Sublineages of Beijing Strain of *Mycobacterium tuberculosis* in Sri Lanka

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Abstract Strains of the Beijing/W genotype of *Mycobacterium tuberculosis* have been responsible for large outbreaks of tuberculosis around the world, sometimes involving multi-drug resistance. It has been shown that more recently evolved Beijing sublineages are prone to cause outbreaks. Furthermore Beijing is the single predominant cluster in Sri Lanka. The present study identifies that recently evolved sublineages of Beijing strains are present in the study population. The majority of Beijing isolates (92.85%) were pan-susceptible. However, these findings may have important implications for the control and prevention of tuberculosis in Sri Lanka.

Keywords Beijing strain · *Mycobacterium tuberculosis* · Sri Lanka

Molecular epidemiological studies that have been carried out so far hypothesize that Beijing strains of *Mycobacterium tuberculosis* (*Mtb*) have evolved acquiring unique properties [1]. Some of these properties are the ability to evade the protective effect of *Mycobacterium bovis* BCG vaccination [2], more efficient dissemination compared to non-Beijing strains [3] and the ability to acquire drug resistance more frequently as a consequence of single nucleotide polymorphisms in mismatched repair genes [4].

Mycobacterium tuberculosis strains with a Beijing genotype are members of the principal genetic group 1 and are characterised by a distinct spoligotype pattern, closely related IS6110 DNA fingerprints and identical variable-

number tandem repeat sequence [1]. Comparative genomics have shown that Beijing strains have evolved through several mechanisms including IS6110 transposition [5] and deletions of chromosomal domains (regions of differences [RD]) [6].

The initial spoligotyping results of *Mtb* in Sri Lanka revealed that Beijing (ST1) is the most predominant single cluster [7]. As part of the ongoing molecular epidemiological study, an attempt was made to determine the evolutionary sublineage of Beijing strain isolates to determine the implications for tuberculosis control in Sri Lanka.

The study was conducted on *Mtb* isolates collected at the Department of Microbiology (DM), Faculty of Medicine, University of Colombo over an 18 month period from July 2005. All samples received within 24 h of collection are processed for PCR, culture and subsequent phenotypic drug sensitivity testing. Isolates are preserved at -70°C in 15% glycerol phosphate broth. During the study period DM collected 210 isolates and 100 were selected by random sampling. Spoligotyping was carried out according to manufacturer's guidelines using a commercially available kit (Isogen, Maarsen MV, The Netherlands). Fourteen isolates that confirmed to be Beijing strain were further characterised.

The ethical approval for the study was granted by Ethical Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka.

Each Beijing strain was subjected to PCR amplification in a reaction mixture containing 0.2 μg DNA template, 5 μl Q buffer, 2.5 μl 10 $\times$ buffer, 2 μl 25 mM MgCl_2 , 4 μl of 10 mM dNTPs, 1 μl of each primer (50 pmol/ μl) (Table 1) and 0.125 μl HotStarTaq DNA polymerase (QIAGEN, Germany) and made up to 25 μl with H_2O . Primers 1–4 were used in combination with the Universal Forward primer and the internal control primers to

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determine the presence/absence of *IS6110* elements at specific chromosomal loci [5, 8]. The primers 5–7 were used to determine the presence of RDs (181,150 and 142) [6] while primer 4 was specifically used to identify the relationship to Beijing-220 cluster [8].

All 14 patients were in the age group of 15–45 years (Mean = 35.08). Majority of them ($n = 8$) were from Colombo (Western province) and rest from Galle (Southern province). Almost all isolates ($n = 13$, 92.85%) demonstrated pan susceptibility to anti-TB drugs. The drug resistant isolate was from a re-treatment patient and demonstrated resistance to isoniazid. This isolate did not belong to Beijing-220 cluster [8] and further sequencing revealed a new mutation at *katG*315 AGC → GGC (Ser → Gly).

Seven isolates (50%) including the isoniazid resistant strain belonged to sublineage-6, defined by the presence of RD150 and *IS6110* insertion at chromosomal position 3379025 (Table 2). Further six isolates belonged to sublineage-5 (35.7%) with *IS6110* insertion at chromosomal position 888990 while the remaining isolate to sublineage-4 (Table 2) [4]. None of the isolates belonged to sublineage-7 (defined by RD150 deletion) [4] nor demonstrated RD142 deletion [4, 6]. Furthermore, all isolates belonged to sublineage-2 or above (confirmed by RD181 deletion) [4].

This is the first study carried out so far to further characterise Beijing strain isolates in Sri Lanka. A recent report indicated Beijing strain as the predominant strain in Sri Lanka [7]. Further to previous study, we observed that more recently evolved sublineages of Beijing are prevalent in our study population with a majority of isolates ($n = 13$, 92.85%) belonging to either sublineage-6 or 5 [4].

Beijing strain is known for its ability to evade protective effect of *M. bovis* BCG vaccination [3]. Sri Lanka is

recognized as a country with high coverage of BCG vaccination [9]. Although the role of recently evolved sublineages in evading BCG protection is unknown, one cannot disregard the high selection advantage the Beijing strain would have in Sri Lanka. It is also interesting to note that all the patients were from two major cities with high population density, tuberculosis burden [10] and high BCG coverage [9].

It has long been known that recently evolved strains of Beijing adapt to spread and cause tuberculosis efficiently. This was aptly demonstrated by Hanekom et al. [4] with predominance of these sublineages in their sample. They also noted that sublineages-5, 6 and 7 are associated with multi-drug resistant tuberculosis [4]. In contrast to their findings [4], almost all our isolates ($n = 13$) were pan-susceptible probably indicating that Beijing strains in our study were naïve. The isolate that demonstrated resistance to isoniazid was further probed to see whether it belonged to the much-dreaded Beijing-220 cluster; a fully drug susceptible strain which consequently acquired drug resistance by sequential selection (initially isoniazid followed by rifampicin) [8]. However, none of the isolates in our sample belonged to 220 cluster.

The results of our study revealed the presence of recently evolved Beijing strains among a relatively young cohort of patients. These highly transmissible strains of *Mtb* in population dense areas of a country with high BCG coverage may pose threat to community, calling urgent action for setting up a molecular epidemiological surveillance program, ideally combined with molecular typing. This would appear even more important in the light of drug resistant mutations as inadequate treatment regimes may lead to selection of resistant strains, which can cause deleterious consequences especially in the presence of

Table 1 Primer sequences^a

Primer set	Primer name ^d	Sequence (5' to 3')
Internal control	Internal control	TCC CAG TGA CGT TGG CTT C
	Internal control	GAG CAG CAG TGG AAT TTC GC
Universal forward	Universal Forward	TTC AAC CAT CGC CGC CTC TAC
1	IS21-11(3379025 ^b)	GGG RRR RAA AAA AGR CGC TGT GAA CC
2	IS21-10(888990 ^b)	TGA GGG GCC GTC GGC CGC CCT GAA CC
3	IS21-1(1262963 ^b)	GTC GCC GGA GTT GAA GAA GCT GAA CC
4	954.19 R	AAT CGT TGA TGC TGG CGC TAT GAA CC
5	RD181 ^c F	AAA TCC GCC CAT ACC CGT C
	RD181 ^c R	AGC TTC GAC TGG CCA TAG GC
	RD150 ^c F	AGT GCT GGC AAT AGC GGT TG
	RD150 ^c int	CAC CGG CAC TTA CCA TCT CG
6	RD150 ^c R	CCA GCA CTT GTT GCA ACT TCG
	RD142 ^c F	CCG GTG GTA CGG GTA TTT CC
	RD142 ^c int	GCT CGA GCA TGA TCA GCA AAG
7	RD142 ^c R	TAG CAC CAG TAC CGG ATG TCC

^a *Tm* annealing temperature was 62°C

^b Chromosomal position of *IS6110* insertion site (5)

^c RD, regions of differences (6)

^d F, forward primer; R, reverse primer; int, internal primer

Table 2 Analysis of sublineage of Beijing strain isolates obtained from patients with pulmonary tuberculosis^a

Isolate	RD150 ^b	IS6110 at 3379025 ^c	IS6110 at 888990 ^c	IS6110 at 1262963 ^c	RD181 ^b	Sublineage ^d
FMC06	+	+	+	+	–	6
FMC10	+	+	+	+	–	6
FMC18	+	+	+	+	–	6
FMC19	+	+	+	+	–	6
FMC22	+	+	+	+	–	6
FMC40	+	+	+	+	–	6
FMC46	+	+	+	+	–	6
FMC25	+	–	+	+	–	5
FMC32	+	–	+	+	–	5
FMC36	+	–	+	+	–	5
FMC45	+	–	+	+	–	5
FMC48	+	–	+	+	–	5
FMC52	+	–	+	+	–	5
FMC29	+	–	–	+	–	4

^a + present, – absent/deleted^b RD, regions of differences (6)^c Chromosomal position of IS6110 insertion site (5)^d Sublineage (4)

recently evolved Beijing strains, which are more prone to acquire drug resistance. However, the results of the present study cannot be generalised. Larger studies with representative samples employing molecular methods such as those used in this study are needed to elucidate the role of these genotypes in the dissemination and transmission of tuberculosis in Sri Lanka.

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Conflict of interest None.

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