Prevalence and molecular characterization of fluoroquinolone-resistant *Mycobacterium tuberculosis* isolates in China

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Abstract

China is one of the countries with the highest multidrug-resistant (MDR) and fluoroquinolone (FQ)-resistant TB burden globally. Nevertheless, knowledge on the prevalence and molecular characterization of FQ-resistant *Mycobacterium tuberculosis* (*M. tuberculosis*) isolates from this region remains not numerous. In this study, by utilizing agar proportion susceptibility method, the 138 ofloxacin (OFX)-resistant *M. tuberculosis* isolates were enrolled from the national drug resistance survey of China. All these *M. tuberculosis* strains were tested for drug susceptibility to ofloxacin, levofloxacin, moxifloxacin, gatifloxacin and sparflloxacin using the liquid Middlebrook 7H9 medium. The entire gyrA and gyrB genes conferring FQ resistance were sequenced, and the spoligotyping was performed to distinguish different genotypes. Overall, the prevalence of FQ resistance in China was highest for ofloxacin (3.76%), intermediate for levofloxacin (3.18%) and moxifloxacin (3.12%), and lowest for sparflloxacin (1.91%) and gatifloxacin (1.33%). Mutations in gyrA gene were observed in 89 (64.5%) out of 138 OFX-resistant *M. tuberculosis* strains. Position 94 and 90 was the most frequent mutation site conferring FQ resistance in these
strains, accounting for high-level FQ resistance. Furthermore, Beijing genotype showed no association with high-level FQ resistance and distribution in hot spots in QRDR of gyrA. Our findings will provide essential implications into the feasibility of genotypic tests relying on detecting mutations in the QRDR of gyrA and the shorter first-line treatment regimens based on FQs in China.

**INTRODUCTION**

Tuberculosis (TB) remains a major public health threat worldwide, causing 8.8 million new TB cases and 1.45 million deaths each year (1). Although the number of new TB cases and TB death has been declining since 2002 (2), TB control still faces formidable challenges, with the increasingly drug-resistant TB, especially the multidrug-resistant TB (MDR-TB), defined as *Mycobacterium tuberculosis* (*M. tuberculosis*) strains that are resistant to both isoniazid and rifampicin (1).

Fluroquinolones (FQs) have high *in vitro* activity against *M. tuberculosis* (3), which are used as the backbone drugs for MDR-TB (4, 5). In addition, due to their early sterilizing effect in human, the new-generation fluroquinolone has been recommended as first-line drugs to reduce the duration of therapy (6). The binding target of fluroquinolone in *M. tuberculosis* is DNA gyrase, consisting of two A and two B subunits encoded by gyrA and gyrB genes, respectively (7, 8). Missense mutations
within the quinolone resistance-determining region (QRDR), including a conserved region of gyrA (codons 88 to 94) and gyrB (codons 500 to 538), have been identified as the primary mechanism conferring fluoroquinolone resistance (8, 9).

China is the highest MDR-TB burden country globally, estimating to contribute 22% of the global burden of MDR-TB (1, 10, 11). The data from the national drug resistance survey conducted in 2007 indicated that 5.7% of new TB cases and 25.6% of previously treated cases had MDR tuberculosis, respectively (10). Unfortunately, fluoroquinolones, the most effective antimicrobial agent used in the chemotherapy of MDR-TB, have widely been used in the treatment for undiagnosed respiratory bacterial infection for more than 2 decades in China. The poorly controlled use of fluoroquinolones can contribute the emergence of FQ resistance in M. tuberculosis, which may influence the clinical outcome of MDR tuberculosis patients (12-14). Hence, it is meaningful to realize the prevalence of fluoroquinolone resistance in China, which will provide new insights to develop the appropriate regimen for MDR patients. Although the report of the national drug resistance survey of China revealed that approximately one-quarter of MDR tuberculosis had ofloxacin resistance, there is still a lack of knowledge on the drug resistant portion for new-generation fluoroquinolones in China, including moxifloxacin (MOX), gatifloxacin (GAT) and sparfloxacin (SPX).
In this study, the respective *M. tuberculosis* isolates from China were selected to evaluate the incidence rate on *M. tuberculosis* resistant to the later generation fluoroquinolones based on the determination of MIC in the liquid Middlebrook 7H9 medium. In addition, we sought to extend our findings on the relationship between nucleotide alteration and the level of phenotypic susceptibility to different FQs by characterizing genotypic mutations in the FQ target genes, *gyrA* and *gyrB*.

**MATERIALS AND METHODS**

**Bacterial strains and culture Conditions**

OFX-resistant *M. tuberculosis* strains, identified by conventional drug susceptibility testing, were all obtained from national tuberculosis drug resistance survey of China conducted in 2007 (10, 15). The *M. tuberculosis* strains isolated from pilots were all transferred to National Tuberculosis Reference Laboratory (NTRL), and the drug susceptibility was determined by the proportion method in NTRL with OFX concentration of 0.2 µg/ml. The critical growth proportion for resistance was 1%. The NTRL participated in the annual proficiency testing of DST organized by the Hong Kong Supranational tuberculosis Reference Laboratory and has passed each testing since 2003. All bacterial cells were stored in Trypticase soy broth containing glycerol at -70°C. All the strains were recovered on Lowenstein-Jensen medium for 4 weeks at
Genomic DNA extraction

Genomic DNA was extracted from freshly cultured bacteria as previously reported (16). The bacterial cells were transferred into a microcentrifuge tube containing 500 μl Tris-EDTA (TE) buffer, followed by centrifuging at 13,000 rpm for 2 min. After the supernatant was discarded, the pellet was resuspended in 500 μl TE buffer, then heated in a 95°C water bath for 1 hour. The cellular debris was separated by centrifugation, and DNA in the supernatant was used for the polymerase chain reaction (PCR) amplification.

Determination of minimal inhibitory concentration (MIC)

To determine MICs of FQ-resistant *M. tuberculosis* strains identified by conventional DST, a microplate Alamar blue assay (MABA) was performed as described previously (17). Five fluoroquinolones, including OFX, levofloxacin (LFX), moxifloxacin (MOX), gatifloxacin (GAT) and sparfloxacin (SPX), were selected to perform the MIC experiments, and the FQ concentrations were 0.125 to 64 μg/ml. The MICs were defined as the lowest concentration of antibiotic that reduced the viability of the culture by at least 90% as determined by fluorescence measurements at room temperature in top-reading mode, in which the excitation
wavelength and emission wavelength were 530 nm and 590 nm, respectively. The MIC breakpoints concentration was defined as 2 \( \mu \text{g/ml} \) for OFX and LFX, and 0.5\( \mu \text{g/ml} \) for MOX, GAT and SPX, respectively (18, 19).

**PCR amplification and sequencing of the gyrA and gyrB gene**

The entire gyrA and gyrB gene was amplified by PCR, respectively. The primer pairs for gyrA and gyrB were synthesized as previously reported (20). The genomic DNA was used as template to perform PCR amplification as follows: each PCR mixture was prepared in a volume of 50 \( \mu \text{l} \) containing 25 \( \mu \text{l} \) 2\( \times \)PCR Mixture, 2 \( \mu \text{L} \) of DNA template and 0.2 \( \mu \text{M} \) of each primer set. PCR program was done under the following conditions: initial denaturation at 94\( ^\circ \)C for 5 min, and then 35 cycles of denaturation at 94\( ^\circ \)C for 1 min, annealing at 58 for 1 min, and extension at 72\( ^\circ \)C for 2 min, followed by a final extension at 72\( ^\circ \)C for 10 min. PCR products were purified using a QIAquick Qiagen PCR purification kit, and then sent to Qingke Company (Beijing, China) for sequencing service.

DNA sequences were aligned with the homologous sequences of the reference *M. tuberculosis* H37Rv strains using multiple sequence alignments (http://www.ncbi.nlm.nih.gov/BLAST). The QRDR of gyrA ranged from codons 88 to 94 (8, 9); the QRDR of gyrB ranged from
Spoligotyping
A commercially available kit was used to perform spoligotyping according to the manufacturer’s instructions and a published report (Isogen Bioscience BV, Maarssen, Netherlands) (22). The original binary data were submitted to the SITVITWEB database to obtain the spoligotyping type (23).

Multiplex PCR
A multiplex PCR method with two sets of primers for the regions flanking the DR region of Beijing and non-Beijing strains was performed to distinguish the mixed infection of MANU2 family according to previous study (24). A positive amplification product of ~550bp indicated the presence of non-Beijing strain, while a PCR-amplified product of ~250bp indicated the presence of Beijing strains. The two PCR-amplified products of ~250bp and ~550bp indicated the mixed infection of both Beijing and non-Beijing strains.

IS6110 insertion in the NTF region
The IS6110 in the NTF region was analyzed according to methods described as the previous study (25). All Beijing family strains identified
by the spoligotyping method were amplified by PCR to detect the presence or absence of IS6110 in the NTF region. The modern Beijing strains were defined as the Beijing strains with a 1.8-kb DNA fragment by amplification, while those without the insert posed a 700-bp PCR product.

**Statistical analysis**

The Pearson chi-square test was used to compare the proportions of Beijing genotype and non-Beijing genotype *M. tuberculosis* isolates with polymorphisms in specific locations. The difference of MIC value between Beijing genotype and non-Beijing genotype was compared with t-test. Two-sided *P* values of <0.05 was considered statistically significant. All the statistical analyses were performed in the SPSS 15.0 (SPSS Inc.).

**RESULTS**

A total of 3634 smear positive patients included in the study (15), of which 145 isolates were identified as OFX-resistant strains by conventional DST. Out of 145 OFX-resistant strains, 138 could be sub-cultured successfully for subsequent MIC analysis and DNA sequencing of the entire gyrA and gyrB genes.

**Prevalence of fluoroquinolone resistance in China**
The 138 OFX-resistant isolates were tested for susceptibility to OFX, LFX, MOX, GAT and SPF by MIC method. As shown in Table 1, a total of 130 (94.2%) isolates were resistant to OFX, while the other 8 (5.8%) ones were susceptible to OFX, which differed from the results of conventional proportion method. In addition, resistance to LFX, MOX, GAT and SPF was found in 110 (79.7%), 108 (78.3%), 46(33.3%) and 66 (47.8%) isolates among the 138 OFX-resistant strains, respectively. Overall, the prevalence of FQ resistance in China were highest for OFX (3.76%), intermediate for LVX (3.18%) and MOX (3.12%), and lowest for SPX (1.91%) and GAT (1.33%).

**Distribution of gyrA and gyrB mutations among OFX-resistant *M. tuberculosis* strains**

The entire gyrA and gyrB genes of 138 FQ-resistant isolates were sequenced. The DNA sequence chromatogram showed that the presence of QRDR mutations in 93 (67.4%) of these 138 isolates. Among these 93 strains, a total of 89 (64.5%) strains carried the mutations in the QRDR of gyrA gene, including codon 88, 89, 90, 91 or 94. Position 94 was the most frequent mutation site conferring FQ resistance in these strains, with six different amino acid substitutions: Asp94His (*n*=3), Asp94Tyr (*n*=5), Asp94Asn (*n*=9), Asp94Ala (*n*=11), Asp94Gly (*n*=16) and Asp94Cys (*n*=1), which accounted for 32.6% of FQ resistance in *M. tuberculosis*. 
isolates. Ala90Val was the next most prevalent mutation, with 22.5% 
(n=31) of isolates harboring this mutant type. In addition, single mutation 
in or near QRDR of gyrB gene associated with OFX resistance was 
observed in 4 out of 138 (2.9%) OFX-resistant strains, including 
Arg485His (n=2), Asp500Asn (n=1) and Asp500Ala (n=1). Double 
substitutions in gyrA and gyrB were detected in 3 strains (2.2%) (Table 2), 
and the M. tuberculosis strains with Asp94Ala and Asp538Thr double 
mutations in gyrA and gyrB, respectively, showed high-level FQ 
resistance (≥16 mg/L), indicating the potential synergy between these two 
high spots.

In addition to the mutations located inside QRDRs, we also detected 31 
and 21 polymorphisms outside QRDR of gyrA and gyrB genes, 
respectively. Out of 52 polymorphisms, 34 single nucleotide 
polymorphisms belonged to non-synonymous SNP, while the other 18 
SNPs turned out to be synonymous. Overall, the majority of SNPs 
(65.4%) resulted in amino acid substitutions, which may be due to a 
relaxed purifying selection during short time periods in M. tuberculosis 
(14, 26) (Table S1).

Spoligotyping

Spoligotyping results for the 138 FQ-resistant isolated showed that a total 
of 113 (81.9%) isolates belonged to Beijing genotype, while 25 (18.1%)
were from non-Beijing families. Strains classified into non-Beijing families included 6 strains from the T1 family (4.3%), 5 from the MANU2 (3.6%), 1 from the T2 family (0.7%), 1 from the T2-UGANDA family (0.7%) and 10 of undefined spoligotype clades (7.2%) (Table 3).

For the 138 *M. tuberculosis* strains genotyped, a total of 19 spolotypes were identified. Clustering analysis revealed that SIT1 was the largest lineage (71.7%), belonging to the classical Beijing genotype, followed by SIT265 (n=10, 7.2%) and SIT54 (n=5, 3.6%), assigned to the Beijing and MANU2 family, respectively (Table 3).

To differentiate the potential mixed infection of MANU2 genotype strains, we performed a multiplex PCR method based on that described by Huang et al. (24). On the basis of the five MANU2 genotype strains, we identified that four strains contained both Beijing and non-Beijing strains, and one strain belonged to non-Beijing genotype.

**Analysis of modern and ancient Beijing genotype strains**

In order to distinguish the sublineages within the Beijing genotype, the insertion of IS6110 in the NTF region was analyzed. Among the 113 Beijing genotype isolates from China, 78 (69.0%) belonged to the modern Beijing sublineage, and 35 (31.0%) to the ancient Beijing sublineage. We further analyzed the proportion of FQ resistant isolates in both modern and ancient Beijing sublineages. As shown in Table 4, there was no
significant difference in distribution of FQ resistance pattern within Beijing genotype sample for ancient versus modern Beijing genotype sublineages.

**Association of the Beijing genotype with gyrA mutation**

Among 113 Beijing genotype strains, a total of 63.7% isolates (n=72) carried the mutations in the QRDR of gyrA gene. 14 (66.7%) out of 21 non-Beijing genotype strains had a gyrA mutation conferring FQ resistance. Statistical analysis revealed that no significant difference was found in the gyrA mutant prevalence between Beijing and non-Beijing genotypes. Similarly, there was also no significant difference among the distribution of the mutant gyrA types between Beijing and non-Beijing genotypes (Table 5).

**DISCUSSION**

FQs have been extensively used for tuberculosis treatment in China, especially for MDR-TB, for more than 10 years, and have been provided routinely as monotherapy for the empirical treatment of numerous outpatient infections. The abuse and overuse of FQs may also contribute to the increasing emergence of FQ-resistant *M. tuberculosis* in China (14). Thus, China may be one of the countries with the highest prevalence of FQ-resistant tuberculosis worldwide. In accordance to this hypothesis,
our data revealed that a high prevalence of FQ resistance, especially OFX, LFX and MOX, was present among the tuberculosis patients in China, while the prevalence of GAT and SPX was relatively low. The low rate of GAT and SPX resistance indicated that these two drugs may serve as a candidate component of therapeutic regimen curing for OFX resistant tuberculosis in China.

Resistance to FQ in clinical M. tuberculosis isolates occurs primarily due to mutations in the QRDR of gyrA (3). According to the previous reports, 50% to 90% of FQ-resistant isolates harbor mutations in the gyrA gene (4, 26, 27). In the present study, mutation at GyrA codons 88, 89, 90, 91 or 94 were observed in 64.5% resistant isolates. The frequency of mutants conferring FQ resistance is similar to that in Beijing (68%) and that in New York (67%), although it is lower than that in Russia (83%) and that in Shanghai (76%), and higher than that in Taiwan (50%) and that in Tunisian (50%) (12, 14, 27, 29, 30). Hence, gyrA mutations may differ from one geographic region to another (3, 31). Recently, several commercial diagnostic tests have been developed to rapidly detect FQ resistance in M. tuberculosis by scanning the mutations in the QRDR of gyrA (32, 33). However, our findings indicate that these tests may have low sensitivity in the patients of China. Even if the drug resistant hot spots of gyrB are also detected meanwhile, more than 30% FQ resistant patients may belong to missed diagnosis, thus resulting in treatment
In consistent to previous reports, substitutions at codon 94 were the most prevalent among the FQ-resistant strains in China (4, 14, 34), and most of mutation in codons 94 and 90, except for Asp94Ala, were linked to higher levels of resistance to all five FQs compared with those in codons 88, 89 and 91. The association may due to the affinity change between GyrA protein and FQs along with the amino acid substitution (35).

Several publications have demonstrated a link between mutations within \textit{gyrB} and FQ resistance (35, 36). In agreement to previous studies, we also found that additional 3% of FQ-resistant strains showed single mutations in the \textit{gyrB} gene. Hence, our findings indicate that inclusion of the QRDR of \textit{gyrB} in rapid molecular testing will provide a more complete picture of FQ resistance than only utilizing mutations within the GyrA QRDR region.

Based on the current study, more than 30% of FQ-resistant strains did not harbor the mutations in QRDRs of \textit{gyrA} and \textit{gyrB}, suggesting that another mechanism likely accounts for the FQ resistance in these strains. Here, we observed numerous non-synonymous SNPs outside of QRDR. It was not clear, however, whether these novel non-synonymous SNPs had the influence on FQ resistance. Further molecular analysis will provide new insight on the functions of the novel non-synonymous SNPs.

In addition to the polymorphisms in areas of \textit{gyrA} or \textit{gyrB} outside of the
QRDR, alternative mechanism including an active drug efflux pumps and decreased cell wall permeability to the drug may also confer the FQ resistant in these strains (37, 38). As previously described, those mechanisms always cause the low-level FQ resistance in *M. tuberculosis* (38). In this study, we found that most of *M. tuberculosis* strains (82.2%, 37/45) without *gyrA* and *gyrB* mutations in the QRDRs showed low-level OFX resistance (≤4 mg/L), while there were still eight strains with high-level OFX resistance, indicating that synergy effect of additional resistance mechanisms other than gyrase mutations may confer FQ resistance to *M. tuberculosis*.

Previous studies in Vietnam and Russia has found that Beijing genotype is significantly associated with high-level FQ resistance (29, 39). In contrast, we found no association between FQ resistance level and Beijing family genotype in China. Similarly, the distributions of *gyrA* mutation also revealed no difference between Beijing and non-Beijing genotype. One possible explanation is that the geographic disparity in prevalence of FQ resistant strains may result in diverse profiles of mutant types and drug resistant level among the *M. tuberculosis* strains from different regions. Consistent to findings from other investigations, the GyrA Gly88Ala mutation is associated with low-level FQ resistance (40).

We acknowledge that there were several limitations in this study. First, all the FQ-resistant strains were identified by conventional DST. Although
the newer FQs with better \textit{in vivo} activity has shown strong
cross-resistance with the OFX, several literatures have reported that
several rare strains resistant to MOX and GAT are susceptible to OFX
(41). Hence, the prevalence of newer FQ-resistance may be
underestimated. Second, we should access the function of efflux pump
mechanism conferring FQ resistance in these non-\textit{gyrA} and non-\textit{gyrB}
FQ-resistant mutants, which will help us to draw a precise picture of the
mechanism of FQ resistance in \textit{M. tuberculosis} isolates.

In conclusion, the prevalence of OFX, LFX and MOX resistance in \textit{M.}
tuberculosis isolates was high in China, compared with the relatively
lower rate of GAT-resistant and SPX-resistant isolates. Our findings also
demonstrated that mutations in \textit{gyrA} QRDR was the most predominant
mechanism accounting for FQ resistance, and a high proportion of
FQ-resistant \textit{M. tuberculosis} isolates did not have previously reported
resistance mutation in China. In addition, Beijing genotype showed no
association with high-level FQ resistance and distribution in hot spots in
QRDR of \textit{gyrA}. Our results will provide essential implications into the
feasibility of genotypic tests relying on detecting mutations in the QRDR
of \textit{gyrA} and the shorter first-line treatment regimens based on FQs in
China (6).

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REFERENCES


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<table>
<thead>
<tr>
<th>Fluoroquinolones</th>
<th>No. of strains with different MIC (mg/L)</th>
<th>Breakpoint MIC for resistance (mg/L)</th>
<th>No. of resistant strains (%)</th>
<th>Prevalence of resistant strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.125 0.25 0.5 1 2 4 8 16 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>0 0 0 8 48 36 36 8 2</td>
<td>2.0</td>
<td>130 (94.2%)</td>
<td>3.76</td>
</tr>
<tr>
<td>LVX</td>
<td>0 3 4 21 73 30 6 0 1</td>
<td>2.0</td>
<td>110 (79.7%)</td>
<td>3.18</td>
</tr>
<tr>
<td>MOX</td>
<td>10 20 21 31 29 15 11 1 0</td>
<td>0.5</td>
<td>108 (78.3%)</td>
<td>3.12</td>
</tr>
<tr>
<td>GAT</td>
<td>29 63 29 13 2 1 0 1 0</td>
<td>0.5</td>
<td>46 (33.3%)</td>
<td>1.33</td>
</tr>
<tr>
<td>SPX</td>
<td>21 51 25 26 13 1 0 1 0</td>
<td>0.5</td>
<td>66 (47.8%)</td>
<td>1.91</td>
</tr>
</tbody>
</table>

*Considering the loss of failure of subculture for 9 strains, the mean prevalence of FQ resistance was estimated according to the formula:

\[ n = \frac{N \times 145}{138 \times 3634} \]

where \( n \) is the prevalence of FQ-resistance, \( N \) is the total number of FQ-resistant strains, 145 is the total number of OFX-resistant strains among the 3634 strains isolated from the national survey of drug-resistant tuberculosis in China.
Table 2 Mutations in the quinolone resistance-determining region (QRDR) of gyrA and gyrB and fluoroquinolone MICs

<table>
<thead>
<tr>
<th>Mutations in QRDR of gyrA</th>
<th>Mutations in QRDR of gyrB</th>
<th>No. of isolates (%)</th>
<th>MIC range (mg/L)</th>
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<tr>
<td></td>
<td></td>
<td>MIC range (mg/L)</td>
<td>OFX</td>
</tr>
<tr>
<td>Gly88Ala</td>
<td></td>
<td>2(1.4)</td>
<td>2.0</td>
</tr>
<tr>
<td>Asp89Asn</td>
<td></td>
<td>3(2.2)</td>
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<tr>
<td>Ala90Val</td>
<td></td>
<td>31(22.5)</td>
<td>2.0-8.0</td>
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<tr>
<td>Ser91Pro</td>
<td></td>
<td>5(3.6)</td>
<td>2.0-4.0</td>
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<tr>
<td>Asp94His</td>
<td></td>
<td>3(2.2)</td>
<td>4.0-32.0</td>
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<td>Asp94Tyr</td>
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<td>5(3.6)</td>
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<td>Asp94Asn</td>
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<td>9(6.5)</td>
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<td>Asp94Ala</td>
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<td>11(8.0)</td>
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<td>Asp94Gly</td>
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<td>16(11.6)</td>
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<td>Asp94Cys</td>
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<td>Arg485His</td>
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<td>2(1.4)</td>
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<td>Asp500Asn</td>
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<td>1(0.7)</td>
<td>8.0</td>
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<td>Ala90Val Ser486Tyr</td>
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<td>Asp94Asn Asp538Thr</td>
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<td>Asp94Ala Asp538Thr</td>
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<td>1(0.7)</td>
<td>8.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>93(67.4)</td>
<td>2.0-32.0</td>
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Table 3 Spoligotypes of 138 FQ-resistant isolates from China

<table>
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<tr>
<th>Spoligotype description octonary</th>
<th>SIT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SpolDB4 ID&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No.&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Prevalence&lt;sup&gt;d&lt;/sup&gt; (%)</th>
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<td>BEIJING</td>
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<td>000000000003711</td>
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<td>54</td>
<td>MANU2</td>
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<tr>
<td>777777777760771</td>
<td>53</td>
<td>T1</td>
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<td>777743777760771</td>
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<td>777761777760771</td>
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<td>T2</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>777777777760730</td>
<td>135</td>
<td>T2-UGANDA</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>000002000003771</td>
<td>1184</td>
<td>-</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>000000000006771</td>
<td>NA&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
<td>2</td>
<td>1.4</td>
</tr>
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<td>1</td>
<td>0.7</td>
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<td>777757777760711</td>
<td>NA</td>
<td>-</td>
<td>1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>SIT from SITVITWEB database
<sup>b</sup>Representing spoligotype families annotated in SITVITWEB database
<sup>c</sup>Number of strains with the same SIT
<sup>d</sup>Prevalence represents the percentage of isolates with a common SIT among all isolates in this study.
<sup>e</sup>NA represents the spoligotyping type which is not found in SITVITWEB database.
Table 4 FQ resistance in ancient versus modern Beijing genotype strains

<table>
<thead>
<tr>
<th>Drug Resistance</th>
<th>Ancient Beijing sublineage (n=35)</th>
<th>Modern Beijing sublineage (n=78)</th>
<th>P value</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFLX</td>
<td>32 (91.4)</td>
<td>75 (96.2)</td>
<td>0.372</td>
<td>0.427 (0.082-2.228)</td>
</tr>
<tr>
<td>LFX</td>
<td>28 (80.0)</td>
<td>66 (84.6)</td>
<td>0.544</td>
<td>0.727 (0.259-2.041)</td>
</tr>
<tr>
<td>MOX</td>
<td>28 (80.0)</td>
<td>64 (82.1)</td>
<td>0.789</td>
<td>0.875 (0.319-2.403)</td>
</tr>
<tr>
<td>GAT</td>
<td>10 (28.6)</td>
<td>30 (38.5)</td>
<td>0.396</td>
<td>0.640 (0.270-1.518)</td>
</tr>
<tr>
<td>SPA</td>
<td>16 (45.7)</td>
<td>42 (53.8)</td>
<td>0.542</td>
<td>0.722 (0.324-1.607)</td>
</tr>
</tbody>
</table>
Table 5 Distribution of different mutations in the QRDR of gyrA gene among Beijing and non-Beijing genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of isolates (%) with different mutant types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gly88Ala</td>
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<tr>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Non-Beijing</td>
<td>(n=21)</td>
</tr>
</tbody>
</table>

*P* value: 0.157 1.000 1.000 0.175 0.403 1.000 0.354 0.378 1.000 1.000 0.796