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## Original article

# Genotyping and drug resistance patterns of *Mycobacterium tuberculosis* strains observed in a tuberculosis high-burden municipality in Northeast, Brazil

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## ABSTRACT

**Objectives:** This study has used a combination of clinical information, spoligotyping, and georeferencing system to elucidate the genetic diversity of the *Mycobacterium tuberculosis* isolates circulating in a TB-prevalent municipality of Northeast Brazil.

**Methods:** A total of 115 *M. tuberculosis* strains were isolated from pulmonary tuberculosis patients from January 2007 to March 2008 in Fortaleza. Drug susceptibility and spoligotyping assays were performed and place of residence of the patients were georeferenced.

**Results:** Of the *M. tuberculosis* strains studied, 51 (44.3%) isolates were resistant to at least one drug (R-TB) and 64 (55.7%) were sensitive to all the drugs tested (S-TB). A high frequency of resistance was found in previously treated cases (84%) and among new cases (16%;  $p < 0.001$ ). A total of 74 (64%) isolates were grouped into 22 spoligotyped lineages, while 41 (36%) isolates were identified as new. Among the predominant genotypes, 33% were Latin American Mediterranean (LAM), 12% Haarlem (H), and 5% U. There was no association of geographic distribution of RT-TB patients as compared to the controls and also the geographic location to the spoligotype patterns. The geospatial analysis revealed that 24 (23%) patients (hot spot zones) either shared the same residence or lived in a close neighborhood of a case. Among these concentration zones, the patients lived in the same residence and shared a common genotype pattern and resistance pattern.

**Discussion:** It was observed that the spoligopatterns family distribution was similar to that reported for South America, prevailing the LAM and H lineages. A high rate-case among the

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resistant TB group occurs as a result of transmitted and acquired resistance. A more effective surveillance program is needed in order to succeed in reducing tuberculosis in Northeast Brazil.

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## Introduction

Drug-resistant *Mycobacterium tuberculosis* (R-TB) is widespread in the world, and of particular concern is the occurrence of multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RIF).<sup>1</sup> Resistance may result either from a selection of drug-resistant organisms (acquired resistance) or infection with a drug-resistant TB strain (primary resistance). Acquired resistance is responsible for the majority of the cases and is well-associated to prior-exposure to anti-TB drugs, which includes those who have relapsed, failed and abandoned treatment.<sup>2</sup>

Among all incident cases of TB globally, 3.6% are estimated to have MDR-TB and 27 countries account for 85% of the MDR-TB cases.<sup>3</sup> Despite continuing to be one of the high TB burden countries, the prevalence of MDR-TB in Brazil has been estimated to be low;<sup>4</sup> surveillance for drug resistance has been limited.<sup>5</sup> In 2009, approximately 87,000 TB cases were reported in Brazil with an incidence rate of 45/100,000,<sup>4</sup> while in the state of Ceará, in 2010, 3430 new cases were reported with an incidence of 40.1/100,000.<sup>6</sup> Of these TB patients, 87.2% were pulmonary TB. Fortaleza is the capital city of Ceará and is the fifth largest city in Brazil, with 2.5 million inhabitants. In Fortaleza, the TB incidence has shown a decrease of 1916 in 2005 to 1651 in 2010.<sup>6</sup> Thus, implementation and intensification of new strategies in the control program is required in this region.

Rapid diagnosis, adequate treatment, and contact tracing to arrest further transmission are important factors in the control of tuberculosis. Molecular genotyping can be applied at the population level, and the obtained clustering of isolates provides clues about the patterns and dynamics of transmission in the population. Moreover when genotyping is coupled with geographic information system (GIS), the generated data can assess the spatial epidemiology of the disease.<sup>7,8</sup> The geographical information can be correlated with other important data, including clinical, drug resistance and genotyping, assisting localization of diseases and graphical analysis of epidemiological indicators.

There are limited available data regarding drug resistance, transmission pattern and strain distribution in Northeast Brazil. Previous studies of TB in Brazil have been mostly based on isolated information, including drug resistance, identifying risk factors or genotyping patterns.<sup>9–11</sup> A combined study based on clinical data, drug resistance patterns, genotyping and GIS has not been previously determined. Thus, we have conducted a study to elucidate the genetic diversity of the *M. tuberculosis* isolates circulating in a TB-prevalent municipality of Northeast Brazil.

## Methods

### Study population

This was a case-control prospective study conducted from January 2007 to March 2008 in Fortaleza, Northeast Brazil. Participants were recruited at Hospital of Messejana, which is a reference hospital for TB treatment. On an average, Hospital of Messejana cares for 130 TB patients per month. The patients were eligible to participate in the study if they were at least 15 years of age, and had respiratory symptoms or chest X-ray results suggesting clinical pulmonary TB.<sup>12</sup> HIV-infected patients, pregnant woman and those with no place of residence were excluded. *M. tuberculosis* was confirmed by culture from all the eligible participants. One hundred fifteen participants entered the study. A reduced number of participants as compared to the expected were due to the outpatient clinic of the research physician being open for only one day a week. No evidence of *M. tuberculosis* growth or culture contamination also reduced the total number of participants. Patients with drug resistant strain to at least one anti-TB drug (R-TB), were considered as cases. The control group consisted of all remaining patients infected by fully sensitive *M. tuberculosis* (S-TB).

A standardized form was used to collect data regarding age, gender, marital status, social behavior and economic status, clinical, radiological and laboratory tests. All these listed factors, and others not listed, had been previously identified to be related to TB.

### Ethical considerations

This project was approved by the Ethical Committee of the Universidade Federal do Ceará, Fortaleza, CE, Brazil. Written consent was obtained from each participant.

### *M. tuberculosis* isolation and drug sensitivity testing

Sputum specimens were examined by microscopy and cultured, after decontamination with NaOH and inoculation in Lowenstein-Jensen medium. All the isolates were identified by microbiological methods.<sup>13</sup> Susceptibility testing for isoniazid (INH) (0.2 µg/mL), rifampicin (RMP) (40 µg/mL), streptomycin (SM) (4 µg/mL) and ethambutol (EMB) (2 µg/mL) was performed by using the standard proportion method.<sup>14</sup>

### DNA extraction and spoligotyping

DNA extraction was performed by using the hexadecyltrimethylammonium bromide (CTAB) method,<sup>15,16</sup> and isolates were subjected to spoligotyping.<sup>17</sup> The spoligotypes patterns were converted into binary and octal formats according to Brudey et al.<sup>18</sup> Spoligopatterns were further analyzed

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