Original Article

Multidrug-resistant *Mycobacterium tuberculosis* at a referral center for infectious diseases in the state of Minas Gerais, Brazil: sensitivity profile and related risk factors*

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ABSTRACT

Objective: To assess the determining factors for *Mycobacterium tuberculosis* multidrug resistance at a referral center for infectious diseases in the state of Minas Gerais, Brazil. **Methods:** A retrospective case-control study was conducted using data collected from September of 2000 to January of 2004. During this period, 473 cultures presenting growth of *M. tuberculosis*, corresponding to 313 patients, were submitted to susceptibility tests at the Central Laboratory of Minas Gerais. Cases presenting resistance to at least rifampin and isoniazid were classified as cases of multidrug resistance and were selected for study. These cases were paired to control group cases of drug-susceptible tuberculosis at a ratio of 1:3. Clinical and demographic data were analyzed using univariate and multivariate analyses. **Results:** During the study period, 12 (3.83%) cases of multidrug-resistant tuberculosis were identified. In the univariate analysis, multidrug-resistant tuberculosis was found to be more common among male patients, as well as among those testing positive in the sputum smear microscopy, those with cavitations larger than 4 cm in diameter and those having been previously treated for tuberculosis (p = 0.10 for all). After the multivariate analysis, only previous treatment for tuberculosis remained statistically significant (p = 0.0374), with an odds ratio of 14.36 (1.96-176.46). **Conclusion:** In the present study, previous treatment for tuberculosis was found to be an independent risk factor for multidrug-resistant tuberculosis.

Keywords: *Mycobacterium tuberculosis*; Tuberculosis, multidrug-resistant; Tuberculosis; Microbial sensitivity tests; Risk factors

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INTRODUCTION

Tuberculosis is a severe infectious disease, although it is curable in practically 100% of new cases, as long as the principles of specific chemotherapy recommended by the Ministry of Health since 1979 are observed. The appropriate doses of the proper drug combinations and the regular use of such medication for a sufficient amount of time, preferably involving supervised treatment, are effective means of avoiding bacterial persistence and the development of drug resistance.[1-2]

The increase in the frequency of multidrug-resistant strains of Mycobacterium tuberculosis, especially in developing countries and in patients infected with the human immunodeficiency virus, has been a worrisome problem within the scientific community. This has resulted in an increased number of studies on the subject.[3]

The frequency of naturally drug-resistant mutants is determined by calculating the ratio of resistant bacilli to susceptible bacilli. For M. tuberculosis, the ratios are 1:106 for isoniazid, 1:108 for rifampin, and less than 1:1014 for the isoniazid-rifampin combination. This drug resistance is due to genetic mutations and can occur naturally in bacillary populations, which has been demonstrated since the appearance of antituberculosis drugs.[4] Although rare, acquired resistance occurs when one of the following is true: the chemotherapy is inadequate to treat susceptible bacilli; there is limited availability or errors in the prescription of antituberculosis drugs; intestinal absorption is deficient; mistakes in the various processes of tuberculosis control programs, resulting in the selection of resistant mutants that predominate over the susceptible populations of bacilli eliminated by the drugs.[4-6]

In Brazil, multidrug-resistant bacilli are defined as those that present in vitro resistance to the rifampin-isoniazid combination and to one other drug from any of the standard regimens. This definition differs from that used in other countries because, since 1979, Brazil has recommended a salvage regimen (Regimen III) for cases in which the first-line regimens (Regimens I, IR and II) fail.[4] The international standard, which was adopted in this study, is to define multidrug resistance as in vitro resistance to the rifampin-isoniazid combination alone.[7]

An increase in the incidence of multidrug resistance and the presence of potentially associated risk factors might function as epidemiologic markers of the effectiveness of tuberculosis control programs and might be useful in devising new strategies.[8] Considering the relevance of tuberculosis in Brazil, together with the scarcity of data related to tuberculosis in the state of Minas Gerais, the objective of this study was to describe the epidemiologic panorama and the factors associated with multidrug resistance at a referral center in this state.

METHODS

Since September of 2000, the standard procedure at the Eduardo de Menezes Hospital has been for every sputum smear testing positive for acid-fast bacilli (AFB) to be sent to the Ezequiel Dias Foundation referral laboratory in the state of Minas Gerais, where the culture of mycobacteria is carried out. If requested by a physician, the smears testing negative for AFB are also sent for culture. At the Ezequiel Dias Foundation, all positive cultures are submitted to further tests for the identification of mycobacteria, as well as to sensitivity testing.

Between September of 2000 and January of 2004 (a period of approximately 40 months), 1519 sputum, bronchoalveolar lavage fluid, lymph node aspirate, liquor, pleural fluid, ascitic fluid, bone marrow and blood samples collected at the Eduardo de Menezes Hospital were cultured for mycobacteria. The results were as follows: 33 (2.2%) contaminated; 956 (62.9%) negative; and 530 (34.9%) positive.

Of the 530 positive cultures, 57 (10.7%) were identified as nontuberculous mycobacteria strains. The 473 remaining positive cultures were of M. tuberculosis strains. After duplicate cultures had been excluded, there were 313 cultures, 60 (19.17%) of which presented strains that were resistant to at least one drug (Table 1). All of the drug-resistant strains were isolated from samples of respiratory material (sputum or bronchoalveolar lavage fluid).

There were 19 strains that were resistant to rifampin only or to a combination of rifampin and another drug or drugs, and there were 32 strains that were resistant to isoniazid only or to a

TABLE 1

Distribution of resistance found in 313 strains of M. tuberculosis isolated between September of 2000 and January of 2004, based on respiratory material samples received from the Eduardo de Menezes Hospital

	No. of cultures
Susceptible to all drugs	253
Monoresistance	
R	5
I	9
P	5
E	1
S	13
Et	1
Multidrug resistance	
R+I	4
R+I+P	3
R+I+Et	2
R+I+P+S	1
R+I+P+E+S	1
R+I+E+S+Et	1
Other resistance patterns	
I+P	2
P+S	1
I+S	1
I+Et	1
R+Et	1
I+P+S	4
I+S+Et	1
R+P+E+S	1
I+P+E+Et	1
I+P+E+S	1

R: rifampin; I: isoniazid; P: pyrazinamide; E: ethambutol; S: streptomycin; Et: ethionamide

combination of isoniazid and another drug or drugs. There were 12 strains (20% of the total number of drug-resistant strains) that were resistant to the rifampin-isoniazid combination. Some of those strains were also resistant to other drugs. Those 12 strains met the international criterion for multidrug-resistant strains and were selected as the study cases.

The inclusion criterion for the selection of cases was presenting at least one culture that tested positive for an M. tuberculosis strain that was resistant to the rifampin-isoniazid combination. The inclusion criterion for the control group was presenting at least one culture that tested positive for an M. tuberculosis strain that was susceptible to all of the drugs tested. The patients were age-matched (within approximately three years of each other). The case/control ratio was 1:3.

The following data were collected: gender; age; ethnicity; level of education; profession; type of dwelling; history of tuberculosis; number of treatments for tuberculosis; regimen used in the first treatment; clinical form; contact with tuberculosis patients; type of contact; history of present illness (new case or not); time since the onset of symptoms; reason for treatment or hospitalization; signs and symptoms at hospitalization or consultation; chest X-ray findings, including the extent of lesions, the size of cavities (smaller or larger than 4 cm in diameter), and other accompanying lesions; concomitant diseases and factors; alcoholism; diabetes; cancer; corticosteroid therapy; use of immunosuppressants; previous lung diseases; psychiatric disorders; serology for human immunodeficiency virus; and the results of AFB tests cross-checked based on the number of bacilli per field examined, in accordance with the criteria established by the Ministry of Health.[9]

The culture for M. tuberculosis was considered positive when there was growth of typical M. tuberculosis colonies, which were submitted to susceptibility testing after identification by p-nitrobenzoic acid test, thiophene-2-carboxylic acid hydrazide test and niacin accumulation test. Lowenstein-Jensen solid medium was used, and the cultures were read on days 45 and 60 after the biological material sample had been prepared and sent for examination.[9] All cultures testing positive for M. tuberculosis were submitted to susceptibility testing using the indirect proportion method on solid medium. Resistance was defined as colony growth in critical drug concentrations: growth of at least 1% in isoniazid (at 0.2 µg/ml), ethambutol (at 2 µg/ml), or rifampin (at 40 µg/ml); and growth of at least 10% in ethionamide (at 20 µg/ml), pyrazinamide (at 100 µg/ml), or streptomycin (at 4 µg/ml).[10-11]

The statistical analysis was made with the objective of evaluating the association among demographic factors, clinical factors, and laboratory testing factors, and multidrug resistance. To build the database and carry out the statistical analysis, the Epi Info 2002 program, version 3.2, was used. After the descriptive analysis and the univariate analysis, the variables presenting a value of $p < 0.10$ were included in the multivariate analysis using the logistic regression technique, and values of $p < 0.05$

were considered statistically significant.

Since this was a retrospective study, based on the review of patient charts, in which the authors pledged to maintain patient anonymity, it was not necessary to obtain written informed consent. The study was approved by the ethics and research committee of the hospital.

RESULTS

The study sample consisted of 32 men (66.7%) and 16 women (33.3%). Ages ranged from 15 to 59 years. The mean age was 37.35 ± 12.09 years (median, 37 years). In the case group, mean age was 37.17 ± 12.90 years (median, 36.5 years), compared with 37.42 ± 11.99 years (median, 37 years) in the control group. The majority of the patients (81.3%) were non-white. In addition, most of the patients had less than 12 years of schooling, and 12.5% were illiterate. In terms of employment, patients were categorized as active (79.2%) or inactive (20.8%). Of the patients comprising the total sample, 91.7% lived in urban areas.

The mean number of previous treatments for tuberculosis was 0.9 (range, 0-6 treatments). The distribution of treatment frequencies is presented in Table 2.

The time since the onset of the tuberculosis symptoms varied from 1 to 30 weeks. The mean time was 8.5 ± 7.3 weeks (median, 4.5 weeks). Alcoholism was presented by 51.1% of the patients, diabetes by 6.7%, other lung diseases by 4.2%, and psychological disorders by 6.3%.

Chest X-rays were normal in 6.3%, unilateral lesions were observed in 33.3%, and bilateral lesions were observed in 60.4%. Small cavities were seen in 35.4%, large cavities were seen in 41.7%, and there were no cavities seen in 22.9%. The following accompanying lesions were observed: pleural presentation (in 6.3%); miliar (in 8.3%); unilateral destroyed lung (in 6.3%); bronchogenic dissemination (in 18.8%); and consolidations (in 25%).

TABLE 2

Reports of previous treatments for tuberculosis in the case group and control group

Previous treatments	Case group	Control group
none	1 (8.3%)	28 (77.7%)
1	3 (25%)	3 (8.3%)
2	4 (33.4%)	1 (2.8%)
3	1 (8.3%)	4 (11.2%)
4	2 (16.7%)	0 (0%)
5	0 (0%)	0 (0%)
6	1 (8.3%)	0 (0%)
Total	12 (100%)	36 (100%)

The distribution of frequencies of the AFB result was 6 negative samples, 13 AFB+ samples, 6 AFB++ samples, and 23 AFB+++ samples. The most common result was AFB+++, corresponding to 47.9% of the patients. In the case group, all samples tested positive for AFB, compared with 83.3% in the control group.

All patients were tested for human immunodeficiency virus, and the results were positive in 8 (1 in the case group and 7 in the control group).

Due to an insufficient number in each category, the following variables were excluded from the analysis: type of dwelling; previous contact with tuberculosis patients; cancer; use of corticosteroids; and use of immunosuppressants.

Table 3 shows the univariate analysis of the associations between individual clinical/radiological characteristics and M. tuberculosis resistance. For the multivariate analysis, the variables presenting significant statistical values ($p < 0.10$) were chosen. The results are presented in Table 4.

The results indicate that the only statistically significant association was between M. tuberculosis resistance and the variable 'previous treatment for tuberculosis' ($p = 0.0374$). In this study, individuals with history of tuberculosis had a fourteen-time greater chance of developing resistance than did individuals with no history of tuberculosis.

DISCUSSION

During the 40-month period evaluated (from September of 2000 to January of 2004), 18,507 new cases of tuberculosis were reported in the state of Minas Gerais, at an average of approximately 6,000 new cases per year (official data obtained from the Minas Gerais State Department of Health in June of 2004). The number of cases of multidrug-resistant tuberculosis reported in Brazil between 2000 and 2005 was 1907 cases, 1246 of which occurred in the southeastern region of the country.[4]

At the Eduardo de Menezes Hospital, a referral center for the treatment of infectious diseases in the state of Minas Gerais, 313 cultures were analyzed,

TABLE 3

Univariate analysis of the associations between individual clinical/radiological characteristics and M. tuberculosis resistance

Variable	Cases n (%)	Controls n (%)	*Odds ratio*	95% CI	p
Male	05 (41.7)	27 (75)	0.2475	0.0603 - 1.0147	0.0524
Non-white	11 (91.7)	28 (77.8)	3.3930	0.3713 - 31.0074	0.2791
Employed	10 (83.3)	28 (77.8)	0.7042	0.1298 - 3.8203	0.6844
Contact with a TB patient	03 (25)	02 (5.6)	3.0000	0.2514 - 35.7943	0.3851
Previous Treatment for TB	11 (91.7)	08 (22.2)	18.9800	2.3812 - 151.282	0.0054
Treatment abandonment	04 (33.3)	00 (0)	0.6916	0.3214 - 1.4879	0.3851
Dyspnea	09 (75)	21 (58.3)	1.6319	0.3789 - 7.0279	0.5109
Fever	09 (75)	31 (86.1)	0.5205	0.1133 - 2.3904	0.4012
Chest pain	04 (33.3)	14 (38.9)	0.9620	0.2524 - 3.6670	0.9548
Night sweats	03 (25)	17 (47.2)	0.7159	0.1588 - 3.2265	0.6635
Asthenia	09 (75)	30 (83.3)	0.5527	0.1203 - 2.5395	0.4460
Lymph node enlargement	01 (8.3)	06 (16.7)	0.4935	0.0492 - 4.9481	0.5482
Emaciation	08 (66.7)	34 (94.4)	0.1381	0.0139 - 1.3733	0.0912
Extensive lesion (bilateral)	08 (66.7)	21 (58.3)	1.8438	0.4789 - 7.0987	0.3737
Cavities > 4 cm in diameter	10 (83.3)	10 (27.8)	7.8990	1.6742 - 37.2690	0.0090
Alcoholism	03 (25)	20 (55.6)	0.9885	0.7314 - 1.3361	0.9402
Diabetes	00 (00)	03 (8.3)	0.0000	0.0000 > 1.0E12	0.9678
Psychological disorder	01 (8.3)	02 (5.6)	1.5000	0.1360 - 16.5407	0.7406
AFB positive	12 (100)	30 (83.3)	1.8929	0.8694 - 4.1214	0.1080

95% CI: 95% confidence interval; TB: tuberculosis; AFB: acid-fast bacilli

60 (19.17%) of which presented resistance to at least one drug. This is similar to the findings of previous studies reviewed in the literature, in which the overall resistance to at least one drug was reported to be approximately 21%.(13-14 Among those 60, 12 were multidrug-resistant strains of tuberculosis. The 3.83% rate of multidrug resistance (12 out of 313 samples) found in the present study is higher than the 0.44% rate found for Brazil as a whole from 2000 to 2005.[4] This is likely due to the selection of patients with more severe forms of the disease, presenting higher rates of treatment abandonment, more often experiencing recurrence, and treated at a referral hospital. Therefore, these data should not be extrapolated to primary health care facilities. Although this number is small, it is worrisome, since a diagnosis of multidrug-resistant tuberculosis not only lowers the probability of a cure but also increases the duration and toxicity of treatment. The cost of treating such patients is approximately 700 times greater than that of treating those with multidrug-susceptible tuberculosis.[14]

Resistance to at least the rifampin-isoniazid combination in the present study (3.83%) was greater than that found in the latest epidemiological survey carried out in Brazil (between 1995 and 1996), which demonstrated a rate of 2.2%.[15] In that survey, the multidrug resistance was acquired in 7.9% and primary in 1.1%. In the present study, primary multidrug resistance was seen in only 1 case (0.3%), compared with 11 cases (3.5%) in which acquired multidrug resistance was seen. Primary multidrug

TABLE 4

Multivariate analysis of the associations between individual clinical/radiological characteristics and M. tuberculosis resistance

Variable	*Odds ratio*	95% CI	p
Cavities > 4 cm in diameter	1.0626	0.2376 - 4.7521	0.9367
Male	0.7390	0.0790 - 6.9105	0.7909
Emaciation	1.2082	0.5870 - 2.4868	0.6076
Previous treatment for TB	14.3616	1.1688 - 176.463	0.0374
AFB positive	1.3153	0.4051 - 4.2713	0.6483

IC95%: intervalo de confiança de 95%; TB: tuberculose; BAAR: bacilo álcool-acido resistente.

resistance, which is not a serious problem in Brazil at present, is high in some countries, such as Latvia (14.4%), Estonia (10.2%), the Dominican Republic (6.6%), the Ivory Coast (5.3%), Argentina (4,4%), and Russia (4%).[16]

In the descriptive analysis, no gender-related or race-related differences were observed. The majority of the patients had less than 12 years of schooling, suggesting that tuberculosis affects individuals with little education, a fact previously reported in other studies conducted in Brazil.[17]

The majority of the patients evaluated in the present study resided in urban areas, a finding similar to those of other studies previously carried out in Brazil. It was not possible to detail the hygienic conditions of the homes (number of bedrooms, presence of running water/sewage system), which is an indirect criterion for poverty, due to scarce information contained in the patient charts.

The mean number of previous tuberculosis treatments was 0.9, varying from 0 to 6, and this number was greater in the case group than in the control group. Only one patient in the case group had not been submitted to previous treatment. In the control group, 77% of the individuals had not had previous treatment for tuberculosis. In another study, it was reported that 85.4% of the patients with multidrug-resistant tuberculosis had had two or more previous tuberculosis treatments, similar to the 91.6% found in the present study.[18-19] The mean number of previous tuberculosis treatments was 2.3 in the case group and 0.47 in the control group. These data are similar to those reported for Brazil as a whole, in which patients with multidrug-resistant tuberculosis had received an average of 2.8 previous tuberculosis treatments.[4]

The interval between the onset of symptoms and the search for medical attention varied from 1 to 30 weeks, with a median of 9 weeks. Longer time since the onset of symptoms (greater than the cut-off point of 9 weeks) was more common among the patients with multidrug-resistant tuberculosis than among those with multidrug-susceptible tuberculosis (in which the mean was less than four weeks). This might be explained by the disease evolution itself, chronicity, previous treatment, treatment abandonment, and a longer delay in seeking medical attention facilitating the progression and dissemination of the disease. This delay in diagnosis can be compared to that reported for the city of São Paulo, where 55.9% of the tuberculosis patients presented symptoms for over 30 days before the diagnosis.[20-22]

The alcoholism rate was higher in the control group than in the case group (55.6% vs. 25%), although this factor was not associated with multidrug-resistant tuberculosis. The evaluation of this finding is important, since a statistically significant association between alcoholism and multidrug resistance has been reported more than once in the literature.[19-21] In the present study, no such association was found, probably due to the greater severity of the patients in the control group, in which the rate of alcohol abuse was 55.6%.

To evaluate the results of the chest X-rays, the findings were subdivided into three categories (extent of the lesions, size of the cavities, and other accompanying lesions). This was done in order to facilitate not only the report but also the data compilation, according to the protocols employed in previously studies.[20,23-24] The final analysis was also made in a manner similar to that used in those same studies. In the univariate analysis, cavities, especially of those larger than 4 cm in diameter, were found to be more common in patients with multidrug-resistant tuberculosis. In the multivariate analysis, this difference was not statistically significant, probably due to the small size of the sample.

Initially, the analysis of sputum showed higher positivity in the case group patients, a fact that is explained by the persistence of positivity in the sputum smear microscopy, due to drug resistance. However, in the multivariate logistic regression analysis, no statistically significant difference was observed, since this test is not a true indicator of resistance but rather an indicator of disease activity.[25]

Although, in our sample, the prevalence of seropositivity for the human immunodeficiency virus was low, our findings are similar to those previously published for Brazil: that there is an association between multidrug-resistant tuberculosis and tuberculosis-human immunodeficiency virus co-infection.[26-28]

Patients previously treated for tuberculosis had a fourteen-time greater chance of developing multidrug-resistant tuberculosis. This result is in consonance with the literature, i.e., multidrug-resistant tuberculosis is not a new disease nor is it

something unknown; it is a disease that affects chronic patients, who are bacilli eliminators. These are consequences of the deficiencies in our basic health system, which is still quite precarious.[1,29]

At the end of the study, the risk factors presenting statistically significant associations with multidrug resistance in the univariate analysis were: gender (resistance being more common in male patients); emaciation (more common in patients with multidrug-susceptible tuberculosis); positivity in sputum smear microscopy (higher positivity in the resistant strain tests); size of cavitations seen on chest X-rays (larger in patients with multidrug-resistant tuberculosis); and previous tuberculosis treatment (associated with an 18.98-time greater chance of developing multidrug-resistant tuberculosis). In the multiple logistic regression analysis, the only variable that remained independently associated with the risk of multidrug-resistant tuberculosis was the presence of previous tuberculosis treatments ($p = 0.0374$), whereas the individuals with a history of tuberculosis presented a fourteen-time greater chance of presenting multidrug resistance than did those with no history of tuberculosis.

The study had its limitations, since it was based only on data collected from patient charts and was limited to a health care facility presenting specific characteristics. The principal limitation was that the patients evaluated presented greater disease severity due to having been selected for treatment at a referral hospital.

However, the study was justified by the need to determine the situation in this referral facility and the relevance of the data described in the literature for this population.

This study underscores the need for investment in the basic public health programs in order to improve treatment and increase patient compliance with treatment regimens, thereby lowering the high rate of treatment abandonment in Brazil, which is in fact the main reason for the appearance of multidrug resistance in our country.

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