Carta ao Editor

Limitations of the use of the mtp40 fragment as a marker of differentiation between *Mycobacterium tuberculosis* and *M. bovis*

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The bacilli that cause tuberculosis (TB) belong to the *Mycobacterium tuberculosis* complex, which is composed of *M. tuberculosis*, *M. bovis* subsp. *bovis*, *M. africanum* and *M. microti,* as well as the *M. bovis* BCG strain used for vaccination. It has been proposed that newly discovered species should also be included in this complex. These new species include *M. canettii*, a variant of *M. tuberculosis* found in the Somalia region[1], *M. bovis* subsp. *caprae*, the etiologic agent of TB in goats[2] and *M. pinnipedii*, which causes TB in sea lions and may also infect humans[3].

Studies involving DNA-DNA hybridization and sequence analysis of the 16S rDNA sequence, the 16S-23S intergenic spacer sequence and the gene encoding the hsp65 heat shock protein[4] have shown that this complex, in fact, constitutes a single species. For reasons essentially related to the medical and veterinary significance of this group of bacteria, as well as to its pathogenic power and the wide spectrum of hosts receptive to each species, the nomenclature remains unchanged.

Within this complex of bacteria, *M. tuberculosis* is the principal pathogen in humans. However, cases of human TB resulting from infection with *M. africanum* and *M. canettii* have been reported, mainly in Africa[1]. In addition, *M. bovis*, the etiologic agent of bovine TB, may also infect humans and other animals. Studies conducted in Argentina and England showed that *M. bovis* is responsible for 0.4% to 1% of human TB cases[5,6]. According to the Pan American Health Organization[7], 7000 new cases of TB appear annually in South America. There are no data available regarding human cases of TB resulting from *M. bovis* infection in Brazil. This species is naturally resistant to pyrazinamide (PZA), a drug used in the treatment of TB in humans, which, in certain cases, makes the differentiation between species relevant. Such cases include those in which epidemiological evidence suggests *M. bovis* involvement and those in which the patient fails to respond to treatment regimes that include PZA.

A diagnosis of TB caused by *M. tuberculosis* or by *M. bovis* can be made through analysis of clinical data and radiological evidence. However, bacteriological diagnosis is necessary in order to confirm the diagnosis and identify the species involved. *M. tuberculosis* and *M. bovis* can be differentiated using phenotyping techniques such as tests for niacin production and nitrate reductase, as well as cultures to assess bacterial growth in the presence of thiophene-2-carboxylic acid hydrazide and PZA[4]. The polymerase chain reaction (PCR) method has been incorporated into the routine of many laboratories as a diagnostic alternative due to its greater speed, sensitivity and specificity. This technique allows the distinction between *M. tuberculosis* and *M. bovis* to be made through differential amplification of the pncA and oxyR gene sequences[8], amplification and analysis of enzymatic restriction within the gyrB sequence[9], multiple amplifications (multiplex PCR) of the DR fragment regions, the insertion sequence 6110 (IS*6110*) and the hsp65 gene[10], as well as amplification of the mtp40 fragment, which is exclusive to *M. tuberculosis* and therefore absent from *M. bovis*[11].

The mtp40 fragment, contained within the plcA gene sequence, which encodes the phospholipase C enzyme of *M. tuberculosis*, has been widely used for specific diagnosis of *M. tuberculosis* in (uncultured) clinical samples[11] and in cultures of isolated strains[12]. The absence of mtp40 from *M. bovis* has been verified by various authors, although the claim that mtp40 is present in all clinically isolated *M. tuberculosis* strains has been challenged[13].

In our laboratory, we analyzed the results from the amplification of the mtp40 fragment in 790 *M. tuberculosis* strains isolated at the *Rijksinstituut voor Volksgezondheid en Milieu* (RIVM, National Institute

for Public Health and the Environment) reference laboratory (Bilthoven, the Netherlands). The primers PT1 (CAACGCGCCGTCGGTGG) and PT2 (CCCCCCACGGCACCGC) were employed. We also evaluated the results of such amplification in 105 *M. africanum* strains and 10 *M. canettii* strains isolated at the *Centre National de Référence des Méningocoques, Institut Pasteur* (National Reference Center for Meningococci, Pasteur Institute, Paris France). Positive amplification results were obtained in 94.6%, 54.6% and 70%, respectively, of the isolated strains of each species (data from the RIVM)[14].

We studied, in detail, a set of 32 strains belonging to these two collections. These strains were chosen because they presented varying PCR results in relation to the mtp40 fragment. In 17 strains, a fragment of the expected size (396 bp) was amplified, no amplification occurred in 13, and a fragment larger than expected (1700 bp) was amplified in 2 of the strains (both *M. tuberculosis*). The presence of genetic polymorphisms in the phospholipase C gene, which would explain the absence of the mtp40 fragment from the isolates, was evaluated. The results show that, in most cases, the lack of mtp40 fragment amplification results from complete deletion of the plcA gene, as well as of the adjacent genes. In addition, insertion of a copy of the IS*6110* element into the mtp40 fragment was observed in 2 of the isolates, thereby impeding amplification of the fragment of the correct size (Figure 1)[15].

There are no available data regarding the relevance of using this marker in Brazilian strains. However, the existence of *M. tuberculosis, M. africanum* and *M. canettii* isolates presenting genetic polymorphisms serves as an indicator that we should be choosing molecular markers that are capable of definitively identifying *M. tuberculosis* and differentiating it from other members of the complex. Curretly, it is advisable to use a combinaton of phenotypic and genotypic markers in the differential diagnosis between *M. tuberculosis* and *M. bovis*.

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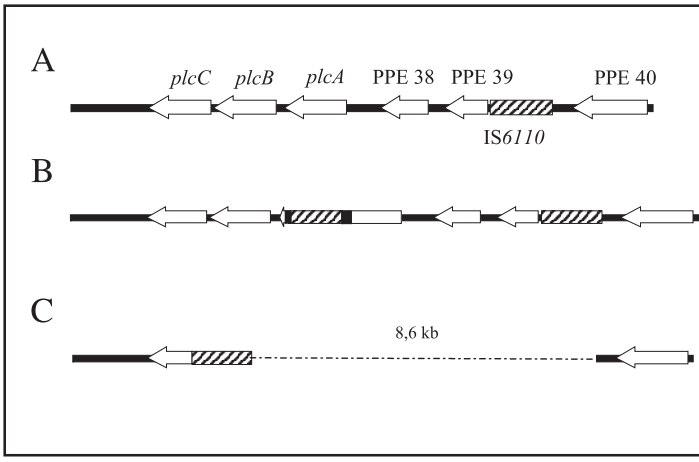

Figure 1. Location of the mtp40 fragment in the *Mycobacterium tuberculo* genome: (A) *M. tuberculosis* H37Rv; (B) insertion of a copy of the IS*6* element into the mtp40 fragment; (C) deletion of the 8.6-kilobase fragm including the mtp40 fragment. The black square within the plcA g represents the mtp40 fragment and the hashed rectangle represents the insert sequence *6110*. This region does not exist within the *M. bovis* genome.

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