

## Original Article

# Spoligotyping and drug-resistance of *Mycobacterium tuberculosis* strains from Shanxi province, China

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**Abstract:** To understand the genotyping characteristics and drug resistant status of *Mycobacterium tuberculosis* (*M. tuberculosis*) circulating in Shanxi province, China. Clinical *M. tuberculosis* isolates were collected from Jan., 2012 to Dec., 2015. The spacer-oligonucleotide typing (Spoligotyping) was performed for the genotyping of the isolates. Drug susceptibility test (DST) was utilized by the proportion method on the Lowenstein-Jensen (L-J) medium using isoniazid, rifampicin, ethambutol, streptomycin, kanamycin, protionamide, p-aminosalicylic acid and Levofloxacin. By means of Spoligotyping, a total of 293 clinical *M. tuberculosis* isolates were differentiated into 10 clusters, in which Beijing family was the most prevalent genotype (82.59%). The DST results showed that 48 (16.38%) strains were MDR strains. There was a difference in the kanamycin resistance between Beijing genotype and non-Beijing genotype strains. We concluded that spoligotyping as the genotyping in our study was applicable for this region and the drug resistant isolates were identified, and Beijing family is the most prevalent genotype in the cities of Shanxi. Meanwhile, Beijing genotyping isolates were more resistant than others. These results prompt for the need to strengthen the current tuberculosis control strategies and surveillance programs.

**Keywords:** *Mycobacterium tuberculosis*, Spoligotyping, genetic diversity, drug resistance

## Introduction

Tuberculosis (TB) remains a major cause of global public health problem, mainly in developing countries [1]. Of 22 high TB burden countries, China has the third, just behind India and Indonesia, and 6.1 million new cases in 2015 [1]. In China, approximately 80% of TB cases are in the rural areas [2]. Recently, the transmission of drug resistant TB, especially the multi-drug resistant (MDR) TB has been a serious matter in China [3].

Shanxi is the inland province, which is located in the middle-east coast of the Yellow River and in the west of the North China Plain. It is in the east of Taihang Mountain, in the west of Hebei, in the south of Shaanxi and across the Yellow River, and adjacent to Inner Mongolia in the north. There are 156 thousand and 700 square kilometers in the total area.

The genotyping of *Mycobacterium tuberculosis* (*M. tuberculosis*) is important for TB control because it allows the detection of suspected outbreaks and the tracing of transmission chains. It is also important to monitor species diversity, as well as to identify secondary infections [4-7]. The genetic markers for genotyping of *M. tuberculosis* should have the genetic stability and the diversity of individuals.

Among the genotyping tools, spacer-oligonucleotide typing (Spoligotyping) is an ideal typing method based on PCR [8] and is the golden standard to identify strains belonging to the Beijing family [9] with the absence of spacers 1-34 in the direct repeat (DR) region of the *M. tuberculosis* genome [8]. In this research, we collected the clinical *M. tuberculosis* isolates for investigating the genotyping characteristics and drug resistant status of *M. tuberculosis* cir-



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**Table 2.** First-line drug resistant frequency among 293 clinical *M. tuberculosis* strains

Drugs	No. (%) of isolates		X <sup>2</sup>	P
	Beijing (n=138)	non-Beijing (n=26)		
Monodrug resistance				
RFP	71 (51.45)	12 (46.15)	0.245	>0.05
INH	94 (68.12)	17 (65.38)	0.075	>0.05
EMB	32 (23.19)	5 (19.23)	0.196	>0.05
SM	105 (76.09)	20 (76.92)	0.008	>0.05
Two-drug resistance				
INH+RFP	42 (30.43)	6 (23.08)	0.572	>0.05
INH+EMB	9 (6.52)	0 (0)		>0.05 <sup>1</sup>
INH+SM	59 (42.75)	8 (30.77)	1.300	>0.05
RFP+SM	42 (30.43)	7 (26.92)	0.129	>0.05
Three-drug resistance				
INH+RFP+EMB	2 (1.45)	0 (0)		>0.05 <sup>1</sup>
INH+RFP+SM	36 (26.09)	6 (23.08)	0.104	>0.05
INH+EMB+SM	2 (1.45)	0 (0)		>0.05 <sup>1</sup>
RFP+EMB+SM	1 (0.72)	0 (0)		>0.05 <sup>1</sup>
Four-drug resistance				
INH+RFP+EMB+SM	20 (14.49)	4 (15.38)		>0.05 <sup>1</sup>

Note. RFP: rifampicin; INH: isoniazid; SM: streptomycin; EMB: ethambutol; 1: Fisher exact probability.

(L-J) medium. One loopful of mycobacterial colonies was resuspended in 200 µL TE buffer (10 mM Tris-Cl, 1 mM EDTA) and was incubated at 80°C for 30 min, using the CTAB (cetyltrimethylammonium bromide)-NaCl method as described previously [11]. Finally, DNA was stored at -20°C for the further use.

### Spoligotyping

Spoligotyping was performed according previously described standard protocol by Kamerbeek et al [8]. Firstly, the direct repeat (DR) region was amplified by the primers DRa and DRb. And then, the PCR products were hybridized to a group of 43 oligonucleotide probes, which were covalently bound to a membrane [8]. Lastly, the Spoligotypes were received by binary format with the SpolDB4.0 database (<http://www.pasteur-guadeloupe.fr:8081/SITVITD>).

### Drug susceptible test (DST)

As for the DST, the L-J medium was impregnated with isoniazid (INH), rifampicin (RFP), ethambutol (EMB), streptomycin (SM), kanamycin (KN), prothionamide (PTH), p-aminosalicylic acid (PAS) and Levofloxacin (LFX) in conforming

to the proportional technique as recommended by World Health Organization (WHO) and Clinical and Laboratory Standards Institute (CLSI) [12]. The concentrations of the drugs INH, RFP, EMB, SM, KN, PTH, PAS and LFX in the media were 0.2 µg/ml, 40.0 µg/ml, 2 µg/ml, 4 µg/ml, 30.0 µg/ml, 40.0 µg/ml, 1.0 µg/ml and 4.0 µg/ml respectively. If the microorganism growing on the particular drug medium was ≥1% or <1% compared to that growing on the control culture, the strain was detected as resistant or sensitive respectively. The standard laboratory strain H37Rv was enclosed for every time. X<sup>2</sup> test and Fisher exact probability were used for analyzing two or more samples within SPSS 17.0 software. The differences were based on α=0.05.

## Results

### Spoligotyping

Spoligotyping results clustering analysis showed that 293 strains could be differentiated into 10 clusters, in which 242 (82.59%) were clustered into Beijing family including typical Beijing genotype (240, 81.91%) and Beijing-like genotype (2, 0.68%), 23 (7.85%) were T family, 1 (0.34%) were U genotype, 2 (0.68%) were MANU2 genotype, 1 (0.34%) was H3, and 24 strains were not identified in the SpolDB4.0 database and referred to 19 'new' genotypes (Table 1).

### The results of the DST

The DST results showed that the proportions of single-drug resistance were 28.33% (83/293) for RFP, 34.47% (101/293) for INH, 3.41% (10/293) for EMB and 42.66% (125/293) for SM. Among all isolates, 48 (16.38%) strains were multi-drug resistant tuberculosis (MDR-TB) including 24 (8.19%) strains resistant to all four first-line drugs. Among the second-line drugs, the proportions of single-drug resistance were 6.83% (20/293) for KN, 3.41% (10/293) for PTH, 13.31% (39/293) for PAS and 17.75% (52/293) for LFX. Among all isolates, one strain was resistant to all the tested antimicrobial agents. Kanamycin resistance rate was the significant difference between Beijing genotype and non-Beijing genotype strains. There was no

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**Table 3.** Second-line drug resistant frequency among 293 clinical *M. tuberculosis* strains

Drugs	No.(%) of isolates		X <sup>2</sup>	P
	Beijing (n=138)	non-Beijing (n=26)		
Monodrug resistance				
KN	13 (9.42)	7 (26.92)		<0.05 <sup>1</sup>
PTH	7 (26.92)	3 (11.54)		>0.05 <sup>1</sup>
PAS	33 (23.91)	6 (23.08)	0.008	>0.05
LFX	48 (34.78)	4 (15.38)	3.802	>0.05
Two-drug resistance				
KN+PTH	2 (1.45)	3 (11.54)		<0.05 <sup>1</sup>
KN+PAS	7 (5.02)	5 (19.23)		<0.05 <sup>1</sup>
KN+LFX	7 (5.02)	1 (3.85)		>0.05 <sup>1</sup>
PTH+PAS	4 (2.90)	3 (11.54)		>0.05 <sup>1</sup>
PTH+LFX	5 (3.62)	1 (3.85)		>0.05 <sup>1</sup>
PAS+LFX	9 (6.52)	1 (3.85)		>0.05 <sup>1</sup>
Three-drug resistance				
KN+PTH+PAS	1 (0.72)	3 (11.54)		<0.05 <sup>1</sup>
KN+PAS+LFX	2 (1.45)	1 (3.85)		>0.05 <sup>1</sup>
PTH+PAS+LFX	2 (1.45)	1 (3.85)		>0.05 <sup>1</sup>
Four-drug resistance				
KN+PTH+PAS+LFX	1 (0.72)	1 (3.85)		>0.05 <sup>1</sup>

Note. KN: kanamycin; PTH: prothionamide; PAS: p-aminosalicylic acid; LFX: Levofloxacin; 1: Fisher exact probability.

**Table 4.** Drug resistance comparison of Beijing genotype and non-Beijing genotype strains in the different areas

Area	Beijing typing strains (n=242) <sup>1</sup>		non-Beijing typing strains (n=51) <sup>2</sup>	
	drug-resistant (n=135) (%)	non-drug-resistant (n=107) (%)	drug-resistant (n=24) (%)	non-drug-resistant (n=27) (%)
Changzhi	108 (80.00)	85 (79.44)	19 (79.17)	25 (92.59)
Lucheng	9 (6.67)	2 (1.87)	3 (12.50)	0 (0)
Jincheng	11 (8.15)	6 (5.61)	1 (4.17)	1 (3.70)
Gaoping	7 (5.19)	11 (10.28)	1 (4.17)	1 (3.70)
Linfen	0 (0)	2 (1.87)	0 (0)	0 (0)
Jinzhong	0 (0)	1 (0.93)	0 (0)	0 (0)

Note. 1: P>0.05; 2: P>0.05.

difference in the percentage of Beijing genotypes among different counties (Tables 2-4).

### Discussion

In this study, 293 *M. tuberculosis* strains were genotyped by Spoligotyping [10]. With Spoligotyping, we found that the strains were divided into 10 clusters, and most of the isolates (accounted for 82.59%) were the Beijing family

strains in Shanxi, China. The results supported the view that Beijing strains are predominant in different areas among China and other countries in the world [13-19]. Recent findings showed the association between the geographical origins of the patients' genotypes of *M. tuberculosis* and clinical manifestations of the disease [20-22].

In our study, MDR rate was 16.38% in Shanxi, China. Those data were higher than the average level of all over China [23]. The MDR rates in Heilongjiang, Liaoning, Henan, Inner Mongolia, Shanghai and Xuzhou were 7.2%, 10.4%, 7.8%, 7.0%, 3.0% and 3.14% respectively [14, 16, 19]. It might be strengthened for the prevention and control work on the spread of tuberculosis, especially drug resistant tuberculosis in Shanxi province. Among the single drug resistance, the resistant rate of *M. tuberculosis* to SM is the highest, the following is to INH, RFP. The single EMB-resistance was the least in this study. It is suggested that physicians could use EMB replaced SM and INH in the clinical work. 42 MDR strains were Beijing genotype among all the resistant isolates. It is demonstrated that Beijing family strains could be more virulence and associated with drug resistance. Considering the regions, it was displayed that the resistant isolates and MDR strains were mainly separated from the city of Changzhi. However, the other regions should be increase the samples in order to obtain more data. Consequently, it is essential to enhance the current TB control strategies and surveillance programs in those areas.

Spoligotyping was helpful for distinguishing the Beijing genotype. However, we still need to explore the other better method for identifying the Beijing family.

In the future, it should be studied the association as well as the special mechanism between the genotypes and drug resistance.

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### Disclosure of conflict of interest

None.

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