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Genetic diversity of the *Mycobacterium tuberculosis* Beijing family in Brazil and Mozambique and relation with infectivity and induction of necrosis in THP-1 cells

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SUMMARY

Introduction: The success of the *Mycobacterium tuberculosis* Beijing (MtbB) lineage in different geographical regions has been attributed to high transmission, increased virulence, drug resistance and rapid adaptation to the host. In some countries of secondary MtbB dispersion like South Africa and Peru, rising prevalence of the Beijing strains is registered. However, in neighboring countries to affected regions such as Mozambique and Brazil, respectively, the prevalence of these strains is still low and this could be due to biological particularities of the circulating MtbB strains and/or differentiated host susceptibility.

Objective: To characterize genetically and phenotypically MtbB strains isolated in Brazil (n = 8) and Mozambique (n = 17).

Methods: This is a descriptive study of genotypes of the MtbB isolates, determined by spoligotyping, MIRU-VNTR typing, analysis of the IS*6110* copy number in the NTF region and screening for mutations in *mut*T2, *mut*T4, *rpo*B, *kat*G and *pks* 15/1 genes. Virulence-associated properties of the studied isolates were verified in the *in vitro* model of infection of human THP-1 cells.

Results: The genotypes defined by the 24VNTRs were distinct for all isolates included in this study and presented an HGDI of 0.997. The VNTR patterns with seven copies of MIRU26 and seven copies of QUB26, representative for the previously described MtbB genotype B0, dominant in Russia, were detected in 38.5% of the studied isolates. In addition, all isolates presented RD105 deletion and a 7 bp insertion in pks15/1 gene. Almost all tested strains belonged to the RD181 sublineage, with the exception of two strains from Mozambique of RD150 sublineage. Combined analysis of the NTF region integrity and mutations in *mut*T genes showed that 62.5% and 47% of isolates obtained in Brazil and Mozambique, respectively, were of the ancestral genotype. The virulence index of the ancient isolates, evaluated in the THP-1 cells, was significantly lower than that of the modern genotype group.

Conclusions: These data demonstrate genotype particularities of the Beijing strains isolated in Brazil and Mozambique, two countries of low prevalence of the MtbB lineage in local Mtb populations. In

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contrast to the neighboring countries with high prevalence of the MtbB strains of modern sublineage, significant proportions of the isolates obtained in Brazil and Mozambique were presented by the strains of the ancient sublineage. Our data suggest that lower virulence of the ancient strains, compared with the modern strains, could be involved in the slow spread of the MtbB strains in some regions.

1. Introduction

Tuberculosis (TB) remains a major global public health problem, more severe in developing countries with insufficient resources for health care. In countries with high burdens of TB, such as Brazil, Mozambique and Russia, the disease cases caused by strains of the East Asian/Beijing *Mycobacterium tuberculosis* lineage (MtbB) are frequently associated with HIV co-infection and/or drug resistance. The MtbB family was first described in 1995 in China [1] but during the following years, strains of this family were isolated in different parts of the world, demonstrating rising prevalence in southeast Asia, southern Africa and northern Eurasia [2]. The emergence of the MtbB family, determined by high level of association of with MDRTB, increased virulence, and more rapid progression of latent infection to active TB, has caused great concern among investigators and public health authorities [3].

Given this scenario, genotyping of Mtb isolates was employed for monitoring of the spread of MtbB strains and definition of epidemiological risk factors predisposing to infection and disease caused by MtbB. Spoligotyping has been the most usual technique in differentiating the MtbB lineage from other Mtb lineages, but it has limited power to differentiate intra-family genotypes. The MIRU-VNTR genotyping procedure has higher discriminatory capacity for the MtbB strains, and it can be further optimized including some additional VNTRs [4–6]. Other procedures for definition of MtbB evolutionary pathways and discrimination of sublineages have been proposed by introducing techniques for the detection of IS*6110* insertions in the NTF locus, capable of differentiating between ancestral or modern MtbB sublineages [2]. Discrimination of modern and ancient MtbB strains is relevant as these two clades seem to have different biological properties [7].

From an evolutionary point of view, the Beijing family can also be defined and divided into distinct subfamilies by analyzing the presence of Regions of Difference (RDs) and Single Nucleotide Polymorphisms (SNPs), confirming that this family is monophyletic [8,9]. While deletion of RD105 was exclusively observed in the MtbB strains and, therefore, is a specific marker of this genotype family, deletions of other specific large genome sequences, including RD207, RD181, RD142 and RD150, were suggested as a markers of MtbB sublineages [8]. Deletion of the RD207 defines the sublineage 2 and with additional deletion of RD181 is the basis of sublineage 3, which further evolved to sublineages 4 and 5 by deletion of RD150 and RD142, respectively. Related studies show that all the MtbB strains, in contrast to many other Mtb genotype lineages, possess intact pks15/1 gene, involved in the synthesis of phenolglycolipids, PGL, that is one of the mycobacterial virulence factors [10]. However, recent study demonstrated that the Beijing strains of sublineages 3, 4 and 5, but not those of the sublineages 1 and 2, are able to produce these antigens, suggesting that this biological property was acquired by MtbB during evolution [11]. These data promoted a new vision on the evolution of MtbB, suggesting that LSP and SNP in the bacterial genome provide new biological attributes, which may influence the virulence and transmission of the Beijing strains [8].

Recent studies demonstrated that in many countries of secondary dispersal of the MtbB, like South Africa [9], or Peru [12], the rising prevalence of the strains of Beijing genotype was associated predominantly with the strains of modern, but not ancient, sublineage. The prevalence of MtbB strains in these countries has reached 25% in South Africa [9] and 9% in Peru [12]. In contrast to these countries, the prevalence of Beijing strains in neighboring countries, like Mozambique and Brazil, is still low, representing only 7% [13,14] and 0.8% in local populations of Mtb strains [15], respectively. In this study, we aimed to investigate genotype characteristics and virulence-associated properties of the Beijing strains isolated in Mozambique and Brazil.

2. Materials and methods

2.1. Samples

We performed genotyping of 26 isolates of MtbB that were collected between 2002 and 2009 and had been characterized by spoligotyping as belonging to the Beijing family. Among the eight isolates obtained from TB cases in Brazil, two had been diagnosed at the University Hospital “Clementino Fraga Filho” (HUCFF) in Rio de Janeiro, while six patients had been diagnosed at the “Instituto Adolfo Lutz” (IAL) in São Paulo. Seventeen isolates were from patients from Mozambique and were provided by the Laboratory of Bacterial Pathogenesis and Molecular Biology, Unicamp, São Paulo. The MtbB clinical isolate from Russia (strain 1471), was used as a reference strain in our experiments and previously characterized as belonging to a large cluster called B0, dominant in an endemic area in Russia and demonstrating MDR profile and high virulence using the THP-1 cell line model [16]. The laboratory Mtb H37Rv strain was used as a control.

2.2. Bacterial culture and DNA extraction

Culturing was performed on Löwenstein–Jensen (LJ) medium and bacterial mass was submitted to thermal lysis in TE (10 mM Tris, 1 mM EDTA, pH 8.0) for liberation of nucleic acids, followed by centrifuging; the supernatant was stored at −20 °C.

2.3. Conventional drug susceptibility assay

Six isolates from Brazil and the Russian isolate 1471 were submitted to the method of proportions at the Adolfo Lutz Institute, while 17 isolates from Mozambique were characterized using the same method at the Laboratory of Bacterial Pathogenesis and Molecular Biology (Unicamp). These isolates were evaluated for resistance to isoniazid, rifampicin, streptomycin and ethambutol.