CHAPTER I

INTRODUCTION

Tuberculosis (TB) remains a major global public health problem in many countries. The World Health Organization (WHO) estimates that 8 million new cases and 3 million deaths directly attributable to the disease each year. The association of TB with human immunodeficiency virus (HIV) infection has proved to be a major new factor in the increase of clinical TB (1-5). Rapid and accurate identification of *Mycobacterium tuberculosis* is critical to ensure that correct decision are made regarding respiratory precautions, antituberculosis drug therapy, and prophylaxis (6). Concurrent with the increased occurrence of TB is the emergence of multidrug-resistant (MDR) isolates of *M. tuberculosis*, which are at least resistant to rifampin and isoniazid (7).

In Thailand, TB was fifth-ranked among over all mortality rates reported in 1995 (8). The annual risk of infection in 1997 is estimated to be 1.40%, while appoximately 100,000 new TB patients of which 50,000 cases are smear positive developed each year. Among these only 40% of total new TB patients and 50-60% of new smear positive patients are diagnosed for treatment (9). Drug resistance surveillance project has been conducted in Thailand, covering 46 provinces, since 1996. The preliminary report revealed overall figure of multidrug-resistant tuberculosis (MDR-TB) of 2.15% and monoresistance to rifampin 6.1% and isoniazid 5.7% in 1998 (10).

Currently, therapy for tuberculosis involves multidrug chemotherapy for a period of several months. The most effective treatment regimens include isoniazid (H) and rifampin (R), the two most effective drugs available in the anti-tuberculosis armamentarium, at least for part if not all of the treatment regimen. Other potent drugs in common use include pyrazinamide (Z), streptomycin (S), and ethambutol (E).

The treatment regimen recommended by WHO is referred to as short course chemotherapy (SCC) and consists of 2 months of H, R and Z plus a fourth drug (usually S or E), followed by 4 months of dual drug therapy with H and R, or alternatively followed by 6 months of H and E (7).

Rifampin was introduced in 1968 as a potent antituberculosis agent. Rifampin is rapidly bactericidal against *M. tuberculosis*, and its use has greatly shortened the duration of chemotherapy necessary for the successful treatment of drug-susceptible tuberculosis. High-level resistance to rifampin occurs at a rate of 10^{-8} in *M. tuberculosis* in vitro and is thought to be a one-step mutational event (11-13). Resistance to rifampin is conferred by the *rpoB* gene (14), which encodes the β subunit of RNA polymerase, an oligomeric enzyme responsible for RNA synthesis. Specific mutation in the *rpoB* gene result in drug resistance by reducing the rifampin binding affinity for RNA polymerase (11,15).

Early diagnosis of the disease and rapid identification of resistance to primary antituberculosis agents are essential for efficient treatment and control of MDR strains (15). Drug resistance is measured currently with a variety of liquid (e.g., Middlebrook broth 7H9 or 7H12) or solid (e.g., Lowenstein-Jensen, Middlebrook 7H10 or 7H11) media by one of three basic method : the resistance ratio, the absolute concentration method (also referred to as minimal inhibitory concentration [MIC] method), or the proportion method (7,16). Broth-based methods are faster than solid media systems and, currently, a commercial radiometric system permits antimicrobial drug susceptibility testing to be performed by the proportional method within 7-14 days following initial growth of the organism (17-19). All methods are affected to different extents by problems relating to variations in the inoculum size and the stability of antimicrobial agents.

The application of molecular biology-based methods has led to marked progress in diagnostics, and identification of the genetic basis of antimicrobial resistance has been used to develop rapid tests for the determination of drug susceptibility (15).

Different molecular biology based methods have therefore been applied to the detection of rifampin-resistant strains by detecting mutations in the *rpoB* gene. More than 95% of rifampin-resistant *M. tuberculosis* strains from different countries appear to harbor specific point mutation located in a 69-bp nucleotide region of the *rpoB* gene (6,14-15,20-23). DNA-based diagnostic assays, such as single-strand conformation polymorphism analysis (SSCP), heteroduplex formation (HDF), dideoxy fingerprinting, line probe assay, and DNA sequencing analysis (6,14,20-31) have been used for the detection of mutations in the polymerase chain reaction (PCR) amplification products of the *rpoB* gene. However, determination of the DNA sequence is the definitive method for detection mutations within the *rpoB* locus.

Prevention of the occurrence and spread of MDR-TB is therefore a major priority of all TB control programs. With traditional techniques, it takes 6 to 12 weeks to obtain susceptibility results. This time lag poses a significant danger to patients, the community, and health care workers. It is therefore important to develop techniques which will shorten the lag period. Recently acquired information on genetics of drug resistance in *M. tuberculosis* has led to the development of rapid tests for detection of resistance to some of the drugs in a relatively short time.

The purpose of this study was to examine the association of mutation in the *rpoB* gene as determined by DNA sequencing with rifampin-resistant *M. tuberculosis* and evaluate the PCR-HDF technique for the rapid detection.