indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam

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Introduction

A *Chlamydia trachomatis* variant has recently been reported from Sweden, characterised by a 377 base pair deletion in open reading frame 1 in the *Chlamydia trachomatis* plasmid (located outside the chromosome) [1]. This deletion is located in the target area of several commercially available PCR tests to diagnose urogenital CT infections, such as the COBAS Amplicor system (Roche Molecular Systems, Branchburg, NJ), which is frequently used in the Netherlands. Due to the deletion, this test will not detect infections caused by this Swedish CT variant. CT tests including the ProbeTec system (Becton Dickinson, Sparks, Maryland) and Aptima Combo 2 (Genprobe) use a CT plasmid target sequence outside the deleted plasmid region and a 16s rRNA target respectively, and are therefore able to detect the Swedish CT variant.

Since we use one of these tests (Roche COBAS Amplicor) at the STI outpatient clinic of the Municipal Health Service Amsterdam for urogenital and anorectal chlamydia diagnostics, we decided to perform a comparative study involving several *C. trachomatis* PCR procedures to assess the prevalence of the Swedish CT variant among visitors to our clinic. This *C. trachomatis* variant was not found among 515 visitors who tested positive for chlamydia.

We wanted to know if the Swedish CT variant was circulating in the Amsterdam population, since this test had failed to detect the variant in Sweden. Since the Swedish discovery was prompted by a decrease in urogenital CT prevalence in their region, we first studied the prevalence rates among the Amsterdam STI clinic visitors from 2000 onwards. We then performed a comparative prospective study using both the COBAS Amplicor test and the Probertec test to detect possible missed CT infections. With molecular analyses, discrepant samples were further evaluated to see if the plasmid deleted CT variant was present among our visitors.
Methods
Separate urogenital CT prevalence rates for men who have sex with men (MSM), heterosexual men, and women, were calculated per 1 000 visitors per year from 2000 to 2006. Data was recorded in our electronic patient filing system, which gives all diagnoses per patient.

To estimate the sample size for the comparative study, we performed a statistical power analysis based on the Swedish data, using Epi Info software. In the Swedish study, 186 CT positive were found in 1 700 consecutively screened samples (overall CT prevalence ca. 11%) [1]. Within this group, 24 Swedish CT variant samples were found (Swedish variant CT prevalence ca. 1.4%). In our clinic, we test all visitors routinely for urogenital CT and find comparable overall CT prevalence rates within the heterosexual population. Since there was no data available, we assumed a prevalence rate around 2%. With 25 000 annual visitors to the clinic, we calculated that one or more cases would be found after screening 363 visitors (95% confidence interval). We hoped to include at least 500 visitors with high-risk behaviour for STIs in this study. Risk behaviour was estimated using our Risk-Based Visitor-Prioritising System, as described elsewhere [2]. A visitor to the STI clinic was defined as showing high-risk behaviour if they:

1. had been referred to the clinic by a physician,
2. reported STI-related complaints,
3. were diagnosed with an STI in the previous six months,
4. if male, had had sex with a man in the previous six months,
5. had been notified by a sex partner with an STI diagnosis.

Two samples for CT testing were collected from each body site: one sample suitable for the Probetec test and one for the COBAS Amplicor test. If one of the tests failed due to missing samples or PCR inhibition, all samples collected from this body site were excluded from the study. Cervical swabs were collected from all female patients. If a female visitor either reported having had sex for money, being pregnant, having abdominal complaints or unusual vaginal discharge, urethral swabs were also taken. A urethral swab (for the Probetec test) and a first void urine sample (for the COBAS Amplicor test) were collected from all male patients to test for urogenital CT infections. An additional ano-rectal swab under proctoscopic supervision was obtained from men and women who reported having receptive anal sex in the previous six months. All samples were processed according to the manufacturer's instructions. Internal controls to monitor inhibition of the amplification reaction were included with each sample in both systems. Since we expected that the Swedish CT variant would be detected by the Probetec test but not by the COBAS Amplicor test, we performed the following additional confirmation tests on all discrepant samples:

1. An in-house test was used to detect the presence of human DNA (HLA target). Since CT is an intracellular living organism, human cells are required for a valid test result, and a second in-house test was used to detect the presence of CT DNA (plasmid target outside the deleted region) [3].
2. A PCR-based assay was developed to distinguish the Swedish CT variant from the non-variant. Two primers were selected around the 377 bp deletion in the CT plasmid: Forward swCT: 5’-TCC GGA TAG TGA ATT ATA GAG ACT ATT TAA TC-3’ and Reverse swCT: 5’-GGT GTT TGT ACT AGA GGA CTT ACC TCT TC-3’. This results in a 475bp PCR fragment for the non CT variant while the Swedish CT variant will generate a 98bp PCR fragment.

Results
No noticeable decrease in urogenital CT prevalence rate was seen in women and heterosexual men from 2000 to 2006 on (mean rate/1000 ± standard deviation respectively 93.1±3.8 and 107.4±5.3). In the same period among MSM, rates of
urogenital CT infections decreased from 74/1000 in 2001 to 47/1000 in 2006 (mean rate/1000 ± standard deviation 56.6±9.8).

**Figure.** Urogenital chlamydia infection rate among women, heterosexual men and men who have sex with men, STI outpatient clinic, Amsterdam, 2000-2006

Discrepancy analysis
A total of 704 samples were collected from 515 participants (152 MSM, 171 heterosexual men and 192 women). Due to missing samples (n=15) and PCR inhibition (n=2), 17 samples were excluded and 687 samples were eligible for our comparative study.

In total, 75 out of 687 samples were positive for CT in the COBAS Amplicor test procedure, whereas 63 out of 687 samples were positive in the Probetec test procedure. In total, 14 sample sites showed discrepant results between the two evaluated tests. Thirteen of the discrepant samples were COBAS Amplicor positive but Probetec negative. Additional confirmation tests revealed six true positive urogenital infections and seven false positive infections based on the initial Roche test result. The overall infection rate was therefore 68 out of 687 samples (9.9%).

In one male patient, the urethral swab was Probetec positive and the urine sample COBAS Amplicor negative. Since we would expect the latter discrepant result to be possibly caused by the Swedish variant, both samples were subjected to the additional tests. This discrepant analysis showed that both samples contained human DNA (indicating that the samples were suitable for detection of CT and that no inhibition was present) and CT DNA. This could be explained either as a sampling error for the COBAS Amplicor test, or because the Swedish variant was present in the sample but not detected by COBAS Amplicor. When we used this CT positive sample together with 10 other CT positive samples from the study (all COBAS Amplicor and ProbeTec positive) and ran the Swedish CT variant PCR on these samples, all 11 samples showed a 475bp PCR fragment after agarose gel electrophoresis. This implies that the Swedish CT variant as found in Halland, Sweden, was not the reason for our discrepant result.
**Discussion**
The Swedish CT variant is an example of a mutation enabling a pathogen to remain undetected in a diagnostic procedure. As long as the test procedure remains unaltered, this mutation offers the organism an evolutionary advantage when compared with non-mutated strains.

In contrast to the Swedish report where a considerable drop in the urogenital CT prevalence in Halland county was noticed over 2005-2006 [1], we did not observe unexpected prevalence changes between 2000 and 2006. We saw a decrease in urogenital CT infections in MSM, but this trend was first noticed in 2001. Only one discrepant result was found in samples from a male patient: the urethral sample was ProbeTec positive, while the corresponding urine sample in the COBAS Amplicor test was negative. With our additional tests, we excluded the possibility that the discrepancy was due to a deletion in the plasmid gene as found in Halland, Sweden. It is possible that a low bacterial load or differences in sample collection (urine versus urethral swab) contributed to discrepant test results.

In 2004, a lymphogranuloma venereum outbreak among MSM was reported in Rotterdam, and was followed by reports of the infection in surrounding European countries, the United States and Australia [4]. A new CT variant L2b seemed to be responsible for this outbreak and its prevalence could be traced back to the beginning of the 1980s [5, 6]. Although the Swedish CT variant does not seem to be circulating in the Amsterdam population, vigilance is required. Both the Swedish CT variant and the LGV outbreaks stress the need for early warning systems and alertness within the STI professional community to (re)emerging infections. Apart from Halland county, to our knowledge the Swedish variant has not been reported in other parts of Sweden, but comparative studies are ongoing [7]. A retrospective study on ca. 8 800 samples for CT screening was recently performed in Ireland, but the Swedish CT variant was not detected [8]. More studies in and outside Sweden are needed to determine whether there has been geographical spread of this variant across Europe.

In the Netherlands, the Centre for Infectious Diseases Control of the National Institute of Health (RIVM) has implemented a working committee that informed microbiologists and STI clinics on the Swedish CT variant. If negative CT test results obtained by Roche PCR are found in patients suspected for urogenital CT infections, physicians are urged to send in the samples to a reference lab (VU University Medical Center, Medical Microbiology and Infection Prevention) for further testing to exclude the Swedish CT variant. CT tests targeting the Swedish CT variant deletion region are currently being redesigned.

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**References:**
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