A Country-Wide Study of Spoligotype and Drug Resistance Characteristics of *Mycobacterium tuberculosis* Isolates from Children in China

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Abstract

**Background:** Tuberculosis (TB) is still a big threat to human health, especially in children. However, an isolation of *Mycobacterium tuberculosis* culture from pediatric cases remains a challenge. In order to provide some scientific basis for children TB control, we investigated the genotyping and drug resistance characteristics of *M. tuberculosis* isolates from pediatric cases in China.

**Methodology/Principal Findings:** In this study, a total of 440 strains including 90 from children (<15 years), 159 from adolescents (15–18 years) and 191 from adults (>18 years) isolated in 25 provinces across China were subjected to spoligotyping and drug susceptibility testing. As a result, Beijing family strains were shown to remain predominant in China (85.6%, 81.1% and 75.4% in three above groups, respectively), especially among new children cases (91.0% vs. 69.6% in previously treated cases, P = 0.03). The prevalence of the Beijing genotype isolates was higher in northern and central China in the total collection (85.1% in northern and 83.9% in central vs. 61.6% in southern China, P < 0.001) and a similar trend was seen in all three age groups (P = 0.708, < 0.001 and 0.025, respectively). In adolescents, the frequencies of isoniazid (INH)-resistant and ethambutol (EMB)-resistant isolates were significantly higher among Beijing strains compared to non-Beijing genotype strains (P = 0.028 for INH and P = 0.027 for EMB). Furthermore, strong association was observed between resistance to rifampicine (RIF), streptomycin (STR) and multidrug resistance (MDR) among Beijing compared to non-Beijing strains in previously treated cases of children (P = 0.01, 0.01 and 0.025, respectively).

**Conclusion/Significance:** Beijing family was more prevalent in northern and central China compared to southern China and these strains were predominant in all age groups. The genetic diversity of *M. tuberculosis* isolates from children was similar to that found in adolescents and adults. Beijing genotype was associated with RIF, STR and MDR resistance in previously treated children.

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Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), still remains a major threat to human health. TB among children is especially important to public health workers since it is an indicator of recent transmission in the community. However, data on pediatric TB is limited and often underesti-mated because of diagnostic challenges. According to WHO tuberculosis report in 2012, there were estimated 0.5 million TB cases and 64,000 deaths among children in 2011 [1]. In the 37 countries providing multidrug-resistant tuberculosis [MDR-TB, characterized by resistance to at least isoniazid (INH) and rifampicine (RIF)] data including children, children represented 1–13% of total enrolments [1]. This was also the first time to include estimates of TB burden among children in the global tuberculosis report.

China is one of the high burden countries, ranking the second of total TB patients globally. However the most recent available data of TB in children came from the fourth national TB survey...
in the media were as following: INH 0.2 µg/mL, RIF 40 µg/mL, EMB 2 µg/mL, and STR 4 µg/mL. The strain was considered resistant to specific drug when the growth rate was more than 1% compared to the control.

Spoligotyping
Total chromosomal DNA of strains was isolated following recommended method described by van Embden et al. [7]. Spoligotyping was performed as described by Kamerbeek et al. [3]. In short, the PCR amplified biotin-labeled DR locus was hybridized against membrane with immobilized 43 different DR spacers using Miniblotter MN45 apparatus (Immunetics, Cambridge, MA, USA). Resulting hybridization signals were revealed by chemiluminescence and visualized as profiles of discrete dots. The spoligoprofiles were entered into Excel spreadsheets and compared with SITVITWEB international spoligotype database [4].

Statistical Analysis
Tests for association between various factors (geographical distribution, drug resistance, new cases or previously treated cases) and genotypes were performed using the chi-square test with IBM SPSS Statistics 19. The level of significance was set at P<0.05.

Results
Study Population
A total of 440 isolates were selected for this study, including 90 from children, 159 from adolescents, and 191 from adults. The male patients were prevalent in all three age groups and constituted 55.6% (50/90), 52.2% (83/159) and 63.9% (122/191), respectively. The average age of patients in each group was 7.3 years, 16.9 years and 40.8 years. Newly diagnosed cases constitute 308 (70.0%) individuals in our collection, and accounted for 74.4% (67/90), 69.8% (111/159) and 68.1% (130/191) among patients from children, adolescents and adults, respectively. All the patients enrolled in this study were proven to be unlinked based on conventional epidemiological investigation.

Distribution of Spoligotypes in Different Groups
In the 440 typed isolates, a total of 66 spoligotypes were identified (Table 1). Of them, 47 spoligotypes were present in the SITVITWEB database, while other 15 spoligotypes were not found in SITVITWEB and were named as New 1 to New 15 (Table 1). Family assignment revealed that 350 isolates (79.5%) belonged to the Beijing family, followed by ill-defined T superfamily (12.7%, 56/440) and Haarlem family (2.0%, 9/440). In addition, strains from other families, such as CAS1-Delhi family, LAM9 family, S family, Manu_ancestor family, MANU2 family, were also found in this study although at very low rate (0.2% for all families except for 0.4% for LAM9 family).

Among 90 strains isolated from children, the Beijing family was predominant (85.6%, 77/90), followed by T family (8.9%, 8/90) and LAM family (1.1%, 1/90) (Table 1). According to the SITVITWEB database, 87 pediatric isolates were assigned to the known SITs, while three isolates presented new patterns (New3, New12 and New13). Seventy-one (78.9%) isolates showed a profile typical of the Beijing genotype (SITI), and six had abridged Beijing-like spoligoprofiles (SIT190, SIT269, SIT621, SIT2413). The remaining 13 isolates were subdivided into 10 genotypes. The isolates from adolescents and adults were divided into five and seven clusters, respectively. The most prevalent family in the two groups was also the Beijing family (81.1% in adolescents, 75.4% in adults), with T family (10.7% in adolescents, 16.2% in adults)
Table 1. Distribution of spoligotype families in different groups.

| SIT* | Family | Children | Adolescents | Adults |
|------|--------|----------|-------------|--------|
|      |        | n(%)     | n(%)        | n(%)   |
|      | Beijing|          |             |        |
| 1    |        | 322 (73.2) | 71 (78.9)   | 118 (74.2) |
|      |        | 190 (2.5)  | 3 (3.3)     | 1 (0.6)  |
|      |        | 250 (1.0)  | 0 (0.0)     | 1 (0.6)  |
|      |        | 260 (1.0)  | 0 (0.0)     | 1 (0.6)  |
|      |        | 265 (1.0)  | 0 (0.0)     | 1 (0.6)  |
|      |        | 269 (1.0)  | 1 (1.1)     | 0 (0.0)  |
|      |        | 621 (1.0)  | 1 (1.1)     | 0 (0.0)  |
|      |        | 632 (1.0)  | 0 (0.0)     | 2 (1.0)  |
|      |        | 1162 (2.0)| 0 (0.0)     | 2 (1.0)  |
|      |        | 1311 (0.2)| 0 (0.0)     | 0 (0.0)  |
|      |        | 1364 (0.2)| 0 (0.0)     | 1 (0.5)  |
|      |        | 1674 (0.2)| 0 (0.0)     | 1 (0.5)  |
|      |        | 2101 (0.2)| 0 (0.0)     | 1 (0.5)  |
|      |        | 2413 (0.2)| 1 (1.1)     | 0 (0.0)  |
|      |        | 2610 (0.2)| 0 (0.0)     | 1 (0.5)  |
|      |        | 2650 (0.2)| 0 (0.0)     | 1 (0.5)  |
|      | T1     | 332 (1.0)| 1 (0.3)     | 13 (0.7) |
|      |        | 118 (0.4)| 0 (0.0)     | 1 (0.6)  |
|      |        | 412 (1.0)| 4 (0.9)     | 1 (0.6)  |
|      |        | 1449 (3.0)| 3 (0.6)    | 1 (0.6)  |
|      |        | 190 (0.4)| 0 (0.0)     | 1 (0.6)  |
|      |        | 250 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 260 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 265 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 269 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 621 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 632 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 1162 (0.2)| 0 (0.0)    | 2 (1.0)  |
|      |        | 1311 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      |        | 1364 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      |        | 1674 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      | T2     | 332 (1.0)| 1 (0.3)     | 13 (0.7) |
|      |        | 118 (0.4)| 0 (0.0)     | 1 (0.6)  |
|      |        | 412 (1.0)| 4 (0.9)     | 1 (0.6)  |
|      |        | 1449 (3.0)| 3 (0.6)    | 1 (0.6)  |
|      |        | 190 (0.4)| 0 (0.0)     | 1 (0.6)  |
|      |        | 250 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 260 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 265 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 269 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 621 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 632 (0.1)| 0 (0.0)     | 1 (0.6)  |
|      |        | 1162 (0.2)| 0 (0.0)    | 2 (1.0)  |
|      |        | 1311 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      |        | 1364 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      |        | 1674 (0.0)| 0 (0.0)    | 1 (0.5)  |
|      | T3     | 332 (1.0)| 1 (0.3)     | 13 (0.7) |
|      |        | 118 (0.4)| 0 (0.0)     | 1 (0.6)  |
|      |        | 412 (1.0)| 4 (0.9)     | 1 (0.6)  |
|      |        | 1449 (3.0)| 3 (0.6)    | 1 (0.6)  |
| SIT* | Family | Spoligoprofile | All | Children | Adolescents | Adults |
|------|--------|----------------|-----|----------|-------------|--------|
|      |        |                | n (%) | n (%) | n (%) | n (%) |
| 44   | T5     |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 47   | H1     |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 49   | H3     |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 50   | H3     |                | 2 (0.4) | 0 (0) | 1 (0.6) | 1 (0.5) |
| 512  | H3     |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 742  | H3     |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 2092 | H3     |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 127  | H4     |                | 2 (0.4) | 0 (0) | 0 (0) | 2 (1.0) |
| 26   | CAS1-Delhi |            | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 803  | LAM9   |                | 2 (0.4) | 1 (1.1) | 0 (0) | 1 (0.5) |
| 34   | S      |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 523  | Manu_ancestor |         | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 54   | MANU2  |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 602  | unknown |                | 1 (0.2) | 1 (1.1) | 0 (0) | 0 (0) |
| 623  | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| 1410 | unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| 2731 | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New1 | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New2 | unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New3 | unknown |                | 1 (0.2) | 1 (1.1) | 0 (0) | 0 (0) |
| New4 | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New5 | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New6 | unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New7 | unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New8 | unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New9 | unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New10| unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New11| unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |
| New12| unknown |                | 1 (0.2) | 1 (1.1) | 0 (0) | 0 (0) |
| New13| unknown |                | 1 (0.2) | 1 (1.1) | 0 (0) | 0 (0) |
| New14| unknown |                | 1 (0.2) | 0 (0) | 0 (0) | 1 (0.5) |
| New15| unknown |                | 1 (0.2) | 0 (0) | 1 (0.6) | 0 (0) |

*SIT and family were defined by comparison with SITVITWEB; ‘new’ designates spoligotype not found in SITVITWEB.
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Previously Treated Cases

Distribution of the Beijing Family Strains in New and Geographical Distribution of Beijing Family Strains in the distribution of the Beijing family strains among the three age groups (Table 1). There was no significant difference in the distribution of the Beijing family strains among the three age groups (P = 0.123).

Geographical Distribution of Beijing Family Strains in Different Age Groups

According to the geographical division suggested by Wan et al. [8], the region of origin of strains were defined as (i) northern China (to the north of Yellow River, and Xizang), (ii) central China (between Yellow River and Yangtze River) and (iii) southern China (to the south of Yangtze River, and Xinjiang). As shown in Table 2, the highest density of the Beijing strains was observed in the northern and central part of China (83.1% and 83.9%, respectively), and the lowest was observed in the southern China (61.6%). The difference was strongly significant (P<0.001). A similar trend of geographical distribution of the Beijing strains was seen in all three age groups, children, adolescents and adults. However, the statistically significant differences among northern, central and southern China were only found for adolescents and adults (P<0.001 and 0.025, respectively), but not children (P=0.700). In addition, in southern China, the prevalence of the Beijing family strains in children was slightly lower than that in adolescents and adults (77.8% vs. 60.4% and 59.5%).

Distribution of the Beijing Family Strains in New and Previously Treated Cases

Table 3 shows the distribution of the Beijing family strains in new and previously treated cases in different age groups. Among 308 new cases of the total sample, the prevalence rate of the Beijing family was almost the same among newly-diagnosed (79.9%, 246/308) and previously treated cases (78.8%, 104/132). After stratification by age, Beijing strains were found to be much more prevalent among new cases in children (91.0% vs. 69.6% in the previously treated cases, P = 0.029). In contrast, the proportion of the Beijing family strains was similar between new and previously treated cases in the adolescents and adults (Table 3).

Comparison of the Beijing Genotype Strains with Drug Resistance

The relationship between the Beijing genotype strains and resistance to anti-TB drugs was analyzed (Table 4). The rates of resistance to the first-line drugs and MDR in the Beijing family strains were higher than those in the non-Beijing strains in the total collection while this difference was statistically significant for EMB resistance (P = 0.009). After stratification by age, the drug resistance rates (all tested drugs and MDR) in the Beijing strains were still higher than those in the non-Beijing strains in all three groups, with the exception of RIF and INH resistance in adolescents (34.0% vs. 38.3% for RIF, 43.8% vs. 46.8% for INH in the Beijing and non-Beijing strains, respectively). In addition, Beijing family strains were also found to be associated with INH (P = 0.028) and EMB (P = 0.027) resistance in adolescent group.

The relationship between Beijing genotype strains and first-line anti-TB drug resistance in new cases and previously treated cases was further analyzed, as shown in Table 5. The distribution of drug resistance between Beijing and non-Beijing strains among new cases and previously treated cases in the total collection was similar, with an exception for EMB resistance in previously treated cases (47.1% in Beijing strains vs. 17.9% in non-Beijing strains, P = 0.009). When the patients were divided into three age groups, no significant difference was noticed in adolescents and adults. However, in the previously treated cases in the children group, the resistance rate to all four first-line drugs and MDR was higher in Beijing genotype strains compared to strains of other genotypes (81.3% vs. 14.3% for RIF, 75.0% vs. 28.6% for INH, 81.3% vs. 14.3% for SM, 50.0% vs. 14.3% for EMB, 75.0% vs. 14.3% for

Table 2. Geographical distribution of Beijing family strains in different age groups.

| Northern | Central | Southern | P value |
|----------|---------|----------|---------|
| Beijing, n(%) | non-Beijing, n(%) | Beijing, n(%) | non-Beijing, n(%) | Beijing, n(%) | non-Beijing, n(%) |
| Children group (n=90) | 55 (87.3) | 8 (12.7) | 15 (83.3) | 3 (16.7) | 7 (77.8) | 2 (22.2) | 0.708 |
| Adolescent group (n=159) | 70 (89.7) | 8 (10.3) | 30 (90.9) | 3 (9.1) | 29 (60.4) | 19 (39.6) | <0.001 |
| Total group (n=440) | 211 (85.1) | 37 (14.9) | 33 (83.9) | 15 (16.1) | 61 (61.6) | 38 (38.4) | <0.001 |

Note: Northern China: Beijing, Gansu, Hebei, Heilongjiang, Jilin, Liaoning, Inner Mongolia, Qinghai, Shanxi, Tianjin, Xizang (Tibet); Central China: Anhui, He’nan, Hubei, Jiangsu, Shandong, Shanxi, Sichuan, Chongqing; Southern China: Fujian, Guangxi, Guizhou, Hu’nan, Jiangxi, Xingjiang.

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Table 3. Distribution of Beijing genotype strains in new cases and previously treated cases in different age groups.

| Children | P value | Adolescents | P value | Adults | P value | Total | P value |
|----------|---------|-------------|---------|--------|---------|-------|---------|
| Beijing | non-Beijing | Beijing | non-Beijing | Beijing | non-Beijing | Beijing | non-Beijing |
| N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| New cases | 61 (91.0) | 6 (9.0) | 0.029 | 87 (78.4) | 24 (21.6) | 0.195 | 98 (75.4) | 32 (24.6) | 1.000 | 246 (79.9) | 62 (20.1) | 0.897 |
| Previously treated cases | 16 (69.6) | 7 (30.4) | 42 (87.5) | 6 (12.5) | 46 (75.4) | 15 (24.6) | 104 (78.8) | 28 (21.2) |

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MDR), and the difference was significant for RIF, SM and MDR ($P = 0.01$, $0.01$ and $0.025$, respectively).

**Discussion**

The present study aimed to investigate the genetic diversity and drug resistance of *M. tuberculosis* strains isolated from children TB patients and compared with adolescent and adult TB patients coming from different regions across China. Although these strains might not be representative of all strains present in the country, they provide a preliminary insight into the population structure of *M. tuberculosis* circulating in these specific groups, especially in view of the total lack of this kind of data on Chinese pediatric TB population. Our study found that the Beijing family strains were prevalent in the total collection (79.5%). This result is similar to the two previous large-scale studies conducted in China [9,10]. Dong et al. analyzed 2,346 *M. tuberculosis* isolates from 13 provinces and showed that Beijing family isolates were prevalent all over the country (74.08%) [9], while Guo et al. reported that 77.8% of 158 strains isolated in five provinces belonged to the Beijing genotype [10]. However, this rate was much lower (62.2%) in a recent study of 4017 isolates from the National Drug Resistance Base-Line Surveillance of Tuberculosis [11]. The explanation of the difference may be that the strains used in the national survey were mainly from the southern China (61.3%), where the Beijing strains showed a lower percentage (discussed below).

Here, the prevalence of the Beijing strains varied in different age groups, ranging from 85.56% in children, 81.13% in adolescents, to 75.39% in adults. Further comparison of the distribution of the Beijing strains between new cases and previously treated cases demonstrated that Beijing strains were significantly associated with new cases in children than in previously treated patients. It is known that the Beijing family strains are worldwide spread clones, some of which represent epidemic clones in some areas [12]. This particular genotype was for the first time identified in strains isolated in the Beijing area in China and thus named accordingly [13]. It was speculated that long-term *Mycobacterium bovis* BCG vaccination may be one of the selective forces implicated in the successful spread of the Beijing genotype at least in East Asia, although the association remains inconclusive [14–17]. Our results provide some evidence in support of the hypothesis that Beijing genotype may possess mechanisms to circumvent a BCG-induced immunity. Firstly, BCG was included in the planned vaccination program in China since mid-1970s, and the vaccination rates had

### Table 4. Drug resistance properties of the Beijing family strains and strains of other genotypes.

| Children |  | Adolescents |  | Adults |  | Total |  |
|----------|---|-------------|---|--------|---|-------|---|
|          | P value | Beijing | non-Beijing |  | Beijing | non-Beijing |  | Beijing | non-Beijing |  | Beijing | non-Beijing |  | Beijing | non-Beijing |  | P value |
|          | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| RIF      | 20 (26.0) | 3 (15.4) | 0.636 | 32 (24.8) | 3 (10.0) | 0.091 | 45 (31.3) | 14 (29.8) | 0.859 | 97 (27.7) | 19 (21.1) | 0.229 |
| INH      | 28 (33.8) | 3 (15.4) | 0.685 | 44 (34.1) | 4 (13.3) | 0.028 | 63 (43.8) | 22 (66.7) | 0.738 | 133 (38.0) | 29 (32.2) | 0.329 |
| STR      | 32 (41.6) | 4 (30.8) | 0.551 | 39 (30.2) | 9 (30.0) | 1 | 64 (44.4) | 19 (40.4) | 0.735 | 135 (38.6) | 32 (35.6) | 0.628 |
| EMB      | 17 (22.1) | 2 (15.4) | 0.857 | 34 (26.4) | 2 (6.7) | 0.027 | 34 (23.6) | 6 (12.8) | 0.148 | 85 (24.3) | 10 (11.1) | 0.009 |
| MDR      | 20 (26.0) | 3 (15.4) | 0.636 | 32 (24.8) | 3 (10.0) | 0.091 | 45 (31.3) | 14 (29.8) | 0.859 | 97 (27.7) | 19 (21.1) | 0.229 |

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### Table 5. Drug resistance properties of the Beijing family strains and strains of other genotypes.

| Children |  | Adolescent |  | Adults |  | Total |  |
|----------|---|-------------|---|--------|---|-------|---|
|          | P value | Beijing | non-Beijing |  | Beijing | non-Beijing |  | Beijing | non-Beijing |  | Beijing | non-Beijing |  | P value |
|          | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| RIF      | 13 (81.3) | 1 (14.3) | 0.010 | 23 (54.8) | 3 (50.0) | 1.000 | 28 (60.9) | 8 (53.3) | 0.764 | 64 (61.5) | 12 (42.9) | 0.088 |
| INH      | 12 (75.0) | 2 (28.6) | 0.102 | 24 (37.1) | 3 (50.0) | 1.000 | 31 (67.4) | 12 (80.0) | 0.546 | 67 (64.4) | 17 (60.7) | 0.825 |
| STR      | 13 (81.3) | 1 (14.3) | 0.010 | 21 (50.0) | 3 (50.0) | 1.000 | 27 (58.7) | 10 (66.7) | 0.763 | 61 (58.7) | 14 (50.0) | 0.520 |
| EMB      | 8 (50.0) | 1 (14.3) | 0.250 | 20 (47.6) | 1 (16.7) | 0.322 | 21 (45.7) | 3 (20.0) | 0.127 | 49 (47.1) | 5 (17.9) | 0.009 |
| MDR      | 12 (75.0) | 1 (14.3) | 0.025 | 22 (52.4) | 3 (50.0) | 1.000 | 25 (54.3) | 7 (46.7) | 0.767 | 59 (56.7) | 11 (39.3) | 0.135 |

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already exceeded 85% after 1990. Although the BCG vaccination information was not available in our study, we can assume that the vaccination rate in children and adolescents is higher than in adults. Therefore, the Beijing family strains might be more prevalent among younger population. Secondly, our data showed that Beijing strains were significantly associated with new cases than in previously treated patients in children. The selection of strains in the previously treated cases mainly comes from the use of anti-TB drugs. However, the newly diagnosed patients did not receive any or very short (less than 1 month) drug therapy. In this sense, the significantly higher proportion of Beijing strains in new cases of children might be explained by other factors, such as higher transmission ability under the pressure of BCG vaccination.

Analysis of the geographical distribution of the Beijing family strains showed similar general trends in all three age groups: the prevalence rate of the Beijing genotype was higher in northern and central China, and lower in southern China. This result is in agreement with a previous study showing that the prevalence of the Beijing genotype exhibited geographical variation from 54.50% to 92.59% across China whereas the highest prevalence was found in northern China, followed by central and southern China [9]. Another study focused on genotypic characteristics of childhood tuberculosis in Chongqing, southern China, found that Beijing lineages accounted for 64.8% of the all strains [18]. The proportion is much lower than in our study, but is in concordance with the previous surveillance in the surrounding areas of Chongqing, such as Sichuan province (57.89%) and Hunan province (66.00%) [9]. Thus a difference between our country-wide study and Chongqing pediatric study may be explained by geographical variation. Pang et al. suggested that the geographical distribution difference of the Beijing genotype strains in China might be caused by the different economic status and climate condition [11]. In addition, compared with adolescents and adults, the prevalence of the Beijing family strains in southern China was relatively high in children. This may be due to the small size of the isolates from south China in children, and the further study including larger samples should be undertaken.

It was reported that Beijing family strains were associated with drug resistance, which might drive the spread of this particular genotype. However, the relationship between the Beijing family strains and drug resistance varied in different studies [11,19–21]. An analysis on the association of drug resistance with Beijing strains revealed that Beijing genotype was significantly associated with resistance in Denmark (rif and emb), Finland (rif and str), the Netherlands (STR), and Russia (all first-line drugs and MDR), while no association was found in most of the Asian studies [19]. Recently, a study analyzing 1,375 M. tuberculosis strains from six provinces in China revealed that Beijing strains were not associated with rif, inh and MDR resistance [20], which was consistent with the above mentioned review [19]. However, Pang and his colleagues using strains from national collection was also geographically representative (25 provinces). The Chinese data included in the comprehensive review were from three single studies (Shanghai, Henan and Hong Kong) [19]. Yang et al. enrolled isolates from six field sites located in six different provinces of China [20]. In contrast, the strains of Pang et al. were from nation-wide survey (31 provinces) [11], and our collection was also geographically representative (25 provinces). Finally, the meta-analysis explored publications that dealt with various sources of isolates [21]. Therefore, the results of the latter two studies [11,21] and this study may better represent a real situation.

Conclusion

In conclusion, this study for the first time analyzed the genetic diversity of M. tuberculosis from children in China at the country-wide level; its results were further compared to those obtained on the isolates from adolescents and adults. As a result, the prevalence of Beijing genotype strains was higher in northern and central China, and the lowest in southern China. The genetic characteristics of M. tuberculosis isolates were found similar in all three age groups, in particular marked with overall predominance of the Beijing family. The Beijing strains were significantly associated with rif, str and MDR resistance in previously treated children.

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Author Contributions

Conceived and designed the experiments: AS KW WJ. Performed the experiments: WJ ZL RH XZ FD HH JT QL LL QY WS. Analyzed the data: WJ ZL RH. Contributed reagents/materials/analysis tools: AS KW. Wrote the paper: WJ AS KW.

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