MIRU-VNTR GENOTYPING OF *MYCOBACTERIUM TUBERCULOSIS* CLINICAL ISOLATES FROM MOSCOW REGION

Shur KV, Maslov DA, Bekker OB, Danilenko VN ✉

Laboratory of Bacterial Genetics, Department of Genetics and Biotechnology,
Vavilov Institute of General Genetics of RAS, Moscow, Russia

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. Sci. (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

ГЕНОТИПИРОВАНИЕ КЛИНИЧЕСКИХ ИЗОЛЯТОВ *MYCOBACTERIUM TUBERCULOSIS*, ВЫДЕЛЕННЫХ В МОСКОВСКОМ РЕГИОНЕ, МЕТОДОМ MIRU-VNTR

К. В. Шур, Д. А. Маслов, О. Б. Беккер, В. Н. Даниленко ✉

Лаборатория генетики микроорганизмов, Отдел генетических основ биотехнологии,
Институт общей генетики имени Н. И. Вавилова РАН, Москва

Под воздействием различных селективных факторов (применение антибиотиков, генетический полиморфизм и разнообразие иммунных статусов хозяина и др.) возбудитель туберкулеза *Mycobacterium tuberculosis* постоянно эволюционирует. Возникают новые линии и сублинии, характеризующиеся набором значимых и незначимых мутаций. Анализ и мониторинг представленности различных линий и их особенностей является важным для понимания эволюции патогена. В данной работе была использована коллекция из 46 клинических изолятов *M. tuberculosis*, выделенных в Московском регионе. Была определена их генотипическая принадлежность к различным линиям и сублиниям типированием по 24 локусам MIRU-VNTR. Было показано преобладание изолятов линии Beijing в коллекции (60,9 %) и изолятов сублинии Beijing-B0/W148 (60,7 % внутри линии Beijing), характеризующихся множественной лекарственной устойчивостью (88,2 % изолятов в данной выборке). Также использованный метод позволил определить один предполагаемый случай смешанной инфекции.

Ключевые слова: *Mycobacterium tuberculosis*, генотипирование, филогенетика, эпидемиология, Beijing, MIRU-VNTR

Финансирование: работа выполнена в рамках двустороннего проекта Российского фонда фундаментальных исследований (№ 13-04-91444 «Токсины–антитоксины и RpsA в лекарственной устойчивости и персистенции микобактерий туберкулеза»).

Благодарности: авторы благодарят коллектив отдела микробиологии Центрального научно-исследовательского института туберкулеза (Москва) и, в частности, д. б. н. Ларису Черноусову за помощь в создании коллекции клинических изолятов *M. tuberculosis*.

✉ **Для корреспонденции:** Даниленко Валерий Николаевич
ул. Губкина, д. 3, г. Москва, 119333; valerid@vigg.ru

Статья получена: 01.02.2017 **Статья принята к печати:** 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 exhibit 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.

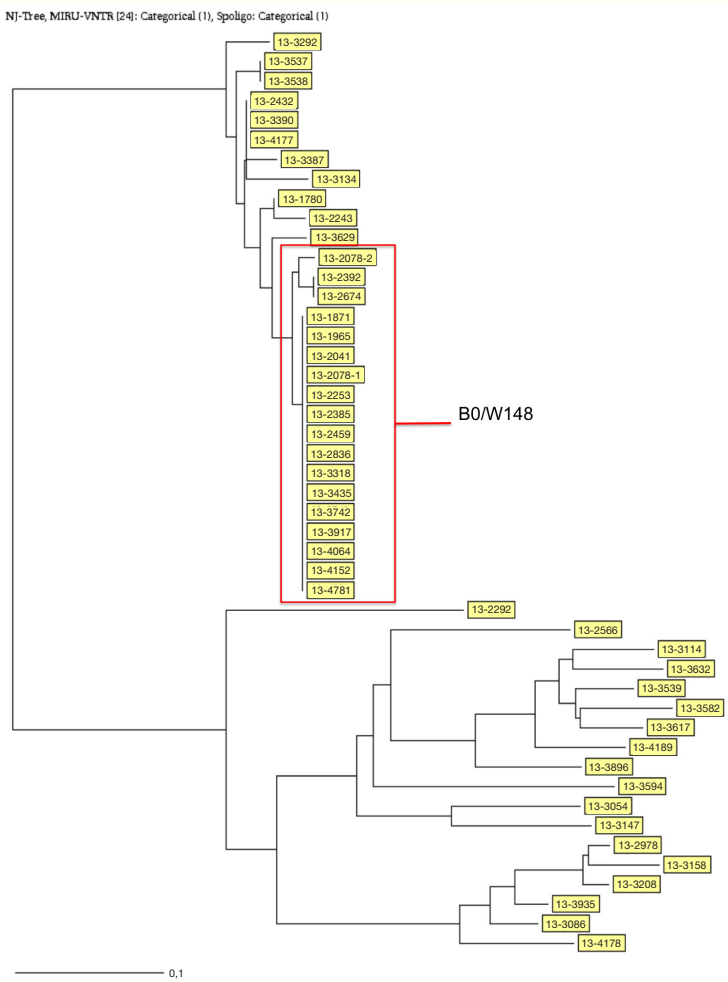
METHODS

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).

Genoptyping 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-



№		SpolDB4 type	MIRU type	24-loci MIRU-VNTR profile
13-3292	1	BEIJING	3856-32	2 4 2 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 6 3 8 2 3
13-3537	1	BEIJING	3844-32	2 4 5 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-3538	1	BEIJING	3844-32	2 4 5 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-2432	1	BEIJING	94-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-3390	1	BEIJING	94-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-4177	1	BEIJING	94-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-3387	1	BEIJING	3843-32	2 4 4 2 3 4 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-3134	1	BEIJING	96-145	2 4 4 2 3 3 3 6 1 6 4 4 4 2 5 1 5 3 3 5 3 8 2 3
13-1780	1	BEIJING	1066-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 5 3 3 5 3 6 2 3
13-2243	1	BEIJING	1066-133	2 4 4 2 3 3 3 5 2 6 4 4 4 1 5 1 5 3 3 5 3 6 2 3
13-3629	1	BEIJING	99-332	2 4 4 2 3 3 3 5 2 6 4 4 2 4 2 5 1 5 3 3 5 3 7 2 3
13-2078	1	BEIJING	10977-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 1 2 3
13-2392	1	BEIJING	1066-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 6 2 3
13-2674	1	BEIJING	1066-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 6 2 3
13-1871	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-1965	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2041	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2078	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2253	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2385	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2459	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2836	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-3318	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-3435	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-3742	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-3917	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-4064	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-4152	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-4781	1	BEIJING	100-32	2 4 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 7 2 3
13-2292			?-15	2 1 3 2 2 3 3 2 2 6 3 4 2 2 5 1 5 3 3 3 3 3 2 2
13-2566			?-66	2 3 4 2 3 2 3 5 2 2 3 4 2 1 5 1 5 3 2 3 3 8 2 2
13-3114	63	T1	?-26	2 3 4 2 4 3 1 2 1 3 1 4 2 2 5 1 5 3 3 3 3 5 2 2
13-3632	118	T2	?-15	2 3 2 1 4 3 1 2 2 3 3 4 2 2 5 1 5 3 3 3 3 3 2 2
13-3539	118	T2	?-16	2 3 4 2 4 3 1 2 2 2 2 4 2 2 5 1 5 3 3 3 6 3 2 2
13-3582	118	T2	?-164	2 3 4 2 4 3 1 2 2 3 2 4 2 2 5 1 5 3 3 3 5 6 0 1
13-3617	118	T2	?-16	2 3 4 2 3 3 1 2 2 2 2 4 2 2 5 1 5 3 4 3 5 5 2 2
13-4189			?-15	2 3 4 2 4 3 1 2 2 3 3 4 2 2 5 1 4 3 3 3 8 5 2 2
13-3896	1252	T1	?-26	2 4 4 2 4 3 3 2 1 5 3 4 2 2 5 1 5 3 3 3 3 5 2 2
13-3594			?-15	2 3 3 2 3 4 3 4 2 6 2 4 4 2 5 1 4 3 3 3 3 8 3 2
13-3055	127	H4	?-15	2 3 4 2 2 11 2 3 2 2 4 4 4 2 5 1 1 3 3 2 3 8 3 2
13-3147	262	H4	?-16	2 4 5 2 5 8 1 3 2 2 4 4 4 2 5 1 1 3 3 2 5 7 3 2
13-2978	42	LAM9	842-52	1 4 2 2 5 4 3 3 2 2 2 4 1 2 5 1 5 3 3 2 2 6 2 2
13-3158	42	LAM9	13916-51	1 4 2 2 4 4 2 3 2 2 2 4 1 2 6 1 5 3 3 2 2 6 2 2
13-3208	42	LAM9	13572-52	1 4 2 2 4 4 3 3 2 2 2 4 1 2 5 1 5 3 3 2 2 4 2 2
13-3935	766	T1	?-52	1 4 2 2 4 4 3 3 2 2 2 4 1 2 5 1 5 3 3 2 2 5 2 2
13-3086			?-52	1 4 2 2 4 4 3 3 2 2 2 4 1 2 5 1 6 3 3 2 2 6 2 2
13-4178	264	T1	11418-51	1 3 2 2 5 6 3 3 2 2 2 4 1 2 6 1 5 3 3 2 2 5 2 2

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

acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, pH 7.6). Results were analyzed using the MIRU-VNTR*plus* web tool [9, 12].

RESULTS

According to the MIRU-VNTR profiles prepared using the MIRU-VNTR*plus* 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 *M. 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 LAM9, 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

1. World Health Organization. Global tuberculosis control: WHO report 2015. Vol. 1. Geneva, Switzerland; 2015.
2. Homolka S, Niemann S, Russell DG, Rohde KH. Functional genetic diversity among Mycobacterium tuberculosis complex clinical isolates: delineation of conserved core and lineage-specific transcriptomes during intracellular survival. PLoS Pathog. 2010 Jul; 6 (7): e1000988. DOI: 10.1371/journal.ppat.1000988.
3. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in guinea-pigs of South Indian and British tubercle bacilli. Tubercle. 1960 Feb; 41: 1–22.
4. Villellas C, Aristimuño L, Vitoria M-A, Prat C, Blanco S, García de Viedma D, et al. Analysis of mutations in streptomycin-resistant strains reveals a simple and reliable genetic marker for identification of the Mycobacterium tuberculosis Beijing genotype. J Clin Microbiol. 2013 Jul; 51 (7): 2124–30. DOI: 10.1128/JCM.01944-12.
5. Mokrousov I, Narvskaya O, Vyazovaya A, Otten T, Jiao WW, Gomes LL, et al. Russian "successful" clone B0/W148 of Mycobacterium tuberculosis Beijing genotype: A multiplex PCR assay for rapid detection and global screening. J Clin Microbiol. 2012; 50 (11): 3757–9. DOI: 10.1128/JCM.02001-12.
6. van Soolingen D, Qian L, de Haas PE, Douglas JT, Traore H, Portaels F, et al. Predominance of a single genotype of Mycobacterium tuberculosis in countries of east Asia. J Clin Microbiol. 1995 Dec; 33 (12): 3234–8.
7. Homolka S, Projahn M, Feuerriegel S, Ubben T, Diel R, Nubel U, et al. High resolution discrimination of clinical Mycobacterium tuberculosis complex strains based on single nucleotide polymorphisms. PLoS One. 2012; 7 (7): e39855. DOI: 10.1371/journal.pone.0039855.
8. Zaychikova MV, Zakharevich NV, Sagaidak MO, Bogolubova NA, Smirnova TG, Andreevskaya SN, et al. Mycobacterium tuberculosis Type II Toxin-Antitoxin Systems: Genetic Polymorphisms and Functional Properties and the Possibility of Their Use for Genotyping. PLoS One. 2015 Dec 14; 10 (12): e0143682. DOI: 10.1371/journal.pone.0143682.
9. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res. 2010 Jul; 38 (Web Server issue): W326–31. DOI: 10.1093/nar/gkq351. Epub 2010 May 10.
10. Maslov DA, Zaichikova MV, Chernousova LN, Shur KV, Bekker OB, Smirnova TG, et al. Resistance to pyrazinamide in Russian Mycobacterium tuberculosis isolates: PncA sequencing versus Bactec MGIT 960. Tuberculosis (Edinb). 2015 Sep; 95 (5): 608–12. DOI: 10.1016/j.tube.2015.05013.
11. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol. 2006 Dec; 44 (12): 4498–510. DOI: 10.1128/JCM.01392-06. Epub 2006 Sep 27.
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

and phylogenetic identification of Mycobacterium tuberculosis complex isolates. J Clin Microbiol. 2008 Aug; 46 (8): 2692–9. DOI: 10.1128/JCM.00540-08. Epub 2008 Jun 11.
13. Cohen T, van Helden PD, Wilson D, Colijn C, McLaughlin MM, Abubakar I, et al. Mixed-strain Mycobacterium tuberculosis infections and the implications for tuberculosis treatment and control. Clin Microbiol Rev. 2012 Oct; 25 (4):708–19. DOI: 10.1128/CMR.00021-12.
14. Mokrousov I. Mycobacterium tuberculosis phylogeography in the context of human migration and pathogen's pathobiology: Insights from Beijing and Ural families. Tuberculosis (Edinb). 2015 Jun; 95 Supple 1: S167–76. DOI: 10.1016/j.tube.2015.02.031. Epub 2015 Feb 24.

Литература

1. World Health Organization. Global tuberculosis control: WHO report 2015. Vol. 1. Geneva, Switzerland; 2015.
2. Homolka S, Niemann S, Russell DG, Rohde KH. Functional genetic diversity among Mycobacterium tuberculosis complex clinical isolates: delineation of conserved core and lineage-specific transcriptomes during intracellular survival. PLoS Pathog. 2010 Jul; 6 (7): e1000988. DOI: 10.1371/journal.ppat.1000988.
3. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in guinea-pigs of South Indian and British tubercle bacilli. Tubercle. 1960 Feb; 41: 1–22.
4. Villellas C, Aristimuño L, Vitoria M-A, Prat C, Blanco S, García de Viedma D, et al. Analysis of mutations in streptomycin-resistant strains reveals a simple and reliable genetic marker for identification of the Mycobacterium tuberculosis Beijing genotype. J Clin Microbiol. 2013 Jul; 51 (7): 2124–30. DOI: 10.1128/JCM.01944-12.
5. Mokrousov I, Narvskaya O, Vyazovaya A, Otten T, Jiao WW, Gomes LL, et al. Russian "successful" clone B0/W148 of Mycobacterium tuberculosis Beijing genotype: A multiplex PCR assay for rapid detection and global screening. J Clin Microbiol. 2012; 50 (11): 3757–9. DOI: 10.1128/JCM.02001-12.
6. van Soolingen D, Qian L, de Haas PE, Douglas JT, Traore H, Portaels F, et al. Predominance of a single genotype of Mycobacterium tuberculosis in countries of east Asia. J Clin Microbiol. 1995 Dec; 33 (12): 3234–8.
7. Homolka S, Projahn M, Feuerriegel S, Ubben T, Diel R, Nubel U, et al. High resolution discrimination of clinical Mycobacterium tuberculosis complex strains based on single nucleotide polymorphisms. PLoS One. 2012; 7 (7): e39855. DOI: 10.1371/journal.pone.0039855.
8. Zaychikova MV, Zakharevich NV, Sagaidak MO, Bogolubova NA, Smirnova TG, Andreevskaya SN, et al. Mycobacterium tuberculosis Type II Toxin-Antitoxin Systems: Genetic Polymorphisms and Functional Properties and the Possibility of Their Use for Genotyping. PLoS One. 2015 Dec 14; 10 (12): e0143682. DOI: 10.1371/journal.pone.0143682.
9. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res. 2010 Jul; 38 (Web Server issue): W326–31. DOI: 10.1093/nar/gkq351. Epub 2010 May 10.
10. Maslov DA, Zaichikova MV, Chernousova LN, Shur KV, Bekker OB, Smirnova TG, et al. Resistance to pyrazinamide in Russian Mycobacterium tuberculosis isolates: PncA sequencing versus Bactec MGIT 960. Tuberculosis (Edinb). 2015 Sep; 95 (5): 608–12. DOI: 10.1016/j.tube.2015.05013.
11. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol. 2006 Dec; 44 (12): 4498–510. DOI: 10.1128/JCM.01392-06. Epub 2006 Sep 27.
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 and phylogenetic identification of Mycobacterium tuberculosis complex isolates. J Clin Microbiol. 2008 Aug; 46 (8): 2692–9. DOI: 10.1128/JCM.00540-08. Epub 2008 Jun 11.
13. Cohen T, van Helden PD, Wilson D, Colijn C, McLaughlin MM, Abubakar I, et al. Mixed-strain Mycobacterium tuberculosis infections and the implications for tuberculosis treatment and control. Clin Microbiol Rev. 2012 Oct; 25 (4):708–19. DOI: 10.1128/CMR.00021-12.
14. Mokrousov I. Mycobacterium tuberculosis phylogeography in the context of human migration and pathogen's pathobiology: Insights from Beijing and Ural families. Tuberculosis (Edinb). 2015 Jun; 95 Supple 1: S167–76. DOI: 10.1016/j.tube.2015.02.031. Epub 2015 Feb 24.