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Short Communication

# Prevalence of *Mycobacterium tuberculosis* with multiple copies of IS6110 elements in Gulbarga, South India

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ABSTRACT

This study was carried out to ascertain the prevalence of *Mycobacterium tuberculosis* by insertion sequence 6110 (IS6110) based DNA fingerprinting method in Gulbarga district belonging to the southern part of India. Results showed that among the 52 *M. tuberculosis* isolates studied, 57.7% exhibited more than 5 copies of IS6110 showing the prevalence of *M. tuberculosis* with multiple copies of IS6110 elements.


Tuberculosis (TB) remains a major infectious disease and causes high morbidity and mortality worldwide. According to the World Health Organization 2011, it is estimated that there were 8.8 million incidence cases and 1.1 million deaths owing to TB in the year 2010. India alone accounted for 26% of all TB cases worldwide [15]. For understanding the molecular epidemiology of the causative agent, an array of molecular typing methods targeting different molecular markers has been recently introduced. Among these, DNA fingerprinting of *Mycobacterium tuberculosis* (MTB) has gained increased acceptance as a useful tool to understanding the molecular epidemiology and phylogenetic investigations [13,14]. Restriction fragment length polymorphism (RFLP) typing by insertion sequence 6110 (IS6110) probe has become the approved standard and most widely used method for differentiating the strains of MTB [5,12]. In the present study, using this fingerprinting methodology, an attempt was made to know the prevalence of MTB in Gulbarga which belongs to the southern part of India.

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Gulbarga is generally considered to be a socioeconomically and educationally backward area. The personal as well as public hygienic standards are poor which can enhance the incidence of infectious diseases, including TB. The MTB isolates ($n = 52$) were collected from sputum specimens of individuals attending designated microscopic centers (DMC) of Gulbarga during the period from February 2005 to March 2006. The specimens were collected after obtaining consent from the study subjects with the help of DMC staff. Equal volumes of 1% cetylpyridinium chloride–2% sodium chloride (CPC–NaCl) solution was used to preserve and transport the sputum specimens to the National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL and OMD), Agra, India for further processing [11]. The specimens processed for culture on Lowenstein–Jensen's medium were confirmed as MTB by a set of biochemical tests. The method described by van Soolingen et al. [13] was followed for the isolation of genomic DNA from MTB cultures. RFLP was performed using the protocol described by van Embden et al. [12]. Briefly, DNA was digested with *Pvu*II restriction enzyme (Genei, Bangalore, India) and the fragments were electrophoretically separated on 1% agarose gel. Later on, the fragments of DNA were transferred overnight onto positively charged nylon membrane and hybridized with digoxigenin (DIG)-labeled 245 bp IS6110 probe. DIG nucleic acid labeling and detection kit (Roche Diagnostics, Germany) was used for detection.

Based on the number of IS6110 elements in the genome, the isolates were classified into three groups, namely I, II and III. The isolate harboring more than 5 copies of IS6110 was considered under group I, between 1 and 5 copies as group II and zero copy as group III. Of the 52 isolates, 57.7% of the strains harbored multiple copies, i.e., more than 5 copies, indicating the prevalence of isolates with multiple copies of IS6110 in Gulbarga. However, 23% of the strains showed 5 or less than 5 copies, and 19.2% of MTB isolates were lacking IS6110 (Fig. 1). The number of IS6110 copies for individual isolates varied from 0 to 14. Comparison of copy numbers among these isolates did not show any clustering, however, samples collected at different time intervals from the same patient showed a similar pattern of IS6110 (Fig. 1, lane 6 and 7).

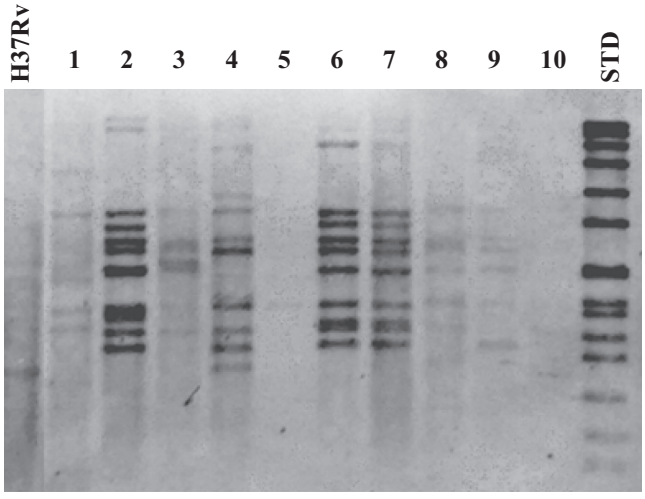


**Fig. 1 – IS6110 hybridization patterns of *M. tuberculosis* isolates of Gulbarga. H37Rv – *M. tuberculosis* standard used for the comparison of IS6110 elements, lanes 1–10 are *M. tuberculosis* isolates collected from pulmonary tuberculosis patients registered at designated microscopic centers, STD-molecular weight standard.**

IS6110-based DNA fingerprinting is considered the gold standard method and is generally used to study the molecular epidemiology of TB owing to its higher strain discriminatory power and reproducibility as compared with other DNA fingerprinting methods [6]. This method has been proved to be useful for molecular typing of MTB in different parts of India. This methodology in this study demonstrated different hybridization patterns of IS6110 probe which suggests the differences in the copy numbers of IS6110 element and also its genomic location. Earlier studies from southern India reported the prevalence of MTB isolates with low or no copy number of IS6110 element. The first study from south India (Madras) revealed a high frequency of single and zero copy isolates in the study population [4]. A study from Kerala reported 62.5% of isolates lacking IS6110 [9]. Later, Narayanan et al. [8] reported 41% of MTB isolates with single copy number in Tiruvallur district of south India. However, in another study on strains collected from different regions of India with zero copy and low copy numbers that are found all over the country and are not restricted to south India alone [3], studies from northern India reported a prevalence of multiple copies of IS6110 varying from 65% to 78% among the MTB isolates [2,3,7,10]. The present study also reports the same trend as that observed in northern India. This is the first study from the southern part of India reporting a higher percentage (57.7%) of MTB isolates harboring multiple copies of the IS6110 element.

Even though the copy number of IS6110 in MTB ranges from 0 to 25, the population-based molecular epidemiological studies report that most strains contain 8–18 copies, a number sufficient to enable discrimination between large numbers of MTB isolates [1]. In the present study, the number of IS6110 copies varied from 0 to 14, which is sufficient to enable discrimination between the majority of isolates. Also, a similar banding pattern of IS6110 in samples collected at different time intervals from the same patient shows the efficiency of this technique in reproducing the results. No clustering among the isolates studied was observed, and the probable reason for this observation could be the low number of samples included in this study. Despite the small sample size, a significant level of polymorphism has been observed, which is due to the prevalence of multiple copies of the IS6110 elements. This fingerprinting method has proved to be very useful in discriminating the MTB isolates at least in this region of south India. Hence, in order to employ this fingerprinting method, further study needs to be carried out on a larger number of samples with detailed demographical data. Also, a study of drug resistance patterns of these isolates will help in better understanding the molecular epidemiology of TB in this part of the country in order to make the TB control program more effective.

## Conflict of interest

The authors report no conflict of interest.

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