Correlation between resistance to pyrazinamide and resistance to other antituberculosis drugs in Mycobacterium tuberculosis strains isolated at a referral hospital*.**

Leila de Souza Fonseca, Anna Grazia Marsico, Gisele Betzler de Oliveira Vieira, Rafael da Silva Duarte, Maria Helena Feres Saad, Fernanda de Carvalho Queiroz Mello

Abstract
The correlation between resistance to pyrazinamide (PZA) and resistance to other first-line antituberculosis drugs was investigated in 395 Mycobacterium tuberculosis strains isolated from clinical specimens, representing 14% of the overall number of M. tuberculosis isolates obtained between 2003 and 2008 at the laboratory of a referral university hospital for tuberculosis. A high correlation was found between resistance to PZA and multidrug resistance, as well as between PZA resistance and resistance to rifampin, isoniazid, and ethambutol (p < 0.01 for all). These results highlight the importance of performing PZA susceptibility testing prior to the prescription of this drug in order to treat drug-resistant and multidrug-resistant tuberculosis.

Keywords: Tuberculosis/drug therapy; Tuberculosis/microbiology; Antibiotics, antitubercular.

Correlação entre a resistência a pirazinamida e a resistência a outros fármacos antituberculose em cepas de Mycobacterium tuberculosis isoladas em um hospital de referência

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Resumo
A correlação entre a resistência à pirazinamida (PZA) e a resistência a outros fármacos antituberculose de primeira linha foi investigada em 395 cepas de Mycobacterium tuberculosis provenientes de espécimes clínicos, que representavam 14% do total de isolados de M. tuberculosis no período entre 2003 e 2008 no laboratório de um hospital universitário de referência para tuberculose. Uma alta correlação foi encontrada entre resistência a PZA e multirresistência, assim como entre resistência a PZA e resistência a rifampicina, isoniazida e etambutol (p < 0,01 para todos). Esses resultados enfatizam a importância da realização do teste de sensibilidade a PZA antes de prescrever a droga para o tratamento de tuberculose resistente e multirresistente.

Descritores: Tuberculose/quimioterapia; Tuberculose/microbiologia; Antibióticos antituberculose.

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Correlation between resistance to pyrazinamide and resistance to other antituberculosis drugs in *Mycobacterium tuberculosis* strains isolated at a referral hospital

During recent decades, patients with drug-resistant tuberculosis are becoming an increasingly common problem in Brazil. Although resistance to other first-line drugs can be easily determined by laboratory susceptibility testing, resistance to PZA remains difficult to determine; PZA is active only in acidic environments (e.g., pH = 5.5), and testing is not routinely performed. However, screening for PZA resistance is essential in order to identify multidrug-resistant (MDR) tuberculosis patients who have been exposed to the drug.

Despite the recent advances in controlling tuberculosis, Brazil ranked 19th among high-burden countries, with 87,000 cases per year and a mortality rate of 7.5 per 100,000 population, according to estimates by the World Health Organization. In 2009, 75,040 cases of tuberculosis were identified. Of those, 10,286 were cases of retreatment. Rio de Janeiro was the Brazilian state that had the largest proportion of retreatment cases (15.2%).

The objective of the present study was to determine the prevalence of PZA resistance in *M. tuberculosis* isolates and to identify a possible correlation between resistance to PZA and resistance to other first-line antituberculosis drugs. The strains used in the present study were isolated in the laboratory of a referral university hospital for tuberculosis, located in the city of Rio de Janeiro, Brazil. To our knowledge, this is the first study to evaluate the correlation between PZA resistance and resistance to other first-line drugs in Brazil.

Drug susceptibility tests were performed by the proportion method on Löwenstein-Jensen medium. Critical concentrations for resistance were as follows: streptomycin, 4 µg/mL; INH, 0.2 µg/mL; RMP, 40 µg/mL; and EMB, 2 µg/mL. We performed PZA susceptibility testing using Löwenstein-Jensen medium (pH = 5.5) containing 100 µg/mL of PZA. We defined MDR *M. tuberculosis* isolates as those resistant to INH and RMP.

Statistical analyses were performed with the Epi Info statistical package, version 6.0. The corrected chi-square test and Fisher’s exact test were used in order to compare resistance to PZA with resistance to the other drugs studied. The level of significance was set at *p* ≤ 0.05. The local research ethics committee approved the study.

Between 2003 and 2008, 2,821 *M. tuberculosis* clinical isolates (from 28,298 clinical samples sent to the laboratory) were submitted to drug susceptibility testing. Of those 2,821 isolates, 395 were selected from stock cultures on the basis of their viability. Of those 395 isolates, 285 (72.2%) were pansusceptible (i.e., susceptible to INH, RMP, EMB, and streptomycin) and 110 (27.8%) were resistant to at least one of the four first-line drugs. Of the 285 pansusceptible *M. tuberculosis* isolates, 22 (7.7%) showed monoresistance to PZA, and 38 (34.5%) of the 110 isolates that were resistant to at least one of the four first-line drugs were also resistant to PZA. There were 53 MDR isolates, 30 (56.6%) of which were also resistant to PZA. Resistance to PZA showed a strong correlation with concomitant resistance to other first-line antituberculosis drugs (Table 1).

<table>
<thead>
<tr>
<th>Drug</th>
<th>PZA Resistance (%)</th>
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<tbody>
<tr>
<td>INH</td>
<td>94.5</td>
</tr>
<tr>
<td>RMP</td>
<td>98.9</td>
</tr>
<tr>
<td>EMB</td>
<td>91.4</td>
</tr>
</tbody>
</table>

The rate of PZA resistance among pansusceptible clinical isolates (7.7%) in the present study was similar to those reported in two studies (range, 6–8%).

We found a high correlation between resistance to PZA and resistance to INH, RMP, and EMB (*p* < 0.01); however, the correlation between resistance to PZA and resistance to streptomycin was lower (*p* = 0.04). This can be explained by the fact that streptomycin is no longer part of the standard treatment for treatment-naïve patients in Brazil.

Recent studies of MDR *M. tuberculosis* strains in South Africa, Thailand, and Taiwan have found the rate of resistance to PZA to be approximately 50%, a finding that is similar to ours (i.e., 56.6%). The fact that studies conducted in different regions of the world (Africa, Asia, and South America) have found similar rates of resistance to PZA among MDR strains despite the use of different methods, such as the proportion method and BACTEC Mycobacteria Growth Indicator Tube, suggests that this is a general phenomenon.
MDR tuberculosis should be based on susceptibility test results in order to avoid disease progression to extensively drug-resistant tuberculosis. Further surveillance studies are needed in order to estimate the prevalence of PZA resistance in \textit{M. tuberculosis} strains in Brazil.

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References


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