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.)

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 2006;11 (11)E061109.2; ** Moghaddam, Reinten
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*

### 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

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### 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 florescence


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

-½ of the probe contains sequences before the deletion of the variant and ½ 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?