

E-1**Isolation of KRM-1648 Resistant Strains with Various *rpoB* Gene Mutations from KRM-1648 Susceptible and Rifampin Resistant *Mycobacterium tuberculosis* Strains**

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Rifampin (RMP) is a most important drug in the treatment of tuberculosis so that resistance to this antibiotic often results in incurable tuberculosis. Over 96% of RMP resistant *M. tuberculosis* have missense mutation, deletion, or insertion within 81bp core region (codons 507 - 533) of RNA polymerase β -subunit gene (*rpoB*). KRM-1648 is considering as an effective drug for RMP resistant *M. tuberculosis*. This study aimed to analyze and compare activity of RMP and KRM-1648 on *rpoB* gene mutation site. RMP resistant strains were obtained from the routine drug susceptibility tests using the conventional method. Twenty-three RMP resistant strains, which had various mutation sites revealed by the Line Probe Assay (INNO-LiPA Rif. TB, Innogenetics Co), were selected for this study. Approximately 3'10⁶ bacilli of RMP resistant strain were inoculated on Middlebrook 7H11 agar media containing KRM-1648 with a range of concentrations, 0.1 mg/mL, 1 mg/mL, and 16 mg/mL and incubated for 4 weeks at 37°C

We found KRM resistant *M.tuberculosis* strains with double mutations in the *rpoB* gene from KRM susceptible and RMP resistant strains. Some progenies derived from a strain with mutation in codon 516 (GAC -> GTC) had various double mutations, in codon 533 (CTG -> GTG), 529 (CGA -> CTA), 526 (CAC -> TAC), or 513 (CAA -> GAA). The other double mutations or novel mutations were also found. Except mutation in codon 526 (CAC -> CCC, TAC, GAC), and 531 (TCG -> TGG, TTG), the other mutation sites of RMP resistant strains showed considerably susceptibility to KRM, but they may be prone to develop resistance to KRM through double mutations.

E-2**Molecular Mechanism of Kanamycin Resistance in *Mycobacterium tuberculosis***

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Control of tuberculosis caused by multiple-drug resistant *Mycobacterium tuberculosis* is one of the major problems throughout the world. Understanding of the molecular mechanisms of drug resistance may offer useful informations to a development of novel and rapid detection methods for drug resistant *M. tuberculosis*. Kanamycin is one of second-line antituberculosis agents. This drug directly binds to ribosomes and inhibits protein synthesis. We have previously shown that nearly 70% of kanamycin resistant *M. tuberculosis* strains possessed a mutation at the A site of the 16S rRNA gene. In this study, we analyzed the molecular mechanisms of the remaining 30% kanamycin resistant strains, which are other than a mutation on the 16S rRNA gene.

We used eleven kanamycin resistant and two kanamycin sensitive clinical *M. tuberculosis* isolates which didn't have any mutation on the 16S rRNA gene. We determined nucleotide sequences of the 23S, 5S rRNA genes and the tap gene encoding a putative efflux pump, and compared them. Ten of eleven kanamycin resistant strains possessed an identical insertional mutation of C residue at the 581st nucleotide position of the tap gene. Therefore, we cloned the mutated and wild type tap genes into *E. coli* plasmid vector, and observed the growth pattern and the morphological change of the *E. coli* transformants possessing the recombinant plasmids. The mutated tap gene offered severe damage onto the growth and caused a bit filament formation of host *E. coli* cell. It is in progress to detect the effects of the genes onto mycobacteria.

E-3**Ambulatory Treatment of Multidrug-resistant Pulmonary Tuberculosis Patients at a Chest Clinic**

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SETTING Retrospective cohort analysis of the multidrug-resistant tuberculosis patients treated at an outpatient chest clinic of the Korean National Tuberculosis Association.

OBJECTIVE: To evaluate treatment outcomes and contributing factors to it.

DESIGN A review with clinical records of 1,011 pulmonary MDR-TB patients treated with individualized regimens selected on the basis of previous chemotherapy and drug susceptibility testing from 1988 to 1996. Patients who had taken second-line drugs or surgical treatment prior to retreatment were excluded.

RESULTS Patients (mean age, 38.6 years) had resistant strains to an average of 3.7 drugs and retreated with 4.2 previously unused, susceptible drugs in regimen. Treatment outcomes were as follows: 487 cases (48.2%) cured, 82 (8.1%) failed, 394 (39.0%) defaulted, 45 (4.5%) transferred out, and 3 (0.3%) expired. Treatment efficacy of those who completed chemotherapy was 85.6%. Favorable response was significantly associated with a greater number of previously unused susceptible drugs in regimen (odds ratio[OR] 3.6; 95% confidence interval[CI] 1.3-9.5), younger age (OR 2.0; 95%CI 1.1-3.9), less number of resistant drugs (OR 1.8; 95%CI 1.1-3.1) in a multivariate analysis. After completion of treatment, 335 cases of those cured were followed-up for an average of 2.1 (\pm 2.1) years ranging from two months to nine years: eight cases relapsed and three died within one year.

CONCLUSION The cure rate of MDR-TB patients treated at an outpatient clinic was 48.2% because of high defaulter rate (39.0%). However 85.6% of those who completed treatment was cured.

E-4**In Vitro Activities of Fluoroquinolones against *Mycobacterium tuberculosis***

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Tuberculosis continues to cause major public health problem around the world and new drugs are urgently needed to combat the increasing problem of resistant organisms. Agents of the fluoroquinolone class have shown distinct activity against *Mycobacterium tuberculosis* and have been used as component drugs in the treatment of multidrug-resistant cases. In this study, six fluoroquinolones: DU-6859a, gatifloxacin (GTFX), moxifloxacin (MXFX), sparfloxacin (SPFX), levofloxacin (LVFX), and ofloxacin (OFLX) were assessed for their *in vitro* activities against *M. tuberculosis*. One hundred and forty-nine clinical isolates, including 31 strains sensitive to all conventional antituberculous drugs (TBS), 42 strains resistant to one or more antituberculous drugs (TBR) and 76 strains resistant to isoniazid and rifampin or more drugs (MDR), were tested using the macro-broth dilution method with Middlebrook 7H9 medium. SPFX was the most active quinolone tested against the group TBS followed by LVFX while DU-6859a, GTFX, MXFX and OFLX yielded identical MIC90 and MBC90. Against the group TBR, DU-6859a, GTFX, MXFX and SPFX showed similar MICs and MBCs and were slightly better than LVFX which has one twofold smaller MICs and MBCs than OFLX. Again, DU-6859a, GTFX, MXFX and SPFX demonstrated comparable *in vitro* activities against the group MDR but the MICs and MBCs were two to four twofolds greater than those of the TBR group. Promising *in vitro* results of newly synthesized fluoroquinolones, such as DU-6859a, GTFX and MXFX merit further evaluation of these agents in *in vivo* studies as well as in combined regimens for the treatment of tuberculosis.

