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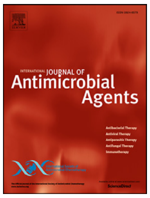

Beijing genotype of *Mycobacterium tuberculosis* is less associated with drug resistance in south China

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ABSTRACT

Mycobacterium tuberculosis Beijing genotype strains are widespread globally. However, there has been no systematic study on the association between Beijing genotype and the characteristics of drug resistance. In this study, 359 *M. tuberculosis* isolates from south China were collected and their background information, genotype diversity and drug resistance was investigated. The results revealed that 66.0% of strains (237/359) were categorised as Beijing genotype. There was no statistical difference between Beijing and non-Beijing genotype strains in terms of patient sex, age, place of residence and treatment history. Drug resistance testing showed that 34.8% (125/359) of isolates were resistant to at least one of the seven drugs tested. The proportions of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis were 17.0% and 1.4%, respectively. Previously treated patients presented a significantly higher risk of developing drug resistance than new cases. Although the prevalence of drug resistance was higher in Beijing genotype than in non-Beijing genotype strains, there was no significant difference between these two genotypes in the multivariate analysis. Even in re-treated patients, the association of Beijing genotype with drug resistance was not significant. This study provides an insight into genotype diversity and demonstrates the characteristics of drug resistance in Beijing genotype strains, which will be useful in generating efficient tuberculosis prevention and control strategies in China.

1. Introduction

Tuberculosis (TB) remains a major global public-health problem. The high mortality rates associated with drug-resistant TB, especially multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), are particularly worrisome. China has the third highest prevalence of TB and the second highest burden of MDR-TB globally, with 895 000 cases of TB and 58 000 cases of MDR-TB in 2016 [1]. Hence, the prevention and control of TB are facing huge pressure in China.

Genotyping of *Mycobacterium tuberculosis (M. tuberculosis)* isolates has significantly improved our knowledge of TB epidemiology and has enabled the development of molecular-guided control strategies [2,3]. Spoligotyping is a reliable and powerful technology to differentiate *M. tuberculosis* complex into various genotypes [4,5]. Molecular typing of *M. tuberculosis* from East Asia demonstrated that a specific family of *M. tuberculosis*, namely the Beijing genotype, was prevalent [6,7]. This genotype is one of the most successful *M. tuberculosis* genotypes with an increasing prevalence in the global population [7,8].

M. tuberculosis Beijing strains have unique properties that explain their widespread distribution, such as efficient dissemination [9], increased virulence [10] and an increased risk of drug resistance [11–13]. Some studies propose that Beijing genotype strains are more likely to develop into MDR-TB [4,14,15]. However, these associations or characteristics have varied among different studies and different regions [16–18]. In China, Beijing genotypes strains are highly prevalent, constituting approximately 80% of *M. tuberculosis* strains [13,19]. However, the proportion of strains of this genotype in north and south China showed a great difference [14]. To date, there has been also no systematic study on the association between Beijing genotype and the characteristics of drug resistance in south China.

Thus, in the present study, the background information, genotype diversity and drug resistance frequencies among 359 *M.*

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Table 1
Characteristics of pulmonary tuberculosis patients included in the study.

Characteristic	Total cases ($N = 359$)		New cases ($N = 256$)		Re-treated cases ($N = 103$)	
	n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
Sex						
Male	260	72.4 (67.8–77.0)	182	71.1 (65.5–76.6)	78	75.7 (67.4–84.0)
Female	99	27.6 (23.0–32.2)	74	28.9 (23.4–34.5)	25	24.3 (16.0–32.6)
Age group						
<30 years	113	31.5 (26.7–36.3)	96	37.5 (31.6–43.4)	17	16.5 (9.3–23.7)
30–59 years	181	50.4 (45.2–55.6)	116	45.3 (39.2–51.4)	65	63.1 (53.8–72.4)
≥60 years	65	18.1 (14.1–22.1)	44	17.2 (12.6–21.8)	21	20.4 (12.6–28.2)
Place of residence						
Rural	180	50.1 (45.0–55.3)	128	50.0 (43.9–56.1)	52	50.5 (40.8–60.1)
Urban	179	49.9 (44.7–55.0)	128	50.0 (43.9–56.1)	51	49.5 (39.9–59.2)
Drug resistance						
Any resistance	125	34.8 (29.9–39.7)	53	20.7 (15.7–25.7)	72	69.9 (61.0–78.8)
INH	103	28.7 (24.0–33.4)	35	13.7 (9.5–17.9)	68	66.0 (56.9–75.2)
RIF	65	18.1 (14.1–22.1)	13	5.1 (2.4–7.8)	52	50.5 (40.8–60.1)
SM	66	18.4 (14.4–22.4)	31	12.1 (8.1–16.1)	35	34.0 (24.8–43.1)
EMB	23	6.4 (3.9–8.9)	4	1.6 (0.0–3.1)	19	18.4 (11.0–25.9)
CAP	6	1.7 (0.3–3.0)	0	0.0	6	5.8 (1.3–10.3)
KAN	5	1.4 (0.2–2.6)	0	0.0	5	4.9 (0.7–9.0)
OFX	28	7.8 (5.0–10.6)	5	2.0 (0.3–3.6)	23	22.3 (14.3–30.4)
MDR	61	17.0 (13.1–20.9)	12	4.7 (2.1–7.3)	49	47.6 (37.9–57.2)
XDR	5	1.4 (0.2–2.6)	0	0.0	5	4.9 (0.7–9.0)

CI, confidence interval; INH, isoniazid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol; CAP, capreomycin; KAN, kanamycin; OFX, ofloxacin; MDR, multidrug-resistant; XDR, extensively drug-resistant.

tuberculosis isolates from south China was investigated to gain an insight into the association between genotype and drug resistance. These data will contribute to developing effective TB prevention and control strategies in China.

2. Materials and methods

2.1. Ethical approval

This study was approval by the Ethics Committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (Beijing, China). Patients with TB were included in this research only after written informed consent was received from the patient or from their parent/guardian if the patient was <18 years of age.

2.2. Study design

This study was conducted between 2009 and 2010 at Hunan Chest Hospital (Changsha) and Fuzhou Pulmonary Hospital (Fuzhou), which serve as the sole specialised TB hospitals in Hunan and Fujian Provinces, respectively. During this period, patients with pulmonary TB and positive cultures identified as *M. tuberculosis* complex were randomly interviewed and enrolled. Strain isolation and identification were performed at the local reference laboratory. Only one isolate per patient was collected and tested.

2.3. Drug susceptibility testing (DST)

For *M. tuberculosis* complex strains, DST was performed using the Lowenstein–Jensen proportion method. The critical concentrations for DST were as follows: 0.2 μg/mL isoniazid (INH); 40.0 μg/mL rifampicin (RIF); 4.0 μg/mL streptomycin (SM); 2.0 μg/mL ethambutol (EMB); 40 μg/mL capreomycin (CAP); 30 μg/mL kanamycin (KAN); and 2.0 μg/mL ofloxacin (OFX) [20]. Quality control was routinely performed during DST using the *M. tuberculosis* reference strain H37Rv (ATCC 27294).

2.4. DNA extraction and genotyping

Genomic DNA was prepared by the cetyltrimethylammonium bromide (CTAB) method as described by Somerville et al. [21]. Strain differentiation was performed by spoligotyping as described by Kamerbeek et al. [22]. The results were compared with the international spoligotyping database SpolDB4.0 [23].

2.5. Statistical analysis

The association of Beijing genotype strains with certain characteristics and drug resistance was assessed using univariate and multivariate logistic regression. To determine whether Beijing genotype was associated with drug resistance, the analysis was also stratified by treatment history. The χ^2 test or Fisher's exact test was used for comparison between groups. Differences were considered statistically significant at $P < 0.05$. All statistical data were analysed using SAS v.9.4 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Demographic characteristics of the patients

During the study period, 359 patients with pulmonary TB were enrolled. Their basic information is summarised in Table 1. Among these patients, 260 (72.4%) were male and 99 (27.6%) were female, with a mean age of 42.55 years (range 14–88 years). The majority (71.3%; 256/359) were newly diagnosed cases, and 50.1% (180/359) were rural residents (Table 1).

3.2. Genotyping results

Among the 359 *M. tuberculosis* isolates, Beijing genotype was predominant, comprising 237 isolates (66.0%). The remaining 122 isolates belonged to non-Beijing genotypes, comprising T in 69 isolates (19.2%), H in 15 isolates (4.2%), U in 7 isolates (1.9%), MANU2 in 4 isolates (1.1%), LAM in 2 isolates (0.6%), EAI in 1 isolate (0.3%) and orphan in 24 isolates (6.7%).

Table 2
Univariate analysis of characteristics associated with *Mycobacterium tuberculosis* genotype.

Characteristic	No. (%) of cases	Genotype [*n* (%)]		*P*-value	OR (95% CI)
		Non-Beijing (*n* = 122)	Beijing (*n* = 237)		
Sex					
Male	260 (72.4)	88 (72.1)	172 (72.6)		Ref.
Female	99 (27.6)	34 (27.9)	65 (27.4)	0.93	0.98 (0.61–1.59)
Age group					
<30 years	113 (31.5)	37 (30.3)	76 (32.1)		Ref.
30–59 years	181 (50.4)	57 (46.7)	124 (52.3)	0.82	1.06 (0.64–1.75)
≥60 years	65 (18.1)	28 (23.0)	37 (15.6)	0.17	0.64 (0.34–1.21)
Place of residence					
Rural	180 (50.1)	63 (51.6)	117 (49.4)		Ref.
Urban	179 (49.9)	59 (48.4)	120 (50.6)	0.37	1.24 (0.77–2.00)
Treatment history					
New cases	256 (71.3)	87 (71.3)	169 (71.3)		Ref.
Re-treated cases	103 (28.7)	35 (28.7)	68 (28.7)	1.00	1.00 (0.62–1.62)
Drug resistance					
Any resistance	125 (34.8)	38 (31.1)	87 (36.7)	0.30	1.28 (0.81–2.04)
INH	103 (28.7)	32 (26.2)	71 (30.0)	0.46	1.20 (0.74–1.96)
RIF	65 (18.1)	17 (13.9)	48 (20.3)	0.14	1.57 (0.86–2.87)
SM	66 (18.4)	15 (12.3)	51 (21.5)	0.03*	1.96 (1.05–3.65)
EMB	23 (6.4)	4 (3.3)	19 (8.0)	0.09	2.57 (0.86–7.73)
CAP	6 (1.7)	1 (0.8)	5 (2.1)	0.38	2.61 (0.30–22.57)
KAN	5 (1.4)	1 (0.8)	4 (1.7)	0.52	2.08 (0.23–18.79)
OFX	28 (7.8)	5 (4.1)	23 (9.7)	0.07	2.52 (0.93–6.79)
MDR	61 (17.0)	16 (13.1)	45 (19.0)	0.16	1.55 (0.84–2.88)
XDR	5 (1.4)	1 (0.8)	4 (1.7)	0.52	2.08 (0.23–18.79)

OR, odds ratio; CI, confidence interval; INH, isoniazid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol; CAP, capreomycin; KAN, kanamycin; OFX, ofloxacin; MDR, multidrug-resistant; XDR, extensively drug-resistant.

* Statistically significant ($P < 0.05$).

3.3. Drug susceptibility profiles and associated factors

DST results indicated that 125 isolates (34.8%) were resistant to at least one of the seven drugs tested, including 103 (28.7%) to INH, 65 (18.1%) to RIF, 66 (18.4%) to SM, 23 (6.4%) to EMB, 6 (1.7%) to CAP, 5 (1.4%) to KAN and 28 (7.8%) to OFX; moreover, 61 isolates (17.0%) were MDR-TB and 5 isolates (1.4%) were XDR-TB (Table 1). The drug resistance percentages of new cases were significantly lower than those of re-treatment cases. Re-treated cases demonstrated an obvious increased risk of developing drug resistance, with an odds ratio (OR) of 8.90 [95% confidence interval (CI) 5.30–14.94; $P = 0.00$]. In addition, the risk increased highly significantly in MDR-TB cases (OR = 18.45, 95% CI 9.19–37.03; $P = 0.00$).

Although resistance to any drug was observed more frequently in Beijing genotype strains than in non-Beijing genotype strains, there was no significant difference except for SM ($P = 0.03$) in the univariate analysis (Table 2). Thus, a multivariate analysis was performed to evaluate the correlation between Beijing genotype and drug resistance. However, in this statistical analysis Beijing genotype was not associated with any drug resistance (Table 3). The analysis was also stratified by new cases ($n = 256$) versus re-treatment cases ($n = 103$) and there was still no association between Beijing genotype and drug resistance (Table 4).

Table 3
Multivariate analysis of drug resistance associated with *Mycobacterium tuberculosis* genotypes.

Drug resistance	Beijing vs. non-Beijing	
	P-value	Adjusted OR (95% CI)
Any resistance	0.65	0.73 (0.19–2.81)
INH	0.84	0.87 (0.24–3.21)
RIF	0.58	2.08 (0.15–28.42)
SM	0.11	2.06 (0.85–5.01)
EMB	0.69	1.33 (0.33–5.34)
CAP	1.00	–
KAN	1.00	–
OFX	0.63	1.54 (0.27–8.81)
MDR	0.76	0.64 (0.04–10.33)
XDR	1.00	–

OR, odds ratio; CI, confidence interval; INH, isoniazid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol; CAP, capreomycin; KAN, kanamycin; OFX, ofloxacin; MDR, multidrug-resistant; XDR, extensively drug-resistant.

3.4. Factors associated with infection with Beijing genotype strain

The characteristics of patients infected with Beijing and non-Beijing strains were also compared (Table 2). There was no obvious difference between Beijing and non-Beijing genotype strains when considering risk factors such as patient sex, age, place of residence and treatment history (Table 2).

4. Discussion

China has the third highest TB burden in the world. Most TB cases are attributed to infection with Beijing genotype strains. Early reports based on clinical isolates in China revealed that the proportion of Beijing genotype varied from 62.2% to 90.5% [13,14,17–19,24,25]. The percentage of Beijing genotype strains in the south of China was lower than in the north [14]. The current study showed that 66.0% of isolates in south China belonged to Beijing genotype, suggesting its dominance in the region. In comparison with surrounding countries, the prevalence of Beijing genotype strains in this study was less than that of northeast Asian countries such as South Korea (80.0%) [26] and Japan (78.5%) [27], but higher than southeast Asian countries such as Vietnam (54.0%) [28], Thailand (44.1%) [29] and Myanmar (31.9%) [30].

The present study revealed that 34.8% of pulmonary TB patients were drug-resistant cases, demonstrating the serious prevalence of drug-resistant TB in south China. The proportions of MDR-TB and XDR-TB cases were 17.0% and 1.4%, respectively, nearly double in comparison with data presented in the national baseline survey in 2007 [31]. The explanation for this difference may be that the isolates included in the current study were obtained from specialised hospitals instead of from an epidemiological survey. In previous

Table 4
Associations between *Mycobacterium tuberculosis* Beijing genotype and drug resistance, stratified by treatment history.

Treatment history and drug resistance	All cases	Beijing genotype (%)	OR (95% CI)	*P*-value
New cases	256	169 (66.0)	–	–
Any resistance	53	35 (66.0)	1.09 (0.68–1.76)	0.73
INH	35	22 (62.9)	0.95 (0.53–1.68)	0.85
RIF	13	8 (61.5)	0.93 (0.38–2.29)	0.87
SM	31	21 (67.7)	1.03 (0.57–1.86)	0.92
EMB	4	3 (75.0)	1.14 (0.25–5.15)	1.00
CAP	0	0 (0.0)	–	–
KAN	0	0 (0.0)	–	–
OFX	5	4 (80.0)	1.22 (0.32–4.60)	1.00
MDR	12	7 (58.3)	0.88 (0.34–2.28)	0.79
XDR	0	0 (0.0)	–	–
Re-treated cases	103	68 (66.0)	–	–
Any resistance	72	52 (72.2)	1.40 (0.69–2.82)	0.35
INH	68	49 (72.1)	1.40 (0.71–2.76)	0.33
RIF	52	40 (76.9)	1.40 (0.76–2.60)	0.28
SM	35	30 (85.7)	1.53 (0.82–2.88)	0.18
EMB	19	16 (84.2)	1.36 (0.64–2.88)	0.42
CAP	6	5 (83.3)	1.28 (0.38–4.38)	0.94
KAN	5	4 (80.0)	1.23 (0.32–4.74)	1.00
OFX	23	19 (82.6)	1.35 (0.67–2.73)	0.40
MDR	49	38 (77.6)	1.40 (0.75–2.58)	0.29
XDR	5	4 (80.0)	1.23 (0.32–4.74)	1.00

OR, odds ratio; CI, confidence interval; INH, isoniazid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol; CAP, capreomycin; KAN, kanamycin; OFX, ofloxacin; MDR, multidrug-resistant; XDR, extensively drug-resistant.

studies, the frequencies of drug-resistant TB in re-treated patients were significantly higher than in new patients [16,31] and this was confirmed in the current study. A higher risk of drug resistance was also observed among re-treated patients. Generally, drug resistance among new cases indicates the transmission of drug-resistant strains, whereas drug resistance among re-treated cases likely indicates acquired drug resistance during treatment. This study implied that appropriate strategies must be implemented to reduce the amount of acquired drug resistance during treatment.

Some studies showed that Beijing genotype was significantly associated with drug resistance and was more likely to develop into MDR-TB [4,14,15]. However, this result is not consistent with several reports in other geographic settings [9,16,17]. In the current study, Beijing genotype strains showed higher drug resistance levels than non-Beijing strains. Univariate logistic regression was conducted to assess the association between Beijing genotype and any drug resistance. To exclude possible confounders in the study, all variables analysed in the univariate regression were also studied in the multivariate model. According to univariate analysis, Beijing genotype was more likely to be resistant to SM compared with non-Beijing genotype. Nevertheless, this association did not reach statistical significance in the multivariate analysis. Hence, the results supported that there was no independent association between Beijing genotype and drug resistance. The conflicting results observed from different studies and different regions are probably because of the amount of Beijing genotype and the proportion of its sublineages (modern or ancient Beijing genotype), which affected the DST results [17,32]. In addition, the difference might be related to the variation in treatment regimens, patient compliance and TB control programmes [16]. Although Beijing genotype was not associated with SM resistance in the multivariate analysis, this genotype still showed a great risk of developing SM resistance (adjusted OR = 2.06, 95% CI 0.85–5.01; $P = 0.11$).

Even in re-treated patients, the association of drug resistance with Beijing genotype was not significant. This result implied that Beijing genotype strains were no more likely to acquire drug resistance than non-Beijing genotype strains. Some reports also suggested that Beijing strains were significantly associated with TB patients of younger ages [9,28]. However, in the current study there was no statistical difference between Beijing genotype and non-Beijing genotype strains in terms of patient sex, age, place of residence or treatment history. Other factors contributing to infection with *M. tuberculosis* strains, such as social behaviours, were not investigated in this study.

This study had several limitations. First, the small sample size of isolated strains may have affected the statistical analysis and the detection of real associations. The results should be validated with a larger sample of strains in the future. Second, the isolates were from specialised TB hospitals in south China, probably leading to a higher rate of drug-resistant TB cases than in an epidemiological survey. Third, the number of second-line drug-resistant isolates, especially for CAP-resistant and KAN-resistant isolates, was relatively small, which prevented an analysis of their association with genotype among new cases.

In summary, these results revealed that Beijing genotype was predominant in south China but showed no independent association with any type of drug resistance. Previously treated patients demonstrated a higher risk of drug resistance and might deserve more attention in the management of TB patients. These findings will be helpful in establishing an appropriate TB control policy to be implemented in China.

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