

## EVALUATION OF THE USEFULNESS OF MIRU-VNTR WHEN EXPLORING CLONAL COMPLEXITY IN CLINICAL *M. TUBERCULOSIS* ISOLATES

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**Purpose of the study:** The application of molecular tools to the analysis of clinical *Mycobacterium tuberculosis* (MTB) isolates has revealed that the infection by this pathogen can be clonally complex and reinfection, coinfection and microevolution are some examples of this complexity. Our purpose was to evaluate whether MIRU-VNTR could aid us to 1) simplify and accelerate the clonal analysis of MTB isolates to enable the clinicians to discriminate quickly between reactivation and reinfection in recurrent cases and to 2) evaluate whether the culture of specimens could modify the clonal composition present in clinical samples.

**Methods:** 1) 32 MTB isolates from 13 patients with recurrent episodes (6 months apart) were retrospectively analyzed by MIRU-VNTR directly from the stored isolates without subculturing them or purifying DNA. 2) 6 pairs of MTB strains with different MIRUtypes were combined in different proportions and then cultured. The purified DNA of each of the mixtures was analyzed by MIRU-VNTR before and after culture.

**Results:** 1) Direct analysis by MIRU-VNTR of stored isolates from recurrent TB cases could detect clonal differences for the sequential isolates in 38.5% (5/13) of the patients. MIRU-VNTR was able to identify cases of exogenous reinfection, microevolution and coinfection. 2) We detected differences in the clonal composition before and after culture of the *in vitro* mixtures which involved two of the MTB strains assayed.

**Conclusions:** 1) MIRU-VNTR succeeded in detecting clonal heterogeneity directly from stored clinical MTB isolates, which allows rapid discrimination between reactivation and reinfection in the clinical setting. 2) Reinfections, microevolution phenomena and coinfection events were efficiently detected. 3) In some cases, culture of the samples led to changes in the clonal composition of the initial specimens.

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