Drug Resistance and Genetic Diversity of Mycobacterium tuberculosis in Luanda, Angola: a Molecular Epidemiological Perspective

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INTRODUCTION

Sub-saharan Africa contributes heavily to the tuberculosis (TB) burden worldwide. According to the World Health Organization (WHO), Africa exhibits the highest TB regional incidence rate (280 cases per 100 000 habitants) and the highest Human Immunodeficiency Virus co-infection rate (34%), both of which mostly driven by sub-saharan African countries (4). Yet, to this date, Angola has no surveillance data regarding drug resistance and the latest WHO estimates point to the occurrence of 69 000 new cases in 2013 and an incidence rate of 320 cases per 100 000 habitants in the same period (4). Additionally nothing is known regarding the genetic diversity and population structure of circulating *Mycobacterium tuberculosis* (*M. tuberculosis*) strains. Its capital city, Luanda, harbours approximately 33.7% of the country's population and is responsible for one-third of the TB cases nationwide. In the present study, we have addressed the genetic diversity and drug susceptibility profiles of circulating *M. tuberculosis* strains recovered from patients followed at a central Luanda hospital unit.

METHODS

Patients and Clinical Isolates

A total of 77 *M. tuberculosis* isolates were recovered from sputum samples from the same number of patients. The patients involved in the study were clinically diagnosed with TB and found to be positive for sputum smear acid fast bacilli in Hospital da Divina Providência in Luanda. Sample collection was performed between March to June 2015.

Sputum samples were frozen upon collection and shipped to the Mycobacteria Laboratory at the Research Institute for Medicines (iMed.ULisboa), under strict Biosafety Level 3 guidelines, where decontamination and culture were performed. Sputum sample decontamination was carried out using the NaOH/N-acetil-L-Cysteine method and cultured on Lowenstein-Jensen slopes and Middlebrook 7H9 medium supplemented with oleic acid, albumin, dextrose and catalase (OADC) and an antibiotic cocktail comprised by carbenicillin (final concentration: 5 µg/ml), trimethoprim (15 µg/ml), amphotericin B (1 µg/ml) and Polymyxin B (20 U/ml).

All isolates were identified as belonging to the *M. tuberculosis* complex by positive PCR amplification of an internal IS*6110* fragment using oligonucleotide primers INS1 (5'-CGTGAGGGCATCGAGGTGGC-3') and INS2 (5'-GCGTAGGCGTCGGTGACAAA-3').

Drug Susceptibility Testing

All isolates were tested for first-line drug susceptibility, at the Institute for Hygiene and Tropical Medicine, by the BACTEC 960 method using standardized critical concentrations, according to the manufacturer's manual.

Genotyping

The isolates were genotyped by 24-*loci* Mycobacterial Interspersed Repetitive Unit – Variable Number of Tandem Repeats (MIRU-VNTR) as described by Supply *et al* (2006) (3). All isolates were also genotyped by Spoligotyping as described by Kamerbeek *et al* (1997) (2). Spoligotyping genotypes were assigned to Shared International Type (SIT) and clade by comparison within the international spoligotyping database SITVIT WEB (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/) (1).

RESULTS

Spoligotyping analysis of a sample of 77 *M. tuberculosis* isolates has enabled the detection of 28 different spoligotype profiles corresponding to 21 different Shared International Types (SIT) and 8 orphan profiles (Figure 1). SIT 42 (LAM9) was the most prevalent SIT found (n=15, 19.5%) followed by SITs 20 (LAM1; n=11, 14,3%) and 53 (T1; n=11, 14.3%). Overall, the *M. tuberculosis* population structure in this sample was dominated by LAM (59.74%) and T (29.9%) strains.

Table 1 – SITs found after spoligotype comparison against the SITVIT WEB database (1).

SIT	Clade	Octal	No. of Isolates (%)
20	LAM1	677777607760771	11 (14.3)
33	LAM3	776177607760771	1 (1.3)
42	LAM9	777777607760771	15 (19.5)
53	T1	777777777760771	11 (14.3)
60	LAM4	777777607760731	3 (3.9)
64	LAM6	777777607560771	3 (3.9)
95	LAM6	777777607560731	1 (1.3)
144	T1	770000003760771	5 (6.5)
194	LAM2	677737607760731	1 (1.3)
244	T1	777777777760601	6 (7.8)
290	LAM8	777777606760771	1 (1.3)
306	T1	777777601560771	1 (1.3)
635	LAM3	000000007560771	2 (2.6)
1321		677777607760731	1 (1.3)
1530	LAM4	777777607760711	1 (1.3)
1535	LAM9	777577607760771	1 (1.3)
1548		777703400000360	2 (2.6)
1755	LAM6	677777607560771	1 (1.3)
1894	LAM9	747777607760771	1 (1.3)
2025		777777777700371	1 (1.3)
2271	LAM2	677717607760771	1 (1.3)

Drug susceptibility data showed that 17 (21.1%) of the 77 clinical isolates were resistant to one or more antibacillary drugs and from these, 3 (3.9%) were multidrug resistant (MDR).

Table 2 – First-line resistance profiles found among the 77 *M. tuberculosis* isolates.

Resistance Profile[a]	No. of Isolates (%)
Susceptible	60 (77.9)
I	5 (6.5)
S	5 (6.5)
R	1 (1.3)
IS	2 (2.6)
IEP	1 (1.3)
IRS	1 (1.3)
IREP	1 (1.3)
IRSEP	1 (1.3)

[a] –Resistance Profile: I – isoniazid, R – rifampicin, S – streptomycin, E – ethambutol, P – pyrazinamide.

Twelve and 24-loci MIRU-VNTR analysis revealed that 47 (15 clusters, data not shown) and 11 (4 clusters) isolates were clustered, respectively (Figure 1). Drug resistant isolates were found across distinct clades and MIRU-VNTR clusters although the largest MIRU-VNTR cluster detected was comprised by 3/5 drug resistant isolates, including one MDR isolate (Figure 1).

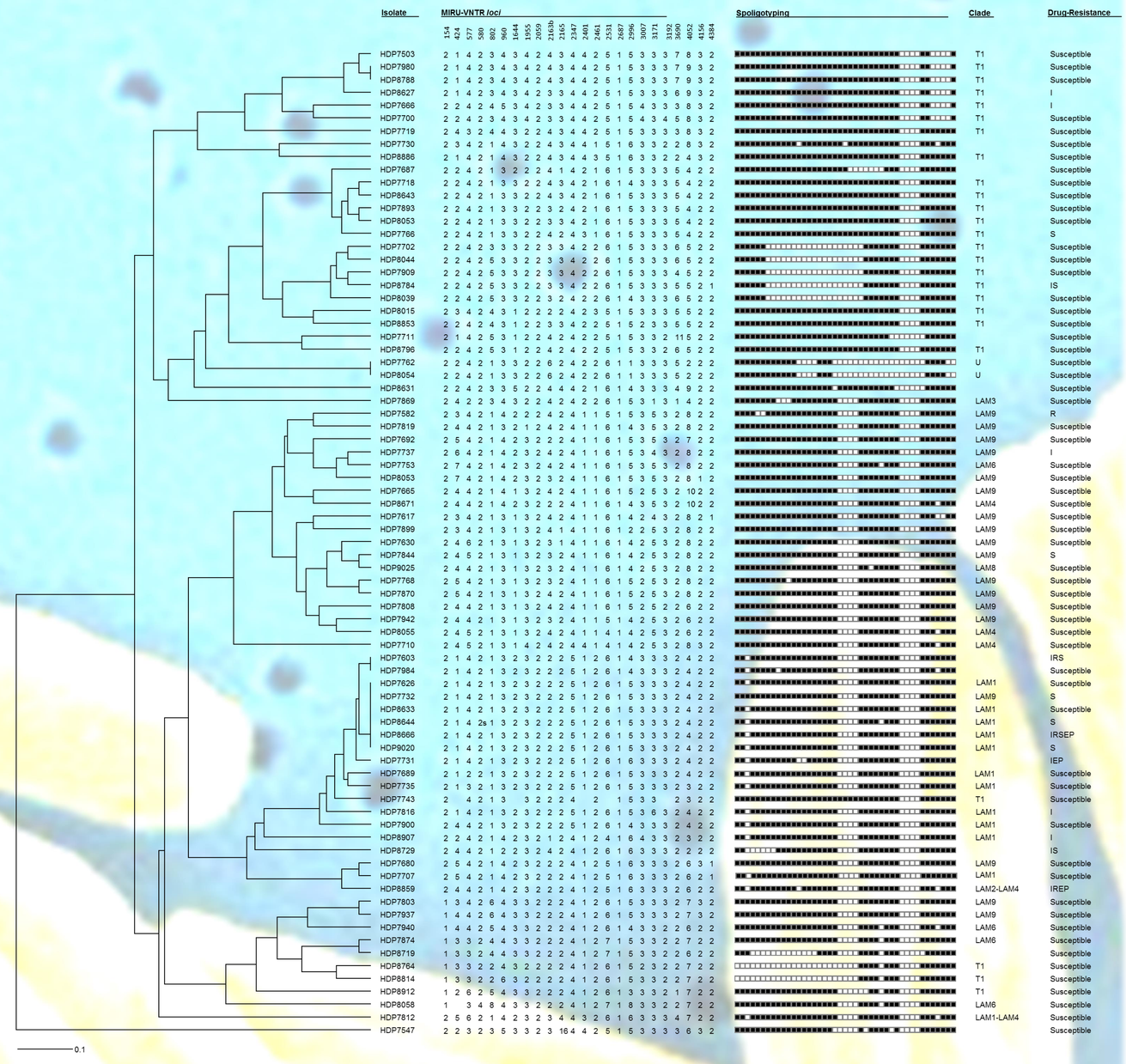

Figure 1 – Twenty-four loci MIRU-VNTR dendrogram of the 77 *M. tuberculosis* clinical isolates analyzed in this study. Resistance profile: I, isoniazid; R, rifampicin; S, streptomycin; E, ethambutol; and P, pyrazinamide.

DISCUSSION AND CONCLUSIONS

- Our data demonstrates a high predominance of LAM strains circulating in Luanda and the presence of recent transmission events that may be masked by complex transmission chains;
- Surprisingly, given the high level of emigrants from Asia in Luanda, no Beijing strain was found;
- Historical ties connect the Community of the Portuguese Speaking Countries and may favor, at a macroepidemiological level, an enhanced strain circulation between these countries. The now unveiled similar Angolan *M. tuberculosis* population structure supports this notion (1);
- The moderate rate of MDR-TB found in this sample has major public health implications and highlights the need for further studies specifically focused on MDR-TB transmission.

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