

## First Reported Case of the Swedish New Variant of *Chlamydia trachomatis* (nvCT) in Eastern Europe (Russia), and Evaluation of Russian Nucleic Acid Amplification Tests Regarding Their Ability to Detect nvCT

Elena Shipitsyna<sup>1</sup>, Ronza Hadad<sup>2</sup>, Olga Ryzhkova<sup>1</sup>, Alevtina Savicheva<sup>1</sup>, Marius Domeika<sup>3</sup> and Magnus Unemo<sup>2\*</sup>

<sup>1</sup>Laboratory of Microbiology, D.O. Ott Research Institute of Obstetrics and Gynaecology, St Petersburg, Russia, <sup>2</sup>Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, SE-701 85 Örebro, and <sup>3</sup>Department of Medical Sciences, Uppsala University, Uppsala, Sweden. E-mail: magnus.unemo@orebroll.se

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*Chlamydia trachomatis* infections are the most prevalent bacterial sexually transmitted infections (STIs) worldwide, and are associated with considerable morbidity and socioeconomic consequences.

The Swedish new variant of *C. trachomatis* (nvCT), first reported in Sweden in late 2006, contains a 377-bp deletion in the cryptic plasmid that covers the single targets originally utilized in the nucleic acid amplification tests (NAATs) manufactured by Roche Diagnostics (Amplicor, Cobas Amplicor, Cobas TaqMan48) and Abbott Laboratories (Abbott *m2000*), which at the time were used worldwide (1, 2). In Sweden, the proportion of nvCT, compared with wild type CT (wtCT), was high (7–64% in the different counties) (3, 4), and nvCT accordingly caused thousands of falsely negative diagnoses. The nvCT prevalence in Sweden is still relatively high, and nvCT has been frequently detected in Norway, Finland and Denmark (5, 6). Although genome sequencing and phenotypic characterization have shown that nvCT, compared with wtCT, has an unaltered biological fitness *in vitro* (7), nvCT is rarely reported outside the Nordic countries (5, 7–9). However, knowledge of the nvCT presence beyond the Nordic countries is limited because few recent studies have been performed, many laboratories still cannot detect nvCT, and the ones that can detect nvCT mainly do not distinguish it from wtCT (5, 10, 11).

The aims of this study were: (i) to investigate the presence of nvCT in St Petersburg, the largest city in the northwest of Russia and in close geographical proximity to Sweden; and (ii) to assess NAATs used in Russia to diagnose *C. trachomatis* infections for their ability to detect nvCT.

## MATERIALS AND METHODS

Between June and December 2010, consecutive urogenital samples (cervical swabs from females and urethral swabs from males) found positive for *C. trachomatis* during routine testing, using a polymerase chain reaction (PCR) that detects the nvCT (AmpliSens *Chlamydia trachomatis*, Central Research Institute of Epidemiology, Moscow, Russia), at the Laboratory of Microbiology, D.O. Ott Research Institute of Obstetrics and Gynaecology, St Petersburg, Russia were collected. For nvCT detection, DNA was subsequently isolated from 200 µl of the primary sample using either the robotized system NucliSens easyMAG (bioMérieux) or QIAamp DNA mini kit (Qiagen). The DNA samples were transported frozen to the Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden, and tested for nvCT with an internationally frequently used nvCT-specific real-time PCR (2). Six PCR tests, which are used in the majority of Russian laboratories performing *C. trachomatis* diagnostics, produced by four Russian companies (Table I) were assessed for their ability to detect nvCT, via testing of purified DNA from the previously genome sequenced nvCT (Sweden2 (7)). Five of these assays (Table I) have been evaluated previously concerning their performance characteristics in comparison with the Cobas Amplicor PCR (Roche Diagnostics) and the LightMix 480HT PCR (TIB MOLBIOL) (12). In the present study, a newly introduced dual-target real-time PCR assay targeting the cryptic plasmid and the *gyrA* gene (Vector-Best, Novosibirsk) was also evaluated.

## RESULTS

During the study period, 9,517 samples from (9,294) patients of gynaecological, urological and STI clinics in St Petersburg were tested for *C. trachomatis*. Of these samples, 275 (2.9%) collected from 198 females and 75 males were *C. trachomatis* positive. Two women submitted 2 positive samples each, at intervals of 3 and

Table I. Nucleic acid amplification tests (NAATs) developed and widely used in Russia for the detection of *Chlamydia trachomatis* and their ability to detect new variant *C. trachomatis* (nvCT)

NAAT (manufacturer, city)	Gene target(s)	Able to detect nvCT?
Conventional PCR (DNA-Technology, Moscow) <sup>a</sup>	Cryptic plasmid	Yes
Real-time PCR (DNA-Technology, Moscow) <sup>a</sup>	16S rRNA gene	Yes
Conventional PCR (Central Research Institute of Epidemiology, Moscow) <sup>a</sup>	Cryptic plasmid	Yes
Real-time PCR (Central Research Institute of Epidemiology, Moscow) <sup>a</sup>	Cryptic plasmid	Yes
Conventional PCR (Lytech, Moscow) <sup>a</sup>	Cryptic plasmid	No
Real-time PCR (Vector-Best, Novosibirsk)	Cryptic plasmid and <i>gyrA</i> gene	Yes

<sup>a</sup>Previously evaluated concerning their performance characteristics in comparison with the Cobas Amplicor PCR (Roche Diagnostics) and the LightMix 480HT PCR (TIB MOLBIOL) (12).

4 weeks, respectively (interpreted as separate cases). The mean age of the patients for whom age data were available ( $n=142$ ) was 26.4 years (median age 25 years: range 19–51 years). nvCT was detected in one sample (0.4%), which was obtained from a 23-year-old Russian woman. It was not possible to trace where the patient was infected or her contact(s). Genotyping using *ompA* sequencing and variable number of tandem repeats (VNTR) typing showed that the nvCT sample was identical to the previously typed nvCT samples from the Nordic countries (genovar E and type 8.7.1, respectively) (13).

Four of the 6 evaluated Russian PCRs targeted the cryptic plasmid only, one targeted additionally the *gyrA* gene, and the remaining PCR targeted the 16S rRNA gene (Table I). The Lytech PCR, frequently used in Russia, could not detect the nvCT DNA. However, all the other PCRs identified the nvCT.

The present study is the first report of an nvCT case in Russia, and in general in Eastern Europe. However, only a few nvCT cases have been reported outside the Nordic countries (5, 7–9). A possible explanation could be that sexual networks of risk groups for the nvCT within Sweden (young heterosexual men and women) are localized, and thus nvCT spreads to other countries only to a limited extent. International spread might be accelerated by the introduction of nvCT in the population of men who have sex with men, which includes large and international sexual networks (5). Since nvCT has unaltered biological fitness compared with wtCT (7), it might be expected to reach equilibrium with wtCT and to further spread to other countries (5, 7). Nevertheless, knowledge regarding the spread of nvCT is limited due to the low number of recent studies and because a substantial number of laboratories are still not able to identify nvCT (5, 10, 11).

## DISCUSSION

The present study is the first assessment of NAATs developed and currently used in Russia for detection of *C. trachomatis* with regards to their ability to detect nvCT. Although it is difficult to say in detail how widely used each of the evaluated NAATs is, in total they are utilized in the vast majority of the Russian laboratories performing *C. trachomatis* diagnostics. Of the six evaluated assays, one conventional PCR (Lytech), which has been used widely in Russia for many years, was not able to detect nvCT.

In conclusion, although the prevalence of nvCT may still be low beyond the Nordic countries, wider geographical spread of nvCT cannot be excluded, and in fact may be expected. Accordingly, laboratories using NAATs that do not detect nvCT should carefully monitor and analyse the *C. trachomatis* incidence and related epidemiological data, and ideally consider replacing

their testing system for a NAAT detecting both nvCT and wtCT (5, 7, 10, 11). Furthermore, regular national and international surveillance, frequent participation in appropriate external quality assessment scheme(s), assessment of diagnostic methods in use for their ability to detect nvCT, and regular review and evaluation of *C. trachomatis* diagnostic guidelines are crucial.

*The authors declare no conflicts of interest.*

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