MOLECULAR CHARACTERIZATION OF RIFAMPICIN AND/ OR ISONIAZID RESISTANT *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

A

DISSERTATION

SUBMITTED TO THE CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

AWARD OF MASTER OF SCIENCE IN MICROBIOLOGY (ENVIRONMENT AND PUBLIC HEALTH)

BY

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2012

RECOMMENDATION

This is to certify that **Mr. Bijay Kumar Sharma** has completed this dissertation work entitled "**Molecular Characterization of Rifampicin and/ or Isoniazid Resistant *Mycobacterium tuberculosis* Complex**" as a partial fulfillment of the requirements of M. Sc. degree in Microbiology (Environment and Public Health) under our supervision. To our knowledge this work has not been submitted for any other degree.

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ABSTRACT

Introduction: The ability to rapidly and accurately detect drug resistance in *Mycobacterium tuberculosis* complex (MTBC) isolates is critical for the appropriate treatment of patients suffering from TB and the effectiveness of TB control programs. GenoType MTBDRplus will allows rapid confirmation of TB through the identification of genetic mutations associated with rifampicin and isoniazid resistance. Therefore, this study provides genetic information of isoniazid and rifampicin drug resistance TB isolates in Nepal.

Objectives: To rapidly identify drug resistant TB in cultured specimen through the identification of genetic mutations associated with rifampin and isoniazid resistance in in *Mycobacterium tuberculosis* complex (MTBC) isolates.

Methods: Fluorescence microscopy was performed on 62 suspected tuberculosis specimen followed by their culture on on Lowenstein–Jensen medium for detection of *Mycobacterium tuberculosis*. Culture specimens were then analysed for identification of rifampicin and /or isoniazid resistance using Genotype MTBDR*plus* assay.

Results: Among the drug resistant isolates, MDR (multidrug resistance i.e. resistant to at least isoniazid and rifampicin) isolates were predominant (51%). Among all rifampicin resistant isolates, 61.76% of them had mutations in the 530-533 region of *rpoB* gene and was the most common mutation observed as detected by the lack of binding to various wild type probes in the presence of D516V and S531L mutation. More RIF-monoresistant strains (16.66%) had a D516V mutation (MUT1 band) compared to MDR strains (3.5%). Of all INH resistant strains 96.96% had a mutation in the *katG* gene and 3.03% had a mutation in the *inhA* gene. All isoniazid resistant strains that have mutation in *inhA* gene were found to be multidrug resistant. None of the strains had mutations in both the *katG* and *inhA* genes. S315T1 and S531L were found as the most common mutation for rifampicin and isoniazid respectively.

Conclusions: GenoType MDTBRplus assay can be effectively used for the detection of mutations in most common genes responsible for isoniazid and/or rifampicin resistance.

Keywords: TB, Genotype MTBDRplus, MDR, MTBC.

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ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
BSL	Biosafety Level
CAT	Category
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Treatment Short Course
DR TB	Drug Resistant Tuberculosis
DRS	Drug Resistance Survey
DST	Drug Susceptibility Testing
GENETUP	German Nepal Tuberculosis Project
GLC	Green Light Committee
HIV	Human Immuno-Deficiency Virus
INH	Isoniazid
LJ	Lowenstein-Jensen
MDR	Multi Drug Resistant
MTB	*Mycobacterium tuberculosis*
MTBC/ MTC	*Mycobacterium tuberculosis* Complex
MUT	Mutation Probe
NaOH-NALC	*N*-acetyl-L-cysteine–NaOH
NCCLS	National Committee for Clinical Laboratory Standards
NTC	National Tuberculosis Centre
NTM	Non Tuberculosis Mycobacteria
NTP	National Tuberculosis Program
PCR	Polymerase Chain Reaction
RIF	Rifampicin
RNA	Ribonucleic acid
TB	Tuberculosis
WHO	World Health Organization
WT	Wild type Probe
XDR	Extensively Drug Resistant