Molecular typing of *Mycobacterium tuberculosis* isolates from different parts of India based on IS6110 element polymorphism using RFLP analysis


*National JALMA Institute for Leprosy & Other Mycobacterial Diseases & *S.N. Medical College, Agra, **S.M.S. Medical College, Jaipur, *K.G. Medical College, Lucknow, ++Postgraduate Institute of Medical Education & Research, Chandigarh, †Military Hospital, Namkum, Ranchi, *+New Delhi Tuberculosis Centre, New Delhi & §Amrita Institute of Medical Sciences & Research, Cochin, India

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**Background & objectives:** IS 6110 based typing remains the internationally accepted standard and continues to provide new insights into the epidemiology of *Mycobacterium tuberculosis*. The aim of the study was to characterize *M. tuberculosis* isolates obtained from different parts of India based on IS6110 element polymorphism using restriction fragment length polymorphism (RFLP) analysis.

**Methods:** RFLP was analyzed among 308 isolates of *M. tuberculosis* deposited in the Mycobacterial Repository Centre, Agra, from different parts of India. DNAs isolated from these strains were restricted with Pvu II, transferred on to nylon membrane and hybridized with a PCR amplified DIG-labeled 245 bp IS6110 probe.

**Results:** Based on the copy number, *M. tuberculosis* isolates were classified into four groups, (i) lacking IS6110 element; (ii) low copy number (1-2); (iii) intermediate copy number (3-5); and (iv) high copy number (6-19). Copy number higher than 19 however was not observed in any of the isolates studied. At the national level, 56 per cent of the isolates showed high copy number of IS6110, 13 per cent showed intermediate copy number, 20 per cent showed low copy number, whereas 11 per cent isolates lacked IS6110 element. At the regional level, there was not much difference in the RFLP profiles of isolates (IS6110 copy numbers/patterns) from different parts of the country.

**Interpretation & conclusion:** IS6110 DNA based fingerprinting could be a potentially useful tool for investigating the epidemiology of tuberculosis in India.

**Key words** Fingerprinting - IS6110 - *M. tuberculosis* - RFLP
In the recent years there has been an increase in the incidence of tuberculosis in both developing as well as developed countries, and the tubercle bacillus continues to claim more lives than any other single infectious agent\(^1\). Among more than hundred known species of mycobacteria, *Mycobacterium tuberculosis* has been considered as the most important cause of morbidity and mortality in human beings. There has been an increase in the tuberculosis cases globally, especially with the increase in the incidence of human immunodeficiency virus (HIV) infected cases\(^2,3\). In an attempt to increase public and political awareness, WHO declared tuberculosis as a global emergency in 1993\(^1\). Reports of outbreaks of multi-drug resistant tuberculosis in acquired immune deficiency syndrome (AIDS) patients was another alarming issue\(^4,5\). To understand the molecular epidemiological trends, the DNA fingerprinting techniques for *M. tuberculosis* are emerging as powerful epidemiological monitoring tools\(^6-12\). This technique permits comparison of results between different laboratories. Such comparisons facilitate investigations into the international transmission of tuberculosis helping identify specific strains with unique properties such as high infectivity, virulence, or drug resistance\(^2,9,13\). IS6110 is being used successfully nowadays to trace exogenous re-infection with multi-drug resistant *M. tuberculosis* in patients with advanced HIV infection\(^9,14,15\). A significant proportion (40%) of south Indian isolates have been reported with single copy or lacked this sequence\(^14,16,17\) and thus, precluded its use in the context of Indian isolates of *M. tuberculosis*. In contrast, our earlier study mainly based on *M. tuberculosis* isolates from north India, revealed substantive polymorphism in drug resistant isolates of *M. tuberculosis* using IS6110 restriction fragment length polymorphism (RFLP) analysis\(^18\). Thus, considering the discrepancy between the data from northern and southern parts of India with respect to the copy number of IS6110, the present study was planned to determine the applicability of this probe for characterization of Indian *M. tuberculosis* isolates.

### Material & Methods

A total of 308 isolates of *M. tuberculosis* deposited in the Mycobacterial Repository Centre at the National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, from different parts of India were included in this study. Among these, 185 were from north India, whereas 24, 57, and 42 were from southern, eastern and western States, respectively. The species level identification of mycobacteria was done by standard biochemical tests (Niacin, Nitrate reduction, Catalase at 68°C, Tween 80 hydrolysis and Aryl sulphatase)\(^19\). For RFLP analysis, the isolates were grown on Lowenstein-Jensen (LJ) media slants at 37°C up to 4 wk. Colonies were scrapped and genomic DNA was isolated using the method described by van Embden \textit{et al}\(^9\). The DNAs (1-2 µg) were digested with restriction enzyme PvuII (Bangalore Genei, India) at 37°C in a shaking water-bath for 4 h. The restriction fragments were separated by a 1 per cent agarose gel electrophoresis run in 1× TBE (Tris-borate EDTA) overnight at 1.2V/cm. The fragments were transferred onto positively charged nylon membrane. A 245 bp probe was synthesized by PCR amplification by using earlier published primers and procedure\(^9,20\) and was labeled non radioactively with DIG nucleic acid labeling-detection kit (Roche Diagnostics, Germany). Hybridization and detection were carried out by following the earlier described method\(^9\).

### Statistical analysis:
Data were analyzed using statistical package STATA-7.0, Texas, USA at 5 per cent level of significance.

### Results

The range of IS6110 copies among isolates varied from 0-19 (Fig.). Based on the copy number, *M. tuberculosis* isolates were classified into four groups, (i) lacking IS6110 element; (ii) low copy number (1-2); (iii) intermediate copy number (3-5);
and (iv) high copy number (6-19), however, copy number higher than 19 was not observed in any of the isolates studied. At the national level, 56 per cent of the isolates studied showed high copy number of IS6110, 13 per cent showed intermediate copy number, 20 per cent showed low copy number, whereas in remaining 11 per cent isolates IS6110 element was found lacking. The discriminatory power has been calculated by using Simpson – index of diversity and its value was found to be approximately 1.0. At the regional level, isolates from all four regions namely, east, west, north, and south showed a similar trend with respect to the copy numbers when compared to the national data.

Statistically significant difference was found in the distribution of IS 6110 copies number in four regions of India ($\chi^2 = 9.41$, $P < 0.025$).

**Discussion**

This study reports no major differences in the proportion of isolates with zero or low copy number among the isolates studied from different regions of the India. However, our observations on isolates from southern region do not exactly match with that of Das et al\textsuperscript{16} who reported as high as 40 per cent of the isolates lacking or having only a single copy of IS6110. This difference could be partially attributed to smaller sample size of southern isolates studied in our study; 83 per cent of the isolates from south India displayed either high or intermediate copy number. As the number of isolates from south India was small, it did not change the national average significantly. Overall percentage for high and intermediate copy number isolates remained nearly the same ($214/308 = 69\%$ versus $194/284 = 68\%$) after excluding south Indian isolates.

In addition, none of the Indian isolates studied showed a copy number of IS6110 higher than 19. Diversity of the IS6110 patterns has been reported to be related to geographical origin of the isolates by others also\textsuperscript{6,21}. The IS6110 based RFLP analysis of *M. tuberculosis* from Tunisia and Denmark has shown interesting findings\textsuperscript{6,8}. About 75 per cent of
strains studied in Tunisia were found to carry 6-10 copies of the IS6110. In Denmark, 50 per cent of the strains investigated carried 11-15 number of IS6110 copies\(^2^1\). The degree of polymorphism of \(M.\) \(tuberculosis\) isolates in Dare-E-Salaam was found to be similar to that observed in New York and San Francisco using IS6110 DNA fingerprinting\(^2^2,2^3\). Our study also confirms a significant level of polymorphism in terms of IS6110 in \(M.\) \(tuberculosis\) isolates from India and clearly indicates a potential for IS6110-RFLP in epidemiological monitoring of tuberculosis in India.

The patterns of IS6110 among the isolates from different parts of India exhibited rather even distribution within a range of 0-19 copies. Significantly, the isolates having no IS6110 copy ranged from 9 to 10 per cent in all four regions of country. The PCR tests targeting this sequence will not be applicable to such cases in India indicating the need to have alternate gene targets for diagnosis and epidemiology in these situations. While a larger number of isolates of \(M.\) \(tuberculosis\) from different parts of India need to be studied using randomized and proper selection of samples, this study as well as earlier studies\(^1^8,2^4\) show the potential usefulness of this technique in molecular characterization of majority of Indian isolates of \(M.\) \(tuberculosis\).

Typing of strains by any DNA fingerprinting system is expected to help to trace the movement of individual strains within the country and even across the country borders for gaining proper understanding of the disease. DNA fingerprinting of \(M.\) \(tuberculosis\) isolates using IS6110 probe has been found to be useful in the investigation of MDR during outbreaks in AIDS patients in USA\(^4,1^5\). While the future scenario of AIDS cases in India cannot be predicted, these studies may help in investigating such phenomena and design proper facilities for management of these cases. The discriminatory power has been calculated by using Simpson–index of diversity and its value was found to be approximately 1.0 which is in the acceptable range >0.9\(^2^5\). In view of the isolates having low copy number or absence of copy, the complementary role of other typing methods like ribotyping, spoligotyping, typing with direct repeat probes, RAPD etc., also needs to be investigated.

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**References**


*Reprint requests*: Dr V.M. Katoch, Director, National JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR)
Taiganj, Agra 282001, India

e-mail: vishwamohan_katoch@yahoo.co.in