RESEARCH ARTICLE

Comparative evaluation of new typing schemes for urogenital *Chlamydia trachomatis* isolates

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Abstract

Thirty urogenital *Chlamydia trachomatis* isolates collected in Moscow in 2005 were typed using newly developed molecular typing approaches: (1) multilocus sequence typing (MLST^7) based on sequences of seven housekeeping genes (http://pubmlst.org/chlamydiales/), (2) MLST^5 based on the investigation of five target regions of the chlamydial genome and (3) *ompA* gene sequencing supplemented with three variable number tandem repeat (VNTR) loci of the genome. *ompA* typing divided all isolates into 11 groups with E serotype dominating, while MLST7, MLST5 and VNTR analysis divided them into eight, 20 and 18 groups, respectively. The discriminatory power of each method calculated using the Hunter–Gaston discriminatory index was found to be 0.83 for the *ompA* typing scheme, 0.82 for MLST^7 and 0.95 for MLST^5. A novel sequence type combining 13% of all strains was discovered, as well as new alleles of genes. This is the first study characterizing the genetic diversity of the urogenital *C. trachomatis* population in Central Russia using MLST. We conclude that the MLST^7 scheme is the best possible choice for global epidemiological purposes, whereas MLST^5 is more appropriate for tracing local outbreaks.

Introduction

Despite intensive efforts to reduce the spread of chlamydial infections, *Chlamydia trachomatis* is still considered to be a major cause of sexually transmitted diseases, and therefore it is a major public health problem worldwide (http://www.who.int/topics/sexually_transmitted_infections/en/; http://www.cdc.gov/std/chlamydia/). Typing of *C. trachomatis* plays an important role in global epidemiological studies, as well as in local cases when questions of infection transmission or recurrence arise. Until recently, the molecular epidemiology of chlamydial infections was based on analysis of *ompA* gene coding of the major outer membrane protein (*ompA* typing) (Rodriguez *et al.*, 1991; Morré *et al.*, 1998; Jurstrand *et al.*, 2001; Lysen *et al.*, 2004). However, to reveal transmission patterns in sexual networks, notify partners and discriminate new cases from cases of persistent infection, a higher degree of strain resolution is required than is provided by *ompA* analysis. Novel schemes of *Chlamydiales* genotyping have been under development for past few years, to provide the best discriminatory capacity of strains and enhance the quality of epidemiological information. Previous studies of different single genes as a potential target for a genotyping system of high resolution have failed due to the low sequence variation, which probably results from the genetic isolation of *Chlamydia* and the low degree of horizontal DNA transfer during evolution (Everett *et al.*, 1999; Stothard *et al.*, 2003; Gomes *et al.*, 2006). Therefore, multilocus sequence typing (MLST) schemes were developed to understand the population genetic structure and diversity of species, and to evaluate the association between genotype and disease. An MLST system based on seven housekeeping genes (MLST^7) has been developed for the entire *Chlamydiaceae* family and is expected to make it possible to analyze evolutionary changes rather than acting as a tool for partner notification (Pannekoek *et al.*, 2008). This system can discriminate reference strains of *C. trachomatis*, but has been evaluated on too few clinical strains to assess its discriminatory capacity. Another scheme based on the investigation of the five most variable regions of the chlamydial genome (MLST^5) provides the highest intraserotype sequence variation and may be more suitable for the typing of closely related clinical strains (Klint *et al.*, 2007). In addition, a method of *C. trachomatis* genotyping using

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ompA gene sequencing supplemented with three new variable number tandem repeat (VNTR) loci of the chlamydial genome, has been developed recently and is expected to provide the best discriminatory capacity in short-term, local epidemiological studies (Pedersen *et al.*, 2008).

The aim of our work was to evaluate a number of newly developed molecular typing approaches to the epidemiological study of a collection of urogenital *C. trachomatis* isolates.

Materials and methods

Chlamydia trachomatis strains and DNA purification

Chlamydia trachomatis strains were obtained from cervical and urethral swabs of the attendees at the Clinico-Diagnostic Center of Lytech Co. Ltd (Moscow, Russia) in 2005. McCoy cells were cultured in 24-well cell culture plates in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine (Gibco, Invitrogen). After growth to monolayer, cells were inoculated with specimens, centrifugated for 1 h at 1700 ***g*** and incubated at 37 °C in 5% CO_2 for 1.5 h. Residual medium was drawn off and replaced by fresh growth medium containing 2 μg mL^{-1} cycloheximide and FBS prescreened for chlamydial antibodies. The infected cells were incubated at 37 °C in 5% CO_2 for 48 h.

Chlamydial inclusions were detected by direct immunofluorescence using fluorescein-labeled antibodies to lipopolysaccharide (Nearmedic Plus, Moscow, Russia) and the major outer membrane protein (CT8P40, Protein Contour, Russia) according to the manufacturer's protocols. To assess inclusions, a Nikon ECLIPSE E800 fluorescent microscope supplied with a confocal ECLIPSE C1 module (Nikon Instruments Inc.) was used. *Chlamydia trachomatis* strains obtained from positive specimens were subcultured in McCoy cells; 2–10 rounds of culture were performed to reach at least 50% of infected cells.

Elementary bodies were purified from McCoy cells in discontinuous density gradients of Renografin-76 (Schering, Germany) following the previously described protocols (Caldwell *et al.*, 1981; Miyashita & Matsumoto, 1992).

A mixture of *C. trachomatis* and McCoy cell genomic DNA was isolated using a standard phenol–chloroform extraction method.

PCR amplification and sequencing

An approximately 930-bp fragment of *ompA* was amplified by PCR using 5′-ATGAAAAAACTCTTGAAATCGG-3′ and 5′-ACTGTAACTGCGTATTTGTCTG-3′ primers (Lysen *et al.*, 2004). Amplification of seven housekeeping gene fragments (*gatA*, *oppA-3*, *hflX*, *gidA*, *enoA*, *hemN* and *fumC*), five regions of the *C. trachomatis* genome 686–2377 bp size [CT046 (*hctB*), CT058, CT144, CT172 and CT682 (*pbpB*)] and VNTR loci (CT1291, CT1299 and CT1335) of the *C. trachomatis* genome was performed using primer sets developed by Klint *et al.* (2007), Pannekoek *et al.* (2008) and Pedersen *et al.* (2008), respectively. Additional primers were used for the amplification of CT682 (*pbpB*) locus (Supporting Information, Table S1). Standard PCR protocol included the reaction in 25 μL of reaction mixture containing 66 mM Tris-HCl (pH 9.0), 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 250 μM of each dNTP, 1 U of *Taq* DNA polymerase (Lytech Ltd) and 10 pmol of each forward and reverse primer. Pfu DNA polymerase (Lytech Ltd) was used for the amplification of the CT1299 locus. A Tetrad DNA Engine thermocycler (MJ Research, Inc.) was used. The results of amplification were confirmed by agarose gel electrophoresis.

All PCR products were treated with 0.5 U of shrimp alkaline phosphatase and 2.5 U of *Escherichia coli* Exonuclease I (Fermentas, Vilnius, Lithuania) at 37 °C for 20 min, to dephosphorylate the 5′-end phosphate groups of deoxynucleoside triphosphates, and to remove residual primers. After that, the enzymes were deactivated at 85 °C for 10 min.

Cycle sequencing reactions were performed using ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism® 3100 Genetic Analyzer (Applied Biosystems; Hitachi, Japan) according to the manufacturer's instructions. Amplified fragments were sequenced on both strands using the same primers as for the PCR step. For most cases, amplicons were short enough to obtain complete double-stranded sequences. In cases of *ompA*, CT058 and the fragments of CT682 (*pbpB*), the sequences obtained created 100–300-bp overlaps in the mid-region. Any doubtful bases were resequenced from the PCR step.

Sequence analysis

VECTOR NTI v. 9.0 software was used for reading the sequences and aligning the forward and backward readings.

Nucleotide sequences of gene fragments used in different typing schemes were aligned and compared using the ALIGNX multiple alignment module implemented in VECTOR NTI v. 9.0. DNASP v. 5.1 and MEGA v. 4.0 were used for examination of sequences and phylogenetic evolutionary analysis. Software package RDP v. 3.41 was used to detect potentially recombinant regions within aligned sequences.

For MLST[7], sequences were submitted to the *Chlamydiales* MLST website (http://pubmlst.org/chlamydiales/) and compared with the existing alleles to determine allele types and sequence types (STs) of the isolates.

Allele profile data were analyzed in EBURST v. 3 (http://eburst.mlst.net/v3/enter_data/single/) to define clonal complexes or groups. Groups were defined as sets of related strains containing pairs of strains that share at least (L–1)

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identical alleles at the L loci with at least one other member of the group.

The discriminatory power of each typing method was calculated using the Hunter–Gaston discriminatory index *D* (Hunter & Gaston, 1988):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$$

where N is the number of unrelated strains tested, S is the number of different types and n_j is the number of strains belonging to the jth type.

Results

Thirty urogenital *C. trachomatis* isolates collected in Moscow in 2005 were typed using four different schemes: the MLST[7] scheme based on sequences of seven housekeeping genes (Pannekoek *et al.*, 2008), the MLST[5] system based on the sequencing of five target regions of the chlamydial genome (Klint *et al.*, 2007), and *ompA* gene sequencing supplemented with three VNTR loci of the genome (Pedersen *et al.*, 2008).

ompA typing

ompA typing was carried out in accordance with Lysen *et al.* (2004). The nucleotide sequence of *ompA* gene was determined for 30 *C. trachomatis* isolates. Serovars were established by comparison with the allele variants available from GenBank.

Sequence analysis of an approximately 930-bp fragment of the *ompA* gene divided all isolates into six serotypes. Serotype E was predominant ($n = 12$), followed by G ($n = 7$), K ($n = 4$), D ($n = 3$), F ($n = 3$) and H2 ($n = 1$). Serotype E was highly conserved: no subtypes were found. Serotype G comprised four gene variants (G1, G2, G3 and Gx): G1 genotype was identical to the reference strain G/UW57/Cx, G3 was different in positions 228, 487, 700 and 1003 (Lysen *et al.*, 2004), and G2 and Gx had a single nucleotide substitution in position 1003: T → G and T → A, respectively. The last variant was not found in GenBank. Two genetic variants of serotype K in the population studied differed by a single nucleotide: one (K) was identical to the reference strain K/UW-31, whereas Kx differed in one position (1063, C → A). Among the two variants of serotype D, D1 genotype was identical to D/B120 reference strain, and Da2 was identical to D/IC-Cal-8 (Lysen *et al.*, 2004). Genotype F showed no variation and was identical to reference strain F/IC-CAL3.

Phylogenetic analysis of the *ompA* sequences was performed on 11 genetic variants from six serotypes obtained in our study (Fig. 1). Three main clusters were identified, according to previous grouping in the B (E, D serotypes), C (K, H serotypes) and intermediate (F, G serotypes) complexes (Stothard *et al.*, 1998).

MLST typing

MLST of *C. trachomatis* using gene sequences of seven housekeeping genes (MLST[7])

Nucleotide gene segment sequences of seven housekeeping genes – *gatA*, *oppA-3*, *hflX*, *gidA*, *enoA*, *hemN* and *fumC* – were determined for 30 *C. trachomatis* isolates following Pannekoek *et al.* (2008). For each isolate, the alleles at each of the seven loci were compared with each other and with alleles from the PubMLST database for *Chlamydiales* (http://pubmlst.org/chlamydiales/). A combination of alleles defines the allelic profile or ST. Eight STs were established for the 30 *C. trachomatis* isolates, with ST-4 predominating (Table 1).

Unique alleles of *hflX* (A → G substitution in position 136 in comparison with reference MLST sequence) and *hemN* (G → A substitution in position 247 in comparison with reference MLST sequence) genes which were not present in databases were revealed. Corresponding STs were designated ST-39 and ST-42.

A novel ST (ST-38) was also discovered in four isolates. This subtype differs from ST-4 by a single allele (*hflX*).

Analysis by eBURST revealed two nonoverlapping groups (clonal complexes) consisting of related strains sharing identical alleles at six of the seven loci with at least one other member of the group (Fig. 2).

Concatenated sequences of seven housekeeping gene fragments were aligned and analyzed using MEGA 4.0 software. A phylogenetic tree was constructed using the neighbor-joining algorithm (Fig. 3). A bootstrap test was conducted for 1000 repetitions.

MLST using gene sequences of five target regions of the chlamydial genome (MLST[5])

Nucleotide sequences of the fragments of five genome regions – CT046 (*hctB*), CT058, CT144, CT172 and CT682 (*pbpB*) – were determined for 30 *C. trachomatis* isolates following Klint *et al.* (2007). As for MLST[7], the numbered alleles at each locus were combined to establish the ST for each isolate. Each ST was numbered in order of identification (K1, K2, etc.). Twenty STs were assigned for the 30 *C. trachomatis* strains.

Analysis by eBURST divided our isolates into six groups – I (K4, K7, K18), II (K8, K15, K20), III (K12, K19), IV (K3, K16), V (K2, K14) and VI (K5, K13), and six singletons (K1, K6, K9, K10, K11 and K17) differing at two or more loci from all other isolates.

A phylogenetic tree based on the concatenated sequences of five loci is presented in Fig. 4.

VNTR typing

VNTR loci – CT1335, CT1299 and CT1291 – were sequenced as described in Materials and methods, and the number of single

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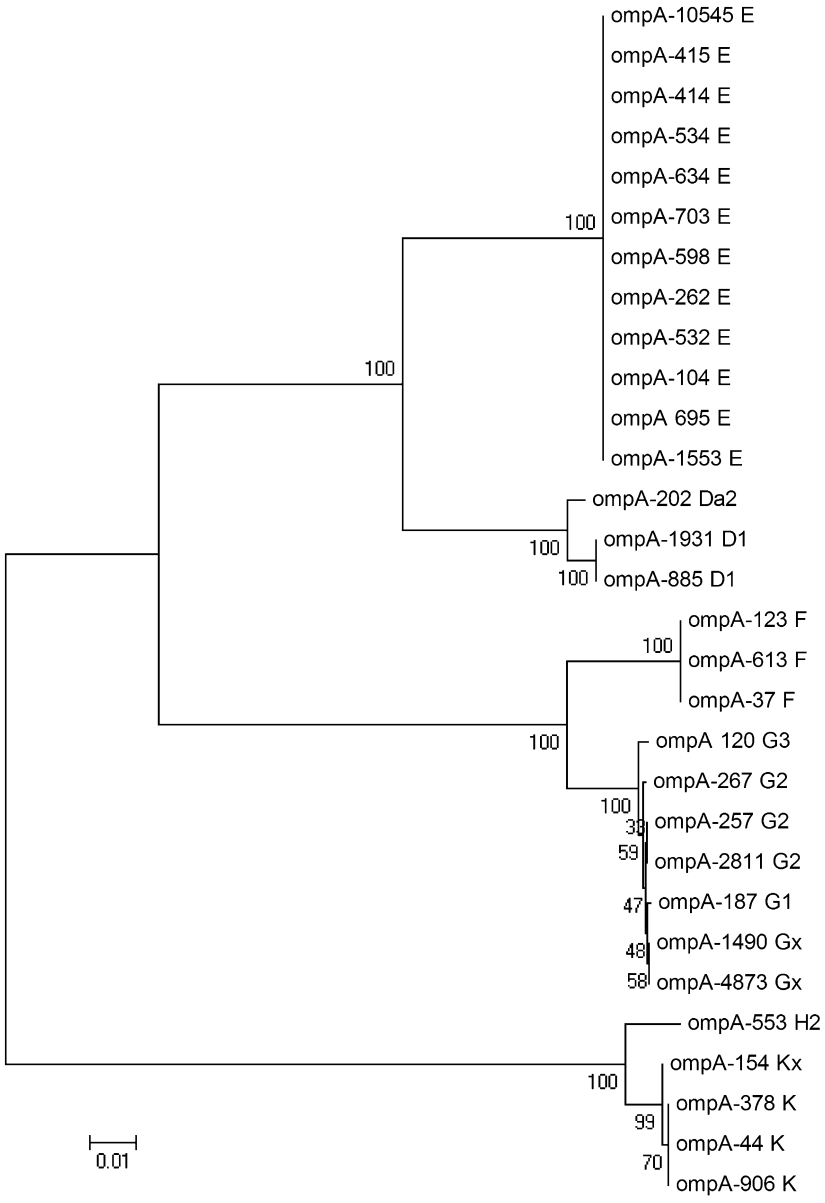

Fig. 1. The distance neighbor-joining tree based on the *Chlamydia trachomatis ompA* nucleotide sequence from 30 strains. Evolutionary distances were computed using the Kimura 2-parameter method. The bootstrap test was for 1000 replicates. Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed.

Table 1. Results of MLST[7] typing based on the analysis of seven housekeeping genes and comparison with *ompA*-typing results

		Allele							
Sequence type	No. of strains	*gatA*	*oppA-3*	*hflX*	*gidA*	*enoA*	*hemN*	*fumC*	*ompA* (no. of strains)
ST-4	11	3	1	1	2	4	2	3	E (11)
ST-38	4	3	1	2	2	4	2	3	Da2 (1), F (3)
ST-12	1	3	4	1	2	4	2	3	E
ST-6	6	3	3	2	5	3	1	3	G2 (1), Gx (2), K (2), Kx (1)
ST-13	4	3	3	2	5	3	2	3	G2 (1), G1 (1),D1 (2)
ST-39	1	3	3	2	5	3	Novel allele (*hemN*-15)	3	G3
ST-9	2	3	3	2	4	3	2	3	G2 (1), H2 (1)
ST-42	1	3	3	Novel allele (*hflX*-19)	4	3	2	3	K

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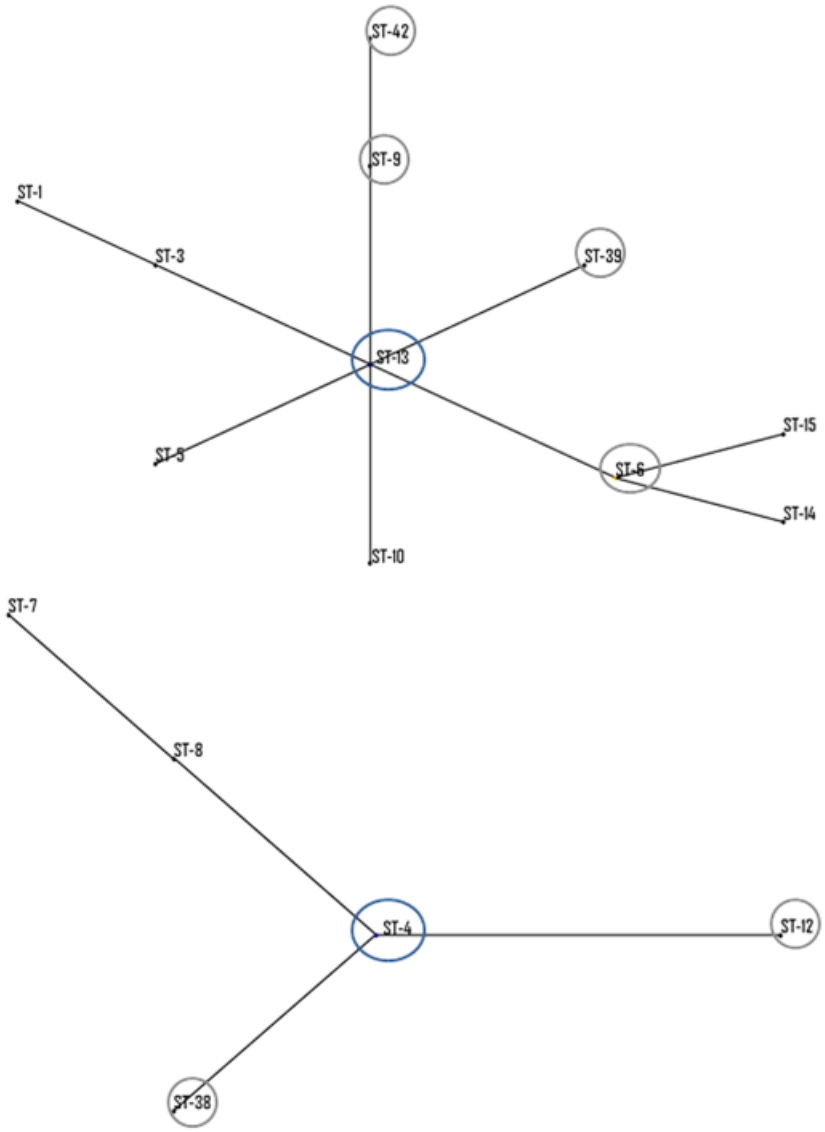

Fig. 2. Clonal grouping of *Chlamydia trachomatis* isolates based on the EBURST analysis. STs from PubMLST/*Chlamydiales* database which belongs to the clonal complexes referred to, are included (STs found in this study are marked with circles).

nucleotide repeats was counted for each locus. Pfu polymerase was used to get a readable sequence of CT1299 fragment. VNTR genotype was determined as a three-digit name composed of the number of the repeat type found within each of the three VNTR loci. As a result, 18 VNTR types were assigned.

Analysis by EBURST revealed one group with P13 as the primary putative founder, defined as the ST that differs from the largest number of other STs at only a single locus, and P7, P4 and P5 as founders of subgroups (Fig. 5).

Discriminatory power

The discriminatory power of each method based on the calculations of the Hunter–Gaston discriminatory index was 0.82 for *ompA* typing scheme, 0.81 for MLST[7], 0.95 for MLST[5] and 0.96 for VNTR. A combination of *ompA* typing scheme and VNTR improved the last value slightly (up to 0.97).

D values were also calculated for all MLST[7] and MLST[5] loci (Table 2). The number of alleles per locus varied between one (*gatA* and *fumC*) and three in MLST[7], and between five and 11 (*pbpB*) in MLST[5].

Sequence variation and recombination

Sequence variations of each chlamydial locus under investigation are summarized in Table 2. Variation among the sequences of all MLST[7] housekeeping loci evaluated for 30 strains was very limited. Only single alleles of *gatA* and *fumC* were revealed in our strains. Variation among the sequences

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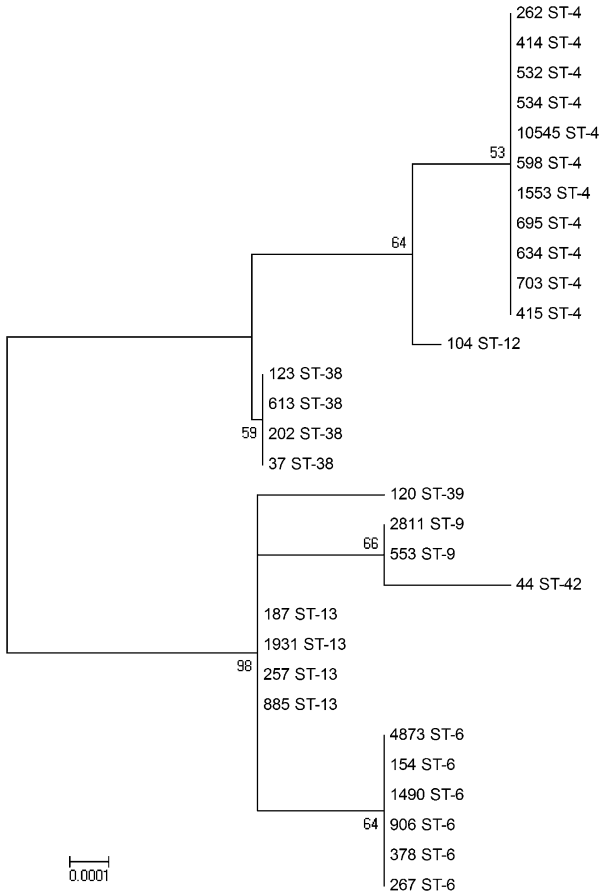

Fig. 3. Phylogenetic analysis of concatenated sequences of seven housekeeping gene fragments. Phylogenetic tree was constructed using the neighbor-joining algorithm. Evolutionary distances were computed using the p-distance method. The bootstrap test was for 1000 replicates. Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed.

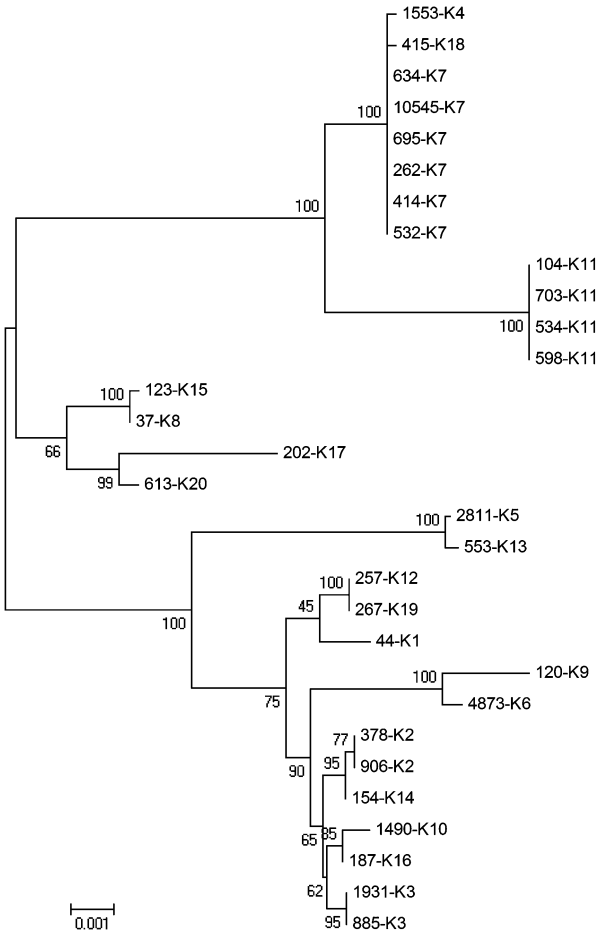

Fig. 4. Phylogenetic analysis of concatenated sequences of five gene fragments. The phylogenetic tree was constructed using the neighbor-joining algorithm. Evolutionary distances were computed using the Kimura 2-parameter method. The bootstrap test was for 1000 replicates. Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed.

of MLST[5] loci was quite high for *hctB*, CT144 and *pbpB*. The d_S values of CT058 and CT172 were in the same range as the corresponding values of the housekeeping genes.

Each of the analyzed gene regions was tested for evidence of recombination using the algorithms included in the RDP software. Recombination events were detected in *hctB*, CT144 and *ompA*. No trace of recombination was detected in other genes.

Discussion

In the past few years, new high-resolution genotyping methods based on nucleic acid amplification techniques and sequencing of various targets in the microbial genomes have been developed, due to simplification and automation, as well as easy access to sequencing. In our work, three newly developed schemes of molecular typing have been adopted and applied for the evaluation of the population heterogeneity of urogenital *C. trachomatis* isolates circulating in the central part of the Russian Federation. Of these schemes, MLST[7] is based on housekeeping genes only; the variation that arises in these genes has been shown to be subject to purifying selection, so this approach is expected to give the best information on the overall population structure. MLST[5] uses genetic variation, which is likely to be subject to selection imposed by environment (human immune system) and is assumed to possess a high discriminatory capacity for the analysis of a contact-tracing chain of *Chlamydia*-infected persons and identification of different chlamydial strains circulated in the community. However, its ability to capture the true relationships between isolates may be poor, like the VNTR scheme using relatively unstable gene markers appearing as a result of DNA polymerase errors during replication. In this study, *ompA* typing served as a starting point.

ompA typing divided all isolates into 11 groups, and MLST[7], MLST[5] and VNTR analysis into eight, 20 and 18

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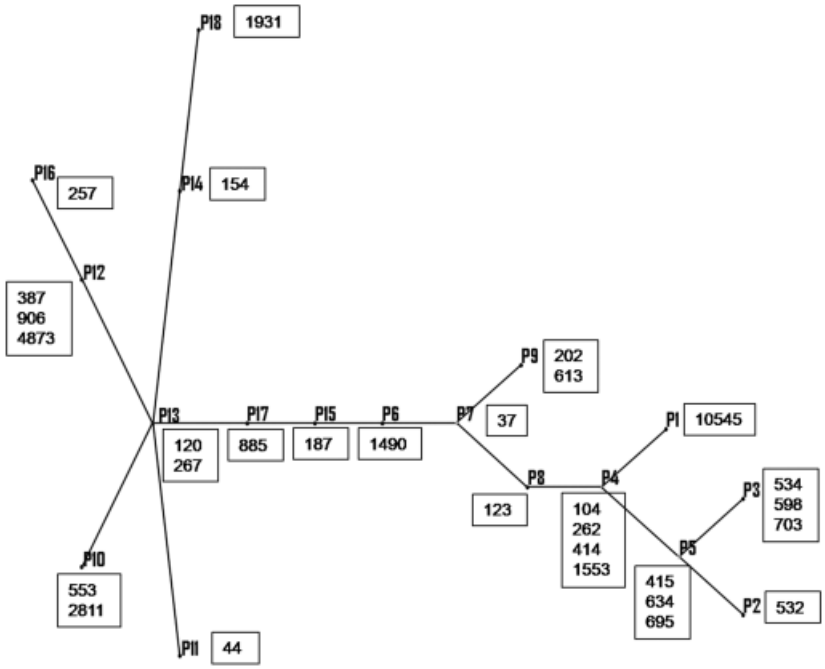

Fig. 5. Results of VNTR typing. Clonal grouping of *Chlamydia trachomatis* isolates based on the EBURST analysis.

Table 2. Nucleotide sequence variation of different loci of *Chlamydia trachomatis* strains and discriminatory index at each locus

Locus*	Size (bp)	S_d	d_S	N_d	d_N	No. of mutations in noncoding sequence regions	d_N/d_S	*D*
hctB	512	4	0.024	4	0.009	5	0.34	0.76
CT058	1263	2	0.003	11	0.007	8	2.50	0.80
CT144	598	17	0.076	18	0.025	–	0.33	0.70
CT172	257	1	0.008	0	0.000	5	0.00	0.80
pbpB	2118	28	0.025	18	0.003	–	0.13	0.84
oppA-3	471	0	0.000	2	0.004	–	–	0.55
hflX	435	1	0.006	2	0.004	–	0.66	0.54
gidA	474	0	0.000	2	0.004	–	–	0.59
enoA	381	1	0.011	0	0.000	–	0.00	0.52
hemN	432	0	0.000	2	0.004	–	–	0.38

*Only single alleles of *gatA* and *fumC* were revealed.

S_d, N_d, number of synonymous and nonsynonymous substitutions, respectively; d_S, d_N, average number of nucleotide differences per site at synonymous and nonsynonymous sites, respectively (Jukes and Cantor corrected); D, discriminatory index at each locus.

groups, respectively (Table 3). The discriminatory index *D* is quite high for both MLST[5] and *ompA*-VNTR schemes (0.95 and 0.97, respectively), satisfying the value of ≥ 0.95 recommended for new typing methods by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM) (van Belkum *et al.*, 2007), in contrast to the *ompA* and MLST[7] typing schemes (0.82 and 0.81, respectively).

According to *D* value, the *ompA*-VNTR method shows the best discriminatory power for the identification of closely related *C. trachomatis* strains. This is not surprising because of the usefulness of mononucleotide tandem repeats chosen for maximal mutability, providing much higher sequence variation than housekeeping genes for evolution studies, as bacterial strain typing has been announced earlier (Diamant *et al.*, 2004; Stratilo *et al.*, 2006). On the other hand, it is difficult to interpret the results of VNTR when there are more than 11 single nucleotide tandem repeats, due to the high probability that DNA polymerases, routinely used in PCR, will generate errors (Clarke *et al.*, 2001). This is why we regard VNTR typing, rather than the routine diagnostics method, as the 'final word' in distinguishing isolates from each other when other typing methods have failed to do so.

In MLST[7], seven housekeeping genes were chosen, which are separated widely along the chromosome and not

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Table 3. Comparison of the results of different typing schemes

ompA (no. of strains)	MLST[7]	MLST[5]	VNTR
E (12)	ST-12 (1)	K7 (6)	P4 (4)
	ST-4 (11)	K-11 (4)	P5 (3)
		K4 (1)	P3 (3)
		K18 (1)	P1 (1)
			P2 (1)
G3 (1)	ST-39 (1)	K9 (1)	P13 (1)
G2 (3)	ST-9 (1)	K5 (1)	P10 (1)
	ST-13 (1)	K12 (1)	P16 (1)
	ST-6 (1)	K19 (1)	P13 (1)
G1 (1)	ST-13 (1)	K16 (1)	P15 (1)
Gx (2)	ST-6 (2)	K6 (1)	P12 (1)
		K10 (1)	P6 (1)
K (3)	ST-6 (2)	K2 (2)	P12 (2)
	ST-42 (1)	K1 (1)	P11 (1)
Kx (1)	ST-6 (1)	K14 (1)	P14 (1)
D1 (2)	ST-13 (2)	K3 (2)	P17 (1)
			P18 (1)
Da2 (1)	ST-38 (1)	K17 (1)	P9 (1)
F (3)	ST-38 (3)	K8 (1)	P7 (1)
		K15 (1)	P8 (1)
		K20 (1)	P9 (1)
H2 (1)	ST-9 (1)	K13 (1)	P10 (1)

adjacent to putative outer membrane, secreted or hypothetical proteins, that might be under diversifying selection. Eight STs were found among 30 *C. trachomatis* strains. Analysis of a phylogeny tree constructed on the concatenated sequences of seven loci allowed division into two large groups: the first group combines ST-4, ST-12 and ST-38, and the second, all others (Fig. 3). eBURST reveals the same evolutionary relationships among genotypes, with ST-4 and ST-13 as the putative founders of the first and second groups, respectively, and ST-6 as the putative founder of a subgroup (Fig. 2). Newly found STs, ST-42 and ST-39 are associated with ST-13 clonal complex, while ST-38 is associated with the ST-4 complex.

As is known, novel genotyping schemes of *C. trachomatis* are limited by the low sequence variation in the chlamydial genome. In MLST[5], five target regions have been selected based on their relatively high variability. A comparison of phylogeny trees constructed on the concatenated sequences of MLST[5] and MLST[7] loci shows their fundamental similarity, with more variants inside the groups in MLST[5] (Figs 3 and 4). The division into two large groups using MLST[5] and MLST[7] fundamentally agrees with the results of VNTR typing (Fig. 5), but is not in a good agreement with *ompA* typing data. *ompA*, the gene encoding the major outer membrane protein, has been most widely used for molecular epidemiology; however, an association between serotypes found by *ompA* and clinical manifestations is still controversial according to many studies. Earlier reports detected the mosaic *ompA* gene structures, indicating that *ompA* or parts of *ompA* do exchange between *C. trachomatis* strains (Hayes *et al.*, 1994; Millman *et al.*, 2001; Brunelle & Sensabaugh, 2006). Recombination in this part of the chlamydial genome could explain why strain typing using the *ompA* gene alone does not correlate with clinical phenotypes (Brunelle & Sensabaugh, 2006; Gomes *et al.*, 2007). Our results confirm the presence of recombination events in *ompA* gene by the RDP test. Moreover, looking at the results of all typing schemes, we can see that strains 553 and 2811 are indistinguishable using MLST[7], MLST[5] or VNTR schemes, and yet have highly divergent *ompA* sequences (serotypes H2 and G2, respectively). Also, strains 37, 123, 202 and 613 are very similar by MLST schemes, but strain 202 has a very different *ompA* gene from the other three (Da2 and F serotypes, correspondingly). The presence of strains that are found to be very similar using MLST/VNTR and yet have very different *ompA* sequences argues strongly for horizontal transfer (recombination) events that have introduced different *ompA* sequences into strains of the same overall genotype.

Sequence variation within the loci generally was expected to be higher for at least three of five MLST[5] gene fragments (*hctB*, CT144 and *pbpB*) than for the MLST[7] ones. Whereas all loci of the MLST[7] scheme have a similar level of variation (excluding *gatA* and *fumC*, in which no allele polymorphisms were revealed), the d_S values within MLST[5] loci varied up to 25-fold. Discriminatory capacity was also higher for all MLST[5] loci. The best discriminatory potential was demonstrated by *pbpB*, the putative outer membrane protein potentially involved in the interaction with the host cell. These results confirm the data obtained by Klint *et al.* (2007).

Thus, we conclude that the MLST[5] scheme, possessing considerable discriminatory potential, is the most appropriate typing of closely related clinical strains, whereas MLST[7] is the best choice for global epidemiological purposes. Our results contribute to the creation of an informative and universal system of the *C. trachomatis* typing, which will allow rapid and reliable strain identification in the future and therefore efficient control of the circulation of strains and their changeability.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. PCR primers used for the CT682 (*pbpB*) fragment amplification.

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