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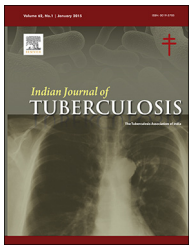

Original Article

Predominance of Central Asian and European families among *Mycobacterium tuberculosis* isolates in Kashmir Valley, India

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ABSTRACT

Background: As there are no data available regarding the strains of *Mycobacterium tuberculosis* circulating in Kashmir Valley, India, the current study aimed at describing the genetic diversity of *M. tuberculosis* strains in this region, by spoligotyping and 12-locus-based MIRU-VNTR typing (Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat).
Methods: Sputa from 207 smear positive cases with newly diagnosed pulmonary tuberculosis were subjected to culture for *M. tuberculosis*. Eighty-five isolates confirmed as *M. tuberculosis* were subjected to drug susceptibility testing and molecular typing by spoligotyping and MIRU-VNTRs.
Results: Drug susceptibility results of 72 isolates revealed 76.3% as fully sensitive while 5.5% as multidrug resistant (MDR). Spoligotyping of 85 isolates detected 42 spoligotypes with 50 isolates (58.8%) clustered into seven spoligotypes. SIT26/CAS1_Del was the major spoligotype (23, 27%) followed by SIT127/H4 (12, 14.1%); CAS lineage (37.6%) was predominant, followed by Haarlem (25.8%) and ill-defined T clade (23.5%). MIRU-VNTR analysis displayed 82 MIRU patterns from 85 strains, including 3 small clusters and 79 unique. MIRU 26 was found to be the most discriminatory locus.
Conclusions: Kashmir Valley has CAS as the predominant lineage of *M. tuberculosis* similar to the rest of the Indian sub-continent, while it is peculiar in having Euro American lineages such as Haarlem and ill-defined T clade.

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1. Introduction

Tuberculosis (TB) is a disease of antiquity and continues to remain a major public health problem in the developing world. Globally, in 2015 there were an estimated 10.4 million incident cases of TB with 1.8 million TB deaths. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa had the largest numbers of cases. India, with the incidence of 2.8 million has the distinction of being a country with the highest burden of TB disease in the world.[1] Despite the high TB burden in India, there are limited data available pertaining to the epidemiological typing of the strains circulating in various parts of the country. The situation is further complicated by the lack of available genotypic epidemiological tools that would allow contact tracing and identification of transmission patterns within the country.

In the past, drug susceptibility tests (DSTs) and phage typing were the only biomarkers available for epidemiological studies of *Mycobacterium tuberculosis*, and both of which have serious limitations. Drug susceptibility results can change in a strain as it acquires resistance to one or more antimicrobials during treatment. Bacteriophage typing of *M. tuberculosis* is of limited value for epidemiologic studies as only a few phage types can be recognized and thus, it cannot adequately distinguish between different strains.[2]

Nucleic acid-based genotyping methods based on the variations in sequence in bacterial genomes allow us to accurately distinguish between different strains of *M. tuberculosis*. The most widely used methods of genotyping employ the IS*6110* element to fingerprint strains. However, IS*6110* fingerprinting is of limited use because a significant proportion (40–44%) of *M. tuberculosis* isolates from several regions of India have been reported to either lack or have low copy numbers of IS*6110*.[3] Spacer oligotyping (spoligotyping) is a polymerase chain reaction (PCR)-based fingerprinting method that detects the presence or absence of 43 defined spacers (36–41 bp) in the direct-repeat (DR) sequences in the genomes of the members of the *M. tuberculosis* Complex (MTC).[4] Although, less discriminatory than IS*6110* RFLP typing, spoligotyping is more rapid and easier to perform. In addition, it has been demonstrated that the results are highly reproducible.[5] A high resolution typing method based on the Variable Number Tandem Repeats (VNTRs) of Mycobacterial Interspersed Repetitive Units (MIRUs) based on 12 specific intergenic regions of the *M. tuberculosis* genome has been proposed.[6] With a discriminatory power close to IS*6110* fingerprinting, MIRU-VNTR is a PCR-based method that differentiates between strains by identifying the number and length of exact tandem repeats present in an isolate, independently of the IS*6110* polymorphisms.[7]

Although some studies have been conducted in other parts of India, nothing is known about the genetic diversity of *M. tuberculosis* in Kashmir Valley till date. The Kashmir Valley, belonging to the state of Jammu and Kashmir, is spread over 16,000 km^2 and consists of 10 districts (Fig. 1). It is culturally, geographically and climatically distinct from the rest of India. Hence, there is a need to know the prevalence of various genotypes in this region. The current study aimed at describing, along with the pattern of drug resistance the genetic diversity of *M. tuberculosis* strains isolated from cases of pulmonary TB, in Kashmir Valley.

2. Material and methods

2.1. Study population

The study was conducted in the department of Microbiology, SKIMS in collaboration with the National JALMA Institute for Leprosy and other Mycobacterial Diseases (NJIL & OMD) Agra, where molecular typing was done. A total of 300 sputum samples from pulmonary TB cases belonging to Cat I – previously untreated cases were collected during 3 years, from May 2007 till May 2010. These patients attended the Designated Microscopy Centres (DMCs) of various District Tuberculosis Centres of Kashmir. Consent was taken from all

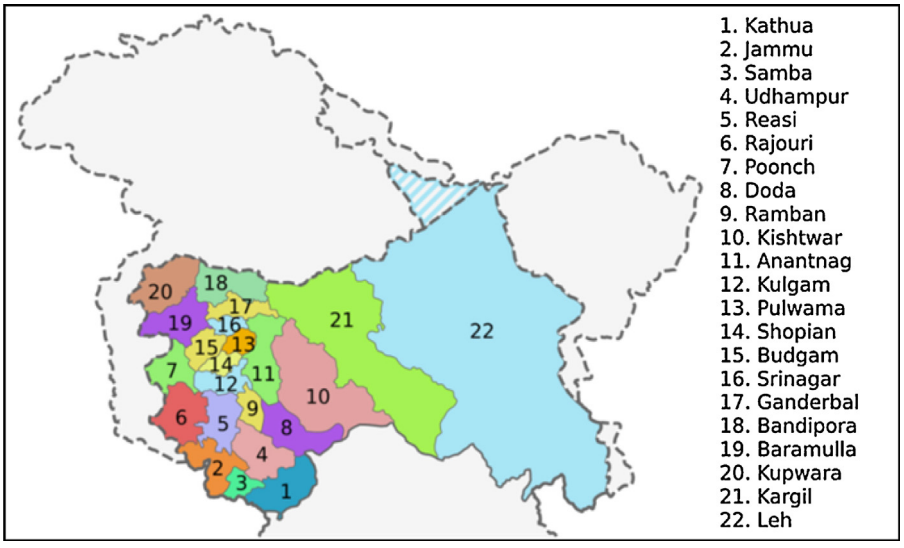

Fig. 1 – J&K is divided into 3 divisions; Jammu with 10 districts (1–10), Kashmir Valley with 10 districts (11–20) and Ladakh with 2 districts (21–22).

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