# DISTRIBUTION AND ANTIBIOTIC SUSCEPTIBILITY TESTING OF MYCOBACTERIUM SPECIES PRESENT IN THE SPUTUM OF SUSPECTED PULMONARY TUBERCULOSIS PATIENTS

A

Dissertation

Submitted to the Central Department of Microbiology Tribhuvan University

In Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Microbiology (Environment and Public Health Microbiology)

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### RECOMMENDATION

This is to certify that Mr. Ujjal Wagley has worked under our supervision and guidance on the thesis entitled "Classification and antibiotic susceptibility testing of *Mycobacterium* species present in the sputum of suspected pulmonary tuberculosis patients" as a partial fulfillment for award of M. Sc Degree in Microbiology. To the best of our knowledge this is an original research work of him and has not been submitted for any other degree.

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#### **CERTIFICATE OF APPROVAL**

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#### ABSTRACT

The study was carried out at National TB Centre, Thimi, Bhaktapur from September 2010 to August 2011 in collaboration with Central Department of Microbiology, Tribhuvan University with the objective to distribute the mycobacteria isolated from sputum of suspected PTB patients and to obtain their drug susceptibility pattern.

A total of 200 suspected new PTB patients were selected for cross-sectional study, on the basis of Bartlett's Pulmonary Specimen Culture Criteria, by observing sputum samples from 1500 patients. After their consent, Questionnaire was administered and sputum was subjected to ZN, Fluorescent microscopy and Culture; and biochemical and antibiotic susceptibility tests were performed on culture positive isolates. A total of 69% (n=138) sputum samples were positive for Mycobacterium either singly or multiply by ZN microscopy, fluorescent microscopy or culture; of which 90.58% (n=125) were positive by ZN microscopy, 92.75% (n=128) were positive by fluorescent microscopy and 93.48% (n=129) were positive by culture. Among culture positive 87.60% (n=113) strains belong to M. tuberculosis complex, all M. tuberculosis strains; and 12.40% (n=16) belong to NTM of which 81.25% (n=13) were Non-photochromogens, 12.50% (n=2) were Scoto-chromogens, and 11.11% (n=1) rapid grower identified as *M. vaccae*. Among M. tuberculosis complex only 7.96% (n=9) M. tuberculosis isolates were resistant to antitubercular drugs, all MDR, of which 11.11% (n=1) was resistant to INH and RMP only, 22.22% (n=2) were resistant to INH, RMP and STR, and remaining 66.67% (n=6) were resistant to all four anti-tubercular drugs INH, RMP, STR and EMB. Among 16 NTM isolates, 6.25% (n=1) isolate was resistant to INH and STR only, but all other 93.75% (n=15) isolates were resistant to all four anti- tubercular drugs, INH, RMP, STR and EMB. Hence, mycobacterial isolates obtained from suspected pulmonary TB patients, were distributed and drug susceptibility pattern determined.

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## LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AFS	Acid Fast Staining
AIDS	Acquired Immune Deficiency Syndrome
AST	Antibiotic Susceptibility Testing
BACTEC	Becton Dickinson and Company
BCG	Bacilli Calmette Guerin
CDC	Center for Disease control and Prevention
CMI	Cell Mediated Immunity
DNA	Deoxyribo-Nucleic Acid
DOTS	Directly Observed Treatment, Short course
DST	Drug Susceptibility Testing
EMB/E	Ethambutol
EPI	Squamous epithelial cells
FS	Fluorescent/Fluorochrome Staining
HIV	Human Immunodeficiency Virus
IFN	Interferon
IL	Interleukin
INH/H	Isoniazid
IS	Insertion Sequence
IUATLD	International Union Against tuberculosis and Lung Diseases
LJ	Lowenstein Jensen
MDR	Multi-drug resistant
MDR-TB	Multi-drug resistant Tuberculosis
MHC	Major Histo-compatibility Complex
MHC-I	Major Histo-compatibility Complex class I
MHC-II	Major Histo-compatibility Complex class II
MIC	Minimum Inhibitory Concentration
MOTT	Mycobacteria Other than Tubercle bacilli
MTC	Mycobacterium tuberculosis Complex
NALC-NaOH	N-Acetyl-L-Cysteine Sodium Hydroxide

NTC	National Tuberculosis Centre
NTM	Non-tuberculous Mycobacteria
NTP	National Tuberculosis Programme
PCR	Polymerase Chain Reaction
PNB	Para- Nitrobenzoic Acid
PPD	Purified Protein Derivative
PTB	Pulmonary Tuberculosis
PZA/Z	Pyrazinamide
RMP/R	Rifampicin
RNI	Reactive Nitrogen Intermediates
RNTCP	Revised National Tuberculosis Control Programme
ROI	Reactive Oxygen Intermediates
SAARC	South Asian Association for Regional Cooperation
SEAR	South East Asia Region
STR/S	Streptomycin
SPSS	Statistical Package for Social Sciences
STC	SAARC Tuberculosis and HIV/AIDS Centre
ТВ	Tuberculosis
TCH	Thiophen- 2-Carboxylic acid Hydrazide
TNF	Tumour Necrosis Factor
TST	Tuberculin Skin Test
WBC	White Blood Cells
WHO	World Health Organization
XDR	Extensively Drug Resistant
ZN	Ziehl Neelsen