Multi-locus sequence analysis reveals a novel sequence type of *Chlamydia trachomatis* in Saratov Region, Russia

V. A. Feodorova[1], S. S. Zaitsev[1], Y. V. Saltykov[1], S. S. Ulyanov[1,2] and V. L. Motin[3]

1) *Laboratory for Molecular Biology and NanoBiotechnology, Federal Research Center for Virology and Microbiology (FRCViM), Branch in Saratov,* 2) *Department for Medical Physics, Saratov State University (SSU), Saratov, Russia and* 3) *Department of Pathology, Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, USA*

Abstract

Chlamydia trachomatis is the causative agent of a variety of chlamydial infections in humans with a predominantly (up to 80%) asymptomatic course of disease. In this study, a potentially novel *C. trachomatis* sequence type (ST) was detected in an asymptomatic man who has sex with a man among the nine STs revealed in urogenital swabs from individuals with chlamydia (n = 18). Phylogenetically this ST270 clustered separately as a single clade to an ST13-founded group of *C. trachomatis* strains and differed from the latter by a single allele, *hflX*. This finding emphasizes the importance of careful investigation of individuals with asymptomatic chlamydia infections.

Keywords: *Chlamydia trachomatis*, men-who-have-sex-with-men, multilocus sequence typing, *ompA*, sequence type
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Corresponding author: V.A. Feodorova, Laboratory for Molecular Biology and NanoBiotechnology, Federal Research Center for Virology and Microbiology (FRCViM), Branch in Saratov, Saratov, Russia.
Corresponding author: V.L. Motin, Department of Pathology, Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, USA.
E-mails: feodorovav@mail.ru **(V.A. Feodorova),** vlmotin@utmb.edu **(V.L. Motin)**

Chlamydia trachomatis is one of the most successful pathogens worldwide. It can cause a variety of chlamydial infections in humans, for instance trachoma, urogenital infection and lymphogranuloma venereum [1]. Chlamydial infection is a leading cause of serious complications such as pelvic inflammatory disease, ectopic pregnancy, inflammation, scarring and infertility [2]. Most chlamydial infections in humans could be asymptomatic (up to 80%) with no clinical manifestations, but the rate of transmission could remain extremely high [2–5]. Here, we describe a 38-year-old man, single who has sex with a man with an asymptomatic chlamydial genital infection. Sexual debut had occurred by age 16 years. He declared few additional irregular sexual contacts during the last 6 months in parallel with his regular sex partner. He denied any pernicious habits, except smoking, and had no addiction to alcohol. Previous regular annual physical and laboratory examinations by a family physician found neither clinically apparent genital manifestations of chlamydial infection nor any other sexually transmitted infections. However, there were chlamydial IgG titres in serum of 1:160, registered with an ELISA kit. Therefore, he was referred to our Diagnostic Centre for laboratory confirmation of chlamydia infection. Indeed, further routine diagnostic PCR demonstrated that his clinical sample (urethral swab) contained *C. trachomatis* DNA. Molecular analysis based on *ompA* and multilocus sequence typing (MLST), conducted as we described recently [6], assigned this DNA to the *C. trachomatis* genovar G, and a novel ST270 of *C. trachomatis* that was previously absent in the PubMLST database (http://pubmlst.org/chlamydiales/). Then, we studied other chlamydial clinical specimens to identify the frequency of occurrence of this novel ST270 in our region. DNA obtained from cervical and urethral swabs from women and men (n = 18) was analysed for the presence of major *C. trachomatis* genovars and sequence types (ST). The *ompA*-based genotyping revealed seven variants (Fig. 1a), while MLST analysis identified nine well-known STs, including the novel ST270, which was assigned as SaratovG/61.21-M156 (Fig. 1b).

The GrapeTree analysis of our strains with those obtained from the PubMLST/Chlamydiales database grouped the Saratov variants into three different clonal complexes (Fig. 1c). The first complex consisted of ST6, ST9, ST10, ST270 and ST13 (ST13 as the founder) and the second one included ST4, ST12, ST38 and ST118 (ST4 as the founder). Both of the complexes consisted of international *C. trachomatis* strains and were assigned earlier as Group I and Group III, respectively [7]. The strains from each Group differed in four alleles (*oppA*, *hplX*, *gidA* and *enoA*) (see Supplementary material, Table S1). SaratovG/61.21-M156 demonstrated identical alleles with six out of the seven loci found in ST13 and differed from the latter by a single allele *hflX* (see Supplementary material, Table S1). This *C. trachomatis*

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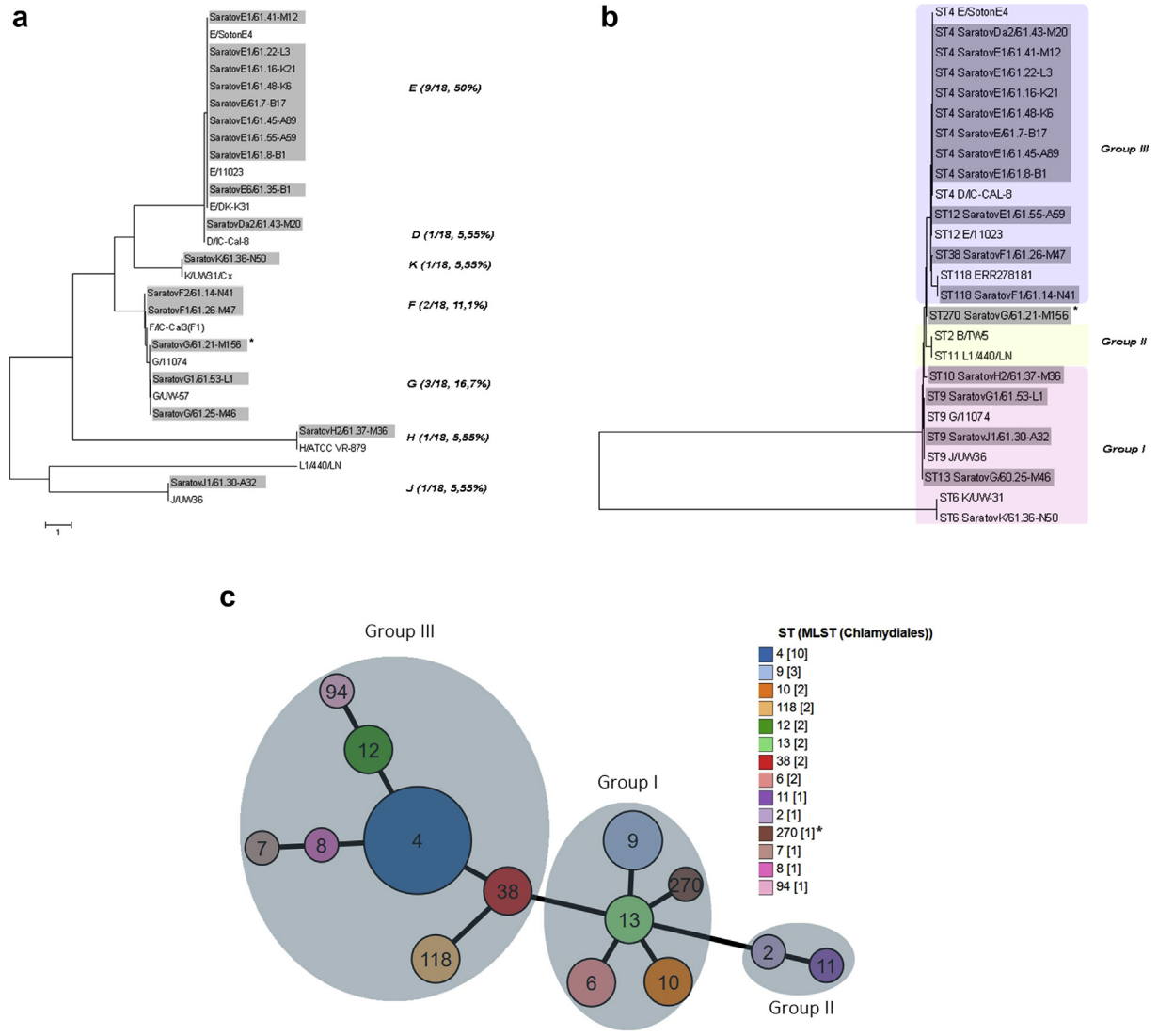

FIG. 1. Phylogenetic analyses of *Chlamydia trachomatis* strains identified in the Saratov Region (highlighted in grey) combined with the reference strains. Novel sequence type (ST) strain SaratovG/61.21-M156 is marked with an asterisk. The trees were constructed using the UPGMA hierarchical clustering method model based on (a) polymorphism in *ompA* sequences; (b) concatenated partial sequences of seven housekeeping gene fragments, such as *gatA*, *oppA*, *hfiX*, *gitA*, *enoA*, *hemN* and *fumC*. The concatenated sequences were aligned and analysed in MEGA 7 [8]. Thick branches, subtending all large clades, represent 100% bootstrap support; and (c) GrapeTree clustering of 14 STs available in the *Chlamydiales* PubMLST database (https://pubmlst.org/chlamydiales/). Each node corresponds to a single ST marked with an individual colour. The numbers in square brackets indicate *C. trachomatis* representatives in the MLST database. The novel ST270 belongs to Group I with ST13 as founder.

strain on *ompA*-typing formed a single additional clade to the Group I *C. trachomatis* strains and was located in an intermediate position between Groups 1/3 and Group 2 (Fig. 1b).

In conclusion, individuals with asymptomatic chlamydia infections should be systematically tested independently of their sexual behaviour. Molecular methods including MLST typing are effective approaches to support identification and differentiation of existing and novel *C. trachomatis* variants. This will provide new insights into the molecular evolution and phylogenetic relationships of *C. trachomatis* worldwide.

PubMLST sequences accession number. The ST sequence was deposited in PubMLST/Chlamydiales database under accession numbers 4434–4450 (https://pubmlst.org/).

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Conflicts of interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2019.100584.

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