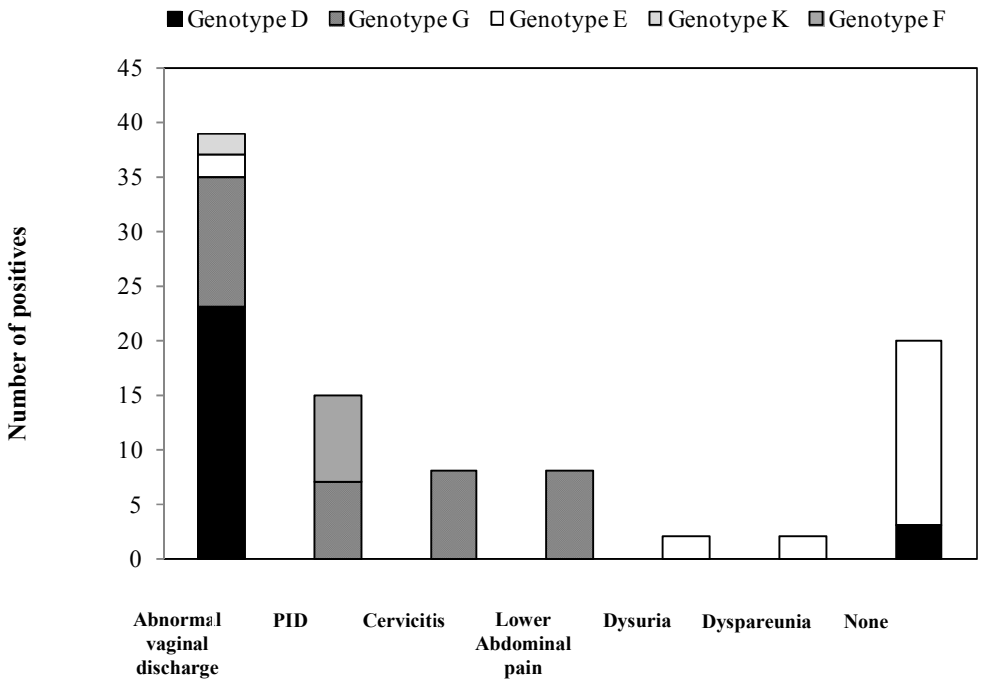

Fig. 4. Association between *C. trachomatis* genotypes and symptoms

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 rutine 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 infertility 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.

REFERENCES

1. Department of Reproductive Health and Research World Health Organization, Global Strategy for the Prevention and Control of Sexually Transmitted Infections: 2006-2015, WHO Press Geneva, Switzerland; 2007.
2. Kaushic C, Zhou F, Murdin AD, et al. Effects of estradiol and progesterone on susceptibility and early immune responses to *Chlamydia trachomatis* infection in the female reproductive tract. Clements JD, ed. Infection and Immunity. 2000;68(7): 4207-4216.
3. I Simms, K Eastick, H Mallinson et al. Associations between *Mycoplasma genitalium*, *Chlamydia trachomatis* and pelvic inflammatory disease. Sex Transm Infect. 2003;79(2):154-156.
4. Owus-Edusei K, Cheson HW, Gift TL, et al. The estimated direct medical cost of selected sexually transmitted infections in the United States 2008. Sex Transm Dis. 2013;40:197-201.
5. Lysén M, et al. Characterization of *OmpA* Genotypes by sequence analysis of DNA from all detected cases of *Chlamydia trachomatis* infections during 1 year of contact tracing in a Swedish County. J Clin Microbiol. 2004;42:1641-7.
6. Malhotra M, Sood S, Mukherjee A, et al. Genital *Chlamydia trachomatis*: An update. The Indian Journal of Medical Research. 2013;138(3):303-316.
7. Faro S, Soper DE, eds. Infectious diseases in women. Philadelphia: WB Saunders 2001;261-2.
8. Benítez C, Mejuto P, Otero L, Margolles M, Leiva P, Vázquez F. Prevalence of genital *Chlamydia trachomatis* infection among young men and women in Spain. BMC Infectious Diseases. 2013;13(1):388.
9. Corbeto EL, Lugo R, Martró E, et al. Prevalence and determining factors of acquiring *C. trachomatis* infection among adolescents and young adults in Catalonia. Enferm Infecc Microbiol Clin. 2011;29:96–101.
10. Villagrana Zesati JR, López Hurtado M, Flores Salazar VR, et al. Persistence of *Chlamydia trachomatis* in endometrium and peritoneal fluid of infertile patients with negative cervical cultura. Ginecol Obstet Mex. 2013;81(1):23-8. (Spanish)
11. Ingrid G, Rours JG, Duijts L, et al. *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: A population-based prospective cohort study. Eur J Epidemiol. 2011; 26:493–502.
12. Hernández-Trejo M, González Herrera NE, Escobedo MR, et al. Reporting detection of *Chlamydia trachomatis* DNA in tissues of neonatal death cases. J Pediatr (Rio J). 2014;90(2):182-9.
13. Broeze KA, Opmeer BC, Coppus SF, et al. Chlamydia antibody testing and diagnosing tubal pathology in subfertile women: An individual patient data meta-analysis. Hum Reprod Update. 2011;17(3):301-10.
14. Machado ACS, Gulmarães EMB, Sakurai E, et al. High titers of *Chlamydia trachomatis* antibodies in Brazilian women with tubal oclussion or previous ectopic pregnancy. Infect Dis Obstet Gynecol. 2007;2007:24816.
15. Price MJ, Ades AE, De Angelis D, et al. Risk of pelvic inflammatory disease following *Chlamydia trachomatis* infection: Analysis of prospective studies with a multistate model. American Journal of Epidemiology. 2013;178(3):484-492.
16. Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol. 2002;16:847-64.
17. Andrews WW, Goldenberg RL, Mercer B, et al. The preterm prediction Study: Association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183:662-8.
18. MacDonald N, Wong T. Canadian guidelines on sexually transmitted infections. CMAJ. 2007;176(2):175-176.
19. Workowski KA, Berman SM. Centers for disease control and prevention: Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep. 2006;55(RR-11):1-94.
20. Force USPST: Screening for chlamydial infection: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007;147(2):128-134.
21. European Center for Disease Control and Prevention (ECDC): Technical report:

Review of Chlamydia control activities in EU countries. Stockholm; 2008.

22. Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG): College statement C-Obs 3(b): Routine antenatal assessment in the absence of pregnancy complications. Melbourne: RANZCOG; 2012.
23. Honey E, Augood C, Templeton A, et al. Cost effectiveness for screening for *Chlamydia trachomatis*: A review of published studies. Sex Transm Infect 2002;78:406-412.
24. Ostergaard L, Andersen B, Moller JK, et al. Home sampling versus conventional swab sampling for screening of *Chlamydia trachomatis* in women: A cluster-randomized 1-year follow-up study. Clin Infect Dis. 2000;31(4):951-7.
25. Ljubin-Sternak S, Meštrović T. *Chlamydia trachomatis* and Genital Mycoplasmas: Pathogens with an Impact on Human Reproductive Health. Journal of Pathogens. 2014;2014:183167.
26. Honey E, Templeton A. Prevention of pelvic inflammatory disease by the control of *C. trachomatis* infection. Int J Gynaecol Obstet. 2002;78(3):257-61.
27. Oakeshott P, Kerry S, Aghaizu A, et al. Randomised controlled trial of screening for *Chlamydia trachomatis* to prevent pelvic inflammatory disease: The POPI (prevention of pelvic infection) trial. BMJ 2010;340:c1642.
28. Nelson HD, Helfand M. Screening for chlamydial infection. Am J Prev Med 2001;20(3 Suppl):95-107.
29. O'Neil D, Doseeva V, Rothmann T, et al. Evaluation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection in urine, endocervical, and vaginal specimens by a multiplexed isothermal thermophilic helicase-dependent amplification (tHDA) assay. J Clin Microbiol. 2011;49:4121-5.
30. Falk L, Coble BI, Mjornberg PA, et al. Sampling for *Chlamydia trachomatis* infection—a comparison of vaginal, first-catch urine, combined vaginal and first-catch urine and endocervical sampling. Int J STD AIDS. 2010;21:283-7.
31. Schachter J, Chernesky MA, Willis DE, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: Results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis. 2005;32:725-8.
32. Schachter J, Mc Cormack WM, Chernesky MA, et al. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. J Clin Microbiol. 2003;41:3784-9.
33. Shafer MA, Moncada J, Boyer CB, et al. Comparing first-void urine specimens, self collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. J Clin Microbiol. 2003;41:4395-9.
34. Byrne GI. *Chlamydia trachomatis* strains and virulence: Rethinking links to infection prevalence and disease severity. The Journal of infectious diseases. 2010; 201(Suppl 2):S126-S133.
35. Morré SA, Rozendaal L, van Valkengoed IG, et al. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection: An association with clinical manifestations? J Clin Microbiol. 2000;38(6):2292-6.
36. R Waalboer, E M van der Snoek, W I van der Meijden, et al. Analysis of rectal *Chlamydia trachomatis* serovar distribution including L2 (lymphogranuloma venereum) at the Erasmus MC STI clinic, Rotterdam. Sex Transm Infect. 2006;82:207–211.
37. Anttila T, Saikku P, Koskela P, et al. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA. 2001;285(1):47-51.
38. Geisler WM, Suchland RJ, Whittington WL, et al. The relationship of serovar to clinical manifestations of urogenital *Chlamydia trachomatis* infection. Sex Transm Dis 2003;30(2):160-5.

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