

Thesis Title DNA Fingerprinting of *Mycobacterium tuberculosis*
Isolated From HIV-positive Patients in
Bamrasnaradura Hospital

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ABSTRACT

Tuberculosis, caused by *Mycobacterium tuberculosis*, is an important infectious disease. Because of the increased incidence of this disease, in 1992, WHO declared tuberculosis as a "global emergency". It was estimated that 1.7 billion people were latently infected with *M. tuberculosis*. Tuberculosis was increasing especially in Human Immunodeficiency Virus (HIV)-infected patients. Studying outbreaks and tracing transmission of particular strains of *M. tuberculosis* are important to control tuberculosis. In this study, the restriction fragment length polymorphism (RFLP) using IS6110 as probe was used to analyze the genotypic variation of *M. tuberculosis* which were isolated from patients in Bamrasnaradura Hospital (117 isolates) and the Central Chest Hospital (48 isolates).

M. tuberculosis isolates had 0-21 IS6110-hybridized bands. Only one isolate did not hybridize to IS6110 probe. The IS6110 hybridization patterns can be used to divide the isolates into 4 groups. They were single-banded isolates (20%), the Beijing family (38%), the Nonthaburi group (15%) and the heterogeneous group (27%). We found 5 different lengths of the hybridized

band in the single-banded isolates. They were 1.4, 1.45, 1.5, 5.0 and 5.5 kb long with the most common one being 1.45 kb long and second most common one being 5.0 kb long. Confirmation that the single-banded isolates were *M. tuberculosis*, was done by using primers specific to species-specific fragment, *mtp40*. The polymorphic GC rich sequence (PGRS) probe and 36 bp direct repeat (DR) probe were used to analyze the relationship of the single-banded isolates. The PGRS hybridization patterns and DR hybridization patterns were polymorphic. Only 3 clusters, each with 2 isolates, were found. DNA of the isolates in the Beijing family and the Nonthaburi group were rehybridized with the PGRS and DR probes. The PGRS hybridization patterns of both groups were less polymorphic than the patterns of the single-banded isolates. The DR patterns of Nonthaburi group were less polymorphic too, while all the member of the Beijing family had only one DR-pattern.

Most of the patients were male (80%) and had middle age (median age=34.8). Most patients stayed in Bangkok and Nonthaburi. The most common type of specimens was sputum and the second most common was lymph node. 67% of the patients in this study were HIV-seropositive. 72% of the isolates in this study were sensitive to all tested antimicrobial drugs. There was statistical relationship between drug resistance and the hybridization patterns. The other clinical and demographic data were not significantly different between each *IS6110*-hybridization group.

There were ten *IS6110* clusters with 7 being the members of the Beijing family. One isolate in a cluster had a different PGRS pattern from the other isolates in the same cluster. The members of the Beijing family were significantly more likely to be clustered than the heterogeneous group. HIV-seropositivity was significant more common among the clustered patients.

This study suggested that the strains with single *IS6110*-banded should be hybridized with the other DNA probes such as the PGRS and DR probes.

Isolates with identical IS6110-bands should also be hybridized again with other probes.