

SHORT PAPER

Seventy percent of the *Mycobacterium tuberculosis* isolates in Hong Kong represent the Beijing genotype

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SUMMARY

We used spoligotyping to study 500 randomly selected pretreatment *Mycobacterium tuberculosis* (MTB) strains isolated in Hong Kong during the 2 year period 1998–9. It was found that amongst all MTB strains studied, the ‘Beijing’ genotype strains were highly prevalent in our geographic area, representing about 70% of the isolates. Unlike previous observations in Vietnam, no significant associations were found either between ‘Beijing’ genotype strains and all other anti-tuberculosis drug resistance phenotypes, or with particular patients’ age groups, except for a weak association with isoniazid susceptibility. Eighteen of these strains exhibited spoligotype patterns that were similar but not identical to the ‘Beijing’ specific pattern. This is the first geographical area where genetic diversity among ‘Beijing’ genotype of MTB strains has been observed on this scale.

In the investigation of the molecular epidemiology of *Mycobacterium tuberculosis* isolates from China and Mongolia, a distinct group of strains which share more than two-thirds of their IS6110 containing *Pvu*II restriction fragments was revealed and was designated as ‘Beijing’ genotype [1]. This ‘Beijing’ genotype of *M. tuberculosis* strains have recently been found to predominate in East Asian countries [1, 2], whereas lower prevalences of such strains were reported in other countries. In the Beijing region of China, about 80% of the *M. tuberculosis* isolates were of this genotype, and in East Asian countries such as Mongolia, Thailand, Vietnam and Republic of Korea, the prevalence was around 40–50% [1]. A recent study in Vietnam revealed that the ‘Beijing’ genotype strains are emerging in this area, as this type of *M. tuberculosis* strains were found more prevalent among

active transmission cases (young patients) than among endogenous reactivation cases (older patients) [3]. Moreover, as in other areas, a significant association between the Beijing genotype strains and resistance was observed [1, 4, 5]. In this study we used spoligotyping method to examine the prevalence of ‘Beijing’ genotype strains in Hong Kong and we examined possible associations with bacteriological or clinical factors.

A total of 500 *Mycobacterium tuberculosis* isolates were selected at random from the specimens received at the Tuberculosis Reference Laboratory in Hong Kong in the 2 years period 1998–9. These non-duplicate strains were isolated from pretreatment patients belonging to five different age groups (less than 25 years, 25–34 years, 35–44 years, 45–54 years, older than 54 years); with 100 strains for each age group. The bacterial cells were suspended in TE buffer, heat-killed for 40 min at 80 °C and were analysed by spoligotyping [6].

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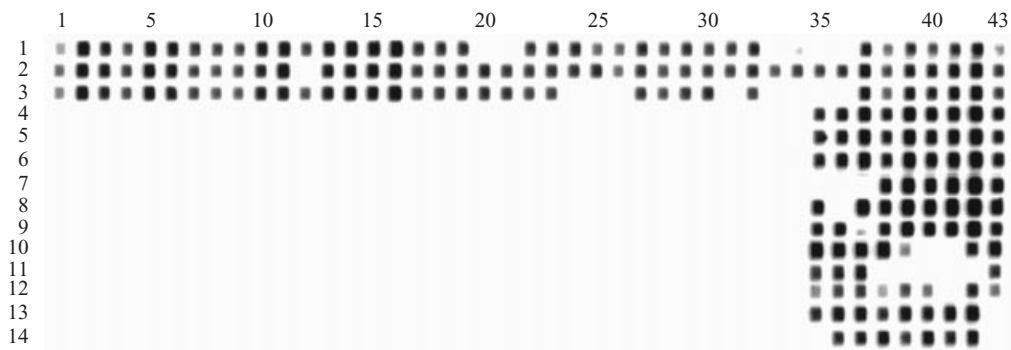


Fig. 1. Spoligotypes of 13 *M. tuberculosis* strains in Hong Kong. Numbers on the top correspond to the 43 applied spacers [3] while numbers at the left represent the lane number. Lane 1: *M. tuberculosis*, H37Rv; lanes 2–3: non-‘Beijing’ genotype; lanes 4–6: ‘Beijing’ genotype; lanes 7–14: ‘Beijing-like’ genotype.

Spoligotyping is a highly reliable method to identify ‘Beijing’ genotype strains which bear the characteristic nine-spacer reaction on the spacers 35 to 43 [1, 4, 5]. This method is based on DNA polymorphism present at one particular chromosomal locus, the DR region, which is uniquely present in *Mycobacterium tuberculosis* complex bacteria.

In vitro susceptibilities to anti-tuberculosis drugs were determined by an absolute concentration method on Lowenstein Jensen medium, using the following breakpoints: streptomycin 32 µg/ml, isoniazid 1 µg/ml, rifampicin 64 µg/ml, ethambutol 4 µg/ml. Determination of these breakpoint values were based on testing methods as previously described [7]. Proficiency of drug susceptibility tests was conducted within the WHO/IUATLD Western Pacific Region Supranational Reference Laboratory Network. Statistical analysis for possible significant association of ‘Beijing’ genotype with different age groups and antibiotic resistance profile patterns was performed using χ^2 test. A two-sided *P*-value of less than 0.05 was taken as cut-off for significance.

Among the 500 strains with spoligotype patterns analysed, the ‘Beijing’-specific genotype pattern was found to be most predominant (337/500; 67.4%). Of the remaining 163 isolates, 18 exhibited ‘Beijing-like’ genotype i.e. patterns were similar, but not identical, to the ‘Beijing’-specific pattern. These consisted of 4–8 positive spacers out of the 9 spacers characteristic of ‘Beijing’ genotype strains (Fig. 1). The distribution of ‘Beijing’ genotype isolates among the five patient age groups was fairly even (61–72%), with only the middle age (35–44 years) group and higher age (> 55 years) group showing a slightly higher percentage (72%). Associations between any resistance (alone or in combination) to streptomycin, isoniazid, rifampicin

Table 1. Association between ‘Beijing’ genotypes and antibiotic resistance profiles to isoniazid, streptomycin, rifampicin and ethambutol in Hong Kong, 1998–9

Genotype	No.	No of strains (%) resistant, alone or in combination, to*			
		INH	SM	RIF	EMB
‘Beijing’	319	20 (6.3)	31 (9.7)	2 (0.6)	2 (0.6)
‘Beijing-like’	18	0 (0.0)	2 (11.1)	0 (0.0)	1 (5.6)
Others	163	21 (12.9)	21 (12.9)	1 (0.6)	3 (1.8)
<i>P</i> value of association†		< 0.05	0.5	–	0.7

* No., number of strains; INH, isoniazid; SM, streptomycin; RIF, rifampicin; EMB, ethambutol.

† *P* value of association were performed between ‘Beijing’/‘Beijing-like’ genotypes versus other genotypes.

and ethambutol and the ‘Beijing’ genotype are shown in Table 1. There was a tendency for the ‘Beijing’ genotype to be associated with isoniazid-sensitive strains ($P < 0.05$). No significant association between ‘Beijing’ genotype strains and all other anti-tuberculosis drug resistance phenotypes were found, or with particular patients’ age groups.

In summary, we used a highly reliable molecular method to identify the ‘Beijing’ genotype of *M. tuberculosis* strains in Hong Kong and found that these were highly prevalent in our patients infected with MTB. In contrast to the results obtained in Vietnam [3], however, there was no significant association between ‘Beijing’ genotype strains and resistance to anti-tuberculosis drugs although slightly fewer ‘Beijing’ genotype strains were resistant to isoniazid. We also did not find any significant correlation between the distribution of ‘Beijing’

genotype strains and the age groups of patients, indicating that in our locality, the 'Beijing' genotype does not seem to be associated with active TB transmission. Our finding of almost equal distribution of these strains in the different patient age categories may reflect the natural distribution of these bacteria in this geographic area for an extended time period. The finding of strains with 'Beijing-like' spoligotype patterns may also point in this direction. In fact, this is the first geographic area in which a genetic divergence has been found among 'Beijing' genotype strains. It would be interesting to conduct similar studies of *M. tuberculosis* strains isolated in different areas in China and those bordering areas of Hong Kong to delineate the extent and possible relationship of this genetic diversity.

Unlike in other geographic areas [3], no association between the nine-spacer 'Beijing' genotype strain and anti-tuberculosis drug resistances, or active transmission was observed in Hong Kong. We postulate that this difference may be due to certain selective advantages of this genotype over other genotypes in particular geographic areas. Further investigation on this postulation is worthwhile in studies of TB drug resistance and transmission.

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