RECHERCHE DU NOUVEAU VARIANT SUÉDOIS DE *CHLAMYDIA TRACHOMATIS* EN TUNISIE

WHAT ABOUT THE PRESENCE OF THE SWEDISH *CHLAMYDIA TRACHOMATIS* VARIANT IN TUNISIA ?

O. Frikha-Gargouri, A. Znazen, S. Hadrich, R. Gdoura, A. Hammami.

Department of microbiology and research laboratory "Microorganismes et Pathologie". University School of Medecine of Sfax.Tunisia

Correspondance :
A. Hammami
Laboratory of microbiology.
University School of Medecine of Sfax.
Avenue Magida Boulila, 3027, Sfax.
Tunisia.
E-mail: adnene.hammami@rns.tn

Résumé :

Un nouveau variant de Chlamydia trachomatis, caractérisé par une délétion dans la région cible du test PCR commercial Cobas Amplicor, a été rapporté en 2006. A cause de cette délétion, ce test ne détecte pas des infections causées par ce variant, produisant de ce fait des résultats faux négatifs. Puisque des taux de détections faibles de C. trachomatis ont été observés en 2008-2009, nous avons recherché la présence de ce nouveau variant en Tunisie en utilisant une PCR nichée montrant une différence de taille entre le phénotype sauvage (630 pb) et le mutant (253 pb). Les prélèvements utilisés provenaient de patients consultants pour des infections sexuellement transmises (n=564) et de prostituées de la Tunisie (n=188). Ces derniers ont été collectés dans le cadre de la surveillance de cette population. Toutes les PCR nichées positives ont montré une bande correspondant à celle de la souche sauvage. Ainsi, nos résultats indiquent qu'actuellement, il n'y a aucune évidence de la présence du nouveau variant de C. trachomatis en Tunisie. Malgré que le test PCR commercial Cobas Amplicor a mis sur le marché un kit détectant la souche sauvage et le variant, nous devons être vigilant devant de nouveaux variants pouvant générer des résultats faussement négatifs.

Mots clés : Chlamydia trachomatis-nouveau variant-délétion.

Abstract:

A Chlamydia trachomatis new variant strain, characterized by a deletion in the target area of the Cobas Amplicor test, has been reported in 2006. Due to the deletion, this affected test will not detect infections caused by this variant, generating thus false negative results. As low detection rates of C. trachomatis were notified in 2008-2009, we assess the presence of the new variant strain in Tunisia using a nested PCR assay showing variation in the amplicon size between the wild type (630 bp) and the mutant strain (253 bp). The specimens used are from patients attending sexually transmission infections clinics (n=564) and from Tunisian prostitutes (n=188). These latter were collected for the surveillance of this population. All PCR positive results showed an amplicon size corresponding to the wild type strain. Thus, our results indicate that, currently, there is no evidence of the presence of the new variant C. trachomatis strain in Tunisia. Although the fact that the Cobas Amplicor test has replaced its old assay by a new one that is able to detect the wild type and the mutant strain, attention must be made for new variants generating false negative results.

Key words : Chlamydia trachomatis-new variant strain-deletion.

INTRODUCTION

Chlamydia trachomatis is the most common cause of sexually transmitted infections all over the world. In males, infection can manifest as urethritis or epididymitis. Infection in women may present with urethritis, cervicitis or salpingitis and, if left untreated, the infection can become chronic and result in ectopic pregnancy, pelvic inflammatory disease and infertility [1]. Up to 70% of women and 50% of men with *C. trachomatis* infections do not show symptoms of the disease, resulting in a silent infection increasing thus the risk of transmission of *C. trachomatis* and its consequences. The method of choice for *C. trachomatis* detection worldwide today is nucleic acid amplification tests (NAAT). Available commercial tests target the cryptic plasmid, the major outer membrane protein gene (MOMP), or rRNA. In 2006, a new variant of C. trachomatis, characterized by a 377 bp plasmid deletion including the targets of the COBAS Amplicor/TaqMan CT/NG (Roche Diagnostics) and Abbott m2000rt RealTime CT/NG (Abbott Laboratories) assays, was discovered in Sweden [2, 3]. The Aptima Combo 2 test (Genprobe), the RealArt CT Kit (Qiagen) and the ProbeTec Strand Displacement Assay (Becton Dickinson), which target rRNA, the omp1 gene and a different region of the cryptic plasmid, respectively, remain unaffected by this deletion. The new variant strain was reported all over Sweden, in proportions of 10%-66% in different counties [4, 5]. This high prevalence in Sweden is the consequence of a high selection pressure, as the Roche test was used almost exclusively throughout the country for the detection of *C. trachomatis* generating thus a high number of false negative results [3]. Failure to detect the new variant strain in Sweden has as a consequence resulted in episodes of complicated infections leading to ectopic pregnancies and infertility [6]. As we used in our laboratory a platform affected by the presence of the new variant *C. trachomatis* strain, the Cobas Amplicor test, we investigated the presence of this variant in our samples.

MATERIALS AND METHODS

Samples received at the laboratory of microbiology in the University Hospital of Sfax, Tunisia, included in this study were divided into two groups: (i) 564 urethral and endocervical swabs collected from patients attending Sexually Transmitted Infections (STI) clinics for urethritis or infertility within the period of 2001 to 2008 including all collected samples in 2007 and 2008. For all these samples direct immunoflorescence was made. (ii) 188 endocervical swabs collected from Tunisian prostitutes during August-September 2007. All samples were frozen and stored at – 80°C until processed.

The Cobas amplicor PCR test (Roche Diagnostic system) was made as recommended by the manufacturer.

For the in-house PCR assays, DNA was extracted from all clinical samples using an in house method based on proteinase K treatment. Briefly, 100 μl of the swab sample was centrifuged at 14.000 rpm for 30 minutes, the supernatant was discarded and the pellet was thoroughly washed twice with Phosphate Buffered Saline (PBS). The DNA was released with Proteinase K by solubilizing the pellet in 50 μl TE buffer (10 mM Tris-HC1 (pH 8), 1 mM EDTA) containing Proteinase K (200 μg/ml). Proteins were digested at 55°C for 1 hour. The enzymatic reaction was then stopped by incubation at 98°C for 10 min and samples were centrifuged at 10,000 rpm for 5 s.

Initially, the extracted DNA was tested for human ß globin gene to check that there were no PCR inhibitors in the samples. Primers ß-GPCO (5'-ACACAACTGTGTTCACTAGC- 3'), and ß-GPCPO (5'-GAAACCCAAGAGTCTTCTCT- 3'), were used to amplify a 209 bp fragment of the human ß globin gene [7]. Samples found to be ß globin negative by PCR were retested after dilution 10 fold in distilled water. Samples shown to be ß globin positive were then examined for the presence of *C. trachomatis* DNA.

A nested based PCR assay was used in this study to differentiate the new variant from the wild type strain. The primers; CTDFO (CGATTTCTAAGCAGGAATGGAC), CTDRO (GACTACAAGCTGCAATCCC), CTDFN (TTGAAGCGCTCCGGATAGTGAA) and CTDRN (GACACGCAACCGCAAAGATGAT) ; spanned the 377 bp deletion in the cryptic plasmid of *C. trachomatis* [8]. They generate in the second round of the PCR, using primers CTDFN and CTDRN, an amplicon of 630 bp in the wild type strain and an amplicon of 253 bp fragments in the new *C. trachomatis* variant.

The PCR mixture, which was made up to 50 μl with sterile water, contained 1x PCR buffer (50 mM Tris-HCl [pH 8.3], 10 mM KCl, 5.0 mM (NH4)2SO4, and 2.0 mM MgCl2); 0.5 mM each primer; 0.2 mM each dATP, dCTP, and dGTP; 0.6 mM dTTP; 1.25 U of Go Taq DNA polymerase (Promega, France); and 10 μl of prepared DNA solution. PCR was performed using the Gene-Amp PCR System 9700 (Perkin Elmer Cetus) under the following conditions: an initial cycle at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and elongation at 72°C for 45 seconds, with a final cycle at 72°C for 7 minutes. The same conditions were maintained in the second round of PCR except for the annealing which was done at 60°C. Furthermore, the reaction in the second round contained 5 μl of amplicons from the first round of PCR. Each PCR run included two positive controls, the wild type and the mutant strains of *C. trachomatis* genotype E, as well as a negative control made with distilled water. Amplicon were analysed in a conventional gel-based assay and PCR products were analysed on ethidium bromide-stained 2 % agarose gel. Sequencing was performed on some PCR fragments to ensure that it targets the correct region of the wild type strain.

RESULTS

The Detection rate of *C. trachomatis* in samples received in the laboratory of microbiology in the University Hospital of Sfax, Tunisia within 2001-2007 indicated no significant decrease (Figure 1). However, during 2008-2009, a significant decrease in the detection rate of *C. trachomatis* was observed.

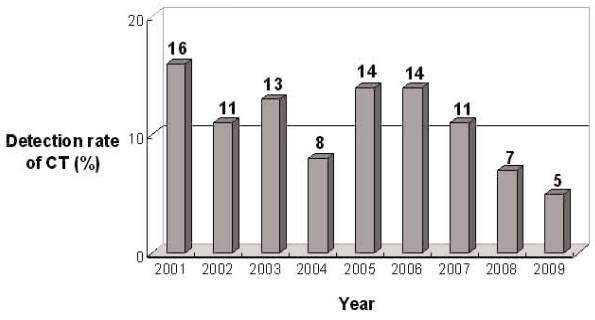

Figure 1 : Detection rate of *C. trachomatis* in samples received received in the laboratory of microbiology Sfax, Tunisia within 2001-2009.
Figure 1 : Taux de détection de *C. trachomatis* dans les échantillons reçus au laboratoire de microbiologie de Sfax, Tunisie durant 2001-2009

In order to explain the decrease of the detection rates of *C. trachomatis* notified in 2008-2009, we assess the presence of the deleted Swedish new variant in Tunisia. The validity of the in house PCR test was first confirmed using, as reference, DNA from the wild type and the mutant strains (Figure 2). Then, the clinical specimens were tested for the presence of the new variant strain of C. trachomatis. A total of 752 clinical specimens were screened for the presence of the new variant strain in Tunisia. 136 samples (18%) generated the 630 bp amplicon which is consistent with the wild type strain. 73 out of the 136 positive samples were from prostitutes. In specimens that failed to generate an amplicon, DNA extracts were tested for PCR inhibition and no inhibition was found for all samples. No new variant strain was identified in any sample. Sequencing the second round amplicon fragments of some specimens confirmed that the PCR spanned the 377 bp target region and that only the wild type *C. trachomatis* strain is present in Tunisia.

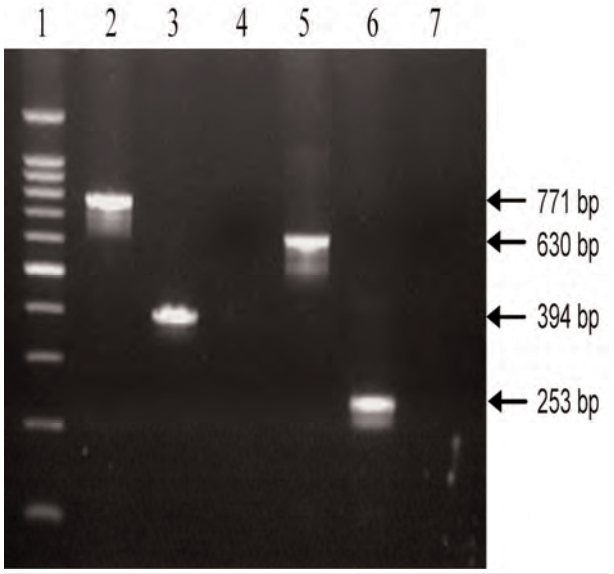

Figure 2 : PCR amplification of the 630 bp and the 253 bp of *C. trachomatis* cryptic plasmid region. Line 1: 100 bp molecular marker (Promega), lane 2: First round PCR of the wild type strain, lane 3: First round PCR of the mutated strain, lane 4: Negative control of the first round PCR, lane 5: Second round PCR of the wild type strain, lane 6: Second round PCR of the mutated strain, lane 7: Negative control of the second round PCR.

Figure 2 : Amplification par PCR de la région de 630 pb et la région de 253 pb du plamside cryptiques de C.trachomatis Ligne 1: marqueurs moléculaires de 100 pb (Promega), piste 2: Première PCR de la souche sauvage, piste 3: Première PCR de la souche mutante, piste 4: contrôle négatif de la première PCR , voie 5: Deuxième PCR de la souche sauvage, une voie 6: Deuxième PCR de la souche mutante, piste 7: contrôle négatif de la deuxième PCR.

DISCUSSION

C. trachomatis is the most common cause of sexually transmitted diseases world wide. In Tunisia, *C. trachomatis* has been reported in 15 % among men with urethritis (data not published), in 59 % among patients with reactive arthritis and undifferentiated oligoarthritis [9], in 35.9% to 43.3 % among infertile men and women [10, 11] and at a higher percentage in prostitutes (data not published). Thus, *C. trachomatis* looks widespread among the Tunisian population.

The new variant *C. trachomatis* strain, characterized by the 377 pb deletion, has been shown to be widespread in Sweden with an estimated prevalence of 10–66% depending on the region [3]. To our knowledge, this paper is the first one to access the presence of this new variant *C. trachomatis* strain in Tunisia. We assume that the specimens examined in our study are representative of the national picture as Tunisian prostitutes represent a high risk population of sexually transmitted diseases including C. trachomatis. Our results indicate the absence of the new variant strain in Tunisia although the decrease in the detection rate of the wild type strains during 2008-2009. Similarly, world wide investigation indicated little evidence for the presence of the new variant strain outside Sweden [4-6, 8, 12-18]. The latest Reports on the presence of the new variant strain in other countries made all in 2007, revealed that it is confined to only few cases mainly of Swedish contacts, in Denmark, Norway, Ireland and France [19-22]. In Denmark, the new variant strain was detected in only 1 of the 691 *C. trachomatis* specimens (0.14%) and from the 121 *C. trachomatis* positive specimens (0.8%) [23]. In the Oslo area in Norway, 2.6% of the *C. trachomatis* infections are caused by the new variant of *C. trachomatis* and mostly found in patients with contact to Sweden [23]. In France, among the 1141 *C. trachomatis* positive samples tested all over the country, only one case of the new Swedish variant *C. trachomatis* was detected. This sample originated from a non French resident consulting a French STI clinic [19]. Finally, in the Dublin area in Ireland, two samples found to be positive by the BD probeTec but negative by the Cobas Amplicor assay were confirmed to be the new variant strain using a PCR that spanned the 377 bp deletion in the cryptic plasmid of *C. trachomatis* [22].

Recent studies indicated that after introduction of an effective new variant *C. trachomatis* diagnostic test in Sweden, one-month sentinel surveillance revealed that the new variant strain proportion has remained high [24] suggesting that the proportion of the new variant/wild-type *C. trachomatis* was already stabilized in Sweden. Thus, as the new variant of *C. trachomatis* may still be in early transmission outside Sweden and as its spread may have as a consequence complicated *C. trachomatis* infections, enhanced surveillance worldwide is crucial to prevent such situation in other countries. Microbiological investigations of the new variant *C. trachomatis* strain isolated in Sweden, Denmark, Norway, Ireland and France indicated that all the isolates are of identical genotype E and are of a single multilocus sequence type and therefore are probably clonal [5, 6, 25]. Further studies indicated that no significant differences between the new variant and the wild-type *C. trachomatis* strains have been identified regarding symptoms and signs, sequelae, growth characteristics, cells/DNA load in NAAT samples [5, 26].

Roche Molecular Diagnostics (COBAS taqMan CT test v2.0) and Abbott Molecular (Abbott RealTime CT assay) have replaced their old assays by new ones that are able to detect both the wild type and the mutant strains. In their tests, they incorporate, in addition to the original targets, new target regions either in the chromosome (the omp1 gene) or in a region of the cryptic plasmid not affected by the deletion respectively for Roche and Abbot tests. Nevertheless, to detect the mutant strain, laboratories using the Cobas Amplicor test must switch to the real time version. At our laboratory, the method of *C. trachomatis* diagnostic has not changed to the real time version of the Cobas amplicor test nor to another NAAT test as we did not detect the mutant strain in Tunisia.

However, as all samples are first assessed by the direct fluorescence antibodies assay (DFA), attention is always made to reproduce PCR positivity of positive DFA specimens.

CONCLUSION

The Swedish *C. trachomatis* variant has not been detected in Tunisia. We recommend monitor the presence of the new strain using culture, DFA and PCR tests on clinical specimens. Furthermore, we should remain vigilant for unexplained changes in *C. trachomatis* prevalence and suspicious negative results.

ACKNOWLEDGEMENTS

We are grateful to Dr. Raouf BEN MAHMOUD from "municipalité de Tunis", Dr. Hechmi KNANI from "direction de santé de base de Sousse" and Dr. Mohamed BEN OTHMEN from "Hôpital régional de Gabes". We thank also, Pr. Gunna CHRISTIANSEN, Pr. Svend BURKILUND and Pr. Bjorn HERRMANN for providing us the DNA from the new variant strain of C. trachomatis.

References

1. Miller K. Diagnosis and treatment of *Chlamydia trachomatis* infection. Am Fam Physician 2006 ; 73 : 1411-6.

2. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid. implications for use of PCR diagnostic tests. Euro Surveill 2006 ; 11: E061109.2.

3. Ripa T, Nilsson P. A *Chlamydia trachomatis* strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic amplification tests. Sex Transm Dis 2007; 34 : 255-6.

4. Söderblom T, Blaxhult A, Fredlund H, et al. Impact of a genetic variant of *Chlamydia trachomatis* on national detection rates in Sweden. Euro Surveill 2006 ; 11 : E061207.1.

5. Unemo M, Olcén P, Agné-Stadling I, et al. Experiences with the new genetic variant of *Chlamydia trachomatis* in Örebro county, Sweden – proportion, characteristics and effective diagnostic solution in an emergent situation. Euro Surveill 2007 ; 12 : E5-6.

6. Herrmann B, Törner A, Low N, et al. Emergence and spread of *Chlamydia trachomatis* variant, Sweden. Emerg Infect Dis 2008 ; 14 : 1462-5.

7. Vogels WH, van Voorst Vader PC, Schröder FP. *Chlamydia trachomatis* infection in a high-risk population. Comparison of polymerase chain reaction and cell culture for diagnosis and follow-up. J Clin Microbiol 1993 ; 31 : 1103-7.

8. Alexander S, Ison C. Is new variant *Chlamydia trachomatis* present in England and Wales? Sex Transm Infect 2008 ; 84 : 29-31.

9. Siala M, Gdoura R, Younes M, et al. Detection and frequency of *Chlamydia trachomatis* DNA in synovial samples from Tunisian patients with reactive arthritis and undifferentiated oligoarthritis. FEMS Immunol Med Microbiol 2009 ; 55 : 178-86.

10. Gdoura R, Keskes-Ammar L, Bouzid F, et al. *Chlamydia trachomatis* and male infertility in Tunisia. Eur J Contracept Reprod Health Care 2001; 6 : 102-7.

11. Gdoura R, Kchaou W, Ammar-Keskes L, et al. Assessment of *Chlamydia trachomatis*, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, and Mycoplasma genitalium in semen and first void urine specimens of asymptomatic male partners of infertile couples. J Androl 2008 ; 29 : 198-206.

12. De Vries HJ, Catsburg A, van der Helm JJ, et al. No indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam. Euro Surveill 2007 ; 12 : E070208.3.

13. Morré SA, Catsburg A, de Boer M, et al. Monitoring the potential introduction of the Swedish *Chlamydia trachomatis* variant (swCT) in the Netherlands. Euro Surveill 2007 ; 12 : E9-10.

14. Savage EJ, Ison CA, van de Laar MJW; ESSTI network. Results of a Europe-wide investigation to assess the presence of a new variant of *Chlamydia trachomatis*. Euro Surveill 2007 ; 12 : E3-4.

15. Sillis M, Skidmore, Mallinson H, et al. No evidence of the Ct variant in the UK. Sex Transm Inf 2007 ; 83 : 488-9.

16. Velicko I, Kühlmann-Berenzon S, Blaxhult A. Reasons for the sharp increase of genital chlamydia infections reported in the first months of 2007 in Sweden. Euro Surveill 2007 ; 12 : E5-6.

17. Gaydos C, Hardick A, Ramachandran P, et al. Development of a specific PCR for detection of the new variant strain of *Chlamydia trachomatis* and surveillance in the United States. 2008 pp. 260 in Proc. Sixth meeting of the European Society for Chlamydia Research. Aarhus (Denmark).

18. Stevens MP, Tan SE, Horvath L, et al. Absence of a *Chlamydia trachomatis* variant, harbouring a deletion in the cryptic plasmid, in clients of a sexually transmissible infection clinic and antenatal patients in Melbourne. Commun Dis Intell 2008 ; 32 : 77-81.

19. de Barbeyrac B, Raherison S, Cado S, et al. French situation concerning the Swedish *Chlamydia trachomatis* variant. Euro Surveill 2007 ; 12 : E11-12.

20. Hoffmann S, Jensen JS. Mutant *Chlamydia trachomatis* in Denmark. Euro Surveill 2007 ; 12 : E7-8.

21. Lynagh Y, Walsh A, Crowley B. First report of the new variant strain of *Chlamydia trachomatis* in Ireland. Epi-Insight 2007 ; 8 : 4.

22. Moghaddam A, Reinton N. Identification of the Swedish *Chlamydia trachomatis* variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill 2007 ; 12 : E070301.3.

23. Westh H, Jensen JS. Low prevalence of the new variant of *Chlamydia trachomatis* in Denmark. Sex Transm Infect 2008 ; 84 : 546-7.

24. Hadad R, Fredlund H, Unemo M. Evaluation of the new COBAS TaqMan CT test v2.0 and impact on the proportion of new variant *Chlamydia trachomatis* by the introduction of diagnostics detecting new variant C trachomatis in Orebro county, Sweden. Sex Transm Infect 2009 ; 85 : 190-3.

25. Klint M, Fuxelius HH, Goldkuhl RR, et al. High-resolution genotyping of *Chlamydia trachomatis* strains by multilocus sequence analysis. J Clin Microbiol 2007 ; 45 : 1410-4.

26. Bjartling C, Johnson A, Persson K. Epidemiology and clinical observations of new variant *Chlamydia trachomatis* infection. 2008 pp.41 in Proc. Sixth meeting of the European Society for Chlamydia Research. Aarhus (Denmark).