Abstract

Purpose

In Taiwan, tuberculosis remains an important treat to public health, and multidrug-resistant *Mycobacterium tuberculosis* is an emerging problem. One of the most important measures to control the prevalence of tuberculosis is strain typing of *M. tuberculosis*. The data of strain typing is helpful in surveillance of the disease and providing knowledge of transmission of the organism in the community. Several techniques are now rather commonly used for typing of *M. tuberculosis*, including IS6110-restriction fragment length polymorphism analysis, the standard method for genotyping of *M. tuberculosis*, spoligotyping, and variable-number tandem repeats (VNTR). The VNTR method is based on the fact that there are variable numbers of tandem repeats among different strains of M. tuberculosis. The purposes of the present project are to develop the VNTR method and to investigate susceptibilities to the first-line antituberculosis drugs in *M. tuberculosis* isolates in southern Taiwan and the genotypes of the drug-resistant isolates.

Materials and Methods

Test organisms included 151 *M. tuberculosis* isolates collected between January 2002 and January 2003 at the Department of Pathology, National Cheng Kung University Hospital, and 150 isolates provided by the Center for Disease Control, Taiwan and originally from four other medical centers in Taiwan. Five targets were selected or VNTR analysis. They were ETR-A, ETR-B, ETR-C, ETR-D, and ETR-E. Polymerase chain reaction assays were performed to amply these genes, followed by agarose gel electrophoresis to estimate the sizes of amplicons and the numbers of tandem repeats. For each target, a number was given according to the number of tandem repeats, and a VNTR type with a five-digital number, which is derived from five numbers of tandem repeats of five targets, is given for each isolate. Susceptibility tests were performed with the proportion method. Test drugs included isoniazid, streptomycin, ethambutol, and rifampin.

Results

Among 299 isolates of *M. tuberculosis*, 62 types were obtained by VNTR. The most common VNTR types in *M. tuberculosis* isolates varied among different regions in Taiwan. VNTR type 42435 was most common (46.0%) among the isolates from northern Taiwan. The most common types among the isolates from central Taiwan were 31433 (36.0%) and 46464(14.0%). The most common types among the isolates from southern Taiwan were 46464, 31433 and 42435 types, with a prevalence rate of 24.5, 17.2, and 16.6%. The most common types among the isolates from eastern Taiwan were 31433 (25.0%) and 32433 (14.6%). *M. tuberculosis* isolates from the National Cheng University Hospital were further subjected to susceptibility tests. Among the 151 isolates, 32 (21.2%) isolates were resistant to at least one of the first-line antituberculosis drugs, and eight isolates (5.3%) were multidrug-resistant. The prevalent rate of multidrug resistance is higher in the isolates from the National Cheng Kung University Hospital than in those from district hospitals in Taiwan. No prevalent VNTR types were found among drug-resistant *M. tuberculosis* isolates from the National Cheng Kung University Hospital.

Conclusions and Suggestions

The present study indicates that VNTR typing is a simple and rapid

method for typing *M. tuberculosis* strains. It should be an acceptable auxiliary method to the standard IS6110-restriction fragment length polymorphism analysis. An automatic typing method based on VNTR should be developed in the future to improve the technique. The variation of genotypes among isolates from different regions needs further investigation to understand the transmission of tuberculosis in Taiwan. The variation of susceptibilities to antituberculosis drugs between isolates from medical centers and district hospitals also need further investigation. The variation of genotypes of drug-resistant isolates suggests that the prevalence drug resistance in *M. tuberculosis* was due to the development of drug resistance among different strains rather than due to the spread of a resistant clone.

Keyword: *Mycobacterium tuberculosis*；restriction-fragment length polymorphism；Variable-Number Tandem Repeat