Antibiotic selection pressure, genetic polymorphism as well as diversity of the immune status of the host and other selection factors continuously prompt *Mycobacterium tuberculosis*, the tuberculosis causative agent, to evolve. Significant or insignificant mutations shape new (sub)lineages of the pathogen whose evolution can be understood only through analyzing and monitoring its genotypic diversity and properties of its lineages. In our study we used a set of 46 *M. tuberculosis* clinical isolates from Moscow region. The samples were typed using the standard 24-loci MIRU-VNTR technique. Beijing family isolates were shown to prevail in the collection (60.9 %), as well as Beijing-B0/W148 subtype (60.7 % of total Beijing type samples); most of them (88.2 %) were multidrug-resistant resistant. The applied technique allowed us to detect one case of a mixed-strain infection.

**Keywords:** *Mycobacterium tuberculosis*, genotyping, phylogenetics, epidemiology, Beijing, MIRU-VNTR

**Funding:** this work was supported by the Russian Foundation for Basic Research (Grant No. 13-04-91444 *Toxin-antitoxins and RpsA in TB drug resistance and persistence*).

**Acknowledgements:** the authors thank the research team of the Department of Microbiology of the Central TB Research Institute (Moscow) for their assistance in preparing a collection of *M. tuberculosis* isolates, with special thanks going to Larisa Chernousova, D. Sc. (Biol).

**Correspondence should be addressed:** Valery Danilenko
ul. Gubkina, d. 3, Moscow, Russia, 119333; valerid@vigg.ru

**Received:** 01.02.2017 **Accepted:** 24.02.2017

Drug resistance of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a major issue in the treatment of this infection. In Russia its annual incidence is estimated as 80 cases per 100,000 population (or a total of 115,000 cases per year). In 20 % of new cases and 50 % of relapses reported in Russia, patients are infected with multidrug-resistant (MDR) strains [1]. Therefore, improvement of treatment strategies largely relies on the identification and study of the most prevalent *M. tuberculosis* strains circulating in the country.

* M. tuberculosis* population can be divided into a number of major lineages; each lineage is geographically associated [2] and carries certain phylogenetic markers that shape the phenotype of the strain [3]. Members of the Beijing family are the most prevalent lineage in Russia; they are highly transmissible.
and virulent, have a higher mutation rate and other properties contributing to their dissemination [4].

Recent research conducted in Russia [5] identified a Beijing-B0/W148 variant of the Beijing lineage. These strains exhibited increased virulence in comparison with the progenitor Beijing family and are multidrug-resistant (there are almost no drug-sensitive strains within this sublineage). Mokrousov et al. called Beijing-B0/W148 “a successful clone” of M. tuberculosis [5].

The lineage of the M. tuberculosis strain/isolate can be determined using a variety of genotyping methods, such as the IS6110-based restriction fragment length polymorphism (RFLP) analysis, spoligotyping [6], differentiation based on the use of single nucleotide polymorphisms (SNPs) of housekeeping genes [7] and type II toxin–antitoxin systems [8]. These methods are different in terms of labor intensity, cost and their discriminatory power. One of the fastest and cheapest methods that nevertheless has a good discriminatory ability is molecular genotyping based on the variable number tandem repeat analysis targeting mycobacterial interspersed repetitive units (MIRU-VNTR) [9].

Previously we analyzed a collection of 64 M. tuberculosis isolates from patients of the Central Research Institute for Tuberculosis, Moscow. Spoligotyping revealed that 70.3% of the isolates belonged to the Beijing lineage [10]. To estimate the proportion of “successful clones” (Beijing-B0/W148) among Beijing strains and to identify the phylogenetic structure across the collection, we genotyped 46 DNA samples using 24-loci MIRU-VNTR. Results are presented below.

METHODS

Collection of DNA samples of M. tuberculosis clinical isolates

We used a collection of DNA samples of M. tuberculosis clinical isolates previously described by Maslov et al. [10]. We have previously spoligotyped the isolates and prepared their drug-resistance profiles using 8 first- and second-line antituberculosis drugs. Then the isolates were distributed into two groups: 1) isolates resistant to any of the antituberculosis drug used in the study (n = 41); 2) controls — drug-sensitive isolates (n = 23). In total, 46 isolates were analyzed (23 from each group).

Genotyping of M. tuberculosis clinical isolates

Genotyping was performed based on 24 MIRU-VNTR loci according to the standard protocol [11]. PCR primers were synthesized by Syntol, Russia. Amplification was performed in 0.2 ml 96-well plates (Bio-Rad, USA) using the Amplification Kit (Dialat, Russia) according to the protocol described in [9] in the T100 Thermal Cycler (Bio-Rad). The obtained fragments were separated by 2% agarose gel electrophoresis in the 1x Tris-

---

**NJ phylogenetic tree of M. tuberculosis isolates from the Moscow region. The tree was constructed using the 24-loci MIRU-VNTR profile of each phylogenetic group. Beijing-B0/W148 is shown in red.**
RESULTS

According to the MIRU-VNTR profiles prepared using the MIRU-VNTRplus web tool, 60.9 % of isolates belonged to the Beijing lineage, 13.0 % — to LAM, 13.0 % — to T1 and T2, 4.3 % — to Ural, 2.2 % — to Cameroon, S and NEW-1 (one isolate per each lineage). One isolate's lineage could not be identified. Isolate 13-2078 was found to have two allelic variants of the QUB26 locus (1 and 7), which may indicate a mixed-strain infection [13].

Based on the MIRU-VNTR profiles, we constructed a dendrogram (see Figure). It clearly shows a cluster of 17 B0/W148 isolates (isolate 13-2078 is a combination of two strains, but both of them belong to the B0/W148 sublineage) accounting for 60.7 % of all Beijing strains. It should be noted that all of those strains were drug-resistant (group A); 15 of them (88.2 %) were multidrug-resistant, of which 3 (20.0 %) exhibited extensive drug resistance (XDR).

DISCUSSION

In our previous work [10] we genotyped isolates of Mycobacterium tuberculosis by spoligotyping. Based on the obtained results, the isolates were distributed into 6 groups: 60.9 % belonged to Beijing family; 21.7 % — to T1 and T2, 6.5 % — to LAM, 6.5 % — to H4 (proportions are specified for 46 isolates studied in this work). Five isolates had a unique genotype [10]. It might be due to accidental spacer deletions or insertions, which are quite typical for the studied gene region due to its high variability.

Analysis of 24 MIRU-VNTR loci allowed us to update the data obtained previously. Thus, we distributed 4 isolates that had been earlier assigned to the T-cluster into 3 lineages: 2 belonged to LAM, one to S and one to Cameroon. Of 3 isolates previously identified as belonging to the H4 lineage, 2 were now assigned to the Ural lineage and 1 — to NEW-1.

We also managed to identify representatives of the Beijing B0/W148 lineage among the isolates of the Beijing family. Therefore, we conclude that MIRU-VNTR typing provides a higher resolution and is capable of identifying mixed-strain infections meaning that it should be preferred over spoligotyping. Still, the best results can be obtained only when combining various genotyping techniques.

Typically, all Beijing-B0/W148 isolates were drug-resistant (88.2 % were MDR), which agrees with the data obtained earlier [5, 14]. This proves the “success” of the Beijing-B0/ W148 sublineage. However, the question remains about the factors that promote selection of this particular phylogenetic group. Perhaps, increased mutational variability resulted in the functional rearrangements that allowed the strains to enhance their virulence and improve survival [4]. Further research is necessary to elucidate this question.

CONCLUSIONS

Assessment and epidemiologic control of the dissemination of successful M. tuberculosis lineages are crucial for the effective diagnosis and treatment of patients with tuberculosis. The results obtained in this study indicate a tendency for increasing dissemination of the Beijing-B0/W148 strains that have a typical MRD phenotype, provide an update of the current epidemiologic data for the central part of Russia and emphasize the importance of combining various genotyping methods for a comprehensive profile of M. tuberculosis clinical isolates.

References

12. Allix-Béguec C, Harmsen D, Weniger T, Supply P, Niemann S. Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data...
Литература