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High frequency of resistance to the drugs isoniazid and rifampicin among tuberculosis cases in the City of Cabo de Santo Agostinho, an urban area in Northeastern Brazil

Alta frequência de resistência às drogas isoniazida e rifampicina em tuberculose na Cidade do Cabo de Santo Agostinho, uma área urbana do nordeste do Brasil

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ABSTRACT

The objective of the present study was to investigate the frequency and risk factors for developing multidrug-resistant tuberculosis in Cabo de Santo Agostinho, PE. This was a prospective study conducted from 2000 to 2003, in which suspected cases were investigated using bacilloscopy and culturing. Out of 232 confirmed cases of tuberculosis, culturing and antibiotic susceptibility tests were performed on 174. Thirty-five of the 174 cultures showed resistance to all drugs. The frequencies of primary and acquired resistance to any drug were 14% and 50% respectively, while the frequencies of primary and acquired multidrug resistance were 8.3% and 40%. Previous tuberculosis treatment and abandonment of treatment were risk factors for drug resistance. The high levels of primary and acquired resistance to the combination of isoniazid and rifampicin contributed towards the difficulties in controlling tuberculosis transmission in the city.

Key-words: Tuberculosis. Drug resistance. Multidrug resistance. Risk factors.

RESUMO

O objetivo do presente estudo foi investigar a frequência e fatores de risco para o desenvolvimento de tuberculose multidroga resistente, na Cidade do Cabo de Santo Agostinho, PE. Este é um estudo prospectivo realizado entre 2000-2003 onde casos suspeitos foram investigados por baciloscopia e cultura. De 232 casos de tuberculose confirmados, 174 tiveram cultura e antibiograma realizados. Trinta e cinco das 174 culturas mostraram resistência a qualquer uma das drogas. A frequência de resistência primária e adquirida a qualquer droga foi 14% e 50% respectivamente enquanto a frequência primária e adquirida para multidroga resistência foi 8,3% e 40%. Tratamento prévio para tuberculose ou abandono de tratamento consistiu em fatores de risco para resistência a drogas. Os altos níveis de resistência primária e adquirida a combinação isoniazida e rifampicina contribuem para as dificuldades no controle da transmissão da tuberculose no Cabo.

Palavras-chaves: Tuberculose. Resistência a drogas. Multidroga resistência. Fatores de risco.

The increasing frequency of drug-resistant tuberculosis (TB), which has been leading to failure to respond to either isoniazid (INH) or rifampicin (RMP) is of great concern in relation to successful implementation of large-scale regional TB control programs. In Brazil, the first-line drugs for TB treatment in use since 1982 are INH and RMP for six months in association with

pyrazinamide (PZA) for the first two months. The drugs are supplied by the government, without cost to the patient, and the treatment is based on self-administration.

The World Health Organization (WHO) defines multidrug-resistant tuberculosis (MDR-TB) as TB involving resistance to at least INH and RMP. However, the Brazilian Consensus

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for Tuberculosis that was agreed in 1997 classifies MDR-TB as tuberculosis caused by *Mycobacterium* that is resistant to more than two drugs including INH and RMP¹¹. Primary resistance refers to the presence of resistant isolates of *Mycobacterium* in patients who, in response to direct questioning, deny having had any prior anti-TB treatment; it is thus a consequence of transmission of resistant isolates. Acquired resistance is defined as the presence of resistant isolates of *Mycobacterium* in patient who, in response to direct questioning, admit having been treated for tuberculosis; it is thus due to selection of the bacterial population by development of mutation(s) that confer(s) survival advantages under antibiotic selective pressure¹⁵. The recommended treatment for multidrug-resistant tuberculosis is more toxic, takes at least three times longer and is a hundred times more expensive than a short course of treatment. It is also less successful¹⁶.

In 2004, WHO published a survey of frequencies of drug-resistant tuberculosis simultaneously in 74 countries around the world. Among new TB cases, the median prevalences of overall resistance, monoresistance and multiresistance were 10.2%, 7% and 1.1% respectively. The prevalence of any monoresistance to streptomycin (SM) was 6.3%, to INH 5.6%, to RPM 1.4% and to ethambutol (EMB) 0.8%. Analysis among previously treated cases was possible for 65 different settings, and it was found that the median prevalence of any resistance was 18.4%, with eleven settings reporting resistance over 50%, while the median prevalence of multidrug resistance was 7%²⁴. The prevalence of any monoresistance was 11.4% for SM, 14.4% for INH, 8.7% for RMP and 3.5% for EMB. The WHO study reported resistance data for 39 more settings than in the previous worldwide survey, but in spite of the great variation in the prevalence of TB resistance, it was possible to identify new hotspots with high prevalence of multidrug resistance among new cases.

Previous treatment for tuberculosis is the predominant determinant for the development of MDR-TB²⁰. Other risk factors are poor adherence to treatment, the presence of cavitory lesions on chest X-ray and HIV infection^{6 18 22}.

In Brazil, culturing and drug susceptibility testing for *Mycobacterium tuberculosis* strains is not performed on a routine basis and, except for a few studies, the information available on drug resistance is scarce. Information from the worldwide survey coordinated by WHO produced the estimate that, in this country, the national prevalence of acquired resistance to any drug was less than 20% and multidrug resistance was less than 10%. Primary resistance to any drug was less than 10% and primary multidrug resistance was less than 1%²⁴. However, Brazil is a very large country with considerable regional differences regarding cultural and socioeconomic conditions. Demographic factors vary also within each region; therefore, heterogeneity is expected in these rates. This has been demonstrated by recent studies such as one performed in São Paulo, Brazil's largest city, with an overall resistance of 15.5%. Among previously treated cases, the prevalence of resistance was 27% and multidrug resistance was 16.7%. Among new cases, the prevalence of any resistance was 11% and multidrug resistance was only 1%²¹.

Cabo de Santo Agostinho, the area of the present study, is an industrial city in northeastern Brazil with an annual incidence

of TB of about 60 per 100,000 inhabitants, a 20% treatment abandonment rate and no information on the frequency of drug resistance until the present study was conducted. The prospective cohort presented here was designed to characterize the drug resistance profile of *Mycobacterium* strains isolated from patients who were attended for TB investigation at public outpatient clinics and to investigate the risk factors for the development of MDR-TB in the city of Cabo de Santo Agostinho.

MATERIAL AND METHODS

Study design and population. A cohort study was designed to analyze newly diagnosed TB cases among 773 patients with TB-like respiratory symptoms who attended public outpatient facilities in Cabo de Santo Agostinho between February 2000 and December 2003. At the beginning of this study, the diagnosing of TB in Cabo was centralized. Thus, all suspect TB patients were referred to the Herbert de Souza Health Center, where they were seen by an experienced physician and were invited to have an appointment with the research team. Patients who were seen by the research team and who agreed to participate in this study signed an informed consent statement prior to answering a clinical and epidemiological questionnaire. Sputa were also collected and sent to the Central Laboratory of Pernambuco, which is the reference laboratory of the State of Pernambuco for tuberculosis diagnosis, for bacilloscopy, culturing and resistance testing. Blood samples were sent to the Public Laboratory of Olinda for HIV infection testing.

TB cases were defined by the physician who attended the patient, based on the clinic findings, X-ray and bacilloscopy. Because not all suspected TB cases were examined by the research team, the study population represented a fraction of the TB cases notified during the study period. Unfortunately, it was not possible to make a comparison between notified TB cases that were included in the research database and the overall number of TB cases, because the variables used in the official notification form were different from those in the questionnaire for the present study, other than age and gender, which were similar. Therefore, the results presented here might be not representative of the whole population of TB cases diagnosed in the City of Cabo de Santo Agostinho during the study period.

The Ethics Committee of the Aggeu Magalhães Research Center of the Oswaldo Cruz Foundation approved this study.

Microbiological analysis. Acid-fast bacilli were detected by means of Ziehl-Neelsen staining. The sputa were then decontaminated in accordance with the Petroff method and aliquots of 0.1ml were placed in Löwenstein-Jensen medium with or without the following antibiotics (Sigma, USA): isoniazid (0.2µg/ml), streptomycin (4µg/ml), pyrazinamide (2µg/ml), ethambutol (2µg/ml), ethionamide (2µg/ml), rifampicin (40µg/ml) or p-nitrobenzoic acid (500µg/ml). The resistance was determined by the proportion method¹¹. In order to increase the bacillary load, all smear-negative or weak positive samples (+) were first grown in the medium without antibiotics for two

weeks and were then subjected to resistance testing. Fast-growing *Mycobacterium* was routinely sent to the “Professor Hélio Fraga” National TB Reference Laboratory (Ministry of Health), in Rio de Janeiro, for species identification when these cases were detected by Regional Laboratories within the Public Laboratory network. When a resistant strain was identified on diagnosis, additional sputum was collected two or three months after the treatment began, for follow-up culturing and resistance testing, which have here been termed control cultures.

Data analysis. A database was constructed containing the clinical, epidemiological and laboratory information obtained from all patients under investigation for TB at the “Hebert de Souza” Public Health Center, using the Epi Info 6.04 software. In order to estimate the number of notified TB cases included in the cohort, we also analyzed official records of tuberculosis cases notified from 2000 to 2003, which were provided by the Health Department of Cabo de Santo Agostinho. Both databases were first reviewed to exclude any inconsistent data. Instances of identical data entries within a 30-day period were taken to be duplicates. Cases already undergoing treatment at the beginning of the study period were excluded.

For the resistance analysis, the frequencies of both monoresistance and multiresistance were determined. To allow comparison with international data, multidrug resistant strains were considered to be those that were resistant to at least INH and RMP, as defined by WHO. To determine the resistance index, files from positive culture on diagnosis were first selected and then any files relating to atypical strains that are normally resistant to multiple drugs and thus not a subject of our study were excluded. Nevertheless, the analysis included other files from patients who developed resistance during the course of their treatment, in the understanding that this represented infection by a new strain based on its resistance profile (Figure 1). The resistance was classified as either primary or acquired and, for primary resistance, it was calculated by dividing the number of patients with resistant strains among new cases by the total number of new cases with culturing and resistance testing. For acquired resistance, it was calculated by dividing the number of patients with resistant strains among previously treated cases by the total number of previously treated cases with culturing and resistance testing. The risk ratios for the development of drug-resistant TB were calculated for biological, clinical and socio-economical factors, including history of previous TB and abandonment of treatment, using logistic regression. The significance of these associations was determined using the chi-squared test and p-values, and Yates correction when indicated.

RESULTS

Cohort characterization. In the present study, 773 symptomatic respiratory cases were investigated for TB (Figure 1). The TB diagnosis was confirmed for 232 cases interviewed, which represented 50.5% of the 459 notified cases during the study period. The official incidence of TB was around 60 new cases per 100,000 inhabitants per year in the city of Cabo de

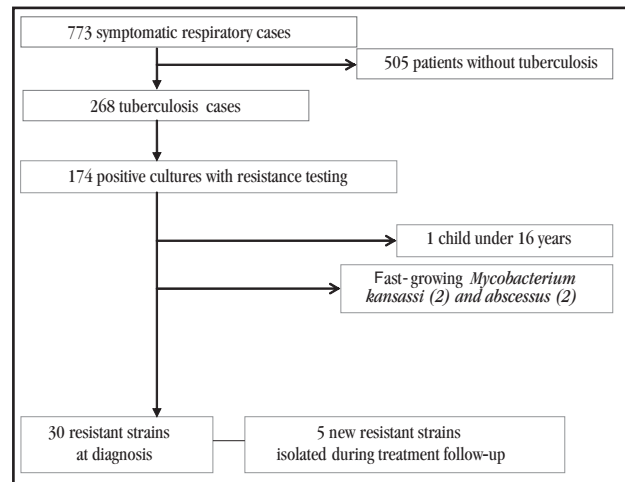


Figure 1 - Selection of the study population. Out of the 268 tuberculosis cases screened, 232 were notified by the Health Department of Cabo de Santo Agostinho during the study period.

Santo Agostinho. Although some fluctuation in the yearly incidence rates could be noticed, no clear upward or downward trend could be observed. A different scenario was apparent when observing two epidemiological characteristics that are associated with drug resistance: abandonment of treatment and disease recurrence (Table 1). A considerably higher number of such events were observed during the first two years of the study, in comparison with the next two years. The mean frequency of abandonment of treatment in 2000-2001 was 15.5%, whereas it decreased to 5% in 2002-2003 (p = 0.004). The mean frequencies of TB recurrence were respectively 5.5% and 2.5% for these consecutive two-year periods, but this difference was not significant (p = 0.346). It is important to point out that two interventions were implemented by the city’s Health Department over the study period: community-based surveillance in 2002 and decentralization of the healthcare system in 2003. These may have had an influence on the fall in the abandonment rate.

Tuberculosis was more frequent among men than among women (2:1), and the majority of the cases (86.6%) occurred within the economically active age group (20 to 64 years). Alcohol consumption and smoking were reported by 65.7% and 59.7% of the cases respectively. Histories of previous TB contact and anti-TB vaccination were present in 32.5% and 59.7% of cases

Table 1 - Coefficients of tuberculosis in Cabo de Santo Agostinho, 2000-2003.

	2000	2001	2002	2003	Total
Coefficients (per 100,000)					
incidence	61.4	58.8	52.3	67.3	
prevalence	81.7	76.5	61.4	80.4	
Total - notified cases	125	117	94	123	459
new cases	94	90	80	103	367
retreatment					
abandonment	19	19	3	8	45
recurrence	8	6	3	3	20
transferred			8	9	17
not classified	4	2			6
Total - studied cases	60	70	42	60	232

respectively. During the analysis, information bias was identified when assessing socioeconomic factors using the variables of employment and income, as measured by the minimum official salary in Brazil. There were records of employment activities with no regular incomes, thereby leading to imprecise quantification. About 70% of the patients were literate.

Culturing results and bacteriological characterization of mycobacterial isolates. Among all of the 773 suspected cases, *Mycobacterium* was isolated by means of Löwenstein-Jensen culturing in 181 (23.4%) samples, while 559 (72.3%) were negative cultures, 10 (1.3%) presented technical problems during the isolation procedure and 23 (3%) were not subjected to culturing. Among the 181 positive cultures, 137 (75.7%) were positive to alcohol-acid staining while 44 (24.3%) were smear-negative. All the negative cultures were also smear-negative. The bacilloscopy performed by the State Laboratory had sensitivity of 75.7%, specificity of 100%, positive predictive value of 100% and negative predictive value of 92.7%.

Thirty out of the 169 isolate samples on which resistance testing was performed at diagnosis showed resistance to at least one drug. However a further five isolates were obtained from patients undergoing treatment and were included in the resistance analysis because they were judged to represent new strains (their resistance testing showed profiles that differed from what was seen at the diagnosis). However, it was not possible to distinguish whether this was due to new infection or to selection of a mutant strain during the course of the treatment. All 35 *Mycobacterium tuberculosis* isolates were resistant to at least isoniazid. Resistance to at least rifampicin was found in 24 (14%) of the isolates. Monoresistance to isoniazid occurred in seven (4%) of the isolates. No monoresistance to rifampicin was found, and an association between isoniazid and rifampicin occurred in 16 (9%) of the cases. Resistance to isoniazid and rifampicin, or multidrug resistance, occurred in 24 (14%) of the cases. The frequency of primary resistance was 14% and of acquired resistance was 50%. The frequency of MDR-TB among new cases was 8% and among previously treated cases was 40%. The proportions of resistance among previously treated cases and new cases were 3.6 for monoresistance, 2.6 for two-drug resistance and 8.0 for resistance to three or more drugs (Table 2). Secondary and primary resistance to the specific combination of isoniazid and rifampicin was 20% and 6.3% with a ratio of 3.2. In a recent visit to the Cabo Health Center, we investigated the outcomes from the patients with drug-resistant TB reported here and found that cases of monoresistance to isoniazid either presented recurrence or were cured using scheme 1. We also observed that most multidrug-resistant cases completed the six-month treatment of scheme 1, even though the culture results were provided by the research team (data not shown).

Risk factors for developing drug-resistant tuberculosis. Comparison of the biological, clinical and epidemiological characteristics of the patient groups with drug-sensitive and resistant isolates showed that there were no statistically significant differences between the groups regarding frequencies of age, gender, literacy, being employed/unemployed, income category or presence of clinical symptoms. However, previous TB treatment and/or presenting a history of abandoning treatment were strongly associated with resistance (Table 3). The risk of developing MDR-TB was higher for those who reported previous TB treatment

Table 2- Frequency of drug-resistant *Mycobacterium tuberculosis* among cases diagnosed in Cabo de Santo Agostinho, 2000-2003.

	Number	Frequency (%)	Prevalence of primary resistance	Prevalence of acquired resistance	Ratio of acquired to primary resistance
<i>Mycobacterium tuberculosis</i> isolates	174		13.9	50.0	3.6
Any resistance	35	20			-
isoniazid (H)	35	20	-	-	-
streptomycin (S)	7	4	-	-	-
rifampicin (R)	24	14	-	-	-
ethambutol (E)	5	3	-	-	-
ethionamide (Et)	4	2	-	-	-
pyrazinamide (Z)	1	1	-	-	-
H, R (MDR)	24	14	8.3	40.0	4.8
H, S	6	3	-	-	-
Specific resistance					
H	7	4	2.8	10.0	3.6
H, S	2	1	9.0	23.3	2.6
H, E	1	1			
H, Et	1	1			
H, R	16	9			
H, R, S	3	2	2.1	16.7	8.0
H, R, E	1	1			
H, R, E, Et	2	1			
H, R, S, E	1	1			
H, R, Z, S, Et	1	1			

Table 3- Risk factors for the development of drug-resistant tuberculosis in Cabo de Santo Agostinho.

	Tuberculosis*				Risk relative	p
	resistant		not resistant			
	n ^o	%	n ^o	%		
Gender ≤ 34						
yes	20	21.3	74	78.7	1.13 [0.62-2.07]	0.82227
no	15	18.8	65	81.3		
Sex female						
yes	11	20.8	42	79.2	1.05 [0.55-1.98]	0.94727
no	24	19.8	97	80.2		
Alcohol consumption						
yes	22	18.6	96	81.4	0.76 [0.42-1.39]	0.49836
no	13	24.5	40	75.5		
Employment						
yes	12	19.7	49	80.3	1.05 [0.53-2.11]	0.94345
no	14	18.7	61	81.3		
Income ≤ 2 national minimum salaries						
yes	25	22.7	85	77.3	1.70 [0.82-3.54]	0.20160
no	8	13.3	52	86.7		
Illiterate						
yes	11	27.5	29	72.5	1.51 [0.81-2.81]	0.28995
no	8	18.2	108	81.8		
Previous treatment						
yes	15	50	50	50.0	3.60 [2.09-6.19]	0.00002
no	20	13.9	15	50.0		
Abandonment			86			
yes	10	52.6	9	47.4	3.26 [1.87-5.70]	0.00057
no	25	16.1	130	83.9		

*missing data was subtracted before statistical analysis.

data relating to retired status was excluded from employment variable.

(RR = 4.9; 95% CI = 2.48-9.77; p = 0.00004) and treatment abandonment (RR = 3.7; 95% CI = 1.82-7.56; p = 0.0033). Among the 232 notified TB cases, 185 underwent HIV testing: three samples were found to be positive and 13 were inconclusive, giving a frequency of TB-HIV coinfection of 1.6%. None of the MDR-TB cases were positive for HIV infection.

DISCUSSION

In the present study, MDR-TB, defined as resistance to at least isoniazid and rifampicin was responsible for 14% of the overall number of TB cases and 69% of the drug-resistant TB cases in the City of Cabo de Santo Agostinho. The prevalence of MDR-TB was as high as 8% among new TB cases and 40% among patients with previous treatment for the disease.

There is still not enough data published on MDR-TB in Brazil. A retrospective study covering one decade (1990-1999) in Ceará, another city in northeastern Brazil, reported an acquired MDR-TB frequency of 0.6%². In Rio de Janeiro, a Brazilian city with high demographic density and a poor situation regarding tuberculosis, the frequency of acquired MDR-TB in the year 1994 was 5.2%¹³. More recently, a study in a district of São Paulo, Brazil's largest city, reported a frequency of 16.7% for the year 2000-2002²¹. Such differences in MDR-TB prevalence as observed in Brazil have also been reported in other countries. One example is Bangladesh, which has an overall MDR rate of 5.5% but considerably higher levels in the regions of Dhara (14.3%) and Matlab (27.3%)²⁵. The resistance rate observed in Cabo is, on the other hand, similar to rates reported in some regions where drug resistance and MDR have been reported to be a major concern such as 54% in Lithuania⁴, 40.2% in Uzbekistan³ and 30.5% in Taiwan⁷.

The primary MDR level of 8% was also higher than the 0.1% observed during the national survey²³ and was comparable with the reported 5.8% in Mozambique¹⁴, 6% in Colombia¹², 9.3% in Lithuania⁴ and 13% in Uzbekistan³. It has been reported that countries with cure rates greater than 85% have primary MDR-TB levels of less than 5% and that the prevalence of MDR-TB is ten times higher among previously treated patients than in new cases¹⁶. This reinforces the idea that primary MDR are related to failing TB control programs, while secondary MDR seems to be a consequence of lack of treatment compliance. In Cabo, the cure rate is currently still below 85%. Before the intervention of the TB Control Program in 2002, the rate of abandonment of treatment was as high as 15%. These epidemiological characteristics may have contributed towards the high levels of both types of resistance in the region, thereby leading to the present prevalence of primary MDR-TB of 8%.

One important finding was the ratio between acquired and primary resistance when considering resistance to one or more drugs: this ratio was 3.6 for monoresistance, 2.6 when there was resistant against any two drugs and 8.0 against three or more drugs. The greater this ratio was, the higher the acquired resistance was, which could be explained by the historical high frequency of treatment abandonment in Cabo, even though the incidence and prevalence rates were shown to be relatively stable (Table 1).

A retrospective study on the prevalence of resistance to at least one drug was performed in Turkey, covering three decades starting in 1972. In this, an increase of 1.4% in the ratio between acquired and primary MDR was observed for each decade. This was accompanied by decreases in the ratio between acquired and primary resistance to any drug over the three decades (6.5, 5.1 and 4.6), thus suggesting that the increase in MDR prevalence was due to rises in primary resistance⁸.

Although the time frame of our study was too short for such an analysis, a lower ratio of acquired and primary MDR-TB (4.8) was observed in comparison with the ratio for resistance to three or more drugs (8.0). In the present study, the ratio between acquired and primary combined resistance to INH and RMP was 3.2, 4.8 or 8.0, depending on whether the cases were only resistant to INH and RMP, were resistant to at least INH and RMP or were associated with resistance to other drugs. This observation could be interpreted to mean that specific resistance to INH and RMP might be occurring as a consequence of lack of effective TB-control strategy in Cabo. This would lead to an increase in the primary resistance to specific drug combinations, thereby lowering the ratio in relation to that of MDR-TB.

We found a level of primary monoresistance against INH of 14% and absence of monoresistance to RMP. The observed pattern of resistance differed from the data presented in a recent review of 21 studies on the levels of primary resistance among *Mycobacterium tuberculosis* isolates in India, a country that accounts for 30% of TB cases worldwide. A high median prevalence of primary resistance to INH of 16.5% (3.2-32.9%) was observed, in comparison with the level of 2.85% (0-11.8%) for RMP and 1.5% (0-5.3%) for combined INH and RMP resistance¹⁷. A population study in Rio de Janeiro, in 1994 showed a similar behavior of resistance frequency: 4.6% and 0.9% for primary monoresistance to INH and RMP respectively and 0.7% for the combined resistance¹³. This was because the mutation rate for genes involved in INH resistance was higher than that reported for rifampicin resistance. Double mutation is therefore a rare event: the development of combined resistance to INH and RMP is unlikely to occur if the patient receives adequate care.

Cases of acquired resistance are reported to be due to inappropriate or irregular drug supply or to patient behavior manifested by nonadherence to treatment, thereby favoring the selection of spontaneously mutated *Mycobacterium tuberculosis* strains¹⁹. In the present study, the patients who had a history of previous treatment at the time of diagnosis had a five times greater risk of presenting MDR-TB. This association has also been found by other authors^{5,6,10,20}. A history of abandonment of treatment was also found to lead to four times greater risk of having MDR-TB in Cabo. In addition to the frequency of abandonment of treatment in Cabo of 15% when the study began, we found a high frequency of alcohol consumption, which has been reported to be a risk factor for treatment abandonment¹.

The frequency of HIV coinfection in TB patients in Cabo was 1.6%. In Brazil, the rate of TB coinfection in patients with HIV/AIDS has been estimated to be 3-4% of cases⁹. All the patients with HIV infection reported here had TB caused by drug-susceptible

Mycobacterium. Development of drug-resistant tuberculosis was, however, associated with HIV in five of the seven European countries reporting this variable in a recent published systematic review⁵.

In conclusion, primary and secondary resistance to both INH and RMP was high in Cabo, thus suggesting that MDR strains are in circulation. This demonstrates the patchy nature of the degree of MDR and suggests that it may be very important to study regional variations. Culturing and resistance testing is recommended for all previously treated cases of TB, for cases without clinical improvement after two months of therapy and for new patients with close contacts that present MDR-TB. Therefore, the main challenge for TB control programs is to provide good laboratory support and to ensure that physicians are informed about the results from the laboratory tests. Decisions regarding adjustment of TB treatment schedules should be discussed in all cases with monoresistance to INH, including the addition of new drugs to the therapeutic schedule and using longer treatment periods. Application of molecular methods to genotype strains, with identification of mutations causing drug resistance among patients who do not respond well to treatment, could be an additional tool for avoiding delayed cure, transmission of MDR strains and mortality.

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