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

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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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.

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.

Pyrazinamide (PZA) is classified as a first-line oral antituberculosis drug and has been widely used in the intensive phase of antituberculosis treatment, which involves the use of isoniazid (INH), rifampin (RMP), ethambutol (EMB), and PZA for two months, followed by the use of INH and RMP for another four months. In addition, chemotherapeutic regimens that include PZA have been associated with the success of the directly observed treatment, short-course strategy. Unlike conventional antibiotics that are active mainly against growing bacteria, PZA appears to kill at least 95% of the semidormant population of *Mycobacterium tuberculosis* that persists in acidic

pH environments inside macrophages.[1] Therefore, PZA susceptibility testing in *M. tuberculosis* isolates is highly recommended.

Although resistance to other first-line drugs can be easily determined by laboratory susceptibility testing, PZA resistance 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.[2] 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%).[3]

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.[4]

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 $p \leq 0.05$. 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). Resistance to PZA correlated most significantly with resistance to INH, EMB, and RMP, as well as with multidrug resistance ($p < 0.01$ for all).

A key drug in the treatment of tuberculosis, PZA acts on dormant bacilli and plays a unique role in killing a subpopulation of semidormant bacilli that are not easily killed by other antibiotics.[1] Concomitant resistance to different first-line antituberculosis drugs, including PZA, is not uncommon. Because of the difficulties in performing PZA susceptibility tests, information regarding PZA resistance is not routinely obtained in clinical settings. 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%).[5]

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[5-7] 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

Table 1 – Pyrazinamide resistance among 110 *Mycobacterium tuberculosis* clinical isolates resistant to at least one first-line drug.[a]

Resistance	Pyrazinamide susceptibility test results		p*
	Resistant (n = 38)	Susceptible (n = 72)	
Streptomycin resistance			0.04
Yes	16 (42.1)	45 (63.4)[b]	
No	22 (57.9)	26 (36.6)	
Isoniazid resistance			< 0.01
Yes	36 (94.7)	43 (59.7)	
No	2 (5.3)	29 (40.3)	
Ethambutol resistance			< 0.01
Yes	22 (57.9)	8 (11.3)[b]	
No	16 (42.1)	63 (88.7)	
Rifampin resistance			< 0.01
Yes	31 (81.6)	25 (34.7)	
No	7 (18.4)	47 (65.3)	
Multidrug resistance		%	< 0.01
Yes	30 (78.9)	23 (31.9)	
No	8 (21.1)	49 (68.1)	

[a]Values expressed as n (%). [b]n = 71 clinical isolates. *Fisher's exact test.

and should be taken into consideration when treatment regimens are devised.

In 2008, the World Health Organization released an emergency update on guidelines for the treatment of drug-resistant tuberculosis.[8] The updated guidelines recommend that treatment of MDR/extensively drug-resistant tuberculosis should include at least four drugs with either certain or almost certain effectiveness.[8] Treatment regimens can be individualized or standardized if resistance patterns for a specific country are known.[8] Finally, PZA must not be counted as one of the four effective drugs.[8] Our data support and reinforce that recommendation, given the strong evidence of high levels of resistance to PZA associated with MDR tuberculosis. If we assume that approximately half of the MDR strains are resistant to PZA, nearly half of those are susceptible to PZA; therefore, it is important to identify the MDR tuberculosis isolates that are susceptible to PZA so that the drug can be added to the combination of antituberculosis drugs for patients with MDR tuberculosis. We conclude that PZA susceptibility tests should be performed prior to starting or adjusting treatment regimens for patients with MDR tuberculosis.

Our results indicate that PZA resistance is far more common than is currently appreciated. Therefore, the inclusion of PZA in the treatment of 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 *M. tuberculosis* strains in Brazil.

Acknowledgments

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