

Original Article



Prevalence and Risk Factors of Primary Drug-Resistant Tuberculosis in China*

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Abstract

Objective To investigate the prevalence of primary drug-resistant tuberculosis (TB) and associated risk factors in China. We also explored factors contributing to the transmission of multidrug-resistant tuberculosis (MDR-TB).

Methods A total of 2794 representative, *Mycobacterium tuberculosis* isolates from treatment-naïve patients were subjected to drug susceptibility testing, and risk factors for drug-resistant TB were analyzed. We also analyzed MDR-TB strain sublineages, drug-resistance-conferring mutations, and risk factors associated with clustered primary MDR strains.

Results Among 2794 *Mycobacterium tuberculosis* isolates from treatment-naïve patients, the prevalence of any resistance to first-line drugs was 33.2% and the prevalence of MDR-TB was 5.7%. We did not find any risk factors significantly associated with resistance to first-line drugs. The 93 primary MDR-TB isolates were classified into six sublineages, of which, 75 (80.6%) isolates were the RD105-deleted Beijing lineage. The largest sublineage included 65 (69.9%) isolates with concurrent deletions of RD105, RD207, and RD181. Twenty-nine (31.2%) primary MDR strains grouped in clusters; MDR isolates in clusters were more likely to have S531L *rpoB* mutation.

Conclusion This study indicates that primary drug-resistant TB and MDR-TB strains are prevalent in China, and multiple measures should be taken to address drug-resistant TB.

Key words: *Mycobacterium tuberculosis*; Primary drug resistance; Multi-drug resistant tuberculosis

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INTRODUCTION

Drug-resistant tuberculosis (TB) is an increasing health problem worldwide, with China being one of the countries most burdened with drug-resistant TB. In 2007, it was estimated that in China there were 110,000 incident cases of multidrug-resistant tuberculosis (MDR-TB) and 8200 incidence cases of extensively drug-resistant (XDR) TB^[1]. Generally, people consider

the main causes of MDR-TB to be poor patient management, non-adherence to the prescribed regimen, a substandard national program, or some combination of these three. Successful treatment of TB has benefits for the patient and reduces the drug-resistant TB burden of a country. It is recommended that the treatment of drug-resistant TB should be guided by drug susceptibility test (DST) results^[2]. In China, according to current guidelines, culture and DST for *Mycobacterium tuberculosis* (*M.*

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tuberculosis) are recommended only in specific patients considered to be at risk for drug-resistant TB^[3]. Access to culture and DST for diagnosis of drug-resistant TB in new TB cases remains unavailable in many areas of China, and new patients with pulmonary TB are often treated empirically with standardized short-course chemotherapy regimen^[4].

Previous studies covering part areas of China showed that some patients had primary drug-resistant TB and the prevalence of such resistance among the Chinese population has shown an upward trend^[5-6]. Reports from previous studies indicated patients with initial drug resistance who were treated with only standardized regimens showed incidences of failure, relapse, and acquired drug resistance were substantially increased^[7-8]. According to Guidelines of World Health Organization^[9], some Chinese provinces carried out surveys on drug resistance in tuberculosis patients-the frequency of drug resistance varies greatly across different regions. In addition, no studies using standardized methodology have been performed to evaluate primary drug resistance in new cases. Therefore, the objective of the present study was to investigate prevalence of primary drug-resistant TB and to contribute new data on the transmission of primary drug-resistant TB in China.

MATERIALS AND METHODS

Study Objects and Data Collection

We performed this study using representative *M. tuberculosis* strains isolated from a national survey of drug-resistant TB in China^[1]. During the survey, data on social and demographic characteristics, clinical characteristics, exposure to TB, and TB treatment history were collected from each of the newly diagnosed, smear-positive TB patients through a standard questionnaire. The patient's treatment history was based on patient interview and validated by review of the patient's medical record. Patients who had no history of TB treatment or evidence of TB treatment on medical record were included into this study. Patients who had been treated for TB, or there was evidence of TB treatment on their medical records were excluded from this study.

Ethics Statement

The study protocol was approved by the Ethical Review Committees of Chinese Center for Disease

Control and Prevention. Written informed consent was obtained from the study participants.

Drug Resistance Testing and Molecular Fingerprinting

The smear-positive specimens were cultured and subjected to identification of *M. tuberculosis* by *p*-nitrobenzoic acid method. Susceptibility to rifampicin (RFP), isoniazid (INH), streptomycin (SM), ethambutol (EMB), kanamycin (KM) and ofloxacin (OFX) was performed on Löwenstein-Jensen (L-J) medium, according to WHO standard proportional method. DNA specimens were extracted from strains, and all isolates were characterized by spoligotyping according to standard methods^[10].

Sequencing of Drug Resistance Genes

DNA from primary MDR-TB isolates was amplified and sequenced for the drug resistance-determining regions of *rpoB* and *katG* using primers *rpoB* F (5'-TACGGTCGGCGA GCTGATCC-3') and *rpoB* R (5'-TACGGCGTT TCGATGAACC-3'), *katG* F (5'-AACGACGTCGA AACAGCGGC-3'), and *katG* R (5'-GCGAACTCGTCG GCCAATTC-3'). Sequences were compared with published sequences for *M. tuberculosis* H37Rv by using DNASTar and BioEdit software.

Determination of Strain Lineages and Clusters

Multiplex PCRs were performed to identify lineages of the primary MDR-TB strains as reported previously^[11-12], and the lineages were defined based on the presence or absence of the region of difference (RD) 105, RD181, RD207, RD150, and RD142. Isolates were considered to belong to the same cluster if the strains had the same phenotypic drug resistance profile, the same *M. tuberculosis* lineage, and the same drug resistance-conferring mutations. Isolates were considered unique if any of these criteria were not met. Cluster analysis was performed by BioNumerics Software (Version 5.1, Belgium).

Statistical Analysis

To explore the relationship between drug resistance or strain cluster with demographic and bacterial variables, univariate analysis using Chi-square test or Fisher's exact test, odds ratios (OR) were calculated with 95% confidence intervals (95% CI). Continuous variables were compared using the Student's *t*-test or non-parametric test. Differences

were considered as significant if $P < 0.05$. Statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, USA).

RESULTS

Characteristics of Study Objects and Drug Resistance Profiles

During the TB baseline survey, 3037 new smear-positive cases of tuberculosis were enrolled, of whom 240 patients had taken anti-tuberculosis drugs for less than one month and 2797 new cases had not reported treatment history for TB (3 cases were excluded as they did have treatment history for TB on their medical records). Ultimately, 2794 new cases with no history of using anti-tuberculosis drugs were included into this study. There were 1937 males (69.3%) and 857 females (30.7%), with the age of the patients ranged from 14 to 93 years and a median age of 44.46 (± 18.9) years.

Table 1 shows the profile of drug resistance in the patients. Among the 2794 new TB cases studied,

1867 (66.8%) excreted bacilli that were susceptible to all four first-line drugs, and 927 (33.2%) were resistance to any first-line drugs. When each drug was considered separately, the highest rates of any resistance to first-line drugs were observed for Streptomycin (26.8%) and Isoniazid (15.7%), followed by Rifampin (6.6%) and Ethambutol (5.0%). Highest monoresistance was observed against Streptomycin (14.6%), followed by Isoniazid (3.7%). One hundred and sixty (5.7%) new cases were infected by MDR strains.

Among strains tested in this study, 2679 (95.9%) were susceptible to the second-line drugs Ofloxacin and Kanamycin. Any resistance to Ofloxacin, Kanamycin was 73 (2.6%) and 55 (2.0%), respectively, compared with 17 (0.6%) and 7 (0.3%) for monoresistance to Ofloxacin and Kanamycin, respectively. Prevalence of XDR TB was 0.4%.

Of 2744 strains with available spoligotyping results, 1748 (63.7%) belonged to Beijing genotype, and for 158 spoligotyped MDR strains, 93 (58.9%) were Beijing genotype and 65 (41.1%) were non-Beijing genotype strains.

Table 1. Prevalence of Primary Drug Resistant Clinical Strains of *M. tuberculosis* Isolated in China from 2007-2008

Pattern	N	Prevalence % (95% CI)
First-line drugs		
Susceptibility to all four first-line drugs	1867	66.8 (65.0-68.6)
Resistance to first-line drugs		
Any resistance to first-line drugs	927	33.2 (31.4-35.0)
INH	438	15.7 (14.4-17.1)
RFP	185	6.6 (5.7-7.6)
EMB	140	5.0 (4.2-5.9)
SM	748	26.8 (25.1-28.5)
Monoresistance to first-line drugs		
INH	104	3.7 (3.1-4.5)
RFP	15	0.5 (0.3-0.9)
EMB	16	0.6 (0.3-1.0)
SM	408	14.6 (13.3-16.0)
Multidrug resistance	160	5.7 (4.9-6.7)
INH+RFP	17	0.6 (0.4-1.0)
INH+RFP+EMB	19	0.7 (0.4-1.1)
INH+RFP+SM	59	2.1 (1.6-2.7)
INH+RFP+EMB+SM	65	2.3 (1.8-3.0)
Second -line drugs		
Susceptibility to OFX and kM	2679	95.9 (95.1-96.6)
Any resistance to second -line drugs		
OFX	73	2.6 (2.1-3.3)
kM	55	2.0 (1.5-2.6)
Monoresistance to second -line drugs		
OFX	17	0.6 (0.4-1.0)
kM	7	0.3 (0.1-0.5)
Extensive drug resistance	12	0.4 (0.2-0.8)

Risk Factors for Primary Drug-Resistant TB

Table 2 shows an analysis of the variables potentially associated with drug resistance in this study. None of the variables studied were found to be statistically associated with TB patients with any resistance to first-line drugs. Female patients were more likely to have primary MDR-TB (OR=1.64, 95% CI 1.18-2.27).

Strain Lineage and Drug Resistance-conferring Mutations of Primary MDR-TB

By screening lineage-specific genetic markers, 93 primary MDR-TB strains were classified into six sublineages. 75 (80.6%) of the strains were RD105-deleted East Asian or Beijing lineage, of which, 65 (86.7%) isolates were in sublineage 1 with concurrent deletions of RD105, RD207, and RD181. No significant difference was observed between lineages with respect to the associations with S315T *KatG* and S531L *rpoB* mutations, (Fisher's 2-tailed exact

test: $P=0.802$, $P=1.000$, respectively), and there was no significant difference between lineages and age ($F=0.678$, $P=0.642$) or sex (Fisher's 2-tailed exact test: $P=0.306$). (Table 3).

Risk Factors for Clustering Primary MDR-TB

As seen in Table 4 and Figure 1, clustering analysis revealed the presence of ten clusters (5, 5, 4, 3, 2, 2, 2, 2, 2, 2) in 93 MDR strains, where a total of 29 (31.2%) patients were included over the ten clusters. In sublineage 1, there were three larger clusters with S315T *KatG* and S531L *rpoB* mutations and similar drug resistance profiles, except for ethambutol or streptomycin resistance. Risk factors associated with patients whether or not they were grouped in clusters revealed that those patients in clusters were more likely to have the S531L *rpoB* mutation. We did not find any other factors such as age, sex, ethnic, contact history or S315T *KatG* associated with clustering.

Table 2. Univariable Analysis for Risk Factors for Primary Drug Resistant TB and MDR-TB

Variables	N	Sensitive to 1 st Line Drugs [n (%)]	Any Resistance to 1 st Line Drugs [n (%)]	Crude OR (95% CI)	Non MDR [n (%)]	MDR [n (%)]	Crude OR (95% CI)
Age							
<30	828	561 (67.8)	267 (32.2)	1.0	776 (93.7)	52 (6.3)	1.0
30-	1296	834 (64.4)	462 (35.6)	1.16 (0.97-1.40)	1218 (94.0)	78 (6.0)	0.96 (0.67-1.37)
60-	670	472 (70.4)	198 (29.6)	0.88 (0.71-1.10)	640 (95.5)	30 (4.5)	0.70 (0.44-1.11)
Sex							
Male	1937	1297 (67.0)	640 (33.0)	1.0	1843 (95.1)	94 (4.9)	1.0
Female	857	570 (66.5)	287 (33.5)	1.02 (0.86-1.21)	791 (92.3)	66 (7.7)	1.64 (1.18-2.27)
Ethnic group							
Han	2500	1666 (66.6)	834 (33.4)	1.0	2359 (94.4)	141 (5.6)	1.0
Other	293	200 (68.3)	93 (31.7)	0.93 (0.72-1.20)	274 (93.5)	19 (6.5)	1.16 (0.71-1.90)
TB contact [*]							
No	2527	1695 (67.1)	832 (32.9)	1.0	2385 (94.4)	142 (5.6)	1.0
Yes	262	168 (64.1)	94 (35.9)	1.14 (0.87-1.49)	244 (93.1)	18 (6.9)	1.24 (0.75-2.06)
TB contact ^{**}							
No	2077	1382 (66.5)	695 (33.5)	1.0	1963 (94.5)	114 (5.5)	1.0
Yes	265	180 (67.9)	85 (32.1)	0.94 (0.71-1.23)	245 (92.5)	20 (7.5)	1.41 (0.86-2.30)
genotype							
Non Beijing	996	666 (66.9)	330 (33.1)	1.0	931 (93.5)	65 (6.5)	1.0
Beijing	1748	1164 (66.6)	584 (33.4)	1.01 (0.86-1.19)	1655 (94.7)	93 (5.3)	0.80 (0.58-1.11)

Note. * Patient state having been in contact with a person with active TB who is his/her family member.
 ** Patient state having been in contact with a person with active TB who is his/her classmate, neighbor or colleague.

DISCUSSION

We found that the prevalence of primary drug-resistant TB was high in China and 5.7% of the patients were primary MDR-TB. Previous studies from Jiangsu, Shanghai China also indicated a high prevalence of primary MDR-TB^[13-14]. Additional

findings show transmission of drug-resistant TB strains among the general population in China^[15]. The rate of primary MDR-TB from this study was comparable to reports from Peru^[16] and Vietnam^[17], lower than some reports from India, Russian Federation and Saudi Arabia^[18-21], and higher than that of Brazil and Ethiopia^[22-24].

Table 3. Characteristics of Sublineages and Resistance Mutations Found in 93 Primary MDR-TB Patients in China

Sublineage	Isolated Strains N (%)	Age (mean±SD)	Male (%)	katG Mutation			rpoB Mutation		
				S315T	Other Mutation	No Mutation	S531L	Other Mutation	No Mutation
1	65 (69.9)	42.83±17.08	39 (60.0)	35 (53.8)	4 (6.2)	26 (40.0)	33 (50.8)	25 (38.4)	7 (10.8)
2	6 (6.5)	49.33±26.35	1 (16.7)	4 (66.7)	0	2 (33.3)	3 (50.0)	2 (33.3)	1 (16.7)
3	4 (4.3)	39.75±16.26	3 (75.0)	3 (75.0)	0	1 (25.0)	2 (50.0)	2 (50.0)	0
4	11 (11.8)	45.82±17.08	5 (45.5)	4 (36.4)	0	7 (63.6)	5 (45.5)	5 (45.5)	1 (9.1)
5	5 (5.4)	35.80±23.66	2 (40.0)	3 (60.0)	0	2 (40.0)	2 (40.0)	2 (40.0)	1 (20.0)
6	2 (2.2)	58.50±4.95	1 (50.0)	1 (50.0)	0	1 (50.0)	1 (50.0)	1 (50.0)	0

Note. * Sublineage 1: RD105(-),RD207(-),RD181(-),RD150(+),RD142(+); Sublineage 2: RD105(-),RD207(-),RD181(-),RD150(-),RD142(+); Sublineage 3: RD105(-),RD207(-),RD181(+),RD150(+),RD142(+); Sublineage 4: RD105(+),RD207(+),RD181(+),RD150(+),RD142(+); Sublineage 5: RD105(+),RD207(-),RD181(+),RD150(+),RD142(+); Sublineage 6: RD105(+),RD207(-),RD181(-),RD150(+),RD142(+).

Table 4. Univariable Analysis for Risk Factors for Grouping in Cluster of Primary MDR-TB

Characteristics	Grouped in Cluster		P	OR 95% CI
	Yes (n=29) n (%)	No (n=64) n (%)		
Age (years), mean±SD	45.2±16.9	42.6±18.3	0.512	
Sex				
Female	11 (26.2)	31 (73.8)	0.651	0.649 (0.266-1.594)
Male	18 (35.3)	33 (64.7)		
Ethnic group				
Han	25 (29.8)	59 (70.2)	0.291	0.530 (0.131-2.138)
Others	4 (44.4)	5 (55.6)		
TB contact				
Yes	7 (36.8)	12 (63.2)	0.551	1.379 (0.479-3.968)
No	22 (29.7)	52 (70.3)		
katG mutation				
S315T	19 (38.0)	31 (62.0)	0.126	2.023 (0.815-5.021)
Other or no mutation	10 (23.3)	33 (76.7)		
rpoB mutation				
S531L	23 (50.0)	23 (50.0)	0.000	6.833 (2.431-19.206)
Other or no mutation	6 (12.8)	41 (87.2)		

At present, the China National TB control program implement DOTS (directly observed therapy, short course) strategy, and standardized, short-course chemotherapy is recommended for patients with previously untreated tuberculosis^[4]. Considering the high prevalence of primary drug-resistant TB among new smear-positive cases, standardized short-course chemotherapy may result in inadequate treatment, generating MDR-TB, which in turn increases the transmission of drug resistant TB strains^[25-26].

According to data from national drug resistant TB baseline survey^[1], the detected total number of cases who are resistant to first-line drug and MDR were 1503 and 401, respectively, and among new smear-positive, untreated cases, the counterpart number of cases was 927 and 160, respectively. If these new cases cannot access DST test, more than 60% of drug-resistant TB and 40% of MDR-TB patients will not be correctly diagnosed. The results here are in accordance with the results of the previous study^[27]. Therefore, in conclusion to resulting data of this study, DST tests should be used to screen all new smear-positive TB cases.

In this study, 31.2% of the primary MDR-TB grouped in clusters. None of the factors showed statistically significant association with clustering patients except the S531L *rpoB* mutation. The S531L *rpoB* mutation is the most prevalent rifampin resistance-conferring mutation in clinical strains. For these clinical isolates, it was suggested that the *rpoB* S531L mutation incurred low or no fitness cost in these particular strains' genetic backgrounds^[28]. Another study indicated that secondary mutations were acquired that mitigate the initial low fitness cost of the *rpoB* S531L mutation and contributed to the spread of drug resistance strains^[29].

The study has some limitation: only patients attending clinics of tuberculosis prevention and control system were included, although most of the TB patients were treated in this system in the survey period, TB cases that were treated in hospital were not included in this study, and this study may promote a selection bias. Despite this limitation, this study investigated a large number of TB isolates representative of the tuberculosis prevention and control system of the country, and developed profile of the status of primary drug-resistance in China.

In summary, this study provides evidence that prevalence of primary drug-resistant TB and MDR-TB was high in China, and drug-resistant *M. tuberculosis* strains had disseminated across general populations.

These findings emphasize that all new smear-positive TB cases should be screened with DST to identify drug-resistant TB and MDR-TB, and, if possible, rapid DST diagnostics should be implemented to reduce transmission of drug-resistant TB. The findings of this study consolidate that infection control should be considered as priority strategy to address drug-resistant TB in China.

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