

ABSTRACT

In the last years, various genotyping techniques were developed for isolation of *Mycobacterium tuberculosis* complex (MTBC) that has demonstrated a high discriminatory power. In this study, after the identification of selected strains at level of species by Genotype® MTBC technique, we evaluated the profit of the simplified amplified-fragment Length Polymorphism technique (AFLPs) and the Mycobacterial Interspersed Repetitive Units technique (MIRU-15). A total of 131 mycobacterium tuberculosis isolates were analyzed, 68 isolates were collected in Ecuador, from the Clinical Laboratory of Hospital Alli Causai located in city of Ambato, and the Laboratory of Bacteriology in Carlos Andrade Marín Hospital located in the capital city Quito. The remaining 63 isolates were harvested in Spain and belong to microorganism's collection of Microbiology Services of Consorcio Hospital General Universitario and Hospital Clínico Universitario of the city of Valencia. Among these isolates, 126 were identified by conventional methods such as molecular MTBC, corresponding to 106 patients. The *Mycobacterium tuberculosis* control strain ATCC 25177 was also identified as such by this method.

The AFLPs technique allowed as to group the strains in twelve patterns (P1 to P8, P10, P12, P13, P14), of which the most prevalent were patterns P1 with 77 (61.1%) and P2 with 27 (21, 4%) isolates, representing 82.5% of the same. These were followed by the pattern P5 with 5 (3.9%) isolates, the patterns P3, P4 and P6 grouped 3 isolates each one (2.4%), the patterns P8 and P12 with 2 isolates (1.6%) and finally the patterns P7, P10, P13 and P14 with 1 isolated each one (0.8%). The control strain *M. tuberculosis* ATCC 25177, showed a restriction profile that prevented their inclusion in any of the patterns described. The discriminatory power of the Hunter-Gaston discriminatory index (HGDI) method was 0.5812 against to 0.9843 of the MIRU-15 technique, which grouped 69 strains (54.8%) in 20 clonal complex and 57 unique patterns (45.2%). In the case of Spain, the strains were related mostly to the lineage 4 or Euro-American including: Cammeroon (1.59%), Haarlem (36.51%), S (31.75%), and LAM (19.05%); the lineage 6 or West Africa I (9.53%), the lineage 1 or EIA (1.59%) In the case of Ecuador the strains were related to the lineage 4: Haarlem (42.86%), S (33.33%) and LAM (22.22%) and Beijing lineage 2 (1.59%) from Asia. The MIRU-VNTR Variable-Number Tandem Repeats (15 loci) technique proved to be a stable system, reproducible and high discriminatory power in comparison with AFLPs system, allowing the use of it to conduct prospective population studies with the aim of contributing to the public health programs to control Tuberculosis (TB).

Keywords: AFLPs, MIRU-VNTR, genotyping, *Mycobacterium tuberculosis*, lineage