

Mycobacterium tuberculosis Beijing Strains Favor Transmission but Not Drug Resistance in China

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Background. The *Mycobacterium tuberculosis* Beijing strains are widespread globally. We aimed to determine whether Beijing strains in China are more likely than other strains to spread, and whether they are more likely to become drug resistant. We also sought to determine whether different Beijing sublineages have distinct phenotypic characteristics.

Methods. We conducted a population-based molecular epidemiologic study in 6 provinces in China from 2009 to 2010. We analyzed data and specimens from culture-confirmed pulmonary tuberculosis patients. Each patient's isolate was genotyped using 16-loci variable number of tandem repeats and 6 single-nucleotide polymorphisms.

Results. By genotyping, 75.0% (1031/1375) of the strains of *M. tuberculosis* were Beijing strains. Beijing strains were more likely than non-Beijing strains to be in a genotypic cluster (odds ratio, 2.40, $P < .001$), and were significantly associated with younger age ($P_{\text{trend}} < .05$). There was no significant difference in the proportion of Beijing strains and non-Beijing strains that were drug resistant, even when stratified by new vs retreatment patients. We identified 6 sublineages of Beijing strains in the study population. The modern sublineage of Beijing strains were more likely than the ancient sublineages to be clustered (odds ratio, 2.27, $P < .001$).

Conclusions. Beijing strains of *M. tuberculosis* were significantly associated with genotypic clustering, reflecting recent transmission, and younger age, but were not associated with drug resistance. Future studies of Beijing family strains should avoid assuming and attributing characteristics to the entire family and should assess strains of specific sublineages and/or settings.

Tuberculosis is a global health concern. Almost one-third of individuals worldwide are infected with the pathogen *Mycobacterium tuberculosis* and are at risk of developing tuberculosis disease during their lifetime [1]. China has the second-highest tuberculosis burden in the world, with an estimated 1.4 million new tuberculosis cases annually and >44.5% of the population infected with *M. tuberculosis* [2].

There are many different strains of *M. tuberculosis*, but the Beijing family genotype is widespread and is a major concern. The Beijing genotype was first described in 1995, and >80% of strains from Beijing, China, were of this genotype [3]. Later, the Beijing genotype was detected in other parts of the world, and it is widespread in East Asia [3, 4]. Researchers hypothesized that Beijing strains have unique properties that might explain their widespread distribution, such as an escape from the protective effect of the BCG vaccine [3, 5], efficient dissemination or increased virulence [6], rapid and increased expansion in younger populations [7], and an increased risk of drug resistance [8, 9]. Some studies claimed that Beijing strains were more likely to develop multidrug resistance (MDR), defined as resistance to at least isoniazid (INH) and rifampicin (RIF) [10–12].

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However, the results from different studies comparing Beijing strains and non-Beijing strains are inconsistent [9, 10, 13–16]. Despite the different research study designs, sampling schemes, and biases, these studies suggested that there is variability among Beijing strains that may influence the results and conclusions of different studies [17–19]. Recent phylogenetic studies showed that Beijing family strains can be subdivided into several divergent sublineages by using different genomic markers [18–22]. For example, the presence or absence of IS6110 insertion(s) in the so-called *NTF* region divides Beijing strains into “modern” (typical) and “ancient” (atypical) sublineages [23]. Modern Beijing sublineage strains are widely distributed worldwide, leading to speculation that this sublineage has hypervirulent features [24, 25]. Meanwhile, studies from Japan and Taiwan reported that sublineages of Beijing strains differed in their associations with drug-resistant tuberculosis [19, 26]. Nevertheless, few studies identified different phenotypic characteristics of the sublineages of Beijing strains.

In China, most tuberculosis cases are attributed to infection with Beijing strains. China also has the highest number of MDR tuberculosis cases [1]. First, we tested the hypothesis that Beijing strains of *M. tuberculosis* were more likely to be drug resistant than non-Beijing strains of *M. tuberculosis*. Next, we tested the hypothesis that tuberculosis in patients who were infected with a Beijing strain was more likely to be caused by recent transmission, as measured by genotypic clustering, than tuberculosis in patients who were infected with a non-Beijing strain. Finally, we compared the characteristics of tuberculosis patients who were infected with a modern sublineage of a Beijing strain and tuberculosis patients who were infected with an ancient sublineage of a Beijing strain. We used a population-based molecular epidemiologic study in 6 different geographical areas of China to test the hypotheses and to characterize the patients with tuberculosis and their mycobacterial strains.

MATERIALS AND METHODS

Study Population

We performed a population-based molecular epidemiologic study in 6 field sites in China from 1 June 2009 to 31 December 2010 (Figure 1). The field sites cover a total population of about 5.8 million inhabitants.

All suspect tuberculosis patients were screened using symptoms including cough for at least 2 weeks, fever, chest pain, weight loss, night sweats, and abnormal chest radiograph. Patients with suspected tuberculosis from general hospitals, community health centers, and countryside healthcare programs were referred to designated tuberculosis hospitals for confirmation and diagnosis. Three sputum samples taken at different times were collected from each individual and used for

light microscopy to detect acid-fast bacilli and for bacterial culture. All culture-confirmed tuberculosis cases were included in the analysis, and the patient’s clinical and demographic information were collected. A new tuberculosis case was defined as patient who had never had anti-tuberculosis treatment or had taken anti-tuberculosis drugs for <1 month. A retreatment tuberculosis case was defined as a patient who received ≥ 1 month of anti-tuberculosis drugs in the past. The study protocol was approved by the Ethics Committees of the Shanghai Municipal Center for Disease Control and Prevention (CDC), and the Institutes of Biomedical Sciences in Fudan University.

Drug Susceptibility Testing

All of the *M. tuberculosis* isolates were sent to the provincial CDC to perform drug susceptibility testing (DST) to detect resistance to RIF and INH using the proportion method on Lowenstein-Jensen media at the following concentrations: RIF, 40 $\mu\text{g}/\text{mL}$ and INH, 0.2 $\mu\text{g}/\text{mL}$. Multidrug-resistant tuberculosis was defined as resistance to at least INH and RIF. The 6 provincial CDCs participated in the China CDC and/or the World Health Organization Global Project on Anti-Tuberculosis Drug Resistance Surveillance [27].

DNA Extraction and Genotyping

The deactivated isolates were shipped to Fudan University for genotyping analysis. Genomic DNA was obtained from isolates by the boiled lysis method. We used a polymerase chain reaction (PCR)-based method to rapidly identify the Beijing strains [28]. The Beijing strains were further confirmed and classified into sublineages by a real-time PCR-based single-nucleotide polymorphism (SNP) genotyping method with 6 SNPs in genes including *ligD*, *recR44*, *recX59*, *ogt37*, *mutT4*, and *mutT2* [20, 29]. In this study, strains with a mutation in *mutT2* were defined as the modern Beijing sublineage [8, 19, 24]. Strains without a mutation in *mutT2* were classified as ancient sublineages. We also used a high-resolution 16-loci variable number of tandem repeat (16-VNTR) method to genotype the *M. tuberculosis* isolates [30]. For patients from the same study field site, 2 or more isolates from different patients who shared the same 16-VNTR genotype patterns were considered clustered. Other isolates were classified as unique. We assumed that genotypic clustering represented recent transmission of a strain of *M. tuberculosis* [31].

Computer-Assisted and Statistical Analysis

The VNTR genotyping data were analyzed by BioNumerics software (version 5.0, Applied Maths, Sint-Martens-Latem, Belgium). Statistical analyses were performed using Stata software (version 10.1/SE, Stata Corp, College Station, Texas). We used the χ^2 and Fisher exact tests, as appropriate, for univariate analysis of categorical variables. Patients’ age was



Figure 1. Map of China showing the distribution of the tuberculosis patients whose isolates of *Mycobacterium tuberculosis* were included in the study. The selected study field sites were Pingguo county, Guangxi Province; Wusheng county, Sichuan Province; Weishi county, Henan Province; Songjiang District, Shanghai; Fei county, Shandong Province; and Wuchang county, Heilongjiang Province. The markers on the map indicate the relative location of the 6 study sites. The star represents the national capital, Beijing city.

categorized (<25, 25–44, 45–64, and ≥65 years) for analysis of the distribution of the Beijing strains by age. A χ^2 test for trend was used to identify changes in the frequency of Beijing strains, by age group. To determine whether Beijing strains were associated with drug resistance, we also stratified by treatment history. We used multivariate logistic regression models to determine whether covariates that were statistically significant in the univariate analysis were independently associated with Beijing strains. A backward stepwise model was used in multivariate analysis and the interaction terms were retained in the multivariate model if they were significant. A *P* value of <.05 was considered statistically significant.

RESULTS

Characteristics of the Study Patients and Strains

We included 1448 patients with culture-positive pulmonary tuberculosis reported during 2009–2010 from 6 field sites (Figure 1). One strain per patient was genotyped. Thirty-nine isolates were identified as nontuberculous mycobacteria and were excluded. Of the remaining 1409 *M. tuberculosis* isolates, 1375 (97.6%) had valid DST and genotyping results. We excluded 29 patients whose isolates had a failure in the DNA extraction and an additional 5 patients who lacked reliable DST results.

Table 1. Characterization of Tuberculosis Cases and Mycobacterial Strains in 6 Study Sites in China, 2009–2010

Region	Total Cases, No.	Male Sex, %	Median Age, y (range)	New Cases, No. (%) ^a				Retreatment Cases, No. (%) ^a			
				Total	INH	RIF	MDR	Total	INH	RIF	MDR
Sichuan	216	76.4	44 (15–83)	172	16 (9.3)	15 (8.7)	10 (5.8)	32	12 (37.5)	14 (43.8)	10 (31.3)
Guangxi	176	72.7	43 (15–86)	137	8 (5.8)	6 (4.4)	2 (1.5)	26	11 (42.3)	11 (42.3)	9 (34.6)
Shanghai	396	65.9	34 (15–88)	359	39 (10.9)	16 (4.5)	11 (3.1)	37	11 (29.7)	8 (21.6)	8 (21.6)
Shandong	206	76.7	55 (17–87)	167	32 (19.2)	17 (10.2)	13 (7.8)	9	5 (55.6)	4 (44.4)	4 (44.4)
Henan	197	73.1	55 (16–93)	122	13 (10.7)	5 (4.1)	4 (3.3)	35	10 (28.6)	9 (25.7)	8 (22.9)
Heilongjiang	184	67.9	49 (16–85)	148	11 (7.4)	6 (4.1)	4 (2.7)	16	2 (12.5)	2 (12.5)	1 (6.3)
Total	1375	71.3	44 (15–93)	1105	119 (10.8)	65 (5.9)	44 (4.0)	155	51 (32.9)	48 (31.0)	40 (25.8)

Abbreviations: INH, isoniazid resistant; MDR, multidrug resistant, resistant to at least isoniazid and rifampin; RIF, rifampin resistant.

^a Data on tuberculosis treatment history were available for 1260 (91.6%) cases.

The patient demographics are shown in Table 1. The median age across 6 sites was 44 years (range, 15–93 years), and most patients were male (71.3%). Of 1260 patients with information on their treatment history, 1105 (87.7%) were newly diagnosed cases.

Factors Associated With Drug Resistance

Overall, 224 tuberculosis patients (16.3%) had a strain that was resistant to at least 1 drug. There were 194 tuberculosis patients (14.1%) whose isolate was resistant to INH, 122 (8.9%) with resistance to RIF, and 92 (6.7%) with MDR. The odds of having resistance to at least 1 drug were 4 times higher among retreatment cases, compared to new cases (odds ratio [OR], 4.24; 95% confidence interval [CI], 2.87–6.22). Retreatments cases also had a higher odds of MDR, compared to new cases (OR, 8.39; 95% CI, 5.08–13.75). New cases still accounted for 52.4% (95% CI, 36.6%–58.8%, 40/84) of the MDR tuberculosis cases (Table 1).

We tested the hypothesis that Beijing strains were more likely than non-Beijing strains to be resistant to at least 1 drug, have any RIF resistance, have any INH resistance, and have MDR. The association of drug resistance with a specific genotype could be caused by 2 mechanisms: the strain was more likely to develop drug resistance during treatment, or the strain was more likely to spread after drug resistance developed. Generally, drug resistance among new cases indicates the transmission of drug-resistant strains, whereas drug resistance among retreated cases likely indicates acquired drug resistance during treatment. Overall, we did not observe that Beijing strains were significantly associated with INH resistance, RIF resistance, or MDR in either univariate or multivariate analysis (Tables 2 and 3). When we stratified the analysis by new tuberculosis cases (n = 1105) vs retreatment cases (n = 155), or by field site, there was still no association between Beijing strains and INH resistance, RIF resistance, and MDR

(Table 4). The proportions of MDR strains were similar between Beijing and non-Beijing genotypes (4.0% vs 3.8% in new cases and 25.6% vs 26.5% in retreatment cases, $P = .8$).

Factors Associated With Infection With a Beijing Strain

Based on the genotyping results, 75.0% (1031/1375) of the tuberculosis patients were infected with a Beijing strain of *M. tuberculosis*. Comparing the 6 field sites, the proportion of patients infected with a Beijing strain ranged from 51.9% to 89.8%, and varied significantly among different provinces (Table 2). To determine whether Beijing strains were associated with recent transmission of *M. tuberculosis*, we compared the proportion of Beijing vs non-Beijing strains that had a clustered genotype. Using the 16-VNTR genotyping analyses, 27.9% (384/1375) of the strains were grouped in 145 clusters, ranging in size from 2 to 17 strains. The largest cluster (n = 17) was formed by a Beijing strain. Overall, the proportion of clustered strains was significantly higher for Beijing strains (31.8%) than non-Beijing strains (16.3%, $P < .001$). In the multivariate analysis, tuberculosis patients infected with a Beijing strain had almost twice the odds of being in a cluster, compared to patients infected with a non-Beijing strain (adjusted OR, 1.95; 95% CI, 1.40–2.71, $P < .001$; Table 3).

We compared the characteristics of patients infected with a Beijing strain vs patients infected with a non-Beijing strain. The 2 groups did not differ with respect to sex or treatment history. Interestingly, tuberculosis patients of younger age were more likely to be infected with a Beijing strain; the proportion of patients infected with a Beijing strain decreased with increasing age (Table 2) and the trend was significant ($P_{\text{trend}} < .05$).

Factors Associated With Infection by Beijing Strain Sublineages

Phylogenetic analysis based on 6 SNPs grouped the 1031 Beijing strains into 6 sublineages (Table 5). The modern sublineage was the most prevalent (74.7%), followed by 5 ancient

Table 2. Univariate Analysis of the Characteristics Associated With Infection With a Beijing Strain, China, 2009–2010

Characteristic	Beijing Genotype, No. (%)	Non-Beijing Genotype, No. (%)	Beijing vs Non-Beijing	
			OR (95% CI)	P Value
Regions				
Sichuan	112 (51.9)	104 (48.1)	1.00	<.001
Guangxi	109 (61.9)	67 (38.1)	1.51 (1.00–2.27)	.045
Shanghai	314 (79.3)	82 (20.7)	3.56 (2.44–5.18)	<.001
Shandong	160 (77.7)	46 (22.3)	3.23 (2.08–5.01)	<.001
Henan	177 (89.8)	20 (10.2)	8.22 (4.57–14.78)	<.001
Heilongjiang	159 (86.4)	25 (13.6)	5.91 (3.45–10.11)	<.001
Sex				
Male	731 (74.8)	246 (25.2)	1.00	
Female	300 (75.4)	98 (24.6)	0.97 (.74–1.27)	.829
Age (y)				
<25	210 (81.1)	49 (18.9)	1.00	.02 ^a
25–44	333 (75.7)	107 (24.3)	0.73 (.50–1.06)	.098
45–64	274 (71.4)	110 (28.6)	0.58 (.40–.85)	.005
≥65	204 (73.6)	73 (26.4)	0.65 (.43–.98)	.040
Unknown	10 (66.7)	5 (33.3)	0.47 (.15–1.43)	.173
Treatment history				
No prior treatment	819 (74.1)	286 (25.9)	1.00	
Prior treatment	121 (78.1)	34 (21.9)	1.24 (.83–1.86)	.291
Unknown	91 (79.1)	24 (20.9)	1.32 (.83–2.12)	.240
Genotypic cluster				
No	703 (70.9)	288 (29.1)	1.00	
Yes	328 (85.4)	56 (14.6)	2.40 (1.74–3.30)	<.001
Drug resistance				
No	862 (74.9)	289 (25.1)	1.00	
Yes	169 (75.4)	55 (24.6)	1.03 (.74–1.44)	.861
INH	149 (76.8)	45 (23.2)	1.11 (.77–1.63)	.568
RIF	90 (73.8)	32 (26.2)	0.94 (.61–1.49)	.786
MDR	70 (76.1)	22 (23.9)	1.07 (.64–1.84)	.799

Abbreviations: CI, confidence interval; INH, isoniazid resistant; MDR, multidrug resistant, resistant to at least isoniazid and rifampin; OR, odds ratio; RIF, rifampin resistant.

^a P value of χ^2 test for trend.

sublineages (25.3%): Bmyc2 (5.8%), Bmyc4 (4.4%), Bmyc6 (1.0%), Bmyc25 (12.3%), and Bmyc26 (1.5%).

We compared the proportion of tuberculosis patients that were clustered, per sublineage of Beijing strain. The odds that Beijing strains in the modern sublineage were clustered were significantly higher than the odds that Beijing strains in any of the ancient sublineages were clustered (Table 5; OR 2.27; 95% CI, 1.60–3.25, $P < .001$). Furthermore, the odds that Beijing strains in the sublineage Bmyc25 were clustered were much lower compared to the odds that Beijing strains in the modern sublineage were clustered (OR 0.35; 95% CI, .21–.59, $P < .001$; Table 5).

We tested for associations between the different Beijing sublineages and drug resistance. No significant differences in the

different drug resistance profiles were observed between ancient and modern Beijing sublineages (Table 5). Interestingly, by univariate analysis the ancient Bmyc2 sublineage was more likely to be resistant to at least 1 drug ($P = .02$) and have MDR ($P = .03$, using Fisher exact test), compared with the modern sublineage (Table 5). However, these associations did not reach statistical significance when stratified by treatment history because of the small numbers for comparisons.

DISCUSSION

The Beijing strains of *M. tuberculosis* are widely distributed around the world, but different mechanisms could contribute to their emergence and prevalence [32]. In our study, Beijing

Table 3. Multivariate Analysis of Characteristics Associated With Infection With a Beijing Strain, China, 2009–2010

Characteristic	Beijing vs Non-Beijing	
	Adjusted OR (95% CI)	P Value
Regions		<.001
Sichuan	1.00	
Guangxi	1.46 (.97–2.20)	
Shanghai	2.95 (2.03–4.29)	
Shandong	3.20 (2.05–4.97)	
Henan	8.30 (4.77–14.44)	
Heilongjiang	5.40 (3.24–9.00)	
Genotypic cluster	1.95 (1.40–2.71)	<.001
Age (y)		.02
<25	1.00	
25–44	0.76 (.51–1.14)	
45–64	0.60 (.39–.90)	
≥65	0.48 (.31–.75)	
Unknown	0.47 (.14–1.60)	

The ORs were adjusted by logistic regression model for all other variables in this table.

Abbreviations: CI, confidence interval; OR, odds ratio.

strains were significantly associated with clustering and with tuberculosis patients of younger ages, suggesting recent transmission of Beijing strains in the population. However, we did not detect a significant association between Beijing strains and drug resistance, including MDR. Furthermore, we noted

Table 4. Associations Between Beijing Strains and Drug Resistance, Stratified by Treatment History (n = 1260), China, 2009–2010

Drug Sensitivity and Anti-Tuberculosis Treatment History	Total Cases, No.	Beijing Genotype, No. (%)	OR (95% CI) ^a
New cases			
Drug susceptible	965	714 (74.0)	1.00
Drug resistant	140	105 (75.0)	1.03 (.67–1.59)
INH	119	92 (77.3)	1.13 (.70–1.83)
RIF	65	46 (70.8)	0.96 (.54–1.72)
MDR	44	33 (75.0)	1.15 (.55–2.39)
Retreated cases			
Drug susceptible	96	76 (79.2)	1.00
Drug resistant	59	45 (76.3)	1.28 (.52–3.17)
INH	51	39 (76.5)	1.16 (.47–2.91)
RIF	48	37 (77.1)	1.56 (.60–4.07)
MDR	40	31 (77.5)	1.44 (.53–3.96)

Abbreviations: CI, confidence interval; INH, isoniazid resistant; MDR, multidrug resistant, resistant to at least isoniazid and rifampin; OR, odds ratio; RIF, rifampin resistant.

^a Adjusted for age, sex, and study settings.

evidence of genotypic and phenotypic differences for the Beijing strain sublineages.

The 16-VNTR genotyping method used in this study had high discriminatory power, comparable to the IS6110-RFLP method [30]. We assumed that *M. tuberculosis* strains that had identical genotype patterns indicated recent transmission [31]. In our study, Beijing strains were significantly associated with clustering and with tuberculosis patients of younger ages, suggesting recent transmission of Beijing strains in the population. However, other factors that could contribute to the transmission of *M. tuberculosis* strains, such as social behaviors, were not investigated in the present study. A study from Vietnam also suggested that Beijing strains were associated with recent transmission and younger age [7]. However, we did not observe a significant difference between the proportions of clustered strains by age groups.

The Beijing strains can be divided into several sublineages [18–21, 24]. Recent molecular epidemiological studies showed that the modern Beijing strains were the most widely disseminated Beijing strains [4, 24, 26], except in Japan and Korea [33, 34]. In the present study, with the modern sublineage strains were overrepresented in the study population, suggesting that this sublineage had an advantage in its ability to spread. The observation was also supported by an association of the modern sublineage with genotypic clustering. Similar findings were observed in South Africa, Japan, and Taiwan [24, 26, 33]. Despite the high prevalence of ancient Beijing sublineages in a study in Japan, the modern Beijing sublineage strains were more likely to be clustered, especially among the homeless population [33]. Currently, it is not known whether the high prevalence of modern Beijing sublineage strains was due to increased transmissibility or was a result of a shorter time in latency and rapid progression to active tuberculosis disease [35], or related to social behaviors of the host (eg, immigration, crowding) or a strong founder effect. However, a founder effect is unlikely to account for recent increases of the modern Beijing sublineage strains in multiple geographic settings. Furthermore, our findings also suggest that different Beijing sublineage strains may have phenotypic differences.

Resistance to 1 or more currently used anti-tuberculosis drugs (eg, MDR) is a major public health concern [1]. Numerous epidemiological studies from various geographic areas suggested that Beijing strains were associated with drug resistance and/or specifically MDR tuberculosis [8, 9, 19, 36–39]. However, the present study did not demonstrate that Beijing strains were more likely than non-Beijing strains to be drug resistant, even among retreatment cases. The data suggest that Beijing strains were no more likely to acquire drug resistance than non-Beijing strains. A previous study suggested that the mutations in putative mutator (*mut*) genes might produce a hypermutator phenotype, and thus enhance the ability of

Table 5. Proportion of Clustered Strains and Drug Resistant Strains Between the Different Sublineages of Beijing Strains, China, 2009–2010

Beijing Sublineage	No. of Cases	Male Sex, (%)	Median Age, y (Range)	Treatment History, No. (%) of Cases ^a			Drug-Resistant Cases, No. (%)			
				New Cases	Retreatment Cases	Unknown	DS	DR	MDR	Cases in Clusters, No. (%)
Total	1031	70.9	43 (15–93)	819 (79.4)	121 (11.7)	91 (8.8)	862 (83.6)	169 (16.4)	70 (6.8)	328 (31.8)
Modern	773	72.2	49 (15–91)	619 (80.1)	87 (11.3)	67 (8.7)	654 (84.6)	119 (15.4)	48 (6.2)	277 (35.8)
Ancient	258	67.1	42 (15–93)	200 (77.5)	34 (13.2)	24 (9.3)	208 (80.6)	50 (19.4)	22 (8.5)	51 (19.8)
Bmyc2	60	73.3	42 (18–84)	46 (76.7)	6 (10.0)	8 (13.3)	44 (73.3)	16 (26.7)	8 (13.3)	18 (30.0)
Bmyc4	46	58.7	52 (15–93)	33 (71.7)	7 (15.2)	6 (13.0)	36 (78.3)	10 (21.7)	2 (4.3)	12 (26.1)
Bmyc6	10	70.0	33 (18–80)	7 (70.0)	2 (20.0)	1 (10.0)	8 (80.0)	2 (20.0)	1 (10.0)	0 (0)
Bmyc25	127	66.9	40 (15–88)	103 (81.1)	16 (12.6)	8 (6.3)	107 (84.3)	20 (15.7)	10 (7.9)	21 (16.5)
Bmyc26	15	66.7	33 (15–67)	11 (73.3)	3 (20.0)	1 (6.7)	13 (86.7)	2 (13.3)	1 (6.7)	0 (0)

Abbreviations: DR, drug resistant; DS, drug susceptible; MDR, multidrug resistant, resistant to at least isoniazid and rifampin.

^a Treatment history was available for 940 (91.2%) of the patients with tuberculosis of known sublineage.

Beijing strains to develop drug resistance [8]. However, no direct correlation between the mutations in *mut* genes and hypermutation of *M. tuberculosis* has been proven, and evidence for an increased mutation rate in Beijing strains is still lacking [40]. The putative mutator (*mut*) was only detected in the modern Beijing sublineages, and there was no association of this sublineage with drug resistance [19, 24, 26]. Together, previous studies and our results suggest that the association of Beijing strains with drug resistance is not due to increased mutability or an increased risk of acquiring drug resistance. One possible explanation for this association might be the clonal spreading of drug-resistant Beijing strains. It is likely that the epidemic burden of drug resistance, specifically MDR tuberculosis, is different in different geographic settings. The tuberculosis epidemic could be accelerated by deteriorating socioeconomic conditions and tuberculosis control systems, the prevalence of human immunodeficiency virus or diabetes, or other factors. Therefore, it is likely that Beijing strains are prevalent in settings where the rates of drug-resistant tuberculosis cases are high. Beijing strains could become the first strains to develop drug resistance, and thus have the greatest time period and opportunity to propagate [11, 37–39]. Thus, an observed association between Beijing strains and drug resistance could be an artifact of the successful transmission of Beijing strains but not reflect an increased mutability or a greater likelihood to acquire drug resistance.

There is variability in the epidemiological and clinical phenotypes of Beijing strains among studies from different geographic settings [9, 10, 13–19]. The spread of Beijing strains may also depend on migrations and movements of human populations [25]. Based on current data, we infer that Beijing strains have adapted to the local host populations, resulting in

the observed phylogenetic diversity of this genotype in different geographic regions. The finding that Beijing strains have a higher odds of drug resistance could be influenced by different treatment regimens and tuberculosis control programs, characteristics of the host population, socioeconomic factors, chance (eg, outbreaks of MDR strains) or any combination of these factors. In addition, the diversity of Beijing sublineages observed in the present study and other studies may also influence the results [19, 26]. Thus, the association of Beijing strains with drug resistance may simply reflect the local tuberculosis epidemic rather than intrinsic properties of the Beijing strains.

CONCLUSIONS

In a population-based molecular epidemiologic study in China, Beijing strains had a higher odds of being in a genotypic cluster, reflecting recent transmission, but were no more likely than non-Beijing strains to be associated with drug resistance. Our findings confirm that the Beijing strains are not homogeneous; there are identifiable sublineages of Beijing strains. Future studies of Beijing family strains should avoid assuming and attributing characteristics to the entire family, and should assess the strains of specific sublineages and/or settings.

Notes

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