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RESEARCH ARTICLE

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Molecular characteristics of ofloxacin mono-resistant *Mycobacterium tuberculosis* isolates from new and previously treated tuberculosis patients

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Background: Ofloxacin (OFX) resistant *Mycobacterium tuberculosis* (MTB) isolates have been increasingly observed and are a major concern in recent years. This study investigated the genetic mutations associated with OFX resistance among clinical OFX mono-resistant MTB isolates from new and previously treated tuberculosis patients.

Methods: A total of 50 unrelated OFX mono-resistant MTB isolates were analyzed. For all isolates, the quinolone resistance determining regions of *gyrA* and *gyrB* were PCR amplified and sequenced.

Results: Single mutations in the quinolone resistance determining regions of *gyrA* (positions D94A, G, N, and Y; A90V; and S91P) and *gyrB* (positions T539A and E540D) were observed in 62% (31/50) and 4% (2/50) of all OFX mono-resistant isolates, respectively. No differences were detected between the proportions of isolates with mutations in *gyrA*/*gyrB* from new and previously treated tuberculosis patients (P=.820).

Conclusions: Although mutations in *gyrB* were rare, they were as important as mutations in *gyrA* in predicting OFX resistance in MTB in Tianjin, China.

KEYWORDS
Fluoroquinolone resistance, *gyrA*, *gyrB*, mutations, *Mycobacterium tuberculosis*, ofloxacin resistance, quinolone resistance determining region

1 | INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) remains a threat to public health and was responsible for ~9.6 million infections and an estimated 1.5 million deaths in 2014.[1] The emergence and spread of drug resistant MTB isolates has worsened the WHO End TB Strategy, especially multidrug resistant MTB, which is resistant to first-line drugs such as rifampicin and isoniazid.[1-3] Ofloxacin (OFX) is a member of the fluoroquinolones (FQs), which are some of the most effective second-line antimicrobial drugs used to treat patients who are infected with drug resistant TB (including multidrug resistant TB) or who are intolerant of current first-line therapy.[4] However, in recent years, OFX resistant MTB isolates have been increasingly observed and are a major concern, which have exacerbated the process of treating and controlling TB.[5]

Molecular methods for the rapid detection of mutations in genes associated with drug resistance are faster than automatic liquid culture systems that require 7-10 days to complete.[2,6,7] The main cellular target for FQs is DNA gyrase encoded by *gyrA* and *gyrB*,[5] in which the mutations in quinolone resistance determining regions (QRDRs) have been associated with FQ resistance.[8] Efflux pumps also confer FQ resistance, which is able to be induced by anti-tuberculosis drug such as rifampicin.[9] Understanding the mainly genetic background of resistance to FQs in MTB is essential, especially in FQ mono-resistant MTB.

In this study, we sought to investigate the genetic mutations in the QRDRs of *gyrA* and *gyrB* among clinical MTB isolates with OFX mono-resistance from new and previously treated patients.

2 | MATERIALS AND METHODS

2.1 | Strains

A total of 2639 MTB isolates were collected by the Tianjin drug-resistance tuberculosis surveillance program from Jan 2013 to Dec 2015. Identification of isolates was performed on Lowenstein-Jensen medium containing p-Nitrobenzoic-acid (PNB) and Thiophene-2-carboxylic hydrazide (TCH).

2.2 | Drug susceptibility test

According to the proportion method recommended by WHO, drug susceptibility tests (DSTs) were performed on Lowenstein-Jensen medium containing streptomycin (4 mg/L), rifampicin (40 mg/L), isoniazid (0.2 mg/L), ethambutol (2 mg/L), OFX (2 mg/L), or kanamycin (30 mg/L), at the Tuberculosis Reference Laboratory of Tianjin Center for Tuberculosis Control.[10]

2.3 | Patient information

The demographic and clinical information of enrolled patients, including gender, age, and TB treatment history, was obtained from the patients' records.

2.4 | DNA sequencing of *gyrA* and *gyrB* genes

DNA was extracted using the cetyltrimethylammonium bromide method, and samples were stored at −20°C. Primers used to amplify and sequence the QRDR of *gyrA* and *gyrB* from MTB isolates were described previously [11] and include GYRA-F: ATCGACTATGCGATGAGCG, GYRA-R: GGGCTTCGGTGTACCTCAT,GYRB-F:AGTCGTTGTGAACAAGGCTGT, and GYRB-R: CCACTTGAGTTTGTACAGCGG. PCR products were sequenced by Thermo Fisher Scientific Inc. (Beijing, China). Sequence data was analyzed using the MUBII-TB-DB database and BLAST on http://blast.ncbi.nlm.nih.gov, using the MTB H37Rv genome as a reference (GenBank accession number: CP003248.2).[12]

2.5 | Statistical analysis

A $\chi 2$ test in IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA) was used to compare the proportions of drug resistance mutations in *gyrA* and *gyrB* between isolates from new and previously treated TB patients. *P* values of <.05 were considered statistically significant.

3 | RESULTS

3.1 | Patients' information

By proportion method, 50 of 2639 isolates were OFX mono-resistant MTB, excluding six repeated OFX mono-resistant MTB isolates. Each of the 50 OFX mono-resistant MTB isolates obtained in this study were collected separately from 50 unrelated pulmonary TB patients (Table 1).

3.2 | Mutations in *gyrA* and *gyrB* genes among MTB strains

As shown in Table 1, 62% (31/50) isolates carried single mutations in the QRDR of *gyrA*. The most frequent mutations in *gyrA* were at positions D94N (n=7), A (n=6), G (n=6), and Y (n=2), followed by A90V (n=5) and S91P (n=5). Of all MTB isolates, 4% (2/50) isolates carried single mutations in the QRDR of *gyrB*, at positions T539A and E540D, based on the findings of Pantel et al.[13] All strains carried S95T mutations in *gyrA* and are not listed in Table 1 because they only showed these specific polymorphisms.[6]

No differences were detected between the proportions of isolates having mutations in *gyrA*/*gyrB* from new (66.7%, 28/42) and previously treated TB patients (62.5%, 5/8), *P*=.820. No differences were detected between the proportions of isolates with mutations in *gyrA*/*gyrB* from male (63.9%, 23/36) and female TB patients (71.4%, 10/14), *P*=.863.

4 | DISCUSSION

In this study, the most common mutations in *gyrA* were found in the QRDR at positions 94, 90, and 91, carried by 62% OFX mono-resistant MTB isolates, which was in agreement with the previously findings from literatures.[5,14] To our knowledge, this is the first report to show that the mutations T539A and E540D in the QRDR in *gyrB* were found from OFX mono-resistant MTB isolates in China. These mutations in *gyrA* and *gyrB* partially accounted for the phenotypic OFX resistance. Other mechanisms of FQ resistance, such as efflux pumps, should be evaluated to improve the prediction of FQ resistance phenotypes.[15]

All OFX mono-resistant MTB isolates were obtained from both new (42 cases) and previously treated TB patients (eight cases). Our results implied that the transmission of isolates that were already resistant to FQs causes the prevalence of FQ resistance and that MTB in TB patients acquires FQ resistance correlated with the patient's previous exposure to FQs.[16] However, no difference was detected between the proportions of isolates with mutations in *gyrA*/*gyrB* from new and previously treated TB patients (*P*=.820). A limitation was that only a small number of MTB isolates that had mutations in *gyrA*/*gyrB* were collected, excluding other OFX resistant MTB isolates.

5 | CONCLUSION

In conclusion, mutations in QRDR in both *gyrA* and *gyrB* were important in predicting OFX resistance of MTB isolates in Tianjin, China. Further research using additional OFX resistant MTB isolates to acquire and analyze the prevalence of OFX genotypic resistance of MTB isolates in this region is needed.

TABLE 1 Distribution of mutations in gyrA/gyrB among 50 OFX mono-resistant MTB isolates from patients

				Mutations in gyrA/gyrB in isolates from patients	
Patient number	**Treatment history**	**Gender**	**Age**	**gyrA**	**gyrB**
13005	New	Male	34	D94N	WT
13137	New	Female	50	D94G	WT
13139	New	Male	60	S91P	WT
13245	New	Male	50	D94N	WT
13497	New	Female	78	S91P	WT
13523	New	Male	69	D94G	WT
13634	New	Male	57	A90V	WT
14015	New	Male	44	D94A	WT
14036	Previously treated	Male	28	D94G	WT
14044	New	Female	54	A90V	WT
14095	New	Male	33	D94N	WT
14179	Previously treated	Male	30	S91P	WT
14374	New	Female	38	D94N	WT
14399	New	Female	29	D94N	WT
14448	New	Male	31	D94A	WT
14466	New	Male	51	D94N	WT
14476	New	Male	45	A90V	WT
14603	New	Male	22	A90V	WT
14823	New	Female	57	D94A	WT
14824	New	Male	58	D94A	WT
14871	New	Male	27	D94A	WT
15043	New	Female	38	D94N	WT
15151	New	Male	82	S91P	WT
15177	New	Female	31	D94G	WT
15182	New	Male	74	A90V	WT
15196	New	Female	25	D94A	WT
15214	New	Male	67	S91P	WT
15219	New	Male	44	D94Y	WT
15426	Previously treated	Male	89	D94G	WT
15457	New	Male	56	D94Y	WT
15786	Previously treated	Male	54	D94G	WT
15369	New	Male	55	WT	T539A
15539	Previously treated	Female	81	WT	E540D
13051	New	Male	61	WT	WT
13356	New	Male	83	WT	WT
13422	New	Male	26	WT	WT
13520	Previously treated	Male	45	WT	WT
13639	New	Male	56	WT	WT
13659	New	Male	66	WT	WT
14096	New	Female	60	WT	WT
14119	New	Male	74	WT	WT
14436	New	Male	55	WT	WT
14739	New	Female	28	WT	WT
14867	New	Male	71	WT	WT

(Continued)

TABLE 1 (Continued)

Patient number	Treatment history	Gender	Age	Mutations in gyrA/gyrB in isolates from patients	
				gyrA	gyrB
15134	Previously treated	Male	41	WT	WT
15297	New	Male	55	WT	WT
15404	New	Male	28	WT	WT
15470	Previously treated	Male	53	WT	WT
15489	New	Female	29	WT	WT
15670	New	Male	62	WT	WT

WT, wild type.

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