

# Comunicação Breve

## Drug resistance in *Mycobacterium tuberculosis* strains isolated from sputum samples from symptomatic outpatients – Complexo de Manguinhos, Rio de Janeiro, Brazil\*

Resistência a drogas em cepas de *Mycobacterium tuberculosis* isoladas de amostras de escarro de pacientes ambulatoriais sintomáticos – Complexo de Manguinhos, Rio de Janeiro, Brasil

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### Abstract

This study aimed to assess drug resistance in *Mycobacterium tuberculosis* strains isolated from sputum samples. To that end, sputum samples were collected from 263 patients suspected of having tuberculosis. All subjects lived in the Complexo de Manguinhos, which is located in the city of Rio de Janeiro, Brazil. Cultures testing positive between October of 2000 and December of 2002 were tested to determine strain susceptibility to isoniazid, rifampicin, streptomycin, ethionamide, and ethambutol. Of the 75 patients diagnosed with tuberculosis, resistance to at least one of the drugs was found in 16 (21.4%). Of those 16 patients, 8 (50%) were new cases, and 8 (50%) had previously been treated. Multidrug-resistant tuberculosis was identified in 8 (10.6%) of the 75 patients, being associated with previous treatment in 6 (8%). The incidence of multidrug-resistant tuberculosis might have been underestimated, since *M. tuberculosis* was not isolated from all of the samples testing positive for acid-fast bacilli. However, at least, our findings shed some light on the problem.

**Keywords:** Tuberculosis; Drug resistance; Drug resistance, multiple.

### Resumo

Para descrever a resistência a drogas em cepas de *Mycobacterium tuberculosis* isoladas de amostras de escarro de 263 pacientes suspeitos de tuberculose moradores do Complexo de Manguinhos, Rio de Janeiro, Brasil, as culturas positivas entre outubro de 2000 e dezembro de 2002 foram submetidas a teste de sensibilidade para isoniazida, rifampicina, estreptomina, etionamida e etambutol. Resistência a qualquer das drogas foi encontrada em 21,4% (16/75) dos pacientes diagnosticados com tuberculose. Destes, 50% (8/16) eram casos novos e 50% (8/16) eram casos com tratamento anterior. A tuberculose multirresistente foi encontrada em 10,6% (8/75) do total de pacientes, estando associada a tratamento anterior em 8% (6/75) deles. Nossos resultados podem ter sido subestimados, pois *M. tuberculosis* não pôde ser isolado em todas as amostras positivas para bacilos álcool-ácido resistentes. Contudo, eles pelo menos revelam parte do problema.

**Descritores:** Tuberculose; Resistência a drogas; Resistência a múltiplas drogas.

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Recebido para publicação em 7/11/2006. Aprovado, após revisão, em 6/2/2007.

Tuberculosis (TB) is the leading cause of mortality from infectious diseases worldwide, accounting for approximately 2 million deaths each year, especially in developing countries.<sup>(1,2)</sup> Despite the existence of effective chemotherapy and the widespread use of the bacillus Calmette-Guérin (BCG) vaccine, the disease has never been properly controlled in these countries.

Brazil ranks fifteenth in the world in the number of estimated cases, with an incidence rate of 62/100,000 inhabitants.<sup>(3)</sup> Higher incidence rates are seen in densely populated cities such as Rio de Janeiro (114/100,000), which has the highest TB-related mortality rate (6.53/100,000).<sup>(4,5)</sup>

The TB situation can be even worse in areas with socially vulnerable populations. One such area is the Complexo de Manguinhos, a slum located in the city of Rio de Janeiro. Although the efforts made by the *Centro de Saúde Escola Germano Silval Faria* (CSEGSF, Germano Sinval Faria School Health Center), working in collaboration with the *Escola Nacional de Saúde Pública* (ENSP, National School of Public Health) of the *Fundação Oswaldo Cruz* (FIOCRUZ, Oswaldo Cruz Foundation) have effectively lowered the TB incidence rate in the Complexo de Manguinhos,<sup>(6,7)</sup> it remains high: 275, 260, and 235/100.000 in 1997, 1998, and 1999, respectively – data obtained from the CSEGSF/ENSP/FIOCRUZ *Programa de Controle da Tuberculose* (PCT, Tuberculosis Control Program). However, drug resistance testing has never been performed. The objective of this study was to describe drug-resistance in *Mycobacterium tuberculosis* strains isolated from adult individuals with respiratory symptoms and suspected of having TB (cough for 3 weeks) who sought treatment at the CSEGSF/ENSP/FIOCRUZ between October of 2000 and December of 2002.

The Complexo de Manguinhos comprises 12 communities with slum-like conditions. However, there are two exceptions, the communities designated CHP2 and Vila Turismo, which have large demographic populations and partial sanitation infrastructure with treated water and a sewage system. Overall, the Complexo de Manguinhos has 42,100 inhabitants distributed in approximately 8,000 houses (Rio de Janeiro Institute for State Management Planning). Physical examinations were carried out by the PCT staff at the CSEGSF/ENSP/FIOCRUZ, at which time sputum specimens were obtained and tested for acid-fast bacilli (Ziehl-Neelsen staining).<sup>(7)</sup> Since

culture and susceptibility tests are not part of the routine of the health care laboratory, they were performed in the Mycobacteriology Laboratory of the FIOCRUZ-operated Evandro Chagas Research Institute. The *M. tuberculosis* strain isolates were tested on Löwenstein-Jensen medium using the standard proportion method for susceptibility to isoniazid (INH), rifampicin (RIF), streptomycin (SM), ethionamide (ETH) and ethambutol (EMB). An isolate was defined as resistant when the percentage of colonies exceeded 1% of the growth on a drug-free medium (control) at the critical concentrations of 0.2, 40, 4.0, 20, and 2.0 µg/mL, respectively.<sup>(7,8)</sup> Multidrug resistance (MDR) was defined as resistance to at least INH and RIF. All TB cases diagnosed in the Complexo de Manguinhos were treated and monitored at the CSEGSF/ENSP/FIOCRUZ.

Between October of 2000 and December of 2002, sputum samples from 263 suspected cases of TB were analyzed by culture, and 80 of them were confirmed as cases of TB. However, 5 samples were contaminated, and therefore only 75 samples were available for susceptibility testing. The mean age of the patients was  $36 \pm 14$  years (range, 18-77 years), and the majority of the patients were male ( $p = 0.04$ ). Serology for HIV was available for 66 of the patients, 4 (6%) of whom were HIV-positive. Data were unavailable for one patient, who was reported to be in prison.

As shown in Table 1, 59 (78.7%) of the 75 patients were infected with strains susceptible to all drugs tested: 55 were new TB cases, and 4 were previously treated TB cases. The overall rate of resistance was 21.3%: 12.7% of the new cases and 66.7% of the previously treated cases. Strains presenting mono- or multiple-resistance to SM were found to infect mainly new cases (4 of the 6 cases, 5.3% of the sample), and no strains showing mono-resistance to RIF or ETH were detected. As expected, most of the patients infected with MDR strains (6 of the 8 cases, 8% of the sample) had been previously treated, none were HIV-positive, and 2 (2.6% of the sample) were new cases, together accounting for 10.6% of the sample. Of the 8 resistant new cases, 3 were found in the largest community in the Complexo de Manguinhos, the CHP2; and MDR cases were found in two communities, Vila Turismo (7324 inhabitants) and Ex-Combatentes (934 inhabitants), although resistant strains infecting previously treated patients

**Table 1** – Susceptibility testing of *Mycobacterium tuberculosis* strains isolated from tuberculosis patients (new and previously treated cases) living in the Complexo de Mangueiros, Rio de Janeiro, Brazil, between October of 2000 and December of 2002.

	n (%)		
	New Cases	Previously treated	All cases
Patients	63 (84)	12 (16)	75 (100)
Susceptible to all drugs tested	55 (73.3)	4 (5.3)	59 (78.7)
Resistant to one or more drugs	8 (10.6)	8 (10.6)	16 (21.3)
To one drug			
INH	2 (2.6)	2 (2.6)	4 (5.3)
SM	3 (4.0)	0 (0.0)	3 (4.0)
To two drugs		0 (0.0)	
INH + SM	1 (1.3)	0 (0.0)	1 (1.3)
Multidrug resistance	2 (2.6)	6 (8.0)	8 (10.6)
INH + RIF	0 (0.0)	4 (5.3)	4 (5.3)
INH + RIF + SM	1 (1.3)	2 (2.6)	3 (4.0)
INH + RIF + EMB	1 (1.3)	0 (0.0)	1 (1.3)

INH: isoniazid; RIF: rifampicin; EMB: ethambutol; and SM: streptomycin.

are more commonly found in communities with more than 4000 inhabitants.

The overall rate of resistance in the Complexo de Mangueiros was 21.4%, and, although the number of strains tested was small, the importance of this datum is that, in addition to being the first time that resistance was determined in the community studied, it was obtained in a high-burden, limited-resource community (in Rio de Janeiro, Brazil) where, despite the existence of a public health care facility that preferentially serves this community, TB incidence rates remain a cause for concern.<sup>(9)</sup> The rate of mono-resistance to INH and SM obtained in this study was high. However, the SM mono-resistance, unlike the INH mono-resistance, which was more frequent among previously treated cases, was detected among new cases (Table 1). Similar results were obtained in TB/HIV co-infected patients in Mozambique.<sup>(10)</sup> This is a concern since treatment regimen 1, which is recommended for untreated patients, does not contain SM, suggesting that older strains (related to endogenous reactivation) continue to circulate in the Complexo de Mangueiros. The number of HIV-positive patients in our sample was low (6%), and the mean rate of TB/HIV co-infection in the study period was 16.3%.<sup>(9)</sup> This might have created a bias in our results, since the isolates for these patients were not available.

The rate of MDR-TB in the Complexo de Mangueiros was similar to that found in

Mozambique.<sup>(10)</sup> The high incidence of drug-resistant strains in Mozambique reflects a pattern found in TB/HIV co-infected patients, who are more susceptible to TB infection, and, according to the authors, is associated with a high risk of exposure to resistant strains due to failure on the part of the PCT. In the Complexo de Mangueiros, most of the patients were HIV-negative, and the resistance problem seemed to be related to treatment non-compliance, as indicated by the high incidence of MDR strains infecting previously treated patients. In addition, although the study population comprised only 36.4% of all TB patients diagnosed at the time, this situation might reflect the reality in the area. With regard to resistant strains infecting new cases, primary resistance should be strongly suspected in at least one patient, since his brother was under treatment for MDR-TB presenting the same resistance pattern, although molecular typing was not performed to confirm the genetic identity of the strains. Other factors might be involved in the resistance situation in the Complexo de Mangueiros. One such factor is that, during the study period, the PCT was closed or worked poorly because of the violence perpetrated by organized crime gangs in the region.

This study is the first to provide information regarding drug resistance in the limited-resource area of the Complexo de Mangueiros, and the results show that the incidence of resistant strains

isolated from new and previously treated cases is high. The highest incidence of resistance in new cases was found in the most densely populated community (CHP2: 8655 inhabitants), and MDR-TB was spread among previously treated patients living in poor and disorganized communities with a high population density, such as Mandela de Pedra, Nelson Mandela, and Parque Oswaldo Cruz, lending credence to the assumption that crowded areas and low socioeconomic conditions favor the spread of resistant strains. However, the incidence might have been underestimated, since *M. tuberculosis* was not isolated from all the samples testing positive for acid-fast bacilli during the study period. We worked with a sample of patients with chronic cough treated at one CSEGSF clinic, and the resistance pattern obtained represents the situation in this group (34.6% of all patients diagnosed during the study period). Nevertheless, our results reveal, at least to some extent, the problem in the Complexo de Manguinhos, clarifying it so that it can be addressed by future PCT interventions.

## References

1. World Health Organization. Anti-tuberculosis Drug Resistance in the world. Report N° 2. Prevalence and Trends. WHO/CDS/TB, 2000.
2. Goldrick BA. Update: Tuberculosis in the United States: the CDC updates its guidelines, as infection rates decline slowly. *Am J Nurs.* 2005;105(7):85-6.
3. World Health Organization. Global tuberculosis control. WHO/CDS/TB, 2005.
4. Hijjar MA. Tuberculose: desafio permanente. *Cad Saúde Pública.* 2005;1(2):348-9.
5. Secretaria de Saúde e Defesa Civil do Estado do Rio de Janeiro [homepage on the Internet]. Rio de Janeiro: [cited 2004 Nov 1]; Available from: <http://www.saude.rj.gov.br/tuberculose/metropolitana.htm>
6. Mendes JM, Santos MO, Esteves MA, Saad MHF, Patrocló MA. Aspectos epidemiológicos da tuberculose no Complexo de Manguinhos, Rio de Janeiro, Brasil. Estudo retrospectivo no período de 1986 a 1994. *Pulmão RJ.* 2002;11(2):46-50.
7. Brasil. Ministério da Saúde. Fundação Nacional de Saúde. Centro de Referência Prof. Hélio Fraga. Sociedade Brasileira de Pneumologia e Tisiologia. Controle da tuberculose: uma proposta de integração ensino-serviço. 5ª Ed. Rio de Janeiro: FUNASA/CRPHF/SNPT, 2002.
8. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ.* 1969;41(1):21-43.
9. Mendes JM, Fonseca LS, Lourenço MC, Ferreira RMC, Saad MHF. Retrospective study: tuberculosis epidemiological aspects in Complexo de Manguinhos an urban slum area in Rio de Janeiro, Brazil, 2000-2002. *J Bras Pneumol.* 2007;33(4):443-7.
10. Nunes EA, De Capitani EM, Coelho E, Joaquim OA, Figueiredo IR, Cossa AM, et al. Patterns of anti-tuberculosis drug resistance among HIV-infected patients in Maputo, Mozambique, 2002-2003. *Int J Tuberc Lung Dis.* 2005;9(5):494-500.