CHAPTER V

DISCUSSION

The aim of this study was to examine the association of mutation in the *rpoB* gene with rifampin susceptibility of *M. tuberculosis*. Drug susceptibility results were determined for the 56 rifampin resistant and 52 rifampin susceptible *M. tuberculosis* by the radiometric method. The techniques used in this study for identifying mutations by direct DNA sequencing and PCR-HDF represent some of the potential tools for genotypic resistance testing.

The direct DNA sequencing of the 305-bp region of *rpoB* was able to detect rifampin resistance with a 100% correlation with the results from radiometric method. This demonstrated that each clinical isolate used in this study contain populations with homogeneous genotype in *rpoB* gene. In case that heterogeneous populations were presented, two bands of susceptible and resistant bases would appear at the same position.

Results of this study essentially confirmed the work of other investigators who found that results of molecular analysis of *rpoB* correlated quite well with those of conventional susceptibility testing. Telenti et al. (20) detected mutations in 97% of 66 rifampin-resistant strains and in none of 56 rifampin-susceptible isolates. The mutation at position Ser-531 was found in 51.5% of rifampin-resistant strains. Although rifampin-susceptible strains were not examined. Morris et al. (30) found *rpoB* mutations in 28 of 29 rifampin-resistant isolates. The most frequent mutation was at position Ser-531 occured in 55% of strains which is similar to this study. By using direct DNA sequencing of the 305-bp segment of *rpoB*, Williams et al. (14) detected genetic alterations in 98.2% of 110 rifampin-resistant strains. Mutations were not detected in the 12 rifampin-susceptible strains from the United States that were tested. Nachamkin et al. (25) detected mutations in 76.2% of 21 rifampin-

resistant strains by HDF, whereas direct DNA sequencing detected all rifampin-resistant strains. The mutation at position Ser-531 was found in 41.7% of rifampin-resistant strains. This is similar to this study which found the mutation at position Ser-531 in 50% of the isolates tested. However, Kim et al. (28) described several characteristic mutations in the *rpoB* gene of *M. tuberculosis* isolated in Korea. High-mutation frequencies of His-526 (37.8%) and Ser-531 (24.4%) were observed in rifampin-resistant strains. In Thailand, Vattanaviboon et al. (23) detected mutations in 3 rifampin-resistant *M. tuberculosis* isolated from TB patiens. All three isolates exhibited rifampin resistance, two of three had a missense mutation at position Ser-531 resulting in an amino acid change from serine (TCG) to leucine (TTG) which is similar to this study and serine (TCG) to phenylalanine (TTT) is not similar to this study. One isolate showed a point mutation at codon 516 causing a change from aspartic acid (GAC) to tyrosine (TAC) which is not similar to this study.

Williams et al. (14) suggested that PCR-HDF was a potential method for detection of rifampin resistance in clinical laboratory. The method described by these investigators was unsuccessful in this study except in one isolate which contained a 3-base insertion resulting in a phenylalanine residue inserted between Phe-514 and Met-515. Two bands in nondenaturing polyacrylamide gel were visualized, which indicated positive result. HDF required long electrophoresis steps under highly controlled conditions and also required expertise to perform and interpret in order to ensure reproducibility. In addition to the technical difficulty of HDF compared with other methods, another disadvantage of this analysis was the inability to distinguish silent from missense mutations. HDF was still less sensitive and specific than direct DNA sequencing (25,55).

The occurrence of geographic variation in the frequency of certain *rpoB* mutations may have important implications for development of molecular strategies designed to rapidly identify mutant *rpoB* alleles. Therefore, it is suggested that extensive sampling of resistant strains from several areas of the country is needed (21). This study may be useful in a setting where rapid detection of *M. tuberculosis* and

rifampin susceptibility by other new techniques such as line probe assay (15,27,29,48) and heteroduplex generator assay (26). This methods are a powerful method for the simultaneous detection of *M. tuberculosis* and rifampin susceptibility directly from clinical specimens. By testing specimens directly, heteroplex generator assay and line probe assay can reduce the time between acquisition of the specimen, determination of rifampin susceptibility, and initiation of appropriate chemotherapy for those individuals with rifampin-resistant tuberculosis. Rapid detection of rifampin susceptibility would improve management and restrict the spread of multiple drug-resistant *M. tuberculosis* strains. One such setting would be helpful serving for populations with a significant prevalence of multiple drug-resistant *M. tuberculosis* and HIV coinfection (26).