

Non-Beijing Strains of *Mycobacterium tuberculosis* in China^{∇†}

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In a 2-year prospective study of tuberculosis (TB) patients in China, the prevalences of non-Beijing strains of *Mycobacterium tuberculosis* varied between Shandong Province (20.6%), Shanghai (27.6%), and Sichuan Province (45.9%) ($P < 0.005$). These differences may be due to factors such as human migration, transmission, or diversification and adaptation of the mycobacteria to different hosts.

In 1995, van Soolingen reported that 86% of *Mycobacterium tuberculosis* strains isolated from tuberculosis patients in the Beijing area of China had highly similar multiband IS6110 restriction fragment length polymorphism (RFLP) patterns and characteristic spoligotyping patterns. These strains were called Beijing family strains (25). Later, the multidrug-resistant “W” strain of *M. tuberculosis* that caused an outbreak in New York City in 1991 (4) was recognized as a member of the Beijing family strains (14). Since then, Beijing family strains have been isolated from many populations throughout the world and have been reported to have a high prevalence (8, 20), high virulence (17, 24), and a high probability of drug resistance (2, 12).

Although Beijing family strains were assumed to be the predominant strains causing active tuberculosis in China, different sublineages of non-Beijing strains have been detected in the countries and regions adjacent to mainland China (1, 3, 22, 23). In Taiwan, strains in the Haarlam (H) sublineage, East African-Indian (EAI) and EAI-like sublineages, and Latin American-Mediterranean (LAM) sublineage are also prevalent (7, 9). Three of the most hypervirulent clinical strains isolated in a study in Shanghai and Hong Kong belonged to families of non-Beijing strains (26). Furthermore, the prevalence of Beijing strains varied from 25% to 91.7% in different studies from different areas in mainland China (5, 16). Taken together, these findings suggest that non-Beijing strains may also contribute to the high tuberculosis burden in Asia.

To determine the prevalence and transmission potential of non-Beijing family strains in mainland China, we performed a population-based prospective study in three geographic areas. Fei County and Yan Zhou City are in Shandong Province, located in the central eastern part of China. Songjiang and Chongming are two districts in the municipality of Shanghai,

south of Shandong Province. Shuangliu County is in Sichuan Province in the southwestern region of China. We collected 1,004 *M. tuberculosis* clinical isolates from 988 culture-positive pulmonary tuberculosis patients from 1 December 2006 to 31 December 2008. We selected the initial isolate from each patient for a total of 988 isolates that were analyzed in this study. *M. tuberculosis* strains from sputum samples were cultured with Lowenstein-Jensen (L-J) medium (18). Of the total of 988 isolates in the present study, 381 (38.6%) were from Shanghai, 315 (31.9%) were from Shandong Province, and 292 (29.5%) were from Sichuan Province. For each of the clinical isolates, genomic DNA was extracted from the mycobacterial culture by the protocol described by Shen et al. (21). Three methods were used to genotype the strains. First, all strains were screened by using a deletion-targeted multiplex PCR (DTM-PCR) method to rapidly identify Beijing family strains, followed by 7-locus and 16-locus variable number of tandem repeats (VNTR) genotyping methods (6, 27). The non-Beijing strains that were identified by DTM-PCR were also spoligotyped (3, 11).

Among the 988 clinical isolates, 304 (30.8%) had non-Beijing strains of *M. tuberculosis*. Of the 988 patients, demographic information was missing from 4 of the tuberculosis patients. We used the nonparametric Wilcoxon rank sum test and the chi-square test of proportions to analyze the data. The median age of tuberculosis patients with a non-Beijing family strain (45.7 years; range, 18 to 87 years) was not significantly different from the median age of tuberculosis patients with a Beijing family strain (48.9 years; range, 15 to 98 years) ($P = 0.66$). The proportion of males among the tuberculosis patients with a non-Beijing family strain of *M. tuberculosis* (73.8%) did not differ from the proportion of males among the tuberculosis patients with a Beijing family strain (74.4%) ($P = 0.86$).

The percentage of non-Beijing strains varied in different geographic areas: 45.9% of the strains isolated from Sichuan were non-Beijing strains, while only 20.6% of the strains in Shandong Province were non-Beijing strains ($P < 0.0005$) (Table 1). The proportion of non-Beijing family strains in Sichuan Province was significantly higher than those in Shandong Province ($P < 0.0005$) and Shanghai ($P < 0.0005$). The proportion of non-Beijing strains in Shanghai was also significantly higher

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TABLE 1. Frequency and percentage of Beijing and non-Beijing family strains of *M. tuberculosis* in different areas of mainland China, 1 December 2006 to 31 December 2008

Sublineage	Frequency or percentage of strains in:						P value
	Shandong		Shanghai		Sichuan		
	n	%	n	%	n	%	
Total	315	100	381	100	292	100	
Beijing family strains	250	79.4	276	72.4	158	54.8	Reference
Non-Beijing family strains	65	20.6	105	27.6	134	45.2	<0.0005
T1	18	5.7	34	8.9	55	18.5	
T2	25	7.9	26	6.8	26	8.5	
H3	1	0.3	3	0.8	9	3.1	
Others ^a	5	1.6	10	2.6	9	3.1	
Unclassified	16	5.1	32	8.4	35	12.0	

^a The category "Others" includes sublineages H1, H4, LAM10_CAM, LAM9, MANU2, S, T1-T2, T2-T3, T3, T5, U (likely T3), and X.

than that in Shandong Province ($P = 0.034$). Therefore, the prevalences of non-Beijing strains were different across the three study areas in mainland China.

Based on the samples isolated in a nationwide random survey in 2000, the data from Li et al. showed that the difference in the prevalence of Beijing strains by region was of borderline significance ($P = 0.06$) (15). In our study, Beijing family strains were more prevalent in the central eastern part of China (Fei County and Yan Zhou County in Shandong Province), areas which have been inhabited for many centuries and have expansive, wide-reaching transportation systems. Throughout Chinese history, large numbers of people have migrated to and from the central eastern regions, likely disseminating the Beijing family strains. In contrast, Sichuan Province in southwestern China has a rather rugged geographical environment, its transportation systems were developed later, and non-Beijing strains are more prevalent in this region.

The spoligotypes of the 304 non-Beijing strains were compared with the data available in the fourth version of the international spoligotype database, SpolDB4 (<http://www.pasteur-guadeloupe.fr/tb/spolddb4/spolddb4.pdf>; accessed 31 January 2010). Altogether, we detected 113 different spoligotypes among the 304 isolates: 75 isolates (24.7%) displayed unique spoligotypes, and 229 (75.3%) had 1 of 38 spoligotypes. The discriminatory power (according to the Hunter-Gaston index [HGI]) of the spoligotyping method among non-Beijing strains in our study areas was 0.92 (13). Spoligotyping has a rather low discriminatory power among Beijing family strains, but when combined with DTM-PCR, it still provides a useful, rapid method to genotype non-Beijing strains in China.

We classified the spoligotypes from 219 isolates (72.0%; 219/304) into 53 shared international types (STs). The remaining 85 strains had 61 different spoligotypes that were not identified in the database. Of the 53 spoligotypes that were in SpolDB4, the most prevalent STs were ST52 in the T2 sublineage (27.9%; 61/219), followed by ST53 of the T1 sublineage

TABLE 2. Spoligotypes of 219 isolates of *M. tuberculosis* with a shared international type number in the SpolDB4 database for mainland China from 1 December 2006 to 31 December 2008

Spoligotype	Sublineage	ST ^a	No. of isolates
77777774020771	H1	47	2
77777770020771	H3	742	2
7777777520771	H3	746	1
7777777720771	H3	50	10
57777777420771	H4	127	1
77777743760731	LAM10_CAM	403	1
737777607760771	LAM9	388	1
77777777763771	MANU2	54	1
57637777760771	S	1211	1
37777777760771	T1	7	2
47777777760771	T1	804	1
57777777760771	T1	334	6
63777777760771	T1	285	1
67777777760771	T1	196	2
73777777760771	T1	205	1
77603777760771	T1	210	1
77677777760771	T1	1129	1
77577777760771	T1	917	1
77761777760771	T1	1214	3
777640007760771	T1	249	1
77767777760771	T1	498	1
77717777760771	T1	131	7
777740007760771	T1	803	1
77774377760771	T1	913	1
77775777760771	T1	393	2
77776377760771	T1	732	1
77777577760771	T1	281	1
77777403760771	T1	1688	2
77777407760771	T1	1800	1
77777707760771	T1	535	1
77777737760771	T1	86	3
7777774760771	T1	353	1
7777776760771	T1	1626	1
7777777760631	T1	888	2
77777777760700	T1	51	3
77777777760740	T1	1583	1
77777777760761	T1	278	1
77777777760771	T1	53	57
77777777760711	T1-T2	78	2
57777777760731	T2	1302	3
73777777760731	T2	848	1
74777777760731	T2	712	2
75777777760731	T2	153	5
77776777760771	T2	118	1
77777737760731	T2	1077	1
77777707760731	T2	1890	2
7777770760731	T2	942	1
77777777760731	T2	52	61
77773777760731	T2-T3	73	5
77773777760771	T3	37	3
77777757760771	T5	44	2
77773770000000	U (likely T3)	56	1
77776777760771	X1	119	1

^a ST, shared international type number.

(26.0%; 57/219), and ST50 of the Haarlem3 (H3) sublineage (4.6%; 10/219) (Table 2). The T family was the most prevalent sublineage, present in 196 (89.5%, 196/219) isolates. Among the strains in the T family, 107 belonged to the T1 sublineage, and 77 belonged to the T2 sublineage. The second largest family of non-Beijing strains was the H sublineage, with 16 isolates (7.3%; 16/219) (Table 2 and Fig. 1).

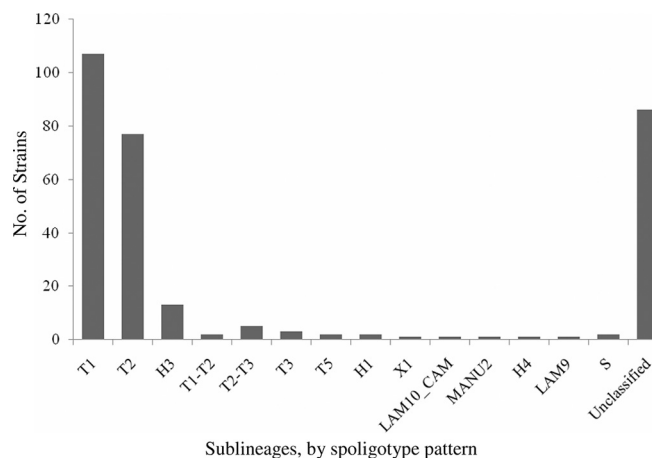


FIG. 1. Number of isolates with non-Beijing family strains of *M. tuberculosis* in three different geographical areas, by sublineages, from 1 December 2006 to 31 December 2008. The major sublineages of non-Beijing strains detected in the study were T1 and T2, which can be found on most continents in the world. “Unclassified” includes a group of strains with spoligotyping patterns that were not classified in SpolDB4.

The same sublineages of non-Beijing family strains were prevalent in our three different study areas (Fig. 2), but the distribution of sublineages of *M. tuberculosis* by spoligotypes varies throughout the world (11). For example, the Beijing family strains are prevalent in the Far East-Asia (19), and the EAI family was predominant in Southeast Asian countries (10, 19). H sublineages of *M. tuberculosis* have been found in Europe, Central America, and the Caribbean; LAM sublineages are prevalent in South America, Africa, the Mediterranean basin, and the Caribbean region (3). In the countries adjacent

to China, EAI family, Central Asia strains (CAS) were reported in neighboring Vietnam and Thailand, Pakistan, and India (1, 3, 22, 23). The predominant non-Beijing strains in our study were in the T family, which remains ill defined and has been reported from all continents (3). Further studies are needed to improve our knowledge of the factors and selective pressures that determine the prevalence of these strains in China.

To determine whether recent transmission of *M. tuberculosis* was likely in our study population, we compared the genotypes obtained by VNTR methods. For the strains with identical VNTR-7 patterns, VNTR-16 was performed. Strains from tuberculosis patients in the same study site (Shanghai, Shandong Province, or Sichuan Province) with identical VNTR-16 and spoligotyping patterns were defined as clustered strains (see Table S1 in the supplemental material). Among 304 non-Beijing strains, we found 15 VNTR clusters with a total of 39 strains. Among the 15 clusters, 3 clusters with 9 strains belonged to the T1 sublineage, 6 clusters with 17 strains belonged to the T2 sublineage, 1 cluster with two strains belonged to the T2-T3 sublineage, and five clusters with 11 strains had a previously unclassified spoligotype pattern. Strains in the T1 sublineage were less likely to be clustered than Beijing family strains ($P = 0.0001$) (Table 3).

In conclusion, despite the high prevalence of Beijing family strains in mainland China, many non-Beijing strains were also isolated from tuberculosis patients. Although the sublineages of non-Beijing strains were similar between different populations, the prevalence of non-Beijing sublineages of strains varied across different geographical areas. The most prevalent non-Beijing family strains in our study population belonged to the T family, which has been reported from all continents.

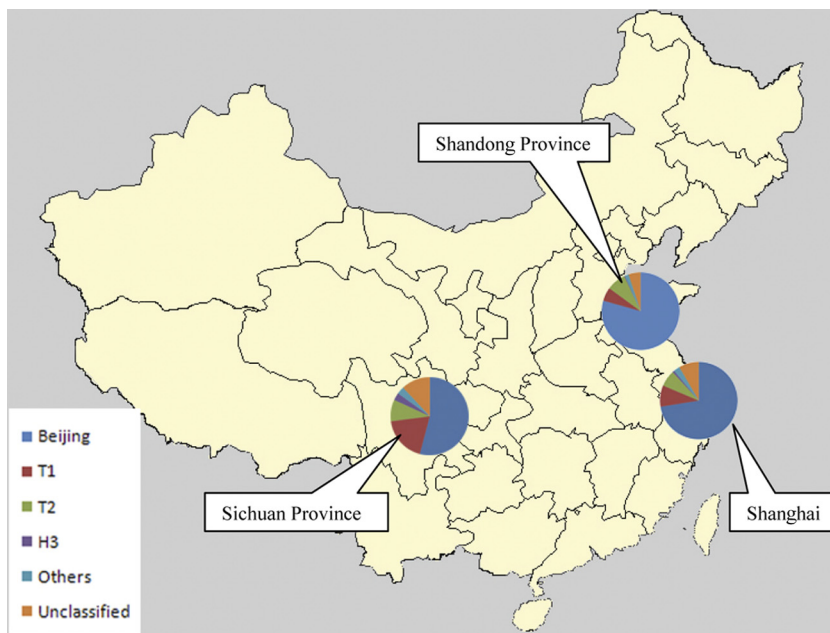


FIG. 2. Map of China showing the percentage of different sublineages of *M. tuberculosis* in three different geographical areas from 1 December 2006 to 31 December 2008. The category “Others” includes sublineages H1, H4, LAM10_CAM, LAM9, MANU2, S, T1-T2, T2-T3, T3, T5, U (likely T3), and X.

TABLE 3. Sublineages of clustered and nonclustered clinical isolates of *M. tuberculosis*, based on spoligotyping and VNTR-7 typing patterns

Spoligotyping lineage or sublineage ^a	VNTR ^b result from:				P value
	Clustered strains		Nonclustered strains		
	n	%	n	%	
Beijing family strains	176	25.7	508	74.3	Reference
Non-Beijing family strains					
T1	9	8.4	98	91.6	0.0001
T2	17	22.1	60	77.9	0.485
T2-T3	2	40.0	3	60.0	0.468
Unclassified	11	12.9	74	87.1	0.010

^a Strains in the T1 sublineage were less likely to be clustered than Beijing family strains ($P < 0.05$). "Unclassified" includes the different spoligotyping patterns that were not identified in the SpolDB4 database.

^b VNTR, variable number of tandem repeats.

Compared with Beijing family strains, strains in the T1 sublineage were less likely to be clustered.

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