Research Article

Chlamydia trachomatis Genotypes and the Swedish New Variant among Urogenital Chlamydia trachomatis Strains in Finland

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1. Introduction

Chlamydia trachomatis, an obligate intracellular bacterium, is worldwide a common cause of sexually transmitted infections. Even asymptomatic infections can lead to serious sequelae such as infertility and ectopic pregnancy. The infections are very prevalent among adolescents, and reinfections are common [1]. Young people with C. trachomatis constitute an important target group for public health interventions. Since 1995, the number of C. trachomatis infections has been officially notified in Finland, and the number of notifications has been increasing. Lately, there has been around 14 000 notified cases annually (250 cases/100 000 inhabitants) [2]. In Finland, C. trachomatis infections are diagnosed mainly with sensitive and specific nucleic acid amplification tests (NAATs), but these tests do not differentiate between genotypes.

The seroimmunological analysis of C. trachomatis major outer membrane protein (MOMP) first leads to the identification of ≥15 different serovars, and sequence differences in the ompA gene, especially in the areas coding the variable domains of MOMP, later confirmed this discrimination [3, 4]. C. trachomatis types A–C cause trachoma, types D–K cause urogenital infections, and types L1–L3 cause lymphogranuloma venereum (LGV). C. trachomatis has also a cryptic plasmid which is commonly used as a target sequence in diagnostic NAATs. In 2006, there was an unexpected fall in C. trachomatis cases in Sweden, which was caused by the appearance of a new variant of C. trachomatis (nvCT) with a 377 base pair deletion in the cryptic plasmid [5]. At the same time, the proportion of urogenital samples that tested positive by Cobas TaqMan CT Test, old version (Roche), decreased slightly in Southern Finland (Dr. Jukka Suni, HUSLAB; personal communication).

The purpose of this work was to set up a real-time PCR-based method for genotyping C. trachomatis (types D–K and L1–L3) in urogenital samples. Additionally, we wanted to set up a method for detection of the Swedish nvCT and to study the occurrence of this variant in Finland.
2. Materials and Methods

2.1. Clinical Samples and DNA Extraction. In 2008, 160 unselected C. trachomatis positive specimens from females and males were collected for genotyping. The first-void urines (N = 82), cervical/vaginal swabs (N = 75), and conjunctival swabs (N = 3) had been sent to HUSLAB, Department of Virology for C. trachomatis nucleic acid testing. HUSLAB, the diagnostic laboratory of the Helsinki University Central Hospital serving the capital area, used at that time both Aptima Combo 2 Assay (Gen-Probe) and Cobas TaqMan CT Test (Roche). Of the 160 samples, 84 tested C. trachomatis positive with Gen-Probe and 76 with Roche test. Additionally, 38 C. trachomatis negative clinical samples were included as negative controls. To estimate the prevalence of the Swedish nvCT in Finland, 495 urogenital samples were studied. Of these samples, 469 were C. trachomatis positive with Aptima Combo 2 Assay (Gen-Probe) (N = 414) or Cobas TaqMan CT Test, new version (Roche) (N = 55), and 26 were negative with Cobas TaqMan CT Test, old version (Roche). As the prevalence of the nvCT at that time was quite high in Sweden [6], we initially planned to collect specimens that tested C. trachomatis negative by the old version of the Cobas TaqMan CT Test (Roche), which fails to detect them. However, the test was soon replaced by a new dual-target version by the manufacturer, and the majority of our material consisted of C. trachomatis positive specimens.

For genotyping, DNA was extracted from a specimen volume of 400 µL with MagNA Pure Compact instrument (Roche) using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) with DNA Bacteria protocol and eluted in 50 µL. For detection of the nvCT, DNA was extracted from a specimen volume of 200 µL with MagNA Pure LC (Roche) instrument using MagNA Pure LC DNA Isolation Kit I (Roche) with DNA I Blood Cells High Performance protocol and eluted in 100 µL. The concentration of total DNA was measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific). DNA extracted from the samples was stored at −70°C until analysis.

2.2. Real-Time PCR. Genotyping was performed according to a method described previously by Jalal et al. [7]. The method is based on the sequence variation of the ompA gene, and it has two primer sets and eleven genotype-specific TaqMan probes for types D–K and L1–L3. In this study, we used the non-nested version of the method. As controls, DNA extracted from C. trachomatis reference strains (types A–K and L2) originally from ATCC (American Type Culture Collection) propagated in McCoy cells (mouse fibroblast cells) [8] was used.

To screen for the Swedish nvCT, we used a method developed by Catsburg et al. [9]. This method has primers and a TaqMan probe flanking the deletion sequence in the cryptic plasmid of C. trachomatis. A clinical sample containing the nvCT, kindly provided by Professor Björn Herrmann, Uppsala, Sweden, was used to construct a positive control for the assay. The sequence flanking the deletion was amplified and cloned into a plasmid vector with Zero Blunt TOPO PCR Cloning Kit (Invitrogen Life Technologies) according to the manufacturer’s instructions. Plasmids were extracted with QIAprep Spin Miniprep Kit (QIAGEN), and the DNA concentration was measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific).

Real-time PCR analyses were performed with an ABI 7500 instrument and Sequence Detection Software version 1.3.1 (Applied Biosystems). The primers and probes used in this study were purchased from Applied Biosystems, Metabion International AG, or TAG Copenhagen A/S. The PCR reactions were performed in a 25 µL volume containing 250 nM primers, 100 nM probes, and either 12.5 µL Platinum Quantitative PCR SuperMix-UDG (Invitrogen Life Technologies) or 12.5 µL TaqMan Universal Master Mix (Applied Biosystems). Thermal cycling conditions were, 50°C 2 minutes, 95°C 10 minutes and 40 cycles of 95°C 15 seconds and 60°C 1 minute. Template volume was 2 µL or 5 µL for genotyping and 2 µL from two different pools pooled together for detection of the nvCT.

3. Results

Of the 160 C. trachomatis positive clinical samples analyzed, 144 (90%) could be genotyped. Ninety % of both first-void urines and swab samples sent for NAAT contained enough chlamydial DNA for the ompA PCR. Sixteen samples (10%) remained without a genotype with the non-nested version of the used method which was most likely due to the low amount of chlamydial DNA present in the specimens. The ompA genotype distribution of the strains is presented in Tables 1 and 2. Genotypes E (N = 57, 40%), F (N = 41, 28%), and G (N = 19, 13%) were the most prevalent in this study and comprised together over 80% of the C. trachomatis findings. The 16 samples that remained negative for genotypes D–K were analyzed for genotypes L1–L3. We did not detect any genotypes L1–L3 in these urogenital samples. All the 38 C. trachomatis negative clinical samples tested also negative in genotyping PCR.

Among the 469 C. trachomatis positive clinical samples screened for the Swedish nvCT, samples from two female patients were found to contain a bacteria with the variant plasmid (0.4%). One of the samples was first-void urine and the other a vaginal swab. Both of the nvCT were of genotype E, as has been described also in Sweden and elsewhere [6, 21]. Both also harbored a cryptic plasmid with a deletion site in the ORF1, and the sequence flanking the deletion in both strains was identical to that of the Swedish nvCT (confirmed by sequencing) [5]. The clinical samples containing the nvCT were both initially tested by the Aptima Combo 2 Assay (Gen-Probe) that is based on detection of the ribosomal RNA and is thus able to detect also this type of variant C. trachomatis.

4. Discussion

Typing of C. trachomatis strains can be used in epidemiologic surveillance locally or internationally, to assess the changes in genotype distribution and to reveal transmission patterns in
5. Conclusions

In this study, we showed that the most prevalent genotype in Finland was E (40%). However, the Swedish variant was rare in Finland despite our close proximity to Sweden and the frequent occurrence of genotype E.
Table 2: The *ompA* genotype distribution (%) of 144 clinical *C. trachomatis* strains in Finland (this study) compared with distribution of types in *C. trachomatis* strains in Sweden, the Netherlands, Portugal and the USA.

| Serotype/ genotype | Finland (this material) | Sweden [12] N = 237 *ompA* sequencing | Sweden [13] N = 678 *ompA* sequencing | The Netherlands [14] N = 438 *ompA* PCR & RFLP | The Netherlands [15] N = 407 *ompA* PCR & RFLP | Portugal [16] N = 795 *ompA* sequencing | The USA [17] N = 11 454 serotyping | The USA* [18] N = 507 *ompA* sequencing | The USA [19] N = 102 *OmpA* sequencing |
|--------------------|-------------------------|----------------------------------------|---------------------------------------|-----------------------------------------------|------------------------------------------|------------------------------------|--------------------------------------|----------------------------------|----------------------------------|
| B/Ba               | ND                      | 0.4                                    | 1                                     | ND                                            | 1                                        | 0.4                               | 2                                    | 1                                | ND                               |
| E                  | 40                      | 47                                      | 39                                    | 40                                            | 33                                       | 40                                | 32                                   | 30/33                           | 30                               |
| D/Da/D-            | 8                       | 14                                      | 9                                     | 12                                            | 13                                       | 13                                | 16                                   | 17/19                           | 14                               |
| **total**          | **48**                  | **61.4**                                | **49**                                | **52**                                        | **53.4**                                 | **50**                            | **48/53**                           | **45**                           | **51**                           |
| F                  | 28                      | 17                                      | 21                                    | 21                                            | 23                                       | 17                                | 18                                   | 20/17                           | 19                               |
| G/Ga               | 13                      | 3                                       | 11                                    | 8                                             | 9                                        | 11                                | 2                                    | 3/4                             | 4                                |
| K                  | 5                       | 9                                       | 4                                     | 2                                             | 1                                        | 1                                 | 3                                    | 3/5                             | 5                                |
| **total**          | **46**                  | **29**                                  | **41**                                | **33**                                        | **34**                                   | **29**                            | **24**                               | **26/26**                       | **28**                           |
| C                  | ND                      | ND                                      | ND                                    | ND                                            | 0.4                                     | ND                                | ND                                   | ND                              | ND                               |
| H                  | 3                       | 3                                       | 2                                     | 4                                             | 8                                        | 3                                 | 3                                    | 2                                | 1                                |
| I/Ia               | 0                       | 3                                       | 1                                     | 4                                             | 7                                        | 6                                 | 10                                   | 12/9                            | 14                               |
| J/Ja               | 2                       | 4                                       | 7                                     | 4                                             | 3                                        | 7                                 | 11                                   | 13/10                           | 12                               |
| **total**          | **5**                   | **10**                                  | **10**                                | **12**                                        | **18**                                   | **16.4**                          | **24**                               | **27/21**                       | **27**                           | **37**                           |

RFLP: restriction fragment length polymorphism.

* Genotypes of women and men separately.

ND: not determined.
Conflict of Interests

The authors declare that they have no conflict of interests.

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