

Evolution of Drug Resistance in Different Sublineages of *Mycobacterium tuberculosis* Beijing Genotype

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We compared the population structure and drug resistance patterns of the *Mycobacterium tuberculosis* strains currently circulating in the Beijing area of China. One hundred thirteen of 123 strains belonged to the Beijing family genotypes defined by spoligotyping. The Beijing genotype strains were further subdivided into old and modern sublineages on the basis of NTF locus analysis. A stronger association with resistance to the more recently introduced antituberculosis drugs has been observed for old versus modern strains of the Beijing genotype, suggesting that its different sublineages may differ in their mechanisms of adaptation to drug selective pressure.

In some areas of the world, the increased rate of multidrug-resistant (MDR) tuberculosis (TB) appears to be linked to a disequilibrium in the local population structure of *Mycobacterium tuberculosis*, manifested as a predominance of particular genetic lineages and sublineages. For example, strains of the Beijing genotype are endemically prevalent in eastern Asia, South Africa, and northern Eurasia (reviewed in references 1 and 9), and new unexpected routes of their transmission are being uncovered (7). This genotype was first identified in *M. tuberculosis* strains isolated in the Beijing area of China, for which it was named (23). Currently, these strains attract great attention worldwide because they demonstrate important pathogenic features (15, 24) and association with drug resistance (4, 9).

China is one of the MDR TB hot spots, along with Russia and India (6). The increased rate of drug-resistant and MDR strains of *M. tuberculosis* remains a serious problem of TB control in China (14, 20). In the present study, we investigated the current population structure of *M. tuberculosis* in the Beijing area of China and compared it with drug resistance patterns in order to gain new insights into the evolution of drug-resistant TB.

One hundred twenty-three *M. tuberculosis* isolates were recovered from 123 adult pulmonary TB patients admitted to the Beijing Chest Hospital in 2002 to 2005. No epidemiological connection of these patients could be detected by standard

investigation. For each patient, only the first available isolate was included in this study. Löwenstein-Jensen medium was used for cultivation of isolates. Testing of susceptibility to rifampin (RIF), isoniazid (INH), streptomycin (STR), ethambutol (EMB), and pyrazinamide (PZA) was done by the method of absolute concentration as previously described (3).

DNA from cultured cells was extracted as described by van Embden et al. (22). Strain differentiation was performed by spoligotyping (11). A PCR approach was used to determine a possible IS6110 insertion(s) in the NTF region of the *M. tuberculosis* Beijing genotype strains (18). Three NTF variants of the Beijing strains are distinguished on the basis of the presence or absence of the IS6110 sequence, thus providing a rough subdivision within this genotype. The W branch prevalent in the United States harbors two head-to-tail IS6110 insertions separated by a 556-bp noncoding spacer (13). Most Beijing strains worldwide harbor only one IS6110 insertion (1, 13); we previously defined them as the NTF::IS6110 “modern” branch (16). Finally, the Beijing strains without an IS6110 insertion in the NTF region (12, 13) were previously defined as atypical (12, 17), ancient/primordial (16), or ancestral (17, 19); here, we assigned these strains to an “old” sublineage of the Beijing genotype.

Odds ratios and *P* values, were calculated with EpiCalc software (8).

Results and discussion. A total of 123 *M. tuberculosis* strains were included in this study. They comprised 40 pansusceptible and 83 resistant isolates randomly selected among those isolated between 2002 and 2005 in Beijing, China. A majority of the resistant strains were multidrug resistant; the distribution of the drug resistance profiles of all of the strains is shown in Table 1.

All strains were subjected to spoligotyping in order to assess their genetic relatedness. This genotyping method subdivided the 123 strains into 14 types (Table 2). The largest cluster

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TABLE 3. Drug resistance in old versus modern Beijing genotype strains

Drug resistance ^a	No. (%) of strains		P value	Odds ratio (95% confidence interval)
	Old Beijing sublineage (n = 27)	Modern Beijing sublineage (n = 86)		
S (1944)	9 (33.3)	32 (37.2)	0.7	0.84 (0.34, 2.10)
H (1952)	17 (63.0)	47 (54.7)	0.5	1.41 (0.58, 3.43)
R (1970s)	21 (77.8)	47 (54.7)	0.03	2.90 (1.07, 7.91)
E (1960s)		9 (10.5)		
Z (1980s)	6 (22.2)	8 (9.3)	0.08	2.79 (0.87, 8.91)
Any drug resistance	21 (77.8)	56 (65.1)	0.2	1.88 (0.68, 5.15)
MDR	17 (63.0)	38 (44.2)	0.09	2.15 (0.88, 5.23)

^a In parentheses is the time of introduction of the particular drug for anti-TB therapy (10). S, STR; H, INH; R, RIF; E, EMB, Z, PZA; MDR, resistance to at least RIF and INH.

(atypical) Beijing genotype strains have been made by Kremer (12) for strains from Hong Kong and Vietnam. Anti-TB chemotherapy was only introduced some 60 years ago (10), and neither old nor modern Beijing strains were subjected to the selective pressure of the drugs before then. A comparison of the order of implementation of the particular drugs used for anti-TB therapy with *P* values in Table 3 revealed an enigmatic and somewhat quantitative association pattern. It shows that resistance to the historically more recently introduced drugs (RIF and PZA) is associated with old Beijing strains and furthermore is the most prominent for the most recently implemented drug, PZA. Contrarily, this difference between old and modern Beijing sublineages is smoothed and even reversed for INH and STR, respectively, which were introduced earlier (Table 3).

It may be that different mechanisms including second-order selection acting on DNA repair systems (19, 21) lie behind mycobacterial adaptation and, in particular, behind the development of drug resistance in different sublineages of the Beijing genotype. The observed variation of the drug resistance patterns in the *M. tuberculosis* Beijing strain populations in different countries may be explained by different ratios of modern to old Beijing genotype subpopulations in the local populations of *M. tuberculosis*. Perhaps the prevalence of the modern Beijing strains in the currently circulating global population of this genotype results not from drug-driven selection but from a real situation prior to the advent of anti-TB therapy 60 years ago, an evolutionarily negligible period of time. It seems reasonable to assume that old (ancestral [17, 19], ancient [16], or atypical [12]) strains were also less numerous than modern "progeny" (typical [12]) strains in that population of the Beijing genotype. Alternatively, the current worldwide dissemination of the modern Beijing genotype strains may be due to their hypervirulent features rather than increased mutability leading to the rapid acquisition of drug resistance, although further studies of this effect are undoubtedly required.

I.M. and W.W.J. contributed equally to this study.

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