Phenotypic Determination of Rifampicin-Resistance by BrothMIC MTB in Correlation with rpoB Gene Mutations in the Isolates of Mycobacterium tuberculosis complex
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Background: BrothMIC MTB (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) is principally based on Middlebrook 7H9 broth microdilutions to determine minimum inhibitory concentrations (MICs) for the isolates of Mycobacterium tuberculosis complex. The MICs to rifampicin (RFP) distribute bipartitely, the most susceptible, wild isolates showing <0.06 mcg/ml and the resistant isolates are >2.0 mcg/ml. As the results, a very few number of isolates showing the MICs between 0.06 mcg/ml to 2.0 mcg/ml are still interpreted and remained as phenotypically indeterminate. With this, we determined the correlation between rpoB gene mutations and MIC interpretations in this study.

Methods: We selected the isolates of which MICs to RFP ranged 0.06 to 2.0 mcg/ml from our laboratory collections and then determined genetic mutations in rpoB gene by direct base-sequencing.

Results: Through the direct base-sequencing, genetic mutation(s) in rpoB gene associated with amino acid substitution(s) were found in 21 of 27 clinical isolates of M. tuberculosis complex. All the isolates with 0.06 mcg/ml of MICs were confirmed as being a wild type, whereas those with >0.06 mcg/ml of MICs have a variety of genetic mutations. There found one exception, that is, a strain with 0.5 mcg/ml of MIC revealed no mutation in rpoB gene. Of twenty-one isolates having mutations in rpoB gene, 13 isolates were characterized as being multi-drug resistants with >2.0 mcg/ml MICs to isoniazid.

Conclusions: With these results, it can be concluded that the interpretive breakpoints for RFP resistance by MICs determined should be revised as follows: susceptible, <0.125 mcg/ml, and resistant, >0.06 mcg/ml.