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Genetic diversity of *Mycobacterium tuberculosis* isolates obtained from patients with pulmonary tuberculosis in Beira city, Mozambique

Abuchahama Saifodine [a,*], *Janet Fyfe* [b], *Aina Sievers* [b], *Elizabeth Coelho* [c], *Khalide Azam* [c], *James Black* [d]

[a] *Faculdade de Medicina, Universidade Eduardo Mondlane, Mozambique*
[b] *Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia*
[c] *Tuberculosis National Reference Laboratory, Ministry of Health, Mozambique*
[d] *Nossal Institute for Global Health, Melbourne School of Population and Global Health, The University of Melbourne, Australia*

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ABSTRACT

Background: Tuberculosis (TB) represents a serious public health problem in Mozambique, with an estimated incidence rate of 548 cases per 100,000 population in 2011. Information on the molecular epidemiology of *Mycobacterium tuberculosis* (MTB) strains circulating in Mozambique is limited. This study provides the first description of the genetic diversity of MTB strains circulating in Beira city, the second largest town in Mozambique.

Methods: A total of 67 MTB isolates were tested to determine genetic lineages and diversity. The genetic lineages were determined using real-time PCR while genetic diversity was assessed by obtaining Mycobacterial Interspersed Repetitive Unit-Variable Numbers of Tandem Repeat profiles.

Results: Only three of the six major lineages were represented, with 41 (61%) strains belonging to lineage 1, 25 (37%) belonging to lineage 4 and the remaining isolate belonging to lineage 3. No lineage 2 strains (containing the Beijing family) were identified. A high degree of diversity amongst the strains from both lineages 1 and 4 were observed. Comparison of the profiles of representative strains with those of reference strains in the MIRU-VNTRplus database revealed that all lineage 1 isolates clustered with the Eastern African Indian (EAI) 5 sub-family. The lineage 4 strains clustered with a variety of different sub-family strains, including the Latin-American-Mediterranean (LAM) 1 sub-family, the Haarlem, Uganda 1 and Cameroon sub-families and the T2-S sub-family.

Conclusions: The TB epidemic in Beira city is caused by a diverse group of MTB strains predominantly belonging to lineages 1 and 4.

* Corresponding author. Tel.: +258 844163052.
E-mail addresses: asaifodine@gmail.com (A. Saifodine), janet.Fyfe@mh.org.au (J. Fyfe), aina.sievers@mh.org.au (A. Sievers), bethhamene@gmail.com (E. Coelho), kie.azam@live.com (K. Azam), jim.black@unimelb.edu.au (J. Black).

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Introduction

Tuberculosis (TB) represents a serious public health problem, and it is estimated that in 2011 more than 8 million cases occurred worldwide [1]. Mozambique, with an estimated incidence rate of 548 cases per 100,000 population in 2011, is one of the countries with the highest TB burden [1]. Mozambique also faces a severe human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) epidemic and in 2009 the HIV prevalence in the adult population was estimated at 11.5% [2]. Among TB patients, the prevalence of HIV is estimated at 64% [3].

The advent of molecular techniques for genotyping has greatly contributed to a better understanding of the epidemiology, evolution and phylogeny of *Mycobacterium tuberculosis* (MTB) [4]. Several molecular typing techniques have been developed, including the *IS6110*-based restriction fragment length polymorphism (RFLP) typing method [5], spoligotyping and Mycobacterial Interspersed Repetitive Units-Variable-Number of Tandem Repeats (MIRU-VNTR) [6]. MIRU-VNTR is based on polymerase chain reaction (PCR) amplification of specific regions of the MTB genome. It identifies specific genotypic profiles by comparing the number of repeats of short deoxyribonucleic acid (DNA) sequences within different loci [6].

Despite the huge TB burden in Mozambique, there is only one published study in the international literature that describes the genetic diversity of MTB in the country [7]. However, that study did not include isolates from any of the four Central region provinces. In this study the genetic lineages of 67 MTB isolates from TB patients, domiciled in Beira city, were determined based on the presence of lineage-defining genomic deletions [6]. Subsequently, MIRU-VNTR profiles were determined to examine the diversity of the MTB strains circulating among this patient population.

Materials and methods

Setting

This study was carried out in Beira city, Mozambique during the month of November, 2009. Beira city is the second largest town in Mozambique and is the capital of Sofala province, located in the Central region of the country. The city has a population of 431,965 inhabitants. TB treatment is only available through the public sector, and it is offered in six health facilities, referred to as TB clinics.

Patients

The patients in this study were sourced from four TB clinics, which accounted for more than 75% of the TB notifications in Beira city. Patients were consecutively recruited for this study if they had pulmonary TB (both smear-positive and smear-negative), if they were at least 18 years old at the time of enrolment, and if they were residents of Beira city. The study included both patients being treated for the first time (new patients) and those with a history of previous TB treatment (re-treatment patients).

Laboratory procedures

All recruited patients were required to provide two sputum samples. After routine microscopy using the Ziehl-Neelsen method, sputum samples were refrigerated at 2–8 °C until they were shipped to the TB National Reference Laboratory (NRL) in Maputo city (capital of Mozambique), within three days of collection.

At the NRL, culture on Lowenstein–Jensen media was performed following World Health Organization (WHO) guidelines [8]. All positive cultures were sent to the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia for molecular testing. DNA was extracted from the original culture isolates using the FastDNA® SPIN Kit and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA). The original cultures were also sub-cultured for purification and storage purposes. The DNA was used to determine the genetic lineage by performing PCR and real-time PCR assays to detect the presence or absence of informative regions of difference (RD9, TBD1, RD239, RD750, *pks*15/1 7 bp deletion), using primer pairs previously described [6]. The basis for lineage assignment is shown in Table 1.

The extracted DNA from the MTB isolates was also used for Mycobacterial Interspersed Repetitive Unit-Variable Numbers of Tandem Repeat (MIRU-VNTR) typing using the most discriminatory loci for the lineages identified, according to an approach proposed by Comas et al. [6]. The loci amplified were Mtub04, QUB-11B, Mtub21, QUB-26, Mtub39, ETRA and MIRU40 for the pink lineage strains while the loci Mtub04, QUB-11B, Mtub21, QUB-26, Mtub30, Mtub39, ETRA, MIRU10 and MIRU40 were amplified to type the red lineage [6]. The amplified fragments were analyzed by electrophoresis through 2% agarose gels, with the number of repeats estimated on the basis of the fragment sizes. Analysis of the MIRU-VNTR profiles using the Bionumerics® software, version 6.5 (2010 Applied Maths, NV, Belgium), enabled a comparison of profiles via the generation of Unweighted Pair group Method using Arithmetic averages (UPGMA) trees and identification of clusters of strains of MTB. A cluster was defined as two or more strains of the same lineage with identical MIRU-VNTR profiles.

In order to compare these strains with those described elsewhere, a sub-sample of 22 strains with representative profiles based on the most discriminatory loci were further typed using the 12 MIRU-VNTR loci described by Supply et al. [9] (MIRU2, MIRU4, MIRU40, MIRU10, MIRU16, MIRU20, MIRU23, MIRU24, MIRU26, MIRU27, MIRU31 and MIRU39). The profiles obtained were compared with those in the MIRU-VNTRplus database (http://www.miru-vntrplus.org/) by performing an Identification by Similarity Search, generating Neighbor-Joining trees [10].

Statistical methods

Laboratory data was double entered using Microsoft Excel and then exported to *Stata Intercooled*, version 10 (Stata Corporation, College Station, Texas) where it was merged with demographic and clinical data. Descriptive statistics such as mean, median and standard deviation were used to summarize numerical variables, and frequency tables were used to summarize discrete variables.