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Prevalence and Molecular Characterization of Fluoroquinolone-Resistant *Mycobacterium tuberculosis* Isolates in China

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China is one of the countries with the highest burdens of multidrug-resistant (MDR) and fluoroquinolone (FQ)-resistant tuberculosis (TB) globally. Nevertheless, knowledge about the prevalence and molecular characterization of FQ-resistant *Mycobacterium tuberculosis* isolates from this region remains scant. In this study, 138 *M. tuberculosis* isolates determined by the agar proportion susceptibility method to be resistant to ofloxacin (OFX) were enrolled from a national drug resistance survey of China. All these strains were tested for susceptibility to ofloxacin, levofloxacin, moxifloxacin, gatifloxacin, and sparfloxacin using liquid Middlebrook 7H9 medium. The entire *gyrA* and *gyrB* genes conferring FQ resistance were sequenced, and spoligotyping was performed to distinguish different genotypes. Overall, the prevalence of resistance in China was highest for ofloxacin (3.76%), intermediate for levofloxacin (3.18%) and moxifloxacin (3.12%), and lowest for sparfloxacin (1.91%) and gatifloxacin (1.33%). Mutations in the *gyrA* gene were observed in 89 (64.5%) out of the 138 OFX-resistant *M. tuberculosis* strains. Positions 94 and 90 were the most frequent sites of mutation conferring FQ resistance on these strains, accounting for high-level FQ resistance. Furthermore, the Beijing genotype showed no association with high-level FQ resistance or distribution in hot spots in the quinolone resistance-determining region (QRDR) of *gyrA*. Our findings provide essential implications for the feasibility of genotypic tests relying on detection of mutations in the QRDR of *gyrA* and the shorter first-line treatment regimens based on FQs in China.

Tuberculosis (TB) remains a major public health threat worldwide: there are 8.8 million new TB cases and 1.45 million deaths attributable to TB each year (1). Although the numbers of new TB cases and TB deaths have been declining since 2002 (2), TB control still faces formidable challenges, especially in the face of increasingly drug-resistant TB, especially multidrug-resistant TB (MDR-TB), caused by *Mycobacterium tuberculosis* strains that are resistant to both isoniazid and rifampin (1).

Fluoroquinolones (FQs) have high *in vitro* activity against *M. tuberculosis* (3) and are used as the backbone drugs for MDR-TB (4, 5). In addition, due to their early eradication effect in humans, the new-generation FQs have been recommended as first-line drugs to reduce the duration of therapy (6). The binding target of FQs in *M. tuberculosis* is DNA gyrase, consisting of two A and two B subunits encoded by the *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 country with the highest MDR-TB burden globally, estimated to contribute 22% of the global burden of MDR-TB (1, 10, 11). The data from a national drug resistance survey conducted in 2007 indicated that 5.7% of new TB cases and 25.6% of previously treated cases were MDR tuberculosis (10). Unfortunately, fluoroquinolones, the most effective antimicrobial agents used in the chemotherapy of MDR-TB, have been widely used in the treatment of undiagnosed respiratory bacterial infections 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 for MDR tuberculosis patients (12–14). Hence, it is meaningful to recognize 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 cases had ofloxacin (OFX) resistance, there is still a lack of knowledge about the resistance to new-generation fluoroquinolones in China, including moxifloxacin (MOX), gatifloxacin (GAT), and sparfloxacin (SPX).

In this study, *M. tuberculosis* isolates from China were selected to evaluate the incidence of *M. tuberculosis* resistance to the later-generation fluoroquinolones based on the determination of MICs in 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 susceptibility testing, were all obtained from a national tuberculosis drug resistance survey of China conducted in 2007 (10, 15). The *M. tuberculosis* strains isolated from clusters were all transferred to the National Tuberculosis Reference Laboratory (NTRL),

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TABLE 1 Distribution and prevalence of fluoroquinolone-resistant strains in China

FQ	No. of strains with indicated MIC (mg/liter)									Breakpoint MIC for resistance (mg/liter)	No. (%) of resistant strains	Prevalence of resistant strains (%)[a]
	≤0.125	0.25	0.5	1	2	4	8	16	32			
OFX	0	0	0	8	48	36	36	8	2	2.0	130 (94.2)	3.76
LVX	0	3	4	21	73	30	6	0	1	2.0	110 (79.7)	3.18
MOX	10	20	21	31	29	15	11	1	0	0.5	108 (78.3)	3.12
GAT	29	63	29	13	2	1	0	1	0	0.5	46 (33.3)	1.33
SPX	21	51	25	26	13	1	0	0	1	0.5	66 (47.8)	1.91

[a] Considering the loss of failure of subculture for 9 strains, the mean prevalence of FQ resistance was estimated according to the formula $n = (N \times 145)/(138 \times 3{,}634)$, where n is the prevalence of FQ resistance, N is the total number of FQ-resistant strains, and 145 is the total number of OFX-resistant strains among the 3,634 strains isolated from the national survey of drug-resistant tuberculosis in China.

and drug susceptibility was determined by the proportion method in the NTRL with an OFX concentration of 0.2 μg/ml. The critical growth proportion for resistance was 1%. The NTRL participated in the annual proficiency testing of drug susceptibility testing (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 37°C.

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 of Tris-EDTA (TE) buffer, followed by centrifugation at 13,000 rpm for 2 min. After the supernatant was discarded, the pellet was resuspended in 500 μl of TE buffer and then heated in a 95°C water bath for 1 h. The cellular debris was separated by centrifugation, and DNA in the supernatant was used for the PCR amplification.

Determination of MIC. To determine MICs of FQ-resistant *M. tuberculosis* strains identified by conventional DST, a microplate alamarBlue assay (MABA) was performed as described previously (17). Five fluoroquinolones, including OFX, levofloxacin (LFX), MOX, GAT, and SPX, were selected to perform the MIC experiments, and the FQ concentrations were 0.125 to 64 μg/ml. The MIC was 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 were defined as 2 μg/ml for OFX and LFX and 0.5 μg/ml for MOX, GAT, and SPX (18, 19).

PCR amplification and sequencing of the *gyrA* and *gyrB* genes. The entire *gyrA* and *gyrB* genes were amplified by PCR. The primer pairs for *gyrA* and *gyrB* were synthesized as previously reported (20). The genomic DNA was used as the template to perform PCR amplification as follows. Each PCR mixture was prepared in a volume of 50 μl containing 25 μl of 2× PCR mixture, 2 μl of DNA template, and 0.2 μM each primer set. PCR was done under the following conditions: initial denaturation at 94°C for 5 min and then 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min, followed by a final extension at 72°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.

DNA sequences were aligned with the homologous sequences of the reference *M. tuberculosis* H37Rv strain 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 codons 500 to 538 (21).

Spoligotyping. A commercially available kit was used to perform spoligotyping according to the manufacturer's instructions (Isogen Bioscience BV, Maarssen, Netherlands) and a published report (22). The original binary data were submitted to the SITVITWEB database to obtain the spoligotype (23).

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

IS*6110* insertion in the NTF region. IS*6110* in the noise transfer function (NTF) region was analyzed according to methods described previously (25). All Beijing family strains identified by the spoligotyping method were amplified by PCR to detect the presence or absence of IS*6110* 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 had a 700-bp PCR product.

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

RESULTS

A total of 3,634 smear-positive patients were included in the study (15); 145 isolates were identified as OFX resistant by conventional DST. Out of the 145 OFX-resistant strains, 138 could be subcultured 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 the MIC method. As shown in Table 1, a total of 130 (94.2%) isolates were resistant to OFX, while the other 8 (5.8%) 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 prevalences 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 the 138 FQ-resistant isolates were sequenced. The DNA sequence chromatogram showed the presence of QRDR mutations in 93 (67.4%) of these 138 isolates. Among these 93 strains, a total of 89 (64.5%) carried mutations in the QRDR of *gyrA*, including codon 88, 89, 90, 91, or 94. Position 94 was the most frequent site of mutation conferring FQ resistance on 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

TABLE 2 Mutations in the QRDRs of *gyrA* and *gyrB* and FQ MICs

Mutation in QRDR of *gyrA*	Mutation in QRDR of *gyrB*	No. (%) of isolates	MIC range (mg/liter)				
			OFX	LFX	MXF	GAT	SPX
Gly88Ala		2 (1.4)	2.0	2.0	0.5–1.0	0.25	0.25
Asp89Asn		3 (2.2)	2.0–4.0	1.0–2.0	0.25–1.0	0.25	0.25–0.5
Ala90Val		31 (22.5)	2.0–8.0	1.0–8.0	0.25–8.0	≤0.125–1	≤0.125–2.0
Ser91Pro		5 (3.6)	2.0–4.0	2.0	0.5–2.0	0.25	0.25
Asp94His		3 (2.2)	4.0–32.0	4.0–8.0	4.0–8.0	0.25–2.0	1.0–2.0
Asp94Tyr		5 (3.6)	4.0–8.0	2.0–4.0	0.5–8.0	0.25–1.0	0.25–1.0
Asp94Asn		9 (6.5)	8.0–16.0	2.0–8.0	2.0–8.0	0.25–4.0	1.0–2.0
Asp94Ala		11 (8.0)	2.0–4.0	1.0–2.0	0.25–4.0	0.25–0.5	0.25–1.0
Asp94Gly		16 (11.6)	4.0–16.0	2.0–4.0	0.5–8.0	0.25–1.0	0.25–2.0
Asp94Cys		1 (0.7)	8.0	8.0	2.0	0.5	0.5
	Arg485His	2 (1.4)	4.0–16.0	4.0–8.0	8.0	0.5–2.0	1.0–2.0
	Asp500Asn	1 (0.7)	8.0	2.0	0.5	0.25	0.5
	Asp500Ala	1 (0.7)	4.0	2.0	1.0	0.5	2.0
Ala90Val	Ser486Tyr	1 (0.7)	4.0	2.0	4.0	0.5	0.5
Asp94Asn	Asp538Thr	1 (0.7)	32.0	32.0	16.0	16.0	32.0
Asp94Ala	Asp538Thr	1 (0.7)	8.0	2.0	4.0	1.0	4.0
Total		93 (67.4)	2.0–32.0	1.0–32.0	1.0–16.0	≤0.125–16.0	≤0.125–32.0

resistance in *M. tuberculosis* isolates. Ala90Val was the next most prevalent mutation, harbored by 22.5% ($n = 31$) of the isolates. In addition, a single mutation in or near the QRDR of *gyrB* gene associated with OFX resistance was observed in 4 out of the 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/liter), indicating the potential synergy between these two hot spots.

In addition to the mutations located inside QRDRs, we also detected 31 and 21 polymorphisms outside the QRDRs of the *gyrA* and *gyrB* genes, respectively. Out of these 52 polymorphisms, 34 single nucleotide polymorphisms (SNPs) were nonsynonymous, 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 periods in *M. tuberculosis* (14, 26) (see Table S1 in the supplemental material).

Spoligotyping. Spoligotyping results for the 138 FQ-resistant isolates showed that a total of 113 (81.9%) isolates belonged to the 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 family (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 spoligotypes were identified. Clustering analysis revealed that SIT 1 was the largest lineage (71.7%), belonging to the classical Beijing genotype, followed by SIT 265 ($n = 10$ [7.2%]) and SIT 54 ($n = 5$ [3.6%]), assigned to the Beijing genotype and the MANU2 family, respectively (Table 3).

To differentiate potential mixed infections with MANU2 genotype strains, we performed a multiplex PCR method based on that described by Huang et al. (24). Of the five MANU2 genotype strains, we found that four strains occurred in mixed infections with both Beijing and non-Beijing strains and one strain occurred in the infection with a non-Beijing genotype.

Analysis of modern and ancient Beijing genotype strains. In order to distinguish the sublineages within the Beijing genotype, the insertion of IS*6110* 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 proportions

TABLE 3 Spoligotypes of 138 FQ-resistant isolates from China

Spoligotype description octonary	SIT[a]	SpolDB4 ID[b]	No.[c]	Prevalence[d] (%)
000000000003771	1	Beijing	99	71.7
000000000003731	190	Beijing	1	0.7
000000000003371	265	Beijing	10	7.2
000000000003711	541	Beijing	2	1.4
000000000003571	632	Beijing	1	0.7
777777777763771	54	MANU2	5	3.6
777777777760771	53	T1	3	2.2
776177777760771	156	T1	1	0.7
777743777760771	913	T1	1	0.7
777761777760771	2114	T1	1	0.7
777777777760731	52	T2	2	1.4
677777777760731	1332	T2	1	0.7
777777777760730	135	T2-Uganda	1	0.7
000002000003771	1184	—	4	2.9
000000000006771	NA[e]	—	2	1.4
700777777760771	NA	—	1	0.7
747777757763771	NA	—	1	0.7
757777777763771	NA	—	1	0.7
777757777760711	NA	—	1	0.7

[a] Spoligotype international type (SIT) from the SITVITWEB database.
[b] Representing spoligotype families annotated in SITVITWEB database. —, undefined.
[c] Number of strains with the same SIT.
[d] Prevalence represents the percentage of isolates with a common SIT among all isolates in this study.
[e] "NA" represents the spoligotyping type which is not found in the SITVITWEB database.

TABLE 4 FQ resistance in ancient versus modern Beijing genotype strains

FQ	No. (%) of strains resistant		*P* value	Odds ratio (95% confidence interval)
	Ancient Beijing sublineage ($n = 35$)	Modern Beijing sublineage ($n = 78$)		
OFX	32 (91.4)	75 (96.2)	0.372	0.427 (0.082–2.228)
LFX	28 (80.0)	66 (84.6)	0.544	0.727 (0.259–2.041)
MOX	28 (80.0)	64 (82.1)	0.789	0.875 (0.319–2.403)
GAT	10 (28.6)	30 (38.5)	0.396	0.640 (0.270–1.518)
SPA	16 (45.7)	42 (53.8)	0.542	0.722 (0.324–1.607)

of FQ-resistant isolates in both modern and ancient Beijing sublineages. As shown in Table 4, there was no significant difference in the FQ resistance patterns within the 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 mutations in the QRDR of *gyrA*. Fourteen (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* mutation prevalence between Beijing and non-Beijing genotypes. Similarly, there was also no significant difference in 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 prevalences of FQ-resistant tuberculosis worldwide. In accordance with this hypothesis, our data revealed that a high prevalence of resistance to FQs, especially OFX, LFX, and MOX, was present among tuberculosis patients in China, while the prevalence of GAT and SPX resistance was relatively low. The low rate of GAT and SPX resistance indicated that these two drugs may serve as candidate components of therapeutic regimens 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 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 codon 88, 89, 90, 91, or 94 was observed in 64.5% of resistant isolates. The frequency of mutations conferring FQ resistance is similar to those in Beijing (68%) and New York (67%), although it is lower than those in Russia (83%) and Shanghai (76%) and higher than those in Taiwan (50%) and Tunisia (50%) (12, 14, 27–29). Hence, *gyrA* mutations may differ from one geographic region to another (3, 30). 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* (31, 32). However, our findings indicate that these tests may have low sensitivity in patients of China. Even if the drug-resistant hot spots of *gyrB* are also detected, diagnosis may be missed in more than 30% of FQ-resistant patients, thus resulting in treatment delay.

Consistent with previous reports, substitutions at codon 94 were the most prevalent among the FQ-resistant strains in China (4, 14, 33), and most mutations at codons 94 and 90, except for Asp94Ala, were linked to higher levels of resistance to all five FQs than were mutations at codons 88, 89, and 91. The association may due to the affinity change between GyrA and FQs along with the amino acid substitution (34).

Several publications have demonstrated a link between mutations within *gyrB* and FQ resistance (34, 35). In agreement with previous studies, we also found that an additional 3% of FQ-resistant strains showed single mutations in the *gyrB* gene. Hence, our findings indicate that inclusion of the QRDR of *gyrB* in rapid molecular testing will provide a more complete picture of FQ resistance than utilizing only mutations within the GyrA QRDR.

Based on the current study, more than 30% of FQ-resistant strains did not harbor the mutations in the QRDRs of *gyrA* and *gyrB*, suggesting that another mechanism likely accounts for the FQ resistance in these strains. We observed numerous nonsynonymous SNPs outside the QRDR. It was not clear, however, whether these novel nonsynonymous SNPs influenced FQ resistance. Further molecular analysis will provide new insight on the functions of the novel nonsynonymous SNPs.

In addition to the polymorphisms in areas of *gyrA* or *gyrB* outside the QRDR, alternative mechanisms, including an active drug efflux pump and decreased cell wall permeability to the drug, may also confer FQ resistance on these strains (36, 37). As previously described, those mechanisms always cause low-level FQ resistance in *M. tuberculosis* (37). In this study, we found that most *M. tuberculosis* strains (82.2%; 37/45) without *gyrA* and *gyrB* mutations in the QRDRs showed low-level OFX resistance (≤4 mg/liter), while there were still eight strains with high-level OFX resistance, indicating that a synergistic effect of additional resistance mechanisms other than gyrase mutations may confer FQ resistance on *M. tuberculosis*.

Previous studies in Vietnam and Russia have found that the Beijing genotype is significantly associated with high-level FQ resistance (28, 38). 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 the Beijing and non-Beijing genotypes. One possible explanation is that the geographic disparity in prevalence of FQ-resistant strains may result in diverse profiles of mutant types and drug

TABLE 5 Distribution of different mutations in the QRDR of *gyrA* among Beijing and non-Beijing genotypes

Genotype	No. (%) of isolates with indicated mutation										Total
	Gly88Ala	Asp89Asn	Ala90Val	Ser91Pro	Asp94His	Asp94Tyr	Asp94Asn	Asp94Ala	Asp94Gly	Asp94Cys	
Beijing ($n = 113$)	0 (0.0)	3 (2.7)	27 (23.9)	3 (2.7)	2 (1.8)	5 (4.4)	9 (8.0)	8 (7.1)	14 (12.4)	1 (0.9)	72 (63.7)
Non-Beijing ($n = 21$)	1 (4.7)	0 (0.0)	5 (23.8)	2 (9.5)	1 (4.8)	0 (0.0)	0 (0.0)	3 (14.3)	2 (9.5)	0 (0.0)	14 (66.7)
P value	0.157	1.000	1.000	0.175	0.403	1.000	0.354	0.378	1.000	1.000	0.796

resistance levels among the *M. tuberculosis* strains from different regions. Consistent with findings from other investigations, the GyrA Gly88Ala mutation is associated with low-level FQ resistance (39).

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 *in vivo* activity have shown strong cross-resistance with OFX, several items of literature have reported that several rare strains resistant to MOX and GAT are susceptible to OFX (40). Hence, the prevalence of resistance to newer FQs may be underestimated. Second, we should assess the function of the efflux pump mechanism conferring FQ resistance on these non-*gyrA* and non-*gyrB* FQ-resistant mutants, which will help us to draw a precise picture of the mechanism of FQ resistance in *M. tuberculosis* isolates.

In conclusion, the prevalence of OFX, LFX, and MOX resistance in *M. tuberculosis* isolates was high in China compared with the rates of GAT and SPX resistance. Our findings also demonstrated that mutation in the *gyrA* QRDR was the most predominant mechanism accounting for FQ resistance, and a high proportion of FQ-resistant *M. tuberculosis* isolates did not have previously reported resistance mutations in China. In addition, the Beijing genotype showed no association with high-level FQ resistance and distribution in hot spots in the QRDR of *gyrA*. Our results 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 (6, 41).

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