CHAPTER VI

DISCUSSION

Ciprofloxacin-resistant isolates of *M. tuberculosis* defined by growth on 7H11 agar containing 2 μg/ml ciprofloxacin and susceptibility testing by radiometric method (BACTEC), were collected and examined for nucleotide sequence changes. The result of susceptibility testing by radiometric method (BACTEC) can be reported on the average of 6-8 days. The results obtained by radiometric method (BACTEC) correlated well with the absolute concentration method (supported by Central Chest Hospital). Therefore, the results can be reported in 4-6 days for identification of *M. tuberculosis* and drug susceptibility test results in another 4-8 days. The radiometric method (BACTEC) is sensitive, rapid and reliable. These developments have made the laboratory diagnosis more useful for patients and have helped in controlling the disease. Since BACTEC method is very sensitive, the preparation of inoculum is very important for the susceptibility test. If the results can be reported in <4 days it means too heavy inoculum, if it can be reported in >12 days it means too low inoculum. In both cases, the experiments were retested.

After amplification, PCR product was run on agarose gel. If it shows the band with smear, it means that the template DNA was too much for amplification. The rest template DNA in the mixture will

interrupt the binding of primer and the sequence that will be analyzed in the sequencing steps. So it is necessary to decrease template DNA in the PCR mixture for the appropriate amplification or purify the PCR product by Wizard DNA minipreps before sequencing.

DNA sequencing showed that 18 isolates had a *gyr*A mutation at codon 94. The rest 3 isolates are interesting because they lacked a mutation in the quinolone-resistance region of *gyr*A. Chen Xu and co-workers also found that 11/13 (84.61%) of fluoroquinolone-resistant *M. tuberculosis* had a mutation in *gyr*A gene and the rest 2 isolates had not a mutation. Since this study did not examine the entire *gyr*A gene, a mutation may reside in another part of *gyr*A, as has been found with other bacteria (100). It is also possible that one or more mutations arose in *gyr*B, *par*C, or *nor*A, other genes to which quinolone resistance has been mapped(54). Additional experiments are necessary to address this study.

The study found that 18/21 (85.71%) of resistance-conferring mutations mapped in the quinolone-resistance-determining region of *gyr*A. Thus, nucleotide sequence determination following polymerase chain reaction-based amplification could be used to identify ciprofloxacin-resistant strains. Strains sensitive to ciprofloxacin (20/20) do not contain the mutations.

HDF can detect sequence variation by noticing a reduced electrophoretic mobility of DNA heteroduplexes following electrophoresis through the gel. Heteroduplexes are formed between

strands from the unknown and the reference DNA by heat denaturation and subsequent cooling to permit reannealing. If the constituent sequences differ by more than 1-2%, heteroduplexes migrate more slowly in polyacrylamide gels than their corresponding homoduplexes(101). In this study, the PCR product was 320 bp in size and there is only one point mutation, so HDF can not detect the differentiation between susceptible strain and resistant strain.