

**STRAIN TYPING OF *Mycobacterium leprae* ISOLATES FROM
NEPAL USING VARIABLE NUMBER OF TANDEM REPEATS**

A

DISSERTATION

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AWARD OF THE DEGREE OF MASTER OF SCIENCE IN
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BY

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ABSTRACT

Poor understanding of leprosy transmission has generated the need for molecular tools for the strain differentiation of *M. leprae* isolates. Tandem repeats, namely microsatellites and minisatellites also known as variable number of tandem repeats (VNTRs) were applied to assess strain variability among *M. leprae* isolates from Nepal to potentially identify epidemiological patterns. VNTR PCR of DNA extracted from 52 elliptical or punch biopsy samples by freeze and boil method, was performed for each six minisatellites (21-3, 27-5, 23-3, 12-5, 18-8 and 6-7) and six microsatellites (GGT5, GTA9, AT17, TA18, AC9 and AC8b) to find out the copy numbers of the repeats within *M. leprae* genome. The amplicons were analyzed by using 8% polyacrylamide gel electrophoresis (PAGE) and band size of PCR positive samples was calculated using a web based programme 'Computation of size of DNA and Protein Fragments from their Electrophoretic Mobility' and VNTR data analysis was performed using Microsatellite tool kit. Locus 27-5 was the least polymorphic with allelic diversity of 0.373; whereas locus AC 8b was found to be the most polymorphic with allelic diversity 0.908. Clustering of the samples identified 7 different clusters in Nepal with no observable relation to geographical distribution, ethnic group and sex. Comparison of VNTR pattern of Nepalese isolates with global data revealed a wide range of genotypes prevalent in Nepal. The study demonstrated the suitability of minisatellites as a genetic marker for potential strain typing and mapping of short range transmission in Nepal. Microsatellite allele differences could not be dependably resolved using PAGE analyses and appeared too hypervariable for clustering within this size population study. In summary, the six member minisatellite PCR panel with PAGE analysis employed in this study has been shown to identify potential strain clusters in Nepalese leprosy patient skin biopsy-derived *M. leprae* samples with important similarities as well as distinctive differences to reported strains from neighboring China and India.

Key Words: Strain typing, Molecular epidemiology, Variable number of tandem repeats, Minisatellite, Microsatellite, polymorphism.

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LIST OF ABBREVIATION

AGE	Agarose Gel Electrophoresis
ALH	Anandaban Leprosy Hospital
AP	Ammonium Persulphate
BG	Bangalore Genei
BI	Bacteriological Index
BPB	Bromophenol Blue
BSA	Bovine serum Albumin
CDR	Central Development Region
dATP	Deoxyadenosine 5'-triphosphate
dCTP	Deoxycytidine 5'-triphosphate
dGTP	Deoxyguanosine 5'-triphosphate
DNA	Deoxyribo Nucleic Acid
dNTPs	Deoxynucleoside 5'-triphosphates
dTTP	Deoxythymidine 5'-triphosphate
EDR	Eastern Development Region
EDTA	Ethylene Diamine Tetra Acetate
EtBr	Ethidium Bromide
FP	Forward Primer
FWDR	Far Western Development Region
Hrs	Hours
IS	Insertion Sequence
MDT	Multi Drug Therapy
MIRU	Mycobacterial Interspersed Repetitive Element

MLVA	Multiple Locus VNTR Analysis
MRL	Mycobacterial Research Laboratory
Min	Minute
MWDR	Mid Western Development Region
NEB	New England Biolab
ng	nano gram(s)=10 ⁻⁹ gram
OD	Optical Density
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PPD	Purified Protein Derivative
PPM	Potential Polymorphic Microsatellite
RFLP	Restriction Fragment length polymorphism
RNA	Ribo Nucleic Acid
RP	Reverse Primer
RXN	Reaction
SDS	Sodium Do Decyl Sulphate
Sec	Second
SSR	Single Sequence Repeats
STR	Short Tandem Repeats
STRP	Short Tandem Repeat Polymorphism
TBE	Tris Borate EDTA
TE	Tris EDTA (buffer)
TEMED	Tetra Ethane Methylene Diamine
TN	Tamil Nadu

T_m	Melting Temperature
TR	Tandem Repeats
UV	Ultra Violet
μl	Micro liter
V	Volt
VNTR	Variable Number of Tandem Repeat
WDR	Western development Region

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