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Identification of mutations conferring streptomycin resistance in multidrug-resistant tuberculosis of China

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ABSTRACT

We investigated the spectrum and frequency of mutations in *rpsL*, *rrs*, and *gidB* among 140 multidrug-resistant tuberculosis (MDR-TB) clinical isolates from China. The association between mutations and different genotypes was also analyzed. Our data revealed that 65.7% of MDR-TB were resistant to streptomycin (STR), and 90.2% of STR-resistant isolates were Beijing strains. STR resistance was correlated with Beijing family ($P = 0.00$). Compared with phenotypic data, detection of mutations for the combination of these 3 genes exhibited 94.6% sensitivity, 91.7% specificity, and 93.6% accuracy. The most common mutations in STR-resistant isolates were *rpsL*128, 262, and *rrs*514, of which *rpsL*128 showed association with Beijing lineage ($P = 0.00$). A combination of these 3 mutations can serve as the reliable predictors for STR resistance, showing the sensitivity, specificity, and accuracy of 85.9%, 97.9%, and 90.0%, respectively. Furthermore, *gidB*A276C, not A615G, was Beijing lineage specific. These findings are useful to develop rapid molecular diagnostic methods for STR resistance in China.

Aminoglycoside antibiotic streptomycin (STR) was the first antibiotic used to control tuberculosis (TB) in 1940s. Since then, it has become the drug of first choice for all forms of TB. However, the high rates of STR resistance and the risk of toxicity including serious neurotoxic reactions contribute to a gradual decrease in the use of STR. In the current World Health Organization (WHO) guidelines, STR is no longer included in the standard treatment regiment for drug-susceptible TB (WHO, 2009). Nevertheless, STR continues to be a component of the retreatment regiments. Also, it is be used to treat the drug-resistant TB cases yet susceptible to aminoglycosides (WHO, 2009).

The mechanism of STR action involves binding to the S12 ribosomal protein (*rpsL*) and 16S rRNA (*rrs*) disrupting protein translation (Honore and Cole, 1994; Meier et al., 1994). Mutations in *rpsL* or *rrs* have been associated with high or intermediate level of resistance (Meier et al., 1996). The most prevalent changes are located in *rpsL* codons 43 and 88 as well as in 2 specific regions, the 530 loop and the 912 loop, of *rrs* (Jagielski et al., 2014; Jnawali et al., 2013). Moreover, mutations in the ribosome methyltransferase (*gidB*) have been correlated with a low level of STR resistance (Wong et al., 2011). Screening for mutations in these 3 genes is the most promising approach for rapid and reliable diagnosis of STR-resistant TB. However, prior publications showed that the type and frequency of resistance-associated mutations varied in different geographical region (Feuerriegel et al., 2012; Jagielski et al., 2014; Jnawali et al., 2013).

In China, STR resistance in TB is a very serious problem. According to the latest data, approximately 27.2% and 37.2% of new and previously treated cases, respectively, were resistant to STR (Zhao et al., 2012). Furthermore, among multidrug-resistant tuberculosis (MDR-TB) of some regions of China, the incidences of STR resistance even reached substantial levels (37.3–87.0%) (Chen et al., 2014; Shi et al., 2011; Xia et al., 2015). The aim of present study was to explore the spectrum and frequency of mutations in the *rpsL*, *rrs*, and *gidB* genes among MDR-TB clinical isolates from China. The frequencies of mutations were also analyzed according to different TB strain genotypes.

1. Materials and methods

1.1. Ethical approval

The study obtained approval from the Ethics Committee of National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The patients with TB included in the present research were given a subject information sheet, and they all gave written informed consent to participate in the study.

1.2. Mycobacterium tuberculosis isolates

In total, 140 MDR-TB samples were randomly selected from all identified MDR-TB clinical isolates stored in the *M. tuberculosis* bank of the National Reference Laboratory of Tuberculosis (Beijing, China) over a

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3-year period from 2008 to 2010. These isolates were recovered from 140 individual patients (97 males and 43 females; age range: 16–83 years; median age: 42.0 years; 55 new patients and 85 previously treated patients) with pulmonary tuberculosis living in 9 provinces (Fujian, Guangdong, Guizhou, Hunan, Liaoning, Beijing, Shaanxi, Shanghai, and Xizang) of China. No epidemiological connection of these patients could be detected. H37Rv (ATCC 27294) was used as a reference.

1.3. Drug susceptibility testing

Drug susceptibility testing was performed with Lowenstein-Jensen (L-J) proportion method (PM) (WHO, 2008). The critical drug concentrations for L-J PM were 0.2 μg/mL for isoniazid, 40 μg/mL for rifampin, 4.0 μg/mL for streptomycin (SM), 2.0 μg/mL for ethambutol (EMB) 30 μg/mL for kanamycin (KAN), and 2.0 μg/mL for ofloxacin (OFX). Results were read 28 days after inoculation of the media. H37Rv was used as a control with each batch of drug susceptibility testing.

1.4. DNA extraction

DNA was extracted from *M. tuberculosis* isolates by the conventional cetyltrimethylammonium bromide (CTAB) method (Somerville et al., 2005). The purified DNA was dissolved in Tris-EDTA (TE) buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0) and stored at −20 °C until used.

1.5. Spoligotyping

All isolates were subjected to spoligotyping as previously described (Kamerbeek et al., 1997). The results in binary format were entered in an Excel spreadsheet and compared with the spoligotyping database SpolDB4 (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/index.jsp).

1.6. PCR amplification and sequencing

The fragments of the *M. tuberculosis rpsL* gene (356 bp) and *rrs* gene (696 bp, containing 530 loop and 912 region) were amplified as described by our previous study (Zhao et al., 2014). A 785-bp fragment containing the complete open reading region of *gidB* was amplified with primers gidB-F (GGAGTGCGTAATGTCTCC) and gidB-R (GTCGGTGGTGTCATTTCC) using the following steps: initial denaturation at 95 °C for 5 min, followed by 31 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were sent for sequencing. All sequencing data were aligned with the corresponding sequences of published H37Rv data (GenBank accession number NC_000962) using BioEdit version 7.05.3.

1.7. Statistical analysis

All data were analyzed with SPSS 16.0 software (SPSS Inc., Chicago, IL). Percentages, confidence intervals (CIs), and odds ratio (OR) value were calculated as appropriate. Chi square test was used for comparison. Difference was considered statistically significant at a P value of <0.05.

2. Results

2.1. Drug-resistant profiles and genotypes

Among 140 MDR-TB isolates, 92 (65.7%; 92/140 isolates) were resistant to STR, 69 (49.3%; 69/140 isolates) were resistant to EMB, 15 (10.7%; 15/140 isolates) were resistant to KAN, 51 (36.4%; 51/140 isolates) were resistant to OFX, and 12 (8.6%; 12/140 isolates) were extensively drug-resistant tuberculosis (XDR-TB) isolates. Beijing family (115 isolates; 82.1%) was the most common in all MDR-TB isolates, followed by T (13 isolates; 9.3%), Orphan (7 isolates; 5.0%), H (3 isolates; 2.1%), the Central Asian (CAS) (1 isolates; 0.7%), and MANU2 (1 isolates; 0.7%). The majority of STR-resistant isolates (90.2%; 83/92 isolates) also belonged to Beijing family. There was a significant association between STR resistance and Beijing family ($P = 0.00$; OR = 4.61, 95% CI [1.85–11.49]).

2.2. Mutations within rpsL

Two different mutated types within *rpsL*, A128G (Lys43Arg) and A262C (Lys88Arg), were observed among 140 MDR-TB isolates. A128G mutation was more prevalent and occurred in 54 (58.7%; 54/92 isolates) STR-resistant isolates, while A262C were identified in 18 STR-resistant isolates (19.6%; 18/92 isolates) and 1 STR-susceptible isolate (2.1%; 1/47 isolates).

2.3. Mutations within rrs

The *rrs* mutations were of 4 types, with A514C, C517T, A554T, and G887T, respectively. Among them, A514C, C517T, and G887T occurred exclusively in STR-resistant isolates, whereas A554T occurred in the STR-susceptible isolate. A514C was the most common mutations in *rrs* gene (7 isolates).

2.4. Mutations within gidB

For *gidB*, both A276C (Glu92Asp) and A615G (Ala205Ala) mutations were present in 115 MDR-TB isolates, including 83 STR-resistant isolates and 32 STR-susceptible isolates. Spoligotyping results indicated that all mutated isolates were Beijing strains. Also, 1 CAS (STR-resistant) strain harbored *gidB*A615G mutation. Thus, A276C and A615G mutations were unlikely involved in STR resistance and not included in Tables 1–3. Moreover, there were 11 MDR-TB isolates that had 9 mutations, with G83A (Gly28Glu), G102del, T176C (Leu59Pro), G178A (Glu60stop), C256T (Leu86Phe), T344G (Val115Gly), C437A (Thr146Lys), C460T (Arg154Trp), and T605C (Val202Ala). The detailed summary of mutations was listed in Table 1. Of them, G102del was detected in both STR-resistant and STR-susceptible isolates. Other mutations consisting of T176C, G178A, T344G, and C460T mutations occurred in STR-resistant isolates, while the remaining Arg738Gln replacement occurred in the STR-susceptible isolate. Interestingly, 6 STR-resistant isolates contained only *gidB* mutations with no additional mutations within *rpsL* or *rrs* (Table 1).

Table 1
Mutations in the *rpsL*, *rrs*, and *gidB* among 140 MDR-TB isolates.

Mutations					No. of isolates	
rpsL		*rrs* DNA	*gidB*		MDR-TB	
DNA	Protein		DNA	Protein	SM-R	SM-S
A128G	Lys43Arg		T605C[a]	Val202Ala	1	0
A128G	Lys43Arg				53	0
A262C	Lys88Arg				18	1
		A514C			7	0
		C517T	G83A[a]	Gly28Glu	1	0
		A554T[a]			0	1
		G887T[a]	C437A[a]	Thr146Lys	1	0
			G102del	34 frameshift	2	1
			T176C[a]	Leu59Pro	1	0
			G178A[a]	Glu60stop	1	0
			C256T[a]	Leu86Phe	0	1
			T344G	Val115Gly	1	0
			C460T[a]	Arg154Trp	1	0
NM	NM	NM	NM	NM	5	44

NM = no mutation.
[a] The mutation was not previously reported.