Eurosurveillance monthly release

Surveillance report

French situation concerning the Swedish Chlamydia trachomatis variant

volume 12
issue 10
publication date October 2007

How to cite this article, click here.

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In 2006, a plasmid deletion mutant of *Chlamydia trachomatis* was identified in Sweden that can not be detected with those commercial tests targeting the deleted area. In order to study the spread of this strain in France, a laboratory-based surveillance system was set up by the National Reference Centre for *Chlamydiae* and the Institut de Veille Sanitaire. Among 1,141 *C. trachomatis*-positive specimens from all over France, the new variant was only detected in one case. This case was a non-French resident consulting a sexually transmitted infections clinic. Although the new variant does not seem to be established in France as yet, surveillance based on the testing of *C. trachomatis*-positive samples from all over France continues.

Introduction

A *Chlamydia trachomatis* variant that harbours a 377 bp deletion in the cryptic plasmid has been identified in patients in Sweden [1]. This deletion is unfortunately located in the region targeted by commercially available PCR tests that diagnose urogenital *C. trachomatis* infections, such as the Cobas Amplicor or Taqman tests (Roche Diagnostics) which are frequently used in France, and the Abbott Real Time CT and CT/NG assays. As a consequence, these commercial kits generate false negative results for patients who are infected with the deletion variant of *C. trachomatis*. Currently, the spread of the new variant to other countries seems to be very limited. It has been detected in two patients in Norway (one Swedish, one Norwegian), and recently in Denmark and in Ireland [2-4]. The new variant had not been detected among 8,797 specimens in an earlier study in Ireland, nor in 515 samples from an outpatient sexually transmitted infections (STI) clinic in Amsterdam, The Netherlands, nor in 1,066 *C. trachomatis*-positive specimens in England and Wales [5-7]. As for other parts of the world, recent studies suggest that the plasmid mutation is not present in the Midwest region of the United States nor in Melbourne, Australia [8,9].

Following the European alert, the French Health Products Safety Agency published an alert bulletin

in February 2007 to inform their health correspondents of the situation [10]. Moreover, both companies, Roche and Abbott, informed their customers that their commercial tests generated false negative results with the new variant strain. They recommended to use a different test that is able to detect this strain in those cases in which *C. trachomatis* infection was suspected but in which the Roche or Abbott tests had given a negative result.
In order to establish whether the Swedish *C. trachomatis* variant was circulating in France, a laboratory-based surveillance system was set up by the French National Reference Centre for *Chlamydiae* (NRC) and the Institut de Veille Sanitaire (InVS). In France, about 1,500 laboratories perform *C. trachomatis* diagnostics on urogenital specimens. A majority (about 70%) of the diagnostic are done using nucleic acid amplification test (NAAT), 70% of which are the Roche tests.

Material and methods

The National Reference Centre for *Chlamydiae* tested samples from three different sources:

1. All consecutive genital specimens from high risk groups consulting four STI centres in two cities (Bordeaux and Paris) in November 2006 that were tested by both Cobas Taqman assay and an in-house real-time PCR assay targeting a 129 bp region of the chromosomal *omp1* gene [11].

2. All samples determined as positive by the Pasteur Cerba laboratory between July 2006 and June 2007 using the CT real-time PCR kit Qiagen Artus targeting the *omp1* gene, a commercial test that is able to detect the new variant *C. trachomatis*. The Pasteur Cerba laboratory is a central laboratory that receives specimens (on average 3,500 specimens per month) from all over the country including the French overseas territories (West Indies, Guyana, Polynesia). The proportion of positive samples is approximately 3.9%. These samples were tested at the NRC using either the new variant-specific real-time PCR described by Ripa or an in-house real-time PCR targeting the deleted region of the plasmid [11,12].

3. All endocervical and male urethral specimens tested routinely in the NCR laboratory located in Bordeaux that were tested by cell culture and Cobas Taqman. Most of those samples came from an STI clinic located in Bordeaux.

Results

A total of 1,141 *C. trachomatis*-positive samples were analysed for the presence of the new Swedish variant:

1. 62 specimens from 784 consecutive genital samples from STI clinics in Paris (n=332) and Bordeaux (n=452) sampled in November 2006. None of those samples contained the Swedish variant *C. trachomatis*.

2. 1,049 samples from 1,040 patients (613 women and 427 men) provided by the Pasteur Cerba laboratory. The Swedish variant was not found among those. However, seven samples failed to amplify and were therefore not typable. This may have been due to differences in the sensitivity of different NAATs, to low concentrations of *C. trachomatis* DNA, or to degradation of the DNA during storage.

3. 30 culture specimens from 650 samples cultivated at an STI centre in Bordeaux since July 2006. Among those, one new variant was detected in an isolate from a woman consulting the centre in March 2007. The endocervical sample had been positive in culture and negative in the Cobas Taqman assay. The strain belonged to serovar E as determined by PCR-RFLP of the *omp1* gene and as described by Ripa for the Swedish strain [13]. The presence of the 377 bp deletion was verified by sequencing and by using the new variant-specific real-time PCR [12]. Unfortunately, since consultations at STI centres in France are anonymous, detailed information about this case is not available and contact tracing was not possible. The only information available is that the patient was a citizen from a northern European country, visiting Bordeaux at the time of consultation.

Discussion

Our results confirm that the new variant *C. trachomatis* seems currently to be restricted to the Scandinavian countries. Among 1,141 *C. trachomatis*-positive samples from all over France, only one case of the new Swedish variant *C. trachomatis* was detected. This sample stemmed from a non-French resident consulting a French STI clinic.
Surveillance of the spread of this variant strain in France was feasible as a result of the cooperation of a private laboratory that performs diagnostics with a technique able to detect the new variant strain. If this had not been the case, it would have been much more difficult to implement a surveillance system, as most (about 50%) of the French laboratories are using assays that are not capable of detecting the new variant.

In Sweden, a decrease of 25% in diagnosed *C. trachomatis* infections was noted at the beginning of 2006. In contrast, the number of *C. trachomatis* infections diagnosed in France, which has been rising between 1998 and 2005 [14], continued to increase in 2006 and 2007 (InVS unpublished data), although the methods of detection of *C. trachomatis* remained the same.
Our current knowledge about the spread of this Swedish strain in France does not permit us to recommend the exclusive usage of tests amplifying other targets than the deleted region of the plasmid. Roche Molecular Diagnostics [15] and Abbott Molecular are developing new assays that will be able to detect wild-type as well as plasmid-mutant strains by incorporating a new target region, either on the chromosome or in a region of the cryptic plasmid not affected by the deletion, in addition to the original primers directed at the mutated region on the cryptic plasmid. These dual target tests will include detection of the Swedish *C. trachomatis* variant but will not allow to identify cases caused by these plasmid-mutant strains specifically. Presently, multiple tests are needed for each specimen to identify the new variant *C. trachomatis* in order to be able to distinguish it from the wild-type and to study its spread.

The new variant-specific real-time PCR test described by Ripa, developed on the LightCycler 1.0, detects only the mutant strain because the FRET probes were designed to bind to the sequence flanking the deletion. The result is positive (presence of deletion) or negative. A negative result is not conclusive because it indicates either the presence of the non-deleted strain, or the absence of any strain, or atechnical problem.

A new method that characterises nucleic acid samples by comparing their dissociation (melting) temperature, High Resolution Melting (HRM), seems to be promising [16]. The HRM profile discriminates amplified fragments according to their sequence, length, and GC content, and can distinguish between wild-type and mutant strains. Our first assays show perfect discrimination between the two. This method will be used in the NRC laboratory on positive *C. trachomatis* samples sent by the Pasteur Cerba laboratory. Continued surveillance based on testing positive samples by this method will be very useful in detecting the variant strain in France.

Acknowledgements

We thank G. Kreplack (LABM Chemin Vert, Paris), A. Bianchi (Conseil général de la Seine–Saint-Denis, Bobigny), and T. Girard (Hôpital Cochin, Espace Santé Jeunes, Paris) for sending their samples, and Lisa King for editorial assistance for English.

References

1. 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(11):E061109.2. Available from: http://www.eurosurveillance.org/ew/2006/061109.asp#2
2. Moghaddam A, Reinton N. Identification of the Swedish Chlamydia trachomatis variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill. 2007;12(3): E070301.3. Available from: http://www.eurosurveillance.org/ew/2007/070301.asp#3
3. Herrmann B. A new genetic variant of Chlamydia trachomatis. Sexually Transmitted Infections 2007;83:253-4.
4. Lynagh Y, Walsh, A., Crowley, B. First report of the new variant strain of Chlamydia trachomatis in Ireland. Epi-Insight 2007;8(7):4. Available from: http://www.ndsc.ie/hpsc/EPI-Insight/Volume82007/File,2424,en.PDF
5. Lynagh Y, Crowley B, Walsh A. Investigation to determine if newly-discovered variant of Chlamydia trachomatis is present in Ireland. Euro Surveill 2007;12(2):E070201.2. Available from: http://www.eurosurveillance.org/ew/2007/070201.asp#2
6. de Vries H, Catsburg A, van der Helm J, Beukelaar E, Morré S, Fennema J, Thiesbrummel H. No indication of Swedish Chlamydia trachomatis variant among STI clinic visitors in Amsterdam. Euro Surveill 2007;12(2):E070208.3. Available from: http://www.eurosurveillance.org/ew/2007/070208.asp#3
7. Alexander S, Ison C. Is new variant Chlamydia trachomatis present in England and Wales? Sex Trans Inf. 2007 Sep 12; [Epub ahead of print].
8. Van der Pol B, Pantone A, Williams JA. No evidence of Chlamydia trachomatis plasmid mutants in Midwestern United States. In: 17th International society for STD research. 10th International Union against STI; 2007 July 29-August 1; Seattle, Washington, USA; 2007. P-091.
9. Tabrizi SN, Stevens MA, Tan S, Horvath L, Fairley CK, Garland SM. Absence of a chlamydia trachomatis variant, harboring a deletion in the cryptic plasmid, among clients of a STI clinic in Melbourne, Australia. In: 17th International Society for STD research, 10th International union against STI; July 29-August1, 2007; Seattle, Washington, USA; July 29-August1, 2007.
10. French Health Products Safety Agency. http://agmed.sante.gouv.fr/ang/indang.htm
11. Clerc M, Ngandjo A, Bébéar C, Barbeyrac de B. Development of real-time PCR for Chlamydia trachomatis quantification of plasmid and elementary bodies. In: Deak J, editor. Proceedings fifth meeting of the european society for Chlamydia Research; 2004 september 1-4; Hungary - Budapest: Pauker; 2004. p.75.
12. Ripa T, Nilsson PA. A Chlamydia trachomatis strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. Sexually Transmitted Diseases 2007;34(5):255-6.
13. Rodriguez P, Vekris A, de Barbeyrac B, Dutilh B, Bonnet J, Bebear C. Typing of Chlamydia trachomatis by restriction endonuclease analysis of the amplified major outer membrane protein gene. J Clin Microbiol 1991;29(6):1132-6.
14. Institut de Veille Sanitaire. The fight against HIV/AIDS and sexually transmitted diseases in France - 10 years of surveillance, 1996-2005. Executive summary. July 2007. http://www.invs.sante.fr/publications/default.htm
15. Krevolin M, Johson J, Lu S-D, Kawa D, Yu J, Kotadia R, et al. Cobas TaqMan CT Test v2.0: detection of the swedish variant by NAAT. In: 17th International Society for STD research , 10th International union againt STI; 2007; Seattle, Washington, USA, July 29-1st August 2007, P-158.
16. Reed GH, Kent JO, Wittwer CT High Resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 2007;8(6):597-608.

Citation style for this article: de Barbeyrac B, Raherison S, Cado S, Normandin F, Clerc M, Clairet V, and al. French situation concerning the Swedish Chlamydia trachomatis variant. Euro Surveill 2007;12(10)[Epub ahead of print]. Available online: http://www.eurosurveillance.org/em/

v12n10/1210-226.asp