

Short Communication

Mycobacterium tuberculosis spoligotypes and drug-resistant characterization from Beira compared to genotypes circulating in Mozambique

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Abstract

Introduction: Mozambique is one of three countries with high prevalence of tuberculosis (TB), TB/human immunodeficiency virus coinfection, and multidrug-resistant TB. We aimed to describe *Mycobacterium tuberculosis* spoligotypes circulating among drug resistant (DR) strains from Beira, Mozambique comparing them with genotypes in the country. **Methods:** We performed spoligotyping of 79 *M. tuberculosis* suspected of DR-TB compared all spoligotype patterns published on the international database and PubMed. **Results:** Both in Beira and Mozambique (n=578), the main clades were Latin-American-Mediterranean, East-African-Indian, Beijing and T, with no extensively DR TB cases. **Conclusions:** Beira and Mozambique share the same population genetic structure of *M. tuberculosis*.

Keywords: Genotyping. Spoligotyping. Tuberculosis.

Despite significant efforts to control tuberculosis (TB), it remains an important public health problem in Mozambique, which is one of the three high-burden countries included in the list of the World Health Organization for TB itself, TB/human immunodeficiency virus (TB/HIV) coinfection, and multidrug-resistant TB (MDR TB), accounting for 85%-89% of TB cases globally¹.

We studied 79 clinical samples diagnosed with pulmonary TB from Beira, which is the second largest town in Mozambique, with 431,965 inhabitants, and the capital of Sofala Province, located in the central region of the country. TB incidence in Mozambique is 551 per 100,000 inhabitants², one of the highest in the world. TB isolates were obtained from the Beira Central Hospital Laboratory from 2013 to 2016.

The deoxyribonucleic acid (DNA) of *Mycobacterium tuberculosis* was extracted by the cetyltrimethylammonium bromide method from Lowenstein-Jensen medium-grown mycobacteria. Subsequently, we performed spoligotyping on the samples using a Luminex 200™ flow cytometry device (Luminex Corp, Austin, TX) and a microbead-based DNA array method³. Drug-resistant (DR) was detected using Genotype MTBDR^{plus}™ 2.0 (Hain Lifescience, Nehren, Germany) for isoniazid (INH) and rifampicin (RIF) resistance⁴; Genotype MTBDRs/ 2.0, another line probe assay, was used to detect fluoroquinolones (FLQs) and injectable second-line drugs (amikacin, kanamycin, and capreomycin) resistance⁵. The project was approved by the Ethics and Research Committee at Clinics Hospital of Ribeirão Preto Medical School, University of São Paulo-Brazil, Mozambique Ministry of Health (Maputo-MZB), and the University of Pittsburgh Institutional Review Board (Pittsburgh, PA-USA).

The total Mozambican database included 578 strains comprising strains from Maputo (132, 22.84%), Matola (93,

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16.09%), Beira (79, 13.67%), Pemba (75, 12.98%), Nampula (65, 11.25%), Inhambane (51, 8.82%), Xai-Xai (47, 8.13%), Lichinga (23, 3.98%), and unknown places (13, 2.25%). **Figure 1** shows the genetic diversity of *M. tuberculosis* complex according to the main spoligotyping clades of Beira and Mozambique as a whole. The clades of the lineages Latin-American-Mediterranean (LAM), East-African-Indian (EAI), T, and Beijing were the most frequent ones. Compared to the study by Perdigão et al.⁶ that described the *M. tuberculosis* genetic diversity in the Portuguese-speaking countries such as Angola, Brazil, Guinea-Bissau, Portugal, and Mozambique (with a lower sampling), we observed that Mozambique has the highest frequency of EAI lineage, which is different than what is observed in other Lusophone countries.

As most of these strains (459/578, 79.41%) are from SITIV2, we may highlight the main analysis previously reported by Couvin et al.⁷, where HIV-positive patients aged between 0 and 20 years were essentially reported in the Peruvian, Spanish, Mozambican, and Nigerian populations. Moreover, among the isolates from Southern Asia, the proportions of EAI and Beijing lineages were relatively higher among HIV-positive patients. In this present study, the HIV status was known for 51.89% (41/79)

of these patients, and 33 had a HIV-positive test. Couvin et al.⁷ suggested that the adaptability of the pathogen to the host could depend both on specific lineages and geographic variations in host immunity^{8,9,10,11}.

Among these isolates from Beira, 17.72% (14/79) were classified as MDR TB due to INH and RIF mutations. **Table 1** displays the total DR profile of these strains. The most important mutations detected in this study included *rpoB_Ser450Leu* (RIF), *katG_Ser315Thr* (INH), and *gyrA_Ala90Val/gyrA_Asp94Gly* (FLQ). There was not a single case of extensively drug-resistant (XDR) TB, and there were three cases of pre-XDR TB due to concomitant resistance to FLQ, INH, and RIF.

Previous studies^{8,9,12,13} have shown that LAM, EAI, T, and Beijing are associated with DR TB in Mozambique and other countries. The EAI lineage was significantly associated with DR TB in North Africa, but it was not the same in Western and Southern Asia, Caribbean area, South America, and Western Europe. Central Asian (CAS) lineage, which could be the ancestor of Beijing family¹⁴, was significantly associated with MDR TB in southern Asia, North Africa, and East Africa (specifically Ethiopia), but not in Western Asia and Northern Europe⁷.

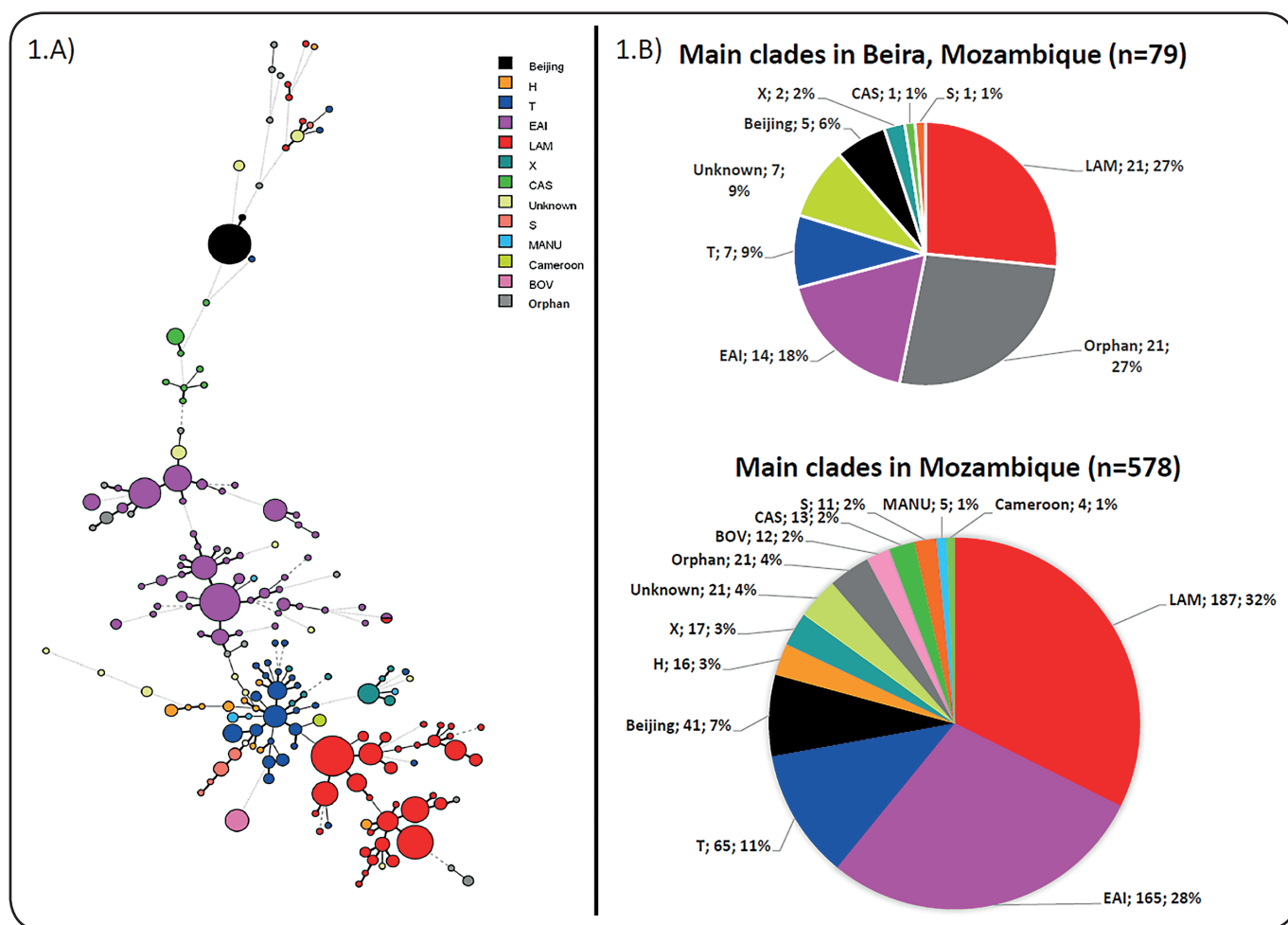


FIGURE 1: Genetic diversity of *Mycobacterium tuberculosis* in Mozambique. **(1.A)** A minimum spanning tree based on shared international typing for 578 Mozambique strains colored according to the main clades. **(1.B)** The main clade frequency distribution in Beira and Mozambique.

TABLE 1: MTBDRplus and MTBDRs/ results of 37/79 patients suspected of drug-resistant tuberculosis from Beira, Mozambique.

| Mutations associated with drug resistance | N |
|---|----|
| Rifampicin (RPM) | 16 |
| rpoB_Ser450Leu | 7 |
| rpoB_His445Tyr | 3 |
| rpoB_Val170Phe | 2 |
| rpoB_Ser441Leu | 1 |
| rpoB_Ser450Trp | 1 |
| rpoB_Asp435Tyr | 1 |
| rpoB_His445Leu | 1 |
| Isoniazid (INH) | 18 |
| katG_Ser315Thr | 14 |
| katG_Ser315Asn | 2 |
| kasA_Gly269Ser | 1 |
| katG_Asp94Gly | 1 |
| Fluoroquinolones (FLQs) | 3 |
| gyrA_Ala90Val | 2 |
| gyrA_Asp94Gly | 1 |
| Total mutations detected by MTBDRplus and MTBDRsl version 2.0 | 37 |

Another study has suggested that *M. tuberculosis* mutation rate estimated from different lineages may predict substantial differences in DR TB emergence¹⁰. To evaluate such result, a whole-genome sequencing (WGS)-based study demonstrated striking differences by lineage in the proportion of disease due to the recent transmission and in transmissibility (highest for Lineage-2 and lowest for Lineage-1)¹¹. It is important to point out that Lineage-4 comprises LAM, T, Haarlem, X, and CAS genotypes; Lineage 1 comprises EAI; and Lineage 2 comprises Beijing.

The study limitation includes the absence of linking epidemiological data to genotyping results, which could be important to evaluate transmissibility and DR lineage association.

In conclusion, both in Beira and Mozambique, the most frequent lineages are LAM, EAI, and T. No XDR TB cases were detected in these isolates. WGS should be performed to significantly understand the phylogenetic association among the strains found in Beira associated with DR and to investigate adaptive compensatory mutations and gene content.

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Conflict of interest

The authors declare that there is no conflict of interest.

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