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Implications of Low Frequency of IS*6110* in Fingerprinting Field Isolates of *Mycobacterium tuberculosis* from Kerala, India

Restriction fragment length polymorphism (RFLP) analysis of IS*6110* in *Pvu*II-digested genomic DNA has been advocated for the fingerprinting of *Mycobacterium tuberculosis* as an approved standard methodology (8). This has proven to be very useful in the developed world, including the tracing of the New York outbreak of multidrug-resistant *M. tuberculosis* (2). The success of the screening hinges on the extent and type of polymorphism of the isolates.

Kerala, a small state in southern India, has moderately good health care facilities and a high rate of tuberculosis. With a view to study IS*6110* polymorphism, we screened 80 isolates obtained from patients at local tuberculosis clinics. The strains were isolated from sputum samples on L-J medium, acid-fast stained, and confirmed to be *M. tuberculosis* by standard biochemical tests. The DNA was isolated by the sand procedure (5) and further purified by phenol-chloroform extractions. The RFLP analysis was performed as described earlier (8) using the [α-^{32}P]dCTP-labeled, amplified 245-bp fragment of IS*6110*. The strains showing an absence of IS*6110* in the RFLP assay were confirmed to be IS*6110* negative by PCR amplification of purified genomic DNA.

Among the 80 isolates, 19 had no copy of IS*6110* and another 31 had only a single copy of the IS*6110* sequence. All the single-copy isolates (except three) showed a band at the 1.3-kb position where an integration hot spot (*ipl*) has been reported earlier (4). Thus, 50 (62.5%) of the 80 strains were not typeable by IS*6110*-based fingerprinting. In addition to this, another eight isolates had only 2 to 5 copies, making definitive fingerprinting difficult. This study clearly shows that IS*6110*-based fingerprinting is not possible in Kerala. While small numbers of IS*6110*-deficient strains have been reported earlier (ranging from less than 1% in San Francisco [1] and 2% in Vietnam [6] to 4% in Chennai [3]), this study is the first of its kind to report a large number of IS*6110*-deficient strains while also demonstrating unequivocally the inability to use the only approved and accepted fingerprinting system for molecular epidemiology of tuberculosis at least in some areas where tuberculosis is endemic.

The ramifications of the study are manifold. There is a clear need for a usable fingerprinting system for areas where IS*6110*-deficient strains abound. While several typing systems such as polymorphic GC-rich sequence typing, variable number of tandem repeat typing, and spoligotyping (7) have been reported, none has the versatility or utility to replace IS*6110*-based fingerprinting nor have they been widely used in any area of endemicity. The second point is that most molecular epidemiology studies of *M. tuberculosis* have been done mainly in the developed world, where the extent and type of variants are likely to be limited, unlike in an area where the disease is endemic. This also raises the fundamental question of whether the strain variations are restricted to IS*6110* distribution or the genetic differences are deeper. Thus, this modest study indicates a need to carry out systematic molecular epidemiological screenings in areas of endemicity to learn more about this pathogen.

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