CHAPTER V

RESULTS

PART I : SUSCEPTIBILITY TESTING BY RADIOMETRIC METHOD (BACTEC)

Twenty one isolates of ofloxacin-resistant *M. tuberculosis*, were first subcultured on 7H11 media supplimented with OADC containing either ciprofloxacin or ofloxacin at concentration of 2 μg/ml. After 4 weeks, all isolates grew on 7H11 media containing either antibiotics. Isolates grew on media containing ciprofloxacin were tested for susceptibility to ciprofloxacin and ofloxacin by radiometric method (BACTEC). It was found that all isolates were resistant to ofloxacin and ciprofloxacin at concentration of 2 μg/ml.

Twenty isolates of *M. tuberculosis* obtained from Department of Microbiology, King Chulalongkorn Memorial Hospital were tested by radiometric method (BACTEC). All isolates were susceptible to ofloxacin and ciprofloxacin at concentration 2 μg/ml.

PART II : POLYMERASE CHAIN REACTION (PCR)

Amplification, by using two oligonucleotide primers highly homologous to DNA sequences flanking the quinolone resistance-determining region in *gyr*A of mycobacteria (corresponding to nucleotides 78 to 397), sequences were shown in Fig 4.

CAGCTACATCGACTATGCGATGAGCGTGATCGTCGG
CCGCGCGCTGCCGGAGGTGCGCGACGGGCTCAAGCC
CGTGCATCGCCGGGTGCTCTATGCAATGTTCGATTCC
GGCTTCCGCCCGGACCGCAGCCACGCCAAGTCGGCC
CGGTCGGTTGCCGAGACCATGGGCAACTACCACCCG
CACGGCGACGCGTCGATCTACGACAGCCTGGTGCGC
ATGGCCCAGCCCTGGTCGCTGCGCTACCCGCTGGTGG
ACGGCCAGGGCAACTTCGGCTCGCCAGACAATGACC
CACCGGCGGCGATGAGGTACACCGAAGCCC

Fig 4. Nucleotide sequence of the *gyr*A FQ resistance region amplified with primers GyrA1 and GyrA2 (underlined)

PCR products were applied on an agarose gel for electrophoresis running. The resulting amplified product was visualized and photographed during UV light exposure as shown in Fig 5.

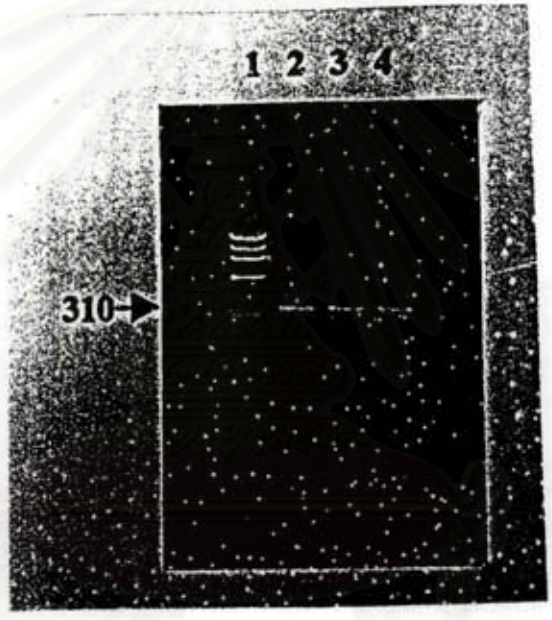


Fig 5. Agarose gel electrophoresis of amplified product (lanes 2-4) compared with ϕx174 marker (lane 1)

PART III : DNA SEQUENCING

The double-stranded DNA was sequenced by the dideoxy chain termination method. Comparison of the nucleotide sequences of the 320 bp fragments of 21 isolates, 18 isolates revealed a point mutation at codon 94 leading to the substitution of Asp to Asn (GAC →AAC) (n=6), Asp to Ala (GAC→GCC) (n=7), Asp to Gly (GAC →GGC) (n=4), Asp to Tyr (GAC→TAC) (n=1) and the rest 3 isolates lacked mutation. Mutation in this region was not found in all 20 sensitive clinical isolates tested.

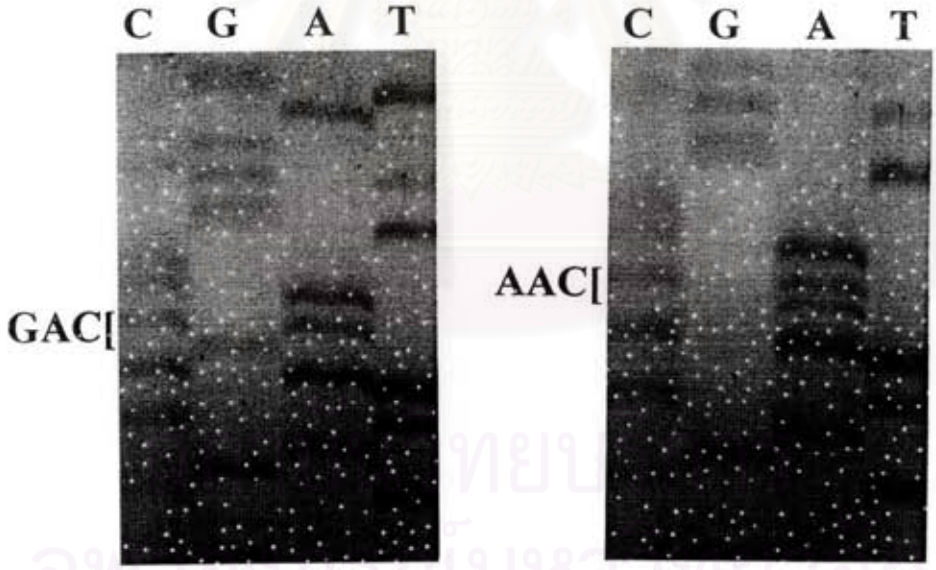


Fig 6. The sequence gel autoradiography showed differentiation sequence between susceptible strain and resistant strain at the position 94 (GAC→AAC)

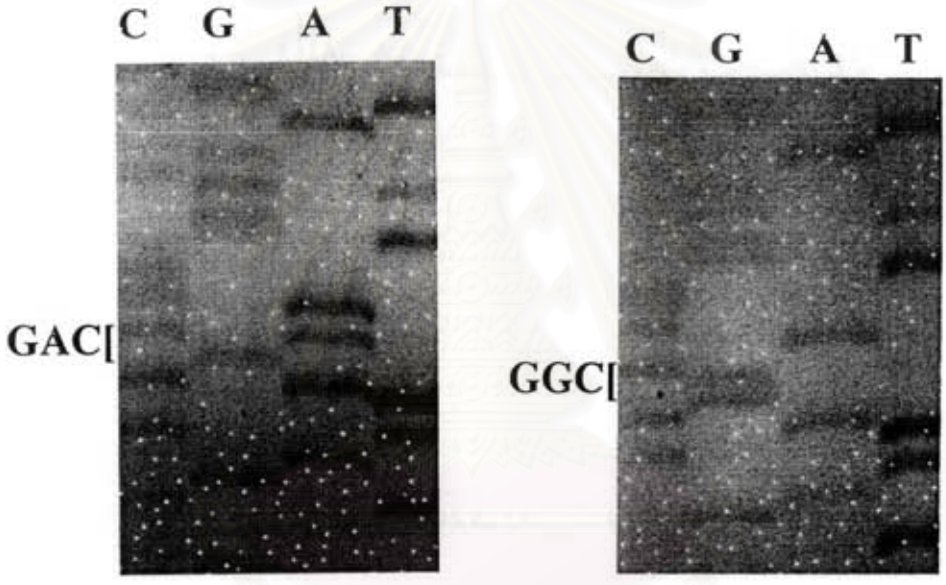
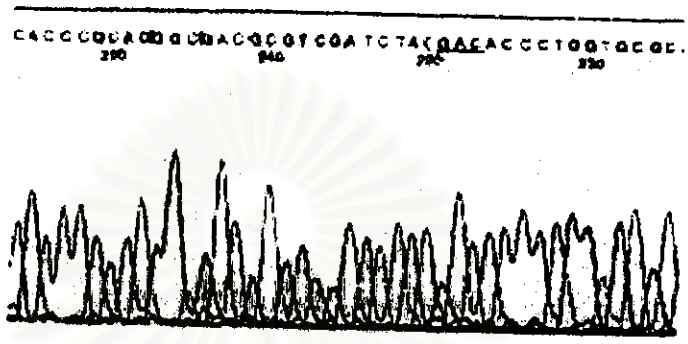


Fig 7. The sequence gel autoradiography showed differentiation sequence between susceptible strain and resistant strain at the position 94 (GAC→GGC)

(a)



(b)

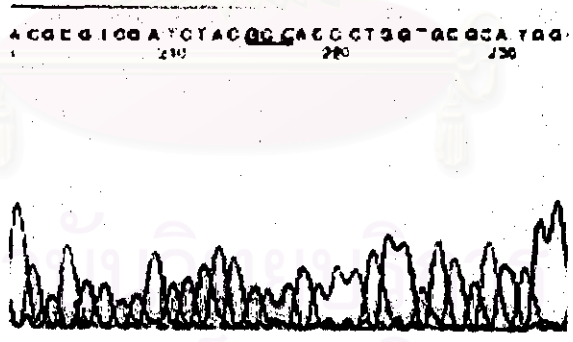
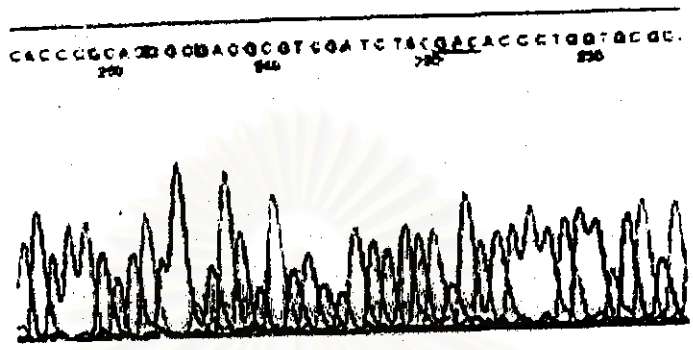


Fig 8. The chromatogram obtained from automate sequencing showed differentiation sequence between susceptible strain (a) and resistant strain (b) at the position 94 (GAC→GCC)

(a)



(b)

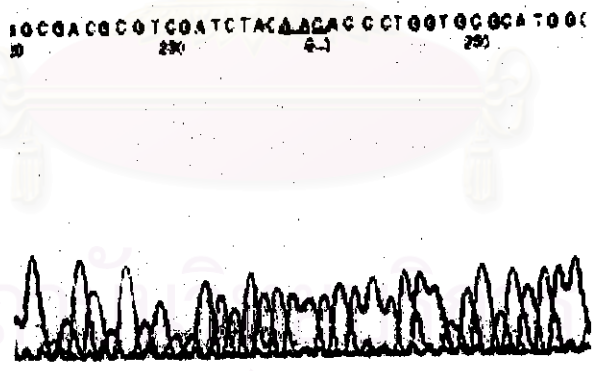
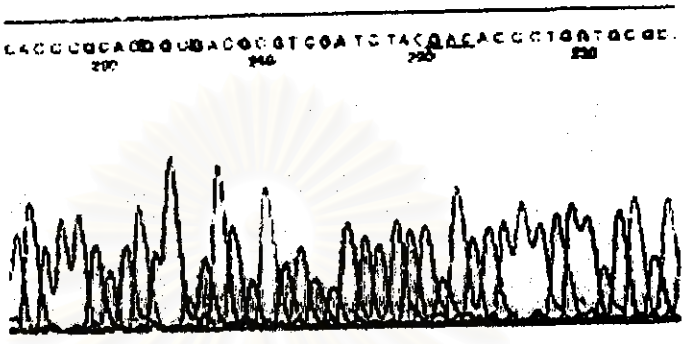


Fig 9. The chromatogram obtained from automate sequencing showed differentiation sequence between susceptible strain (a) and resistant strain (b) at the position 94 (GAC→AAC)

(a)



(b)

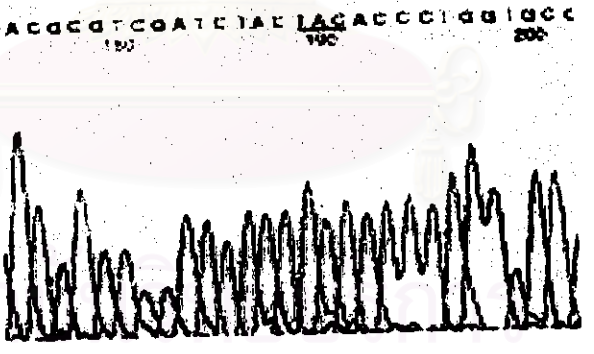


Fig 10. The chromatogram obtained from automate sequencing showed differentiation sequence between susceptible strain (a) and resistant strain (b) at the position 94 (GAC→TAC)

Reference strain and Clinical isolate (number)	Mutation at codon 94	
	Specific mutation (number)	Amino acid change
Reference strain		
H37Rv	No mutation	No
Clinical isolates		
Susceptible (20)	No mutation	No
Resistant (21)	GAC→AAC (n=6)	Asp→ Asn
	GAC→GCC (n=7)	Asp→ Ala
	GAC→GGC (n=4)	Asp→ Gly
	GAC→TAC (n=1)	Asp→ Tyr
	No mutation (n=3)	No

Table 2. The results of detection of *gyr*A gene mutation in reference strain and clinical isolates

PART IV : HETERODUPLEX FORMATION (HDF) ANALYSIS

It is not successful to find the differentiation between susceptible strain H37Rv and resistant strains by using heteroduplex formation technique in this study.

1,2,3 : resistant isolates

Fig 11. Polyacrylamide gel electrophoresis for heteroduplex formation analysis (HDF)