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-naive 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-naive 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

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.*

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