Detection of *Chlamydia trachomatis* in Endocervical Smears of Sexually Active Women in Manaus-AM, Brazil, by PCR

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C*hlamydia trachomatis* is now one of the most prevalent bacteria found in classic sexually transmissible diseases (STD), and as such, constitutes a serious public health problem. We examined the prevalence of *Chlamydia trachomatis*, by polymerase chain reaction (PCR), in 121 sexually active women who sought treatment for STD in the Alfredo da Matta Institute of Dermatology and Venerology and the Institute of Tropical Medicine of Amazonas in Manaus, Brazil. These women were examined by a specific PCR for the chlamydial plasmid, and the nature of the amplicon was determined by restriction analysis and DNA sequencing. The PCR diagnosis revealed a prevalence of 20.7% infected women.

Key Words: ***Chlamydia trachomatis*, PCR, DST.**

Urogenital infections by *Chlamydia trachomatis* are now recognized as highly prevalent sexually transmissible infections. In frequency, they surpass the classic sexually transmissible diseases such as syphilis and gonorrhea, and thus constitute a serious public health problem.

Chlamydia trachomatis has 15 serovars (A- K, L1, L2 L3 and Ba) [1]. The growth of serovars D to K seems restricted to epithelial columnar and transitional cells, while serovars L1, L2 and L3 cause systemic disease (lymphogranuloma venereum - LGV). The location of the infection determines the nature of the clinical disease [2].

Studies made by the Centers for Disease Control – USA have shown that three to four million new cases of chlamydial infection are detected annually in United States [3]. They estimated the cost of this infection is about 1.4 billion dollars / year [4].

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Uyeda et al. [5] found infection rates of over 20% in healthy carriers; and research in family clinics and prenatal planning centers revealed rates of from 5% to 10%. Children may acquire infection during childbirth, with 50% of them developing inclusion conjunctivitis and 20% developing pneumonia.

Several laboratory methods are used for the diagnosis of *C. trachomatis*, these include cytological tests for the detection of intracytoplasmic inclusions, cell culture, immunoassay enzyme analysis (ELISA), direct immunofluorescence, DNA hybridization techniques and DNA amplification – polymerase chain reaction (PCR).

Molecular genetics techniques are useful for the identification of microorganisms that are difficult to cultivate, such as *C. trachomatis*, and for those that grow slowly [6]. PCR is more sensitive test than cell culture; it has a high sensibility and specificity when compared to other tests used for *C. trachomatis* diagnosis, such as direct immunofluorescence and ELISA, which give some false-positive results [7].

Mahony et al. [8] compared five different PCR procedures to identify C. *trachomatis*. Two of these use the chlamydial plasmid as a template for PCR amplification. Two others use the gene of the major outer membrane protein (MOMP) and the fifth uses

ribosomal DNA (rDNA). It was found that plasmid-based PCR reactions are 10 to 1000 times more sensitive than those based on the bacterial chromosome sequences [8, 9].

Shirata et al. made a cytological study of 129 sexually active asymptomatic women in São Paulo, and found that 34.3% were infected with *C. trachomatis*, 32.5% of which were confirmed by immunofluorescence [10]. In Manaus-AM the prevalence was 27.1% (n=199), determined by direct immunofluorescence test [11].

As there have been no previous PCR studies of *C. trachomatis* infection in Amazonia, we decided to study *C. trachomatis* prevalence in endocervical smears of sexually active women in Manaus, Amazonas, Brazil, by this technique.

Materials and Methods

One hundred and twenty one women, between the ages of 14 and 63, were studied at the Sexually Transmissible Disease Services of the Alfredo da Matta Institute of Dermatology and Venereology and in the Institute of Tropical Medicine in Manaus – AM. The criterion for patient selection was simple random sampling.

Collection of Material and Preparation of the Samples

Preparation of the Samples for PCR. Endocervical smears were collected in 400 µl of TE (10mM Tris-HCl pH8.0 and 1mM EDTA). Each sample was supplemented with 4 µl triton 10% (v/v) and 4 µl proteinase K (10µg/ml), followed by incubation at 55ºC for 90 minutes and then at 95ºC for 30 minutes. The samples were maintained at -20 ºC, until used [16].

Amplification Reaction. The primers KL1-5' TCCGGAGCGAGTTACGAAGA 3'; KL2 - 5'AATCAATGCCCGGGATTGGT 3' were used to amplify a chlamydial plasmid 241 bp fragment (KL1 and KL2) [7].

PCR Conditions. A typical reaction system containing a final volume of 50 µl, was composed of 5 µl of the DNA sample; 25 mM of $MgCl_2$; 25 mM dNTP; 1mM of each primer KL1, KL2 and 1.5U of Taq polymerase. The amplification was made in a thermocycler MINI-CICLER TM. MJ. RESERCH. MODEL - 150, using the following 35 cycles program: denaturation at 93ºC for 1 minute, annealing at 64ºC for 1 minute and polymerization at 72ºC for 1 minute, followed by a final PCR extension at 72ºC for 5 minutes. The products were analyzed by electrophoresis in a 2% agarose gel.

DNA Sequencing. PCR products of three positive samples of *Chlamydia trachomatis* were purified with a QIAquick PCR Purification Kit (QIAGEN) and the DNA concentration was estimated using a spectrophotometer GeneQuant (Pharmacia).

The Perkin Elmer Dye Terminator Cycle Sequencing kit was used, the sequencing reaction was run for 25 cycles: denaturation at 96ºC for 30 seconds, annealing at 50ºC for 5 seconds and polymerization at 60ºC for 4 minutes. The products were precipitated with ethanol and dissolved in formamide – EDTA and applied in a 6% polyacrylamide gel in the ABI 373A DNA Sequencer.

Sequence analysis were performed with the GCG (Genetic Computer Group, University of Wisconsin/ USA) FASTA, BESTFIT and CHROMAS programs.

Results

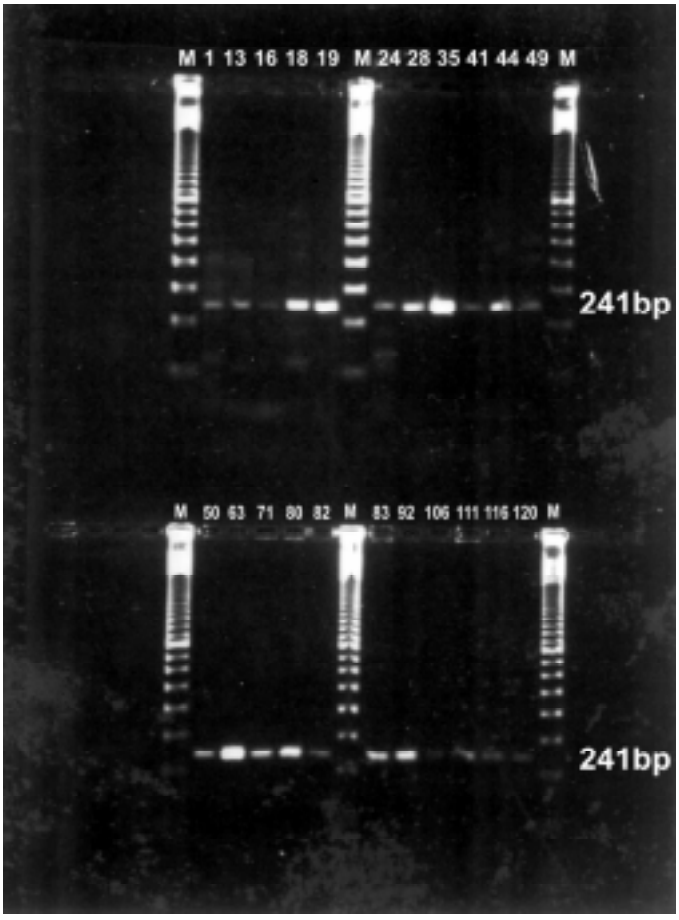
In the population study, 20.7% of the samples were positive for *C. trachomatis* by PCR diagnosis. There was a 241 bp DNA band in agarose gel electrophoresis, indicating presence of the parasite (Figure 1).

The average age of *C. trachomatis*-positive patients was 22 years and 80% of these were ≤ 23 years old.

Analysis of the Amplified DNA Sequences

The amplicon identities were confirmed by sequencing the amplified 241 bp DNA band. All three sequences had high similarity with ORF 2 of the *C. trachomatis* cryptic plasmid pLGV440 (Figure 2).

Figure 1. Analysis by agarose gel (2%) electrophoresis of the PCR products. The 241 bp DNA corresponds to the specific chlamydial amplified plasmid DNA sequence. (M): 100bp ladder (GBICO/BRL).



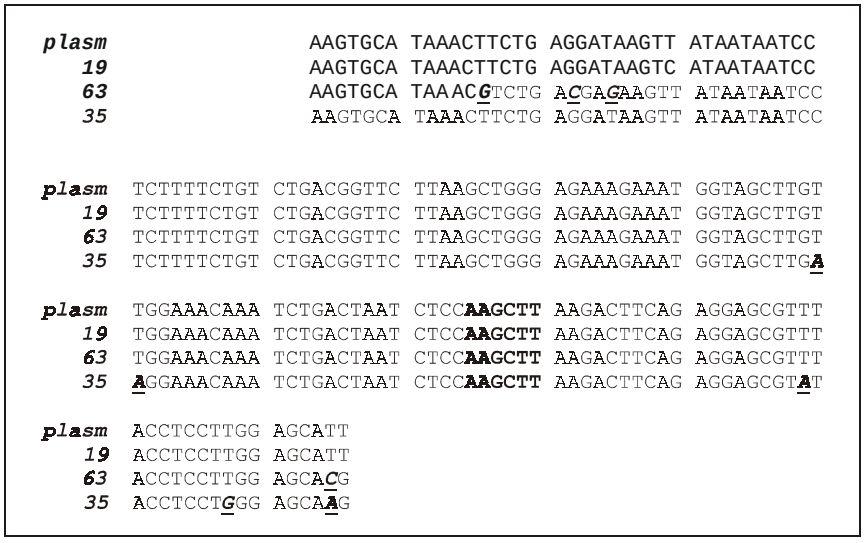
Discussion

Chlamydia trachomatis is a highly prevalent bacteria in many regions, and it seriously affects public health; therefore it needs effective epidemiological control, starting with an adequate method for correct and effective diagnosis.

We found a prevalence of 20.7% women infected with *C. trachomatis*, and 80% of the positive cases were found to be in the ≤ 23 year old age group. These findings are in agreement with most previous reports, which indicate that *C. trachomatis* mainly attacks young women [5, 17-19], apparently because they are at risk due to behaviors such as sex without protection and multiple sexual partners.

The prevalence of 27.1% found in Manaus-AM(Brazil) in a previous study, using a direct immunofluorescence test [11], is somewhat higher than what we found with PCR (20.7%). This discrepancy may be due to the lower specificity of the immunofluorescence method, compared to PCR. Furthermore, the immunofluorescence test gives cross reactions with Gram-negative bacteria, including other species of Chlamydia, thus increasing the number of false-positive cases [7]. PCR gives high specificity and sensibility [7, 12, 20, 21-24], however in some situations, such as blood samples with high concentrations of proteins and lipids, the sensibility of the reaction can be lowered due to inhibition of PCR [25]. In any case, a high prevalence of *C. trachomatis* was found in both studies.

Figure 2. Multiple alignment of nucleotides of the *Chlamydia trachomatis* diagnostic amplicon from samples 19, 35 and 63 with GeneBank sequence number (MI9487). In black: H*ind III* sequence target. In black italic: polymorphic sites among the isolates [17].



The nature of the amplified product was confirmed by DNA sequencing. A low degree of DNA polymorphism was found in the 241 bp DNA fragment.

The high prevalence of *C. trachomatis* and its severe impact on public health suggests the necessity of implementing better diagnostic methods for its detection in the routine of STDs laboratory public services in Manaus – AM, Brazil. PCR could be an adequate alternative method for diagnosis.

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References

1. Jones R.B. Chlamydial diseases. In: Mandell, G.L., Bennett, J.E., Dolin, R. Principles and practice of infectious disease. 4th ed. New York. Curchill Livingstone, **1995**.
2. Mims C. A., Playfair J. H. L., Roitt I. M. et al. Microbiologia Médica. Lição. Editora Manole. São Paulo. **1995**.
3. Centers for Disease Control. *Chlamydia trachomatis* infections: Policy guidelines for prevention and control. MMWR. **1985**; 34 (suppl 3) : 53-74 apud Bowie, W.R., Holmes, K.K. Chlamydial diseases. In: Mandell, G.L.; Douglas JR, G.R.; Ben, J.E. Practice of infectious diseases. 3rd ed. New York. Churchill Livingstone.**1990**.
4. Washington A.E., Johnson R. E., Sanders Jr. L.L. *Chlamydia trachomatis* infection in the United States: What are they costing us? JAMA **1987**;257:2070-2.
5. Uyeda C. T., Welborn P., Ellison-Birang N., et al. Rapid diagnosis of Chlamydial infections with microtrack direct test. J Clin Microbiol **1984**;20(5):948-50.
6. Piémont Y., Jaulhac B. Use of molecular methods for bacteriology diagnosis. Ann Dermatol Venereol **1995**;122:206-12.
7. Schachter J. DFA, EIA, PCR, LCR and other technologies: What tests should be used for diagnosis of Chlamydia infections? Immunological Investigations **1997**;26:157-61.
8. Mahony J.B., Luinstra K.E., Sellors J.W., Chernesky M.A. Comparison of plasmid- and chromosome- based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. J Clin Microbiol **1993**;31(7):1753-8.

9. Tam J.E., Davis C.H., Theresher R.J., Wyrick P.B. Short Communications. Location of the origin of replication for 7.5 Kb *Chlamydia trachomatis* plasmid. Plasmid **1992**;27:231-6.
10. Shirata N.K., Andrea-Filho A. Utilidade do uso combinado de colheita com citobrush e espátula de Ayre para o exame citológico cérvico-vaginal. J Bras Ginecol **1992**;102(1/2):15-8
11. Alencar A.A.F., Ferreira L.C.L., Loureiro J.A.S. Detecção de *Chlamydia trachomatis* por imunofluorescência direta em esfregaços endocervicais. J Bras Ginecol **1993**;103(6):199-203.
12. Domeika M., Bassiri M., Mårdh. Diagnosis of genital *Chlamydia trachomatis* infections in asymptomatic males by testing urine by PCR. J. Clin Microbiol **1994**;32(10):2350-2.
13. Claas H.C.J., Wagenvoordt J.H.T., Niesters H.G.M., et al. Diagnostic value of the polymerase chain reaction for *Chlamydia* detection as determined in a follow-up study. J Clin Microbiol **1991**;29(1):42-5.
14. Dean D., Stephens R. Identification of individual genotypes of *Chlamydia trachomatis* from experimentally mixed serovars and mixed infections among Trachoma patients. J Clin Microbiol **1994**;32(6):1506-10.
15. Cook R.L., Kirsten St. G., Lassak M, et al. Screening for *Chlamydia trachomatis* infection in college women with a polymerase chain reaction assay. CID **1999**;28:1002-7.
16. Griffais R., Thibon M. Detection of *Chlamydia trachomatis* by polymerase chain reaction. Res Microbiol **1989**;140:139-41.
17. Comanducci M, Ricci S., Cevenini R., Ratti G. Diversity of the *Chlamydia trachomatis* common plasmid in biovars with different pathogenicity. Plasmid **1990**;23(2):149-54.
18. Schachter J., Hill E.C., King E.B., et al. *Chlamydia trachomatis* and cervical neoplasia. J Am Med Assoc **1982**;248:2134-8.
19. Jolly A.M., Orr P.H., Hammond G., Yong T.K. Risk factors for infection in women undergoing testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Manitoba, Canada. Sex Transm Dis **1995**;22(5):289-95.
20. Mahony J.B., Luinstra K.E., Sellors J.W., et al. Confirmatory polymerase chain reaction testing for *Chlamydia trachomatis* in first-void urine from asymptomatic and symptomatic men. J Clin Microbiol **1992**.30(9):2241-5.
21. Catry M.A., Borrego M.J., Cardoso J., et al. Comparison of the amplicor *Chlamydia trachomatis* test and cell culture for the detection of Urogenital Chlamydial Infections Genitourin Med **1995**;71(4):247-50.
22. Kuipers J.G., Scharmann K., Wollenhaupt J., et al. Sensitivities of PCr, Microtrack, Chlamydia EIA, IDEIA, and PACE 2 for purified *Chlamydia trachomatis* elementary bodies in urine, peripheral blood, peripheral blood leucocytes, and synovial fluid. J Clin Microbiol **1995**;33(12):3186-90.
23. Näher H., Höchstetter R., Peltzold D. Evaluation of the polymerase chain reaction in the detection of *Chlamydia trachomatis* in urogenital smears. Hautarzt **1995**;46:693-6.
24. Chout R., Vaton S., Quist D., et al. Improvement of cervical *Chlamydia* detection in asymptomatic family planning attendees by using amplicor *Chlamydia trachomatis* assay. Cell Mol Biol **1995**;41(5):731-6.
25. Rossen L., Nørskov P., Holmstrøm K., Rasmussen O.F. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. International Journal of food Microbiology **1992**;17:37-45.