

Title: Molecular and epidemiological characterization of multidrug-resistant *Mycobacterium tuberculosis* isolates in Johannesburg, South Africa

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Summary

South Africa has a heavy burden of tuberculosis (TB) which is exacerbated by the concurrent epidemic of HIV. Molecular techniques have been used in most developed countries to investigate the dynamics of the TB epidemic, but despite the high prevalence of TB in sub-Saharan Africa, little data on strain types are available outside of the Western Cape. This study aims to provide information on the genotypic characteristics of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains in Johannesburg. Patient data obtained from the National Health Laboratory Service (NHLS) referral TB diagnostic laboratory and from Sizwe hospital, a MDR-TB referral hospital, were used to determine the risk factors for treatment outcomes in patients with MDR tuberculosis.

Multidrug-resistant *M. tuberculosis* isolates from over 100 clinics and hospitals in Johannesburg were stored for the study. Spoligotyping and MIRU-VNTR were used to genotype the strains. Drug susceptibility profiles showed that 238 (55%) of the 434 *M. tuberculosis* isolates tested were resistant to streptomycin and ethambutol, in addition to being resistant to rifampicin and isoniazid. A comparison of spoligotyping results with the international spoligotyping database (SITVIT2) showed a total of 50 shared international types (SITs). Forty-five shared types, containing 417 isolates (96%) matched a pre-existing shared type whereas 5 shared types (containing 11 isolates) were newly created. Diverse strain types were noted, with Beijing, LAM, EAI, T, S, H and X families being dominant. Spoligotype defined families were split into sub-clusters by MIRU typing, resulting in 76 MIRU international types (MITs), containing 389 isolates and 45 orphan isolates.

Spoligotyping showed lower discrimination (Hunter-Gaston discriminative index (HGDI) of 0.917) compared with MIRU typing (HGDI = 0.957) but there was no remarkable difference in the discriminatory power of combined spoligotyping and MIRU (HGDI = 0.962) compared with MIRU typing used alone. Twenty-four loci MIRU-VNTR typing was performed on strains from Beijing and CAS, EAI and H families to identify loci with high discriminatory power in our region. The proposed 15 MIRU-VNTR locus combination, together with MIRU 39, was found to be sufficient as a secondary typing method for the routine epidemiological investigation of the Beijing family isolates. Non-Beijing families could be sufficiently differentiated by the 15 MIRU locus combination.

This study also describes the treatment outcomes of 351 MDR-TB patients at Sizwe hospital, who started treatment between 2004 and 2007, and investigates possible risk factors associated with poor outcomes. Final treatment outcome was available for 324 (92%) of the patients. Treatment success (completion and cure) was recorded in 158 (48.8%) of patients, while 73 (22.5%) had poor outcomes and 93 (28.7%) defaulted. Eleven (3.1%) patients were transferred out to another health facility and 16 (4.6%) had no recorded final outcome.

The proportion of successful treatment increased significantly over time. Univariable and multivariable analysis ($P = 0.05$) identified the year of MDR-TB diagnosis and spoligotype-defined families as factors associated with treatment outcome. No associations were found between treatment outcome and HIV status, previous TB and additional MDR resistance to either streptomycin or ethambutol. The patient isolates were also characterised molecularly, complementing the study of isolates from Johannesburg alone, and providing information for the Gauteng Province.

A sub-study illustrating genotypic diversity of the families constituting extensively drug-resistant TB (XDR-TB) strains in South Africa was conducted subsequent to the nosocomial outbreak in KwaZulu Natal (KZN). The results show that multiple, parallel development of resistance, rather than transmission alone, also plays an important role in the incidence of this extended form of resistance.