

Implications of the variant strain of *Chlamydia trachomatis* outside the Nordic Region

Becton Dickinson and ASHA
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Update and Implications of the variant strain of *Chlamydia trachomatis* circulating in Northern Europe



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Background

- The Swedish variant* has a 377 bp deletion in the cryptic plasmid gene of 7501 bp

- The deletion is from 654 to 1030 bp (serovar E)

 - Common serovar in the U.S. and the world

- Some diagnostic tests target the plasmid:

 - Roche Cobas Amplicor PCR/TaqMan 48
 - Abbott m-2000 (not available in the U.S.)
 - Becton Dickinson ProbeTec

- Other tests target other genes/targets

 - GenProbe Aptima: 16S rRNA gene
 - Artus RealArt CT PCR: *omp-1* gene (not in U.S.)
 - Chlamydia Rapid Test, Univ Cambridge: LPS (Not U.S.)

*Ripa and Nilsson. *Eurosurveillance.org* 2006;11 (11)E061109.2; Ripa T. and Nilsson, P.A. *STD* 2007;34:255-256.

Background

- Detection of the variant (mutant) as a positive depends on the kind of the NAAT test, or whether a non-culture test, or culture is performed
- Because of where the primers set down on the plasmid gene target, both the Roche Cobas Amplicor PCR/TaqMan 48 and the Abbott m-2000 will be affected and the test will not identify the variant CT as positive
(Redesigning the primers can fix the test)

Background

- The Becton Dickinson ProbeTec primers are not affected as to where the deletion is located and the test will be positive for the Swedish variant
- The GenProbe Aptima tests (Combo2 and ACT) and Artus test will be positive
- Culture and non-culture tests not targeting the plasmid (i.e. targeting LPS or MOMP) will be positive

Background

- The test of choice for the detection of chlamydia today is a NAAT assay
- U.S public health labs in 2004*, 3.6 M tests
64.4% were NAATs
(0.9% PCR, 31% SDA; 32.5% TMA)
30.9% were direct probe hybridization
3.2% were EIA; 0.1% were culture
- Commercial Labs ~77% NAATs
- CAP Feb-2007 proficiency survey: 857 of NAATs participants; 27.4% used the Roche PCR test; APTIMA 28%; 39.4% ProbeTec

*Dicker L.W. et al STD 2006;34:41-46

Implications

- Although the variant has been found only in Sweden* and Norway** thus far, surveillance outside of Northern Europe and in the U.S. will be necessary
 - None found in Ireland*** or Amsterdam#
 - Initially about 20% of the positives were discovered to be the variant; all serovar E; single clone, thus far

*Ripa and Nilsson. [Eurosurveillance.org](http://www.eurosurveillance.org/ViewArticle.aspx?pubId=11111) 2006;11 (11)E061109.2; **Moghaddam, Reinton <http://www.eurosurveillance.org/ViewArticle.aspx?pubId=11111>; ***Lynagh et al. [Eurosurveillance.org](http://www.eurosurveillance.org/ViewArticle.aspx?pubId=11111) 2007/070201; # de Vries et al. <http://www.eurosurveillance.org/ViewArticle.aspx?pubId=11111>

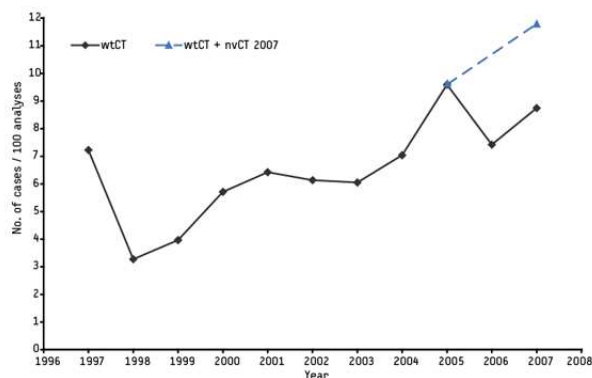
New Results-Sweden*

- nvCT infections in different counties in Sweden have been reported to represent from around 10% of all CT infections up to 64% in Dalarna county (Britta Lore, personal communication)*
- In Södra Älvsborg County, among the 789 samples 69 (8.8%) were positive for wtCT as detected by Roche CTM48*
- An additional 24 were positive for nvCT in the in house PCR. Thus 25% of a total of 93 CT-positive samples were nvCT*

* Björkman, Jonsson, Nilsson; Prevalence of the new genetic variant of *Chlamydia trachomatis* in Södra Älvsborg County, Västra Götaland Region, Sweden <http://www.eurosurveillance.org/ew/2007/070614.asp#4>

FIGURE

Chlamydia trachomatis cases in Södra Älvsborg, 1997-2007 (19 Feb-1 Mar and 29 Mar-2 Apr)



* Björkman, Jonsson, Nilsson; Prevalence of the new genetic variant of *Chlamydia trachomatis* in Södra Älvsborg County, Västra Götaland Region, Sweden <http://www.eurosurveillance.org/ew/2007/070614.asp#4>

Implications

- **De Laar & Ison conducting a Europe-wide investigation to assess variant***;

European Surveillance of Sexually Transmitted Infections network (ESSTI) and the European Center for Disease Prevention and Control (ECDC)

- **SURVEY AIMS:**

- to provide an overview of the current situation in Europe

- to assess the presence of the new strain across Europe

- to compare the diagnostic recommendations made for Europe

The variant was discovered because an epidemiological decrease (25%) in prevalence was noted and because the lab was able to perform different tests on their diagnostic samples**

*<http://www.eurosurveillance.org/ew/2007/070208.asp#3> **Schachter J. STD 2007;34:257

Implications

- **Reasons for concern:**

- False negatives of some commercial NAATs

- Possibility of a positive selective advantage b/c if not being detected by screening tests, it would allow the strain to spread more easily

- **Other potential reasons for caution:**

- We do not know the function of the cryptic plasmid

- Genetic changes in other bacterial genes have led to antimicrobial resistance and loss of enzymes

Schachter J. STD 2007;34:257

New Implications: Another Variant

Recent Publication: [Magbanua, J.P. STI 2007; pub on line June 13.](#)

- CT Variant which is plasmid-free detected
- FVU from 28 y/o male in London, 2006, failed AZ Rx, responded to Doxy
- Sequence analysis *omp-1* indicated serovar I
- Sample negative by all plasmid based NAAT assays: Amplicor PCR, BD ProbeTec ET, research plasmid PCRs
- Sample positive by GenProbe Aptima Combo2, Artus, Univ. Cambridge Chlamydia Rapid test, & Taqman research *omp-1* PCRs

What do these variants mean to the rest of the world?

- Surveillance will be necessary. Europe has been conducting and reporting via Eurosurveillance. Travel is common
- Actions we can take:
 - Surveillance in the US, the remainder of the world, outside Europe
 - Specific molecular test will be required or genotyping of new isolates
 - Performance of multiple NAAT assays or dual target assays

Specific NAAT for the Variant from Sweden-Published

A specific real time research PCR published for Light Cycler using FRET probes

-probes only produce fluorescence when the probes bind to the variant and they set down close to each other b/c the deletion brings them close together; wild type indicates no fluorescence

Ripa T. and Nilsson, P.A. STD 2007;34:255-256.

Second Specific NAAT for the Variant Under Development

A specific real time research PCR under development for the ABI 7900 prism (Applied Biosystems) using a single Taqman probe (109 bp amplicon)

-FAM probe (26bp) only produces fluorescence if probe binds to the variant

- $\frac{1}{2}$ of the probe contains sequences before the deletion of the variant and $\frac{1}{2}$ has plasmid sequences after the deletion

Plans that could be made for surveillance

- Set up a Center or two in the U.S., Europe, and Australia (Others?) that can accept CT samples on an ongoing basis– perhaps a few every month from various participants
- Centers could test samples using multiple NAATs and a variant-specific PCR to detect variants; genotyping may also be necessary
- Committee (s) will be needed to organize such surveillance (CDC? HPA? Manufacturers, Others?)

Remaining Questions

- How widespread is the Swedish variant?
- How widespread is the UK serovar I variant?
- How and why did the variants arise?
- How stable are the variants? Is there a growth advantage or disadvantage? or infectiousness?
- Will antibiotic susceptibility be affected?
- Will dual target NAATs be required or will chromosomal (omp-1, 16S) targets be needed?

Remaining Questions

- Who is at risk for contracting the variant (s)
 - Travelers, those with multiple PN?
- Does nvCt cause the same sequelae as the wtCT?
- How will manufacturers respond to this diagnostic challenge?
- What should they do?
 - Many have responded by reassuring customers and are interested in supporting surveillance

Summary

- Two variants of CT have been recently reported in Sweden and UK
 - We do not know the extent of spread or whether there is a biological advantage
 - The variants have affected our diagnostic capabilities with some assays failing to detect variants
 - Surveillance appears to be prudent in the future
- Who will do it? How will it be done?