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Molecular characteristics of fluoroquinolone-resistant *Mycobacterium tuberculosis* strains from newly diagnosed tuberculosis patients in the Northwest of Russia

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Abstract

Introduction. Fluoroquinolones remain the key second-line anti-tuberculosis drugs.

The aim of the study was the molecular characterization of fluoroquinolone-resistant *Mycobacterium tuberculosis* strains from newly diagnosed tuberculosis patients in the Northwest of the Russian Federation.

Materials and methods. The retrospective study collection included *M. tuberculosis* isolates isolated in 2015–2019 from previously untreated tuberculosis patients. Susceptibility to antituberculosis drugs (including the fluoroquinolone ofloxacin) was determined using the BACTEC MGIT960 or absolute concentration method. Mutations in the *gyrA* gene as a marker of resistance to fluoroquinolones, were detected by real-time PCR. Beijing genotype and its subtypes were detected by PCR and real-time PCR methods. Non-Beijing strains were spoligotyped.

Results and discussion. Phenotypic resistance to ofloxacin was detected in 6.7% (40/599) of strains and in 17.4% (40/230) of MDR strains. 34 of 40 (85%) ofloxacin-resistant strains belonged to the Beijing genotype. 18 (45%) strains were assigned to the Russian epidemic subtype Beijing B0/W148 and 12 (30%) to Beijing Central Asian/Russian. The remaining 6 ofloxacin-resistant strains belonged to the Euro-American phylogenetic lineage. Mutations in the *gyrA* gene were found in 97.5% (39/40) of strains. The most common were mutations in codon 94 (69.2%, 27/39). The *Asp94Gly* substitution was identified in 57.5% (23/40) of ofloxacin-resistant strains and was dominant among Beijing (19/34) and non-Beijing (4/6) strains. The second most common substitution was *Ala90Val* (25%, 10/40). More than half of the ofloxacin-resistant strains, Beijing B0/W148 (10/18) and Central Asian/Russian (7/12), carried the *Asp94Gly* mutation.

Conclusion. In the Northwest of Russia in 2016-2019, primary resistance of *M. tuberculosis* to fluoroquinolones was 6.7% in the total collection and 17.4% of MDR strains, and was mainly caused by the *gyrA Asp94Gly* and *Ala90Val* mutations. Beijing B0/W148 genotype was characterized by the largest proportion of fluoroquinolone-resistant strains.

Keywords: *Mycobacterium tuberculosis, gyrA, drug resistance, fluoroquinolones, ofloxacin, Beijing genotype, Central-Asian/Russian, B0/W148*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the St. Petersburg Pasteur Institute (protocol No. 61, 4 April, 2020).

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Оригинальное исследование
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Молекулярная характеристика фторхинолон-устойчивых штаммов *Mycobacterium tuberculosis* от впервые выявленных больных туберкулёзом на северо-западе России

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Аннотация

Введение. Фторхинолоны остаются ключевыми противотуберкулёзными препаратами 2-го ряда.

Цель исследования — молекулярная характеристика фторхинолон-устойчивых штаммов *Mycobacterium tuberculosis* от впервые выявленных больных туберкулёзом на северо-западе России.

Материалы и методы. Ретроспективная коллекция исследования включала изоляты *M. tuberculosis*, выделенные в 2015–2019 гг. от ранее не леченных больных туберкулёзом, проживающих в различных областях северо-запада России. Чувствительность к противотуберкулёзным препаратам (в том числе к фторхинолону офлоксацину) определяли с применением BACTEC MGIT960 или метода абсолютных концентраций. Мутации в гене *gyrA* как маркере устойчивости к фторхинолонам выявляли методом ПЦР в реальном времени. Принадлежность к генотипу Beijing и его субтипам устанавливали методами ПЦР и ПЦР в реальном времени. Штаммы других генотипов (не-Beijing) сполиготипировали.

Результаты и обсуждение. Фенотипическая устойчивость к офлоксацину установлена у 6,7% (40/599) штаммов и у 17,4% (40/230) штаммов с множественной лекарственной устойчивостью. К генотипу Beijing принадлежали 34 (85%) из 40 устойчивых к офлоксацину штаммов, 18 (45%) штаммов были отнесены к российскому эпидемическому субтипу Beijing B0/W148-кластер и 12 (30%) — к Beijing Central Asian/Russian. Остальные 6 офлоксацин-устойчивых штаммов принадлежали к евро-американской филогенетической линии. Мутации в *gyrA* обнаружены у 97,5% (39/40) штаммов, наиболее часто — в кодоне 94 (69,2%; 27/39). Замена *Asp94Gly* была выявлена в 57,5% (23/40) офлоксацин-устойчивых штаммов и доминировала среди штаммов как Beijing (19/34), так и не-Beijing (4/6). Второй по частоте была замена *Ala90Val* (25%; 10/40). Более половины офлоксацин-устойчивых штаммов Beijing B0/W148 (10/18) и Central Asian/Russian (7/12) несли мутацию *Asp94Gly*.

Заключение. На северо-западе России в 2016–2019 гг. первичная резистентность *M. tuberculosis* к фторхинолонам составляла 6,7% в общей популяции возбудителя туберкулёза и 17,4% у штаммов с множественной лекарственной устойчивостью и была обусловлена преимущественно мутациями *gyrA Asp94Gly* и *Ala90Val*. Наибольшая доля фторхинолон-резистентных штаммов *M. tuberculosis* была у генотипа Beijing B0/W148.

Ключевые слова: *Mycobacterium tuberculosis, gyrA, лекарственная устойчивость, фторхинолоны, офлоксацин, генотип Beijing, Central-Asian/Russian, B0/W148*

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Introduction

The decline in tuberculosis (TB) incidence in Russia (from 57.7 per 100,000 of the population in 2015 to 31.1 in 2022) is accompanied by the continued spread of drug-resistant, primarily multidrug-resistant strains of *Mycobacterium tuberculosis*. The share of multidrug resistance (MDR) among first-time diagnosed patients (primary MDR) increased from 27.5% in 2016 to 34% in 2022.[1]

The World Health Organization's (WHO) classic long-standing definition of MDR is that strains that are simultaneously resistant to two key first-line antibiotics: isoniazid and rifampicin. Treatment of MDR-TB requires the use of 2nd-line drugs, to which mycobacteria has also become resistant. In addition to MDR-TB, in 2006, WHO introduced the definition of broad (extensive resistance to fluoroquinolones and injectable antibiotics; XDR) and pre-broad (extensive resistance to either fluoroquinolones or injectable antibiotics; pre-XDR) drug resistance in 2006 [1]. The declining role of injectable antibiotics and the wider use of newer drugs has led to a modification of the definition of XDR and pre-XDR TB, which is recommended by WHO for use in clinical and surveillance purposes from January 2021. According to the new classification, TB caused by MDR strains of *M. tuberculosis* resistant to any of the fluoroquinolones was designated as pre-XDR-TB[2]. Pre-XDR strains with extensive resistance to bedaquiline or linezolid are defined as XDR-TB.

Thus, fluoroquinolones (previously ofloxacin, currently levofloxacin and the new generation fluoroquinolone moxifloxacin) have retained their importance in the treatment of MDR-TB. The development of resistance has implications for treatment outcomes: a study in Arkhangelsk in 2005-2008 showed that adverse outcomes were more likely among patients with acquired resistance to capreomycin (100% vs. 25.9%), ofloxacin (83.6% vs. 22.7%) or XDR (100% vs. 24.4%) [2].

The target of fluoroquinolones is the DNA-gyrase enzyme, which is necessary for replication and transcription of *M. tuberculosis* in the cell [3]. Resistance to fluoroquinolones in 90% of cases is associated with mutations in the *gyrA* and *gyrB* genes encoding DNA-gyrase. Mutations in the hotspot of the *gyrA* gene (the region determining resistance to quinolones, codons 88-94) represent the main mechanism of resistance, while mutations in the *gyrB* gene are much less common and the role of several of them in resistance to fluoroquinolones is not always obvious [4, 5]. The most common mutations in *gyrA* are *Ala90Val*, *Asp94* (*Gly*, *Ala*, *His*, *Asn* or *Tyr*) and *Ser91Pro*, and the *Gly88Cys* mutation is less common [6–12].

Ofloxacin is currently not used for TB therapy, and a substantial proportion of strains phenotypically resistant to ofloxacin are sensitive to moxifloxacin. At the same time, the rapid development of drug resistance to ofloxacin and, as a result, a significant decrease in therapeutic efficacy led to a policy rejection of the use of this drug in the treatment of TB. In recent years, the WHO recommendations, as well as the Russian guidelines [13], have been significantly amended in terms of drug susceptibility testing of *M. tuberculosis* by bacteriological methods — ofloxacin, cycloserine, para-aminosalicylic acid were excluded from the list of drugs to which testing is recommended.

At the same time, the molecular mechanism of resistance to all fluoroquinolones is mediated by mutations in DNA-gyrase genes, and the new WHO catalog of resistance mutations to antituberculosis drugs contains a list of proven resistance mutations in *gyrA* in relation to the new generation fluoroquinolone — moxifloxacin [14]. At the same time, a number of mutations are defined as leading to a high level of resistance to moxifloxacin: *gyrA Gly88Cys*, *Asp94Asn*, *Asp94Gly*, *Asp94His*, *Asp94Tyr*.

In northwest Russia, the first study of ofloxacin-resistant *M. tuberculosis* strains aimed at investigating the variability of the *gyrA* and *gyrB* genes was conducted in 2008 and was mainly based on strains isolated from previously treated TB patients (85.4%) [15]. Analysis of such a sample does not provide a reliable answer to the question regarding which strains are currently actively circulating; such an analysis requires a cohort of newly diagnosed patients.

M. tuberculosis is characterized by a clonal population structure consisting of large phylogenetic lineages, smaller genotypes and genetically compact clusters of closely related strains. Some genotypes or their subtypes are characterized by association with drug resistance, increased transmissibility or hypervirulence, which determines their clinical significance and the need for more careful monitoring of their spread. Russia is characterized by the dominance of the Beijing genotype in the general population, especially strong among resistant strains. An earlier study of ofloxacin-resistant strains in northwestern Russia showed that 73% of strains from previously treated patients and 71% of strains from newly diagnosed patients belonged to the Beijing genotype [16]. A mutation in the *gyrA* gene was found in 89% of Beijing strains and 69% of strains of other genotypes. This dominance of Beijing strains among those resistant to ofloxacin is significantly higher than the share of the Beijing genotype in northwestern Russia: 52–56% [16, 17] in the general population; 34% among sensitive strains in St. Petersburg [17]. The authors of the 2008 study concluded that, similar to the spread of MDR-TB, the

[1] National Medical Research Center for Phthisiopulmonology and Infectious Diseases. URL: https://nmrc.ru/for_specialists/main-directions/tuberculosis

[2] World Health Organization. Meeting report of the WHO expert consultation on the definition of extensivey drug-resistant tuberculosis // Geneva: World Health Organization; 2021. URL: https://www.who.int/publications/i/item/9789240018662

spread of fluoroquinolone-resistant TB in Russia may be due to the predominance of the Beijing genotype in the *M. tuberculosis* population [15].

In another study in northwestern Russia [18], the Beijing genotype was detected in 70.8% of isolates with a low level of ofloxacin resistance, 84.6% of isolates with a high level of resistance, and 50% of sensitive strains; the proportion of Beijing was significantly higher among strains highly resistant to ofloxacin compared to sensitive strains ($p = 0.03$). However, it is possible that the association of Beijing with high levels of ofloxacin resistance is not so much a matter of Beijing dominance among MDR strains.

In 2006, in various regions of northwestern Russia, the proportion of *M. tuberculosis* strains resistant to ofloxacin ranged from 1.1–1.6% among newly diagnosed TB patients and 4.1–10.3% among previously treated patients [15]. Analysis of the drug susceptibility pattern of *M. tuberculosis* among newly diagnosed TB patients in northwest Russia for 2010–2021 showed a rapid (2.5-fold) increase in drug resistance to rifampicin in combination with fluoroquinolones (from 2.4%; 95% confidence interval (CI) 2.2–2.6 to 6.1%; 95% CI 5.6–6.6) [19].

Due to the spread of MDR-TB and the use of second-line drugs, it is important for clinical practice to test the sensitivity of the pathogen to fluoroquinolones other than isoniazid and rifampicin.

Given the increasing proportion of MDR strains of *M. tuberculosis* isolated from newly diagnosed patients in Russia, it was relevant to study the distribution of mutations in genes that cause resistance to fluoroquinolones in the modern period.

The aim of the present study was the molecular characterization of a retrospective collection of ofloxacin-resistant *M. tuberculosis* strains from newly diagnosed TB patients in northwestern Russia.

Materials and methods

The study collection included 599 *M. tuberculosis* isolates from the working collection of the bacteriological laboratory of the St. Petersburg Research Institute of Phthisiopulmonology, which was isolated in 2015–2019 from previously untreated TB patients living in various regions of northwest Russia.

Cultivation and determination of drug sensitivity of *M. tuberculosis* to the main antituberculosis drugs were performed using the standard indirect method of absolute concentrations on dense nutrient media and with the modified proportion method on liquid medium in a system with automated growth detection for antituberculosis drugs BACTEC MGIT960 (Becton Dickinson). The critical drug concentrations used were 1.0 μg/mL for streptomycin, 0.1 μg/mL for isoniazid, 5.0 μg/mL for ethambutol, 1, 0 μg/mL for rifampicin, 100 μg/mL for pyrazinamide, 1.0 μg/mL for amikacin, 2.5 μg/mL for capreomycin, 2.0 μg/mL for ofloxacin, and 5 μg/mL for ethionamide[3].

DNA was isolated from pure cultures of M. tuberculosis as described previously [15]. To determine genotypic resistance to fluoroquinolones, multiplex PCR was used (Amplitub-MLU-RV and Amplitub-FQ-RV (Syntol) kits).

Belonging to the Beijing genotype and its subtypes B0/W148, Central Asian/Russian, CAO, Beijing 1071-32-cluster, 14717-15-cluster was determined by PCR and real-time PCR methods to identify specific markers [20]. Strains of other genetic groups (non-Beijing) were spoligotyped [21]. The obtained spoligotype profiles were compared with the SITVIT2 international database[4] and the SIT (Spoligotype International Type) number was determined.

Statistical analysis was performed using MedCalc[5]. Differences between groups were determined by the χ^2 criterion; differences were considered statistically significant at $p < 0.05$.

Results and discussion

Drug sensitivity assessment of 599 *M. tuberculosis* strains isolated from newly diagnosed TB patients showed that 292 (48.7%) strains were sensitive to all 1st-line antituberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol), 230 (38.4%) isolates had MDR. Phenotypic resistance to ofloxacin was established in 6.7% (40/599) of strains in the general sample and in 17.4% (40/230) of MDR strains of *M. tuberculosis*. According to the new WHO definition, all 40 ofloxacin-resistant strains were pre-XDR (**Table 1**).

Genotyping showed that in the total collection of *M. tuberculosis* strains, the share of the Beijing genotype was 57.8% (346/599). The remaining 253 strains belonged to different genetic families of the Euro-American phylogenetic lineage of *M. tuberculosis* (another name is lineage 4), predominantly T (31.6%; 80/253), LAM (Latin American Mediterranean) (25.3%; 64), Ural (22.9%; 58), and Haarlem (9.9%; 25). The Central Asian/Russian (including the CAO subtype) and B0/W148 clusters of the Beijing genotype included 34.2% (205/599) and 17.4% (104) of strains, respectively.

Ofloxacin-resistant strains were predominantly (85%; 34/40) of the Beijing genotype. At the same time, 18 (45%) strains were assigned to the best known Russian epidemic subtype Beijing B0/W148-cluster, 12 (30%) to Beijing Central Asian/Russian. Of the latter, 2 strains represented the CAO subtype, which is more characteristic of Central Asia and quite rare in Russia [20, 22]. All Beijing strains had the SIT1 spoligotype, 1 strain belonged to the ancient sublin-

[3] Order of the Ministry of Health of Russia dated December 29, 2014 No. 951 "On approval of methodological recommendations to improve the diagnosis and treatment of respiratory tuberculosis."
[4] SITVIT2. URL: http://www.pasteur-guadeloupe.fr:8081/SITVIT2
[5] MedCalc. URL: http://www.medcalc.org/calc/odds_ratio.php

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Table 1. Spoligoprofiles of 40 ofloxacin-resistant *M. tuberculosis* strains

SIT, family	43-spoligoprofile	Number of strains	*gurA* mutation
SIT1 Beijing	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■	34	19 — *Asp94Gly* 8 — *Ala90Val* 3 — *Asp94Ala* 2 — *Ser91Pro* 1 — no
SIT42 LAM	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■□□□□■■■■■■■	2	1 — *Ala90Val* 1 — *Asp94Gly*
SIT252 LAM	■■■■■■■■■■□□■■■■■■■■□□□□■■■■■■■□□□□■■■■■■■	1	*Asp94Gly*
SIT4 LAM*	□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■□□□□■■■■■■■	1	*Asp94Gly*
SIT53 L4-unclassified	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	1	*Asp94Gly*
SIT251 L4-unclassified	■■■■■■■□□□□□□□□□□□□□□□□□□□□■■■■■□□□□■■■■■■■	1	*Ala90Val*

Note. *Strain SIT4 belongs to the LAM genotype based on 24-MIRU-VNTR typing and clustering with reference profiles from the MIRU-VNTRplus.org database.

eage (cluster 1071-32), and the others to the modern sublineage of the Beijing genotype. We also identified 4 strains of genotype LAM and 2 strains with spoligotypes SIT53 and SIT251, which belong to group T according to the SITVIT2 database. Taking into account the heterogeneity and polyphyletic nature of both the T group as a whole and the SIT53 spoligotype [23, 24], it is more appropriate to define these strains as L4-unclassified.

Mutations in the *gyrA* gene were detected in 97.5% (39/40) of *M. tuberculosis* strains (**Table 2**). The most frequent mutations were found in codon 94 (69.2%; 27/39); they were represented by 3 variants of single-nucleotide polymorphisms, among which the *Asp94Gly* substitution was detected in 57.5% (23/40) of ofloxacin-resistant strains and was dominant among both Beijing (19/34) and non-Beijing (4/6) strains. The second most frequent substitution was *Ala90Val* (25%, 10/40) which was established in 23.5% (8/34) of Beijing strains and 33.3% (2/6) of non-Beijing strains. Cumulatively, *M. tuberculosis* strains with *Asp94Gly* and *Ala90Val* mutations in the *gyrA* gene accounted for 82.5% (33/40). The simultaneous presence of 2 mutations in a single strain was not detected.

When analyzing the polymorphism of the *gyrA* gene, it should be taken into account that not all mutations, even in codons adjacent to hotspots, contribute to resistance. The most well-known phylogenetic substitution is a mutation in *gyrA95*, which was proposed as an evolutionary marker back in 1997 for the very first scheme of dividing *M. tuberculosis* species into major genetic groups [25]. Currently, as a modern source of information on the significance of resistance mutations (or lack thereof), one can refer to the WHO Mutation Catalog, the second edition of which was published in 2023. [14]. The enumeration of this codon *gyrA95* in the same row with mutations in the hotspot of the *gyrA* gene [7, 26] creates an erroneous impression of its correlation with resistance to fluoroquinolones.

Analysis of ofloxacin-resistant strains of different genotypes revealed all variants of the detected mutations in the *gyrA* gene in strains of the Beijing Central Asian/Russian subtype. More than a half of ofloxacin-resistant strains Beijing B0/W148 (10/18) and Cen-

Table 2. Mutations in the *gyrA* gene in ofloxacin-resistant *M. tuberculosis* strains

gyrA mutation	All strains	Beijing, all	Beijing B0/W148	Beijing Central Asian/Russian*	Beijing, other**	non-Beijing
n	40	34	18	12	4	6
Ala90Val	10 (25%)	8	5	2	1	2
Ser91Pro	2 (5%)	2	1	1		
Asp94Gly	23 (57,5%)	19	10	7	2	4
Asp94Ala	3 (7,5%)	3	1	1	1	
Asp94Tyr	1 (2,5%)			1		
Not detected	1 (2,5%)	1	1			

Note. *2 Beijing Central Asian/Russian strains belonged to the CAO subtype and had *Ala90Val* and *Ser91Pro* mutations. **1 strain belonged to the 1071-32 cluster of the ancient sublineage of the Beijing genotype and had the *Ala90Val* mutation.

tral Asian/Russian (7/12) had the *Asp94Gly* mutation. No significant differences in the mutation spectrum depending on the *M. tuberculosis* genotype were found.

In the studied sample of 40 patients from whom ofloxacin-resistant *M. tuberculosis* strains were isolated, clinical forms of infiltrative and disseminated pulmonary TB prevailed (16 and 14 patients, respectively; **Table 3**). Comparison of clinical forms of the disease and genotype of strains did not reveal statistically significant differences. The proportion of disseminated pulmonary TB was higher in patients infected with Central Asian/Russian strains (41.7%; 5/12) than with B0/W148 (27.8%; 5/18; $p = 0.4$). The proportion of patients with infiltrative pulmonary TB was higher in the B0/W148 group (55.6%; 10/18) than Central Asian/Russian (33.3%; 4/12; $p = 0.2$). It is possible that statistically insignificant differences between groups are due to small sample size or different reactivity of patient's body.

We compared our results with previous Russian studies [7, 8, 15, 26, 27]. As in our study, *gyrA* mutations *94Gly*, *90Val*, *94Ala* were the most frequent, regardless of the region of Russia. In the second edition of the WHO Catalog [15] published in 2023 for the new fluoroquinolone moxifloxacin, the most frequent of these *gyrA* mutations, *Asp94Gly*, was identified to be leading with high confidence to a high level of resistance to moxifloxacin, along with *gyrA Gly88Cys*, *Asp94Asn*, *Asp94His*, and *Asp94Tyr*. Other mutations in *gyrA* and *gyrB* have been identified to be resulting in low levels of resistance to moxifloxacin.

Mutations in *gyrB*, which are not included in the test system used in this study, are generally rare [8, 15] or have not been detected at all in some local collections [26], although, for example, found in 10% of ofloxacin-resistant strains in the Leningrad region [18]. Some ofloxacin-resistant strains do not carry mutations in *gyrA* or *gyrB* [15, 18]. Their resistance to ofloxacin can be hypothetically explained by a mutation in another target gene or active efflux [28, 29].

The reverse situation, namely multiple mutations in the same strain, was not observed in the studied sample, but was described earlier in other Russian studies. For example, in a study of a collection of strains from different regions of Russia, 4 isolates carried *gyrA Asp94Gly* and *gyrB Asn538Asp* mutations simultaneously, 1 Beijing B0/W148 strain had *gyrA* (*Ala90Val-Ser91Pro*, *Asp94Asn*) and gyrB (Ala543Val) mutations simultaneously [7]. There is no correlation between the level of phenotypic resistance and the type of mutation or the presence of more than 1 mutation. In the Leningrad region in 2011. 54.3% of isolates with a low level (2 μg/mL) and 76.9% of isolates with a high level (10 μg/mL) of ofloxacin resistance had mutations in *gyrA* [18]. Two isolates with low levels of ofloxacin resistance had mutations in *gyrA* and *gyrB* genes simultaneously, and major mutations in *gyrA* codons 94 and 90 were found among strains with both high and low levels of resistance [18]. This contradicts the statement in the WHO Catalog [14] that "multiple genetically linked mutations with low levels of resistance to moxifloxacin have an additive effect and should be considered as conferring high levels of resistance". Hypothetically, multiple mutations could arise due to mutator (hypermutable) alleles of DNA repair genes in such strains, similar to the situation with rifampicin resistance and multiple mutant alleles of *rpoB* [30].

An interesting result of a previous study in northwestern Russia was the detection of a high proportion of heteroresistant isolates, i.e., those with both mutant alleles and wild-type *gyrA* or *gyrB* alleles [15]. However, this current study did not detect such cases, i.e., strains with already persistently acquired resistance to ofloxacin are actively spreading at present.

Conclusion

The widespread use in the past of phenotypic drug resistance testing based on absolute concentration method data may have led to false results, which further increases the importance and necessity of genetic typing. The new version of the guidelines [13] includes additional provisions that prioritize the proportion method over the absolute concentration method in phenotypic susceptibility testing of *M. tuberculosis* clinical isolates to antituberculosis drugs. Critical concentrations for rifampicin (0.5 mg/L instead of 1 mg/L), levofloxacin (1.0 mg/L instead of 1.5 mg/L), moxifloxacin (0.25 and 1 mg/L instead of 0.5 and 2.0 mg/L) were changed according to WHO recommendations. This complicates retrospective comparison of phenotypic drug suscepti-

Table 3. Distribution of clinical forms of the disease among strain genotypes

Clinical forms of tuberculosis	Total	B0/W148-cluster	Central Asian/ Russian	Beijing, other	non-Beijing
n	40	18	12	4	6
Disseminated	14	5	5		4
Infiltrative	16	10	4	1	1
Fibrous-cavernous	6	2	2	1	1
Cavernous	2	1		1	
Focal	2		1	1	

bility data and further strengthens the role of molecular genetic studies aimed at establishing genetic markers of resistance.

In the studied sample of *M. tuberculosis* strains from different regions of northwestern Russia collected in 2015-2019, primary resistance to fluoroquinolones amounted to 6.7% in the general population and 17.4% in MDR strains and was mainly due to *Asp94Gly* and *Ala90Val* mutations in the *gyrA* gene. The largest proportion of fluoroquinolone-resistant *M. tuberculosis* strains was represented by the Beijing B0/W148 genotype.

In the population of *M. tuberculosis* in northwestern Russia, resistance to fluoroquinolones is currently forming against the background of already existing MDR, and strains with *Asp94Gly* and *Ala90Val* mutations in the *gyrA* gene play a major role in the spread of pre-SDR-TB. It is advisable to detect mutations in genes associated with resistance of *M. tuberculosis* to antituberculosis drugs and monitor the circulation of these genotypes to evaluate their epidemiologic significance.

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