Molecular Characterization of Ofloxacin-Resistant
Mycobacterium tuberculosis Strains from Russia

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In this work, we studied the variation in the gyrA and gyrB genes in ofloxacin- and multidrug-resistant Mycobacterium tuberculosis strains circulating in northwest Russia. Comparison with spoligotyping data suggested that similar to the spread of multidrug-resistant tuberculosis, the spread of fluoroquinolone-resistant tuberculosis in Russia may be due, at least partly, to the prevalence of the Beijing genotype in a local population of M. tuberculosis.

Fluoroquinolones (FQ) are potent second-line drugs recommended to treat multidrug-resistant tuberculosis. The main target of FQ, in Mycobacterium tuberculosis and other pathogens, is DNA gyrase, a type II topoisomerase composed of two A and two B subunits encoded by the gyrA and gyrB genes, respectively; mutations in the quinolone resistance-determining regions (QRDR) in these genes serve as a primary mechanism of the development of FQ resistance (1, 7, 10, 20).

FQ susceptibility testing is not performed in all regional laboratories in Russia, and data on FQ resistance rates are limited. In 2006, the rate of ofloxacin (OFL)-resistant M. tuberculosis strains was in the range of 1.1 to 1.6% for patients newly diagnosed with tuberculosis and 4.1 to 10.5% for previously treated patients in different regions of northwest Russia. Here, we report the results of the first population-based study of the molecular basis of FQ resistance in M. tuberculosis strains currently circulating in Russia.

The bacteriology laboratory in the St. Petersburg Research Institute of Phthisiopulmonology serves as a reference center for northwest Russia. In the present study, we included all OFL-resistant strains isolated from March to November 2006 (see the table in the supplemental material); these strains were recovered from 48 patients among the total of 261 patients screened. The patients were epidemiologically unlinked and originated from different regions of the Russian Federation.

Drug susceptibility testing was done by the absolute concentration method according to the guidelines of the Russian Ministry of Health (order no. 109 of 21 March 2003) by using recommended MIC breakpoints (in particular, 2 μg/ml for OFL). All 48 strains were multidrug resistant (resistant to at least rifampin [RIF] and isoniazid [INH]), while 45 of them were resistant to at least INH, RIF, OFL, and kanamycin (see the table in the supplemental material) and were classified as extensively drug resistant (22).

DNA extraction from Löwenstein-Jensen cultures, spoligotyping, and variable-number tandem repeat (VNTR) typing (using a 24-locus format and three hypervariable loci, QUB-3232, VNTR-3820, and VNTR-4120) were done as described previously (8, 9, 19). The gyrA QRDR was amplified and sequenced using the forward primer 5′-ACCGCAGCCACGCGAAGTTCG and the reverse primer 5′-CCTGGCGAGCCGAA GTTGCC. The gyrB QRDR was amplified and sequenced using primers described by Takiff et al. (20). Mutations in the rpoB hot-spot region (codons 507 to 553) and katG S315T (AGC>ACC) were detected as described previously (12, 15). To minimize the risk of laboratory cross-contamination during PCR amplification, the individual procedures (the preparation of the PCR mixes, the addition of the DNA, the PCR amplification, and the electrophoretic fractionation) were conducted in physically separated rooms. Negative controls (water) were included to control for reagent contamination. EpiCalc software (6) was used for statistical analysis.

The analysis of the gyrA QRDR in 23 OFL-susceptible strains, including H37Rv, revealed no mutations associated with FQ resistance. A gyrA mutation associated with FQ resistance was found in codon 88, 90, or 94 in 40 (83%) of 48 OFL-resistant strains (Table 1; see also the table in the supplemental material). Previously described mutations in gyrA codons 74 and 91 (5, 17) were not found. Otherwise, this overall prevalence of the gyrA QRDR mutations in our collection of Russian OFL-resistant isolates is within the range of the prevalence of these mutations in FQ-resistant isolates in other world regions (2, 5, 17, 18).

A gyrB mutation (NS10K, A515V, A515T, or Q549H) was identified in 6 of the 48 OFL-resistant strains (see the table in the supplemental material). This finding is in agreement with previously published observations that gyrB mutations are less frequent than gyrA mutations (1, 10, 18, 21). At the same time, 4 of 48 OFL-resistant strains still did not have any mutation in
the gyrA or gyrB genes; their OFL resistance may be explained by a mutation in another target gene or by active efflux (4, 7). To the best of our knowledge, a gyrB Q549H mutation located outside of the gyrB QRDR has not been described previously. In the present study, this mutation was detected in an extensively drug-resistant strain with a gyrA wild-type allele; there appears to be a casual link between the gyrB Q549H mutation and OFL resistance, although further studies with more strains are needed to confirm it. It should be noted that four OFL-resistant strains harbored a gyrA mutation alone (A515V, A515T, or Q549H). Given this finding, genotypic testing for resistant strains harbored a proportion of heteroresistant isolates, i.e., those with both mutant and wild-type alleles, would be needed to confirm that they are resistant to other drugs (see the table in the supplemental material). All but one INH-resistant strain had the katG S315T mutation. On the other hand, RIF-resistant strains had a broader spectrum of mutations in the rpoB hot-spot region, although this spectrum was dominated by the rpoB S531L mutation, found in 37 strains (77.1%). Otherwise, no bias in the codistribution of the gyrA and rpoB mutations was observed.

Spoligotyping revealed that 35 (73%) of the total of 48 strains, including 30 (73%) of 41 strains from previously treated patients and 5 (71%) of 7 strains from newly diagnosed patients, belonged to the Beijing genotype family (see the table in the supplemental material). A mutation in gyrA was found in 31 (89%) of 35 Beijing strains and 9 (69%) of 13 non-Beijing strains (Table 1). This difference is statistically insignificant (P = 0.2), due perhaps to the small sample size. However, we note that previous studies have demonstrated that the Beijing genotype strains, at least those circulating in Russia, more readily acquire the most frequently observed resistance mutations in other drug resistance genes, such as rpoB, katG, and embB, than non-Beijing strains (12–14).

Previously, the Beijing genotype was identified in 52 to 56% of M. tuberculosis strains in northwest Russia (13, 16). In particular, the Beijing genotype was identified in 34% of fully susceptible strains in St. Petersburg (16). Consequently, similar to the spread of multidrug-resistant tuberculosis (3, 16), the spread of OFL-resistant tuberculosis in Russia may be due, at least partly, to the prevalence of the Beijing genotype in the local M. tuberculosis population, although further studies in other Russian regions should be carried out in order to test this hypothesis.

To conclude, mutations in the gyrA QRDR were identified in a high proportion of the OFL-resistant strains in this study. At the same time, gyrB mutations were detected in a minor but nonnegligible proportion of the OFL-resistant strains without gyrA mutations. Consequently, genotypic testing for OFL resistance in M. tuberculosis strains circulating in Russia should target both gyrA and gyrB genes.

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REFERENCES

### Notes

- Numbers indicate codons. Mixed alleles were confirmed by resequencing of both DNA strands. Alleles listed as having no changes were wild-type alleles.
- Mutations were detected in a minor but nonnegligible proportion of the OFL-resistant strains without gyrA mutations. Consequently, genotypic testing for OFL resistance in M. tuberculosis strains circulating in Russia should target both gyrA and gyrB genes.
- The OFL-resistant strains were additionally studied for the presence of mutations associated with resistance to other drugs (see the table in the supplemental material). All but one INH-resistant strain had the katG S315T mutation. On the other hand, RIF-resistant strains had a broader spectrum of mutations in the rpoB hot-spot region, although this spectrum was dominated by the rpoB S531L mutation, found in 37 strains (77.1%). Otherwise, no bias in the codistribution of the gyrA and rpoB mutations was observed.

### Table 1. Distribution of the gyrA mutations in 48 OFL-resistant M. tuberculosis strains from Russia

<table>
<thead>
<tr>
<th>Nucleotide change(s) (amino acid change[s]) in gyrA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>All strains (n = 48)</th>
<th>Beijing genotype strains (n = 35)</th>
<th>Non-Beijing genotype strains (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>88-TGC (G88C)</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>90-CTG (A90V)</td>
<td>13</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>94-GGC (D94Q)</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>94-GCC (D94A)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>94-AAC (D94N)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>94-TAC (D94Y)</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>None, 94-AAC (none, D94N)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>None, 94-GGC (none, D94G)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>None, 94-TAC (none, D94Y)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>None, 90-CTG, 94-GGC (none, A90V, D94G)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>None, 94-AAC, 94-GGC (none, D94N, D94G)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>None, 94-AAC, 94-TAC, 94-GCC (none, D94N, D94G, D94Y)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers indicate codons. Mixed alleles were confirmed by resequencing of both DNA strands. Alleles listed as having no changes were wild-type alleles.


