NOVEL MUTATION DETECTION IN *RPOB* OF RIFAMPICIN-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* USING PYROSEQUENCING

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Abstract. Tuberculosis (TB) remains a major global public health problem particularly severe in parts of Asia and Africa, where often it is present in HIV-AIDS patients. Although rifampicin-resistant (RIF^r) TB is slow to emerge due to the low rate of mutation of its target leading to RIF^r being a marker of TB that is already resistant to other anti-TB drugs, and such cases are prone to treatment failure. More than 95% of rifampicin resistance is associated with mutations in *Mycobacterium tuberculosis* (MTB) *rpoB*, with 97% of mutations occurring within the 81 bp rifampicin-resistant determining region (RRDR) of this gene. In this study, we employed pyrosequencing technique to identify mutations in RRDR and 5 codons beyond of 39 MTB strains, comprising of 14 multi-drug resistance TB (MDRTB) and 3 RIF susceptible (RIF^s) MTB from the Center of Disease Control (CDC), Ratchaburi Province, and 19 mono RIF^r MTB, 1 MDRTB and 2 poly-drug resistant MTB from the Chest Institute, Ministry of Public Health, Thailand. Mutations in 8/22 samples from the Chest Institute and 13/14 from CDC were able to be identified. Six point mutations were detected, with Ser531Leu mutation accounting for 13, the silent mutation at Gly536 for 4, deletion of Gly523 for 2, combination of His526Cys and novel Leu533Arg for 1, and a novel Leu538Arg for 1. Mutation analysis of the 81 bp fragment and 5 codons beyond in MTB *rpoB* using pyrosequencing provides a useful approach in predicting RIF^r phenotype allowing early diagnosis and appropriate drug therapy.

Keywords: *Mycobacterium tuberculosis,* mutation, pyrosequencing, rifampicin, *rpoB*, tuberculosis

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