SUPPLEMENTAL DIGITAL CONTENTS

Supplemental Methods

DNA amplification and sequencing

Independent amplification of Mtb DNA, a *katG* segment, was performed on 68 Genotype MTBDRplus failed samples. Speciation was done by amplification and sequencing of 16S rDNA. A 535bp 16S rDNA and 498bp *katG* segments were amplified from crude sputum lysate using outer primers 16SOL/16SOR [1] and katGout_fw:TGATCGTCGGCGGTCACACTTTC/ katGout_rv:CCAGCGGCCCAAGGTATCTCG, respectively, and PCR conditions: 3min at 95^{o}C; 25 cycles: 20sec at 95^{o}C, 10sec at 52^{o}C or 60^{o}C, 3sec at 70^{o}C. Nested PCR was performed on 10uL of 16S rDNA amplification (16SIL & 16SIR [1] as follows 1 cycle: 3min at 95^{o}C; 25 cycles: 20sec at 95^{o}C, 10sec at 55^{o}C, 3sec at 70^{o}C). The nested PCR for *katG* was performed with 10 uL of amplified *katG* DNA (tomkp1 & tomkp2 [2]) as follows 1 cycle: 3min at 95^{o}C; 25 cycles: 20sec at 95^{o}C, 10sec at 65^{o}C. All PCR reactions included 10uL of DNA template, 1X KOD polymerase buffer, 0.2mM dNTP (each), 1.5mM MgSO4, 5% DMSO, 200nM of each primer, 1% KOD Hot Start polymerase (Toyobo, Osaka, Japan). Two clones (Zero Blunt® PCR Cloning Kit, Invitrogen Carlsbad, CA) were sequenced using the BigDye Terminator v1.1 Cycle sequencing kit (Applied Biosystems, Carlsbad, CA) and Invitrogen's M13 primers on an Applied Biosystems 3100 sequencing instrument.

Bibliography

1. Gengvinij N, Pattanakitsakul SN, Chierakul N, Chaiprasert A. Detection of Mycobacterium tuberculosis from sputum specimens using one-tube nested PCR. Southeast Asian J Trop Med Public Health **2001**; 32:114-25.

2. Baker LV, Brown TJ, Maxwell O, et al. Molecular analysis of isoniazid-resistant Mycobacterium tuberculosis isolates from England and Wales reveals the phylogenetic significance of the ahpC -46A polymorphism. Antimicrob Agents Chemother **2005**; 49:1455-64.