

ORIGINAL ARTICLE

A DECADE TREND OF MULTIDRUG RESISTANT TUBERCULOSIS IN SÃO PAULO STATE, BRAZIL

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SUMMARY

The aim of this retrospective study was to review all the notified cases of multidrug-resistant tuberculosis (MDR-TB) in *São Paulo* State (Brazil), as well as to describe and discuss the clinical, microbiological and radiologic aspects in a single reference center, within the same state, from 2000 to 2012. There were 1,097 notifications of MDR-TB in *São Paulo* State over this period, 70% affecting men aged on average 38 years (10-77). There was a significant fall in the MDR-TB mortality rate from 30% to 8% (2000-2003 versus 2009-2012). The same trend was observed in the cases studied at the reference center. The number of notified cases increased and death rate reduced from 37.5% (2000-2005) to 3.4% (2006-2012). Among the 48 drug-resistant TB cases, 17 non-tuberculous Mycobacteria were isolated in the sputum culture of nine patients, without any clinical significance. TB and fungus co-infection was diagnosed in 15% (7/48) of these cases: three with confirmed chronic pulmonary aspergillosis and four with positive serological markers for paracoccidioidomycosis. Overall, the reports show that MDR-TB diagnosis and cure rates have increased, while the mortality rate has decreased significantly in *São Paulo* State including in the studied reference center.

KEYWORDS: Multidrug resistant tuberculosis; Treatment outcome; Non-tuberculous; Mycobacteria; Lung Diseases; Fungal.

INTRODUCTION

Tuberculosis (TB) continues to be an important worldwide public health problem, affecting millions of persons per year. Although it can be treated effectively, it is still the major cause of deaths due to infectious disease in adults¹. Brazil is one of the 22 countries that concentrate 80% of the total number of TB cases, although the incidence and mortality of TB have been slowly and progressively decreasing over the last decades. However, new challenges regarding the management of the disease are increasingly important, especially the TB and human immunodeficiency virus (HIV) co-infection, which affects 12% of the TB cases around the world and 9.8% of cases in Brazil^{1,2}. More recently, there has been an increase in the percentage of multidrug-resistant TB (MDR-TB) notified in the world³.

Increased bacillary resistance is also observed in Brazil according to the data of national surveys of resistance to anti-tuberculous drugs, the first conducted between 1995 and 1997 and the second between 2007 and 2008. An increase of almost 40% in primary resistance to isoniazid (from 4.4% to 6.0%) and of 30% in resistance to combined isoniazid and rifampicin (from 1.1% to 1.4%) were observed^{4,5}. The number of multidrug-resistant tuberculosis (MDR-TB) cases in 2014 was 702, compared with 334 in 2001. There has been a consistent increase in the

number of MDR-TB cases in Brazil in the past 15 years. This increase was particularly high between 2004–2005 and 2009–2010, with 22.6% and 47.3% increments from one year to another, respectively. It is important to consider that in the latter period, the National Tuberculosis Program began to prioritize culture and sensitivity testing for all retreatment cases and in the most vulnerable populations^{1,6}.

The first report of extensively drug-resistant TB (XDR-TB) case was published in 2006 and it was characterized by resistance to rifampicin, isoniazid, fluoroquinolones and to a second-line injectable drug⁷. Over the last 10 years, XDR-TB cases have been reported in many countries, including Brazil. It is estimated that all the XDR-TB cases reported in 2012 corresponded to 9.6% of MDR-TB cases, but this is a condition still highly under diagnosed. Only 23% of the patients with a diagnosis of MDR-TB had samples tested for second-line drugs in 2011⁸⁻¹⁰, and the occurrence of XDR-TB is an indirect indicator of failure in the management of MDR-TB cases¹.

There is a lack of data about the situation of TB resistance currently available in Brazil, with widely diverse reports varying according to the region where the cases were detected¹¹⁻¹⁵. The aim of the present study was to review the notifications of all cases of MDR-TB in the state of *São Paulo*, as well as to describe and discuss the clinical and microbiological

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aspects of all notified cases at a reference center for the northeast and northwest regions of the state from 2000 to 2012.

MATERIAL AND METHODS

This is a retrospective study of MDR-TB cases notified in the state of São Paulo from 2000 to 2012, with a detailed description of the patients with drug-resistant TB seen at the reference center for MDR-TB of the University Hospital, School of Medicine of *Ribeirão Preto* (HCRP), University of São Paulo.

Information for the state of São Paulo

Inclusion criteria

We included data of TB patients infected by *Mycobacterium tuberculosis* resistant to at least rifampicin and isoniazid. These cases had been notified in the state of São Paulo, and the data were available in the system of information on special treatment of TB (SITE-TB - <http://sitetb.org/>), from 2000 to 2012. The following data were collected: age, sex, place and date of notification, and the patient outcome (death, cure, failure and abandonment of treatment). During the study period, some patients were notified more than once (failure, abandonment and/or retreatment), but only patients with a notification interval greater than 18 months, were considered for analysis in this study, as this is the shortest time for full treatment of the MDR-TB cases, whereas other cases would represent recurrence of the disease.

Exclusion criteria

Patients with TB whose bacillus did not show resistance to rifampicin and isoniazid were excluded.

Data source

The SITE-TB website (<http://sitetb.org/>) was the basic source of information.

Drug-resistant of TB cases notified at HCRP reference center

Inclusion criteria

All TB cases with diagnosis of drug resistance seen and followed up at the outpatient clinic of HCRP during the study period were included, as long as they had at least 36 months of follow-up after the diagnosis of resistant TB. The medical records of all patients were reviewed with special attention to clinical, imaging and microbiology recorded information.

The diagnosis of resistance was confirmed by culture, identification and drug-susceptibility testing for all the patients.

Sample collection and processing

The clinical specimens collected on the occasion of the diagnostic investigation of TB were processed according to the routine established by the Mycobacteria Laboratory of HCRP for these cases: direct examination after Ziehl-Neelsen staining and incubation in liquid culture medium by means of the automated system MGIT 960[®]. When the result was positive the sample was submitted again to bacilloscopy in order to confirm the AFB and detect possible false-positivity of the system and samples were cultured on solid Lowenstein-Jensen medium. The identification of *M. tuberculosis* complex was performed by polymerase chain reaction (PCR)

that amplifies a 123 pair fragment of *Mycobacterium tuberculosis*¹⁶. The *M. tuberculosis* isolates suspected of being resistant (previously treated TB patients, patients with treatment failure, health professionals and prisoners) were sent to the Central Reference Laboratory for Mycobacteria to perform the drug-susceptibility testing, as described by Palacci *et al.*¹⁷.

Non-tuberculous mycobacteria were also sent to the Central Reference Lab for species identification by a phenotypic technique and molecular typing (PCR coupled to the Restriction Fragment Length Polymorphism - RFLP, and the analysis of a 441 bp fragment from the hsp65 gene (PRA-hsp65)¹⁸.

Paracoccidioides brasiliensis and *Aspergillus* infection were diagnosed based on mycological investigation (direct observation and culture), histological and serologic methods. A counter immunoelectrophoresis test was used to detect antibodies raised to both fungi.

Exclusion criteria

Patients with *M. tuberculosis* detected, but showing drug susceptibility or a mono-resistance profile to the first-line drugs were excluded from this study.

Data source

Information was obtained from the medical records of the patients and from the notification and follow-up forms of the epidemiological surveillance of HCRP. For the analysis of the patient evolution and outcome according to the case notification, patients were divided into three periods: Period 1 (P1): 2000-2003, Period 2 (P2): 2004-2008 and Period 3 (P3): 2009-2012, for both situations: 1) those cases notified in the state of São Paulo and 2) the subgroup of patients seen at the reference center of HCRP.

Statistical analysis

A comparative analysis of the death risk and the chance of cure during different time periods (P1, P2, P3) was performed using the Pearson chi-square test for the state of São Paulo, and the two-tailed Fisher test for the patients seen at the reference center of HCRP. Based on these analyses it was possible to observe a trend regarding mortality, cure and other outcomes for both groups, São Paulo State and *Ribeirão Preto* reference center.

Ethical aspects

The study was approved by the Ethics Committee of HCRP according to the process number 8595/2011.

RESULTS

MDR-TB in the state of São Paulo

During the study period, 958 patients were notified as having MDR-TB in the state of São Paulo. For this group of patients there were 1,097 notifications, as some of them were notified more than once with an interval of more than 18 months, over a period of 13 years.

The mean age of patients was 38.3 years (range: 10-77 years) and

70% were males. Most cases were notified at outpatient clinics in the capital city of *São Paulo* (54.2%); 26.1% at outpatient clinics in the countryside areas; 17.7% at reference hospitals for drug-resistant TB and 2% at prison hospitals of *São Paulo* State.

Analysis of the course and outcome of the cases notified in the state of *São Paulo* revealed a significant increase in the number of cases notified within a little more than a decade, 266 during the period from 2000 to 2003; 362 from 2004 to 2008 and 469 from 2009 to 2012. The outcomes recorded in the notifications revealed an important fall in mortality due to MDR-TB, of 30%, 12% and, 8% respectively, when the three periods were compared. There was also a significant increase in the proportion of completed treatments and the cure of cases: 53% (2000 to 2003); 56% (2004 to 2008) and 68% (2009 to 2012) (Table 1).

There was a significant reduction in mortality due to MDR-TB in the state of *São Paulo* over the three periods analyzed when comparing Period 2 (2004-2008) with Period 1 (2000-2003), with a relative risk (RR) of 0.40 (0.29-0.56) ($p < 0.001$), and Period 3 (2009-2012) to Period 1 with RR of 0.25 (0.18-0.37) ($p < 0.001$). The same was observed regarding the chance of cure during the three periods. When Period 2 (2004-2008) was compared to Period 1 (2000-2003) RR was 1.25 (1.09-1.42) ($p = 0.001$) and when Period 3 (2009-2012) was compared to Period 1, RR was 1.29 (1.14-1.45) ($p < 0.001$).

Resistant TB in the reference outpatient clinic of HCRP

Until 2012, the reference outpatient clinic of HCRP was the first one regarding the number of cases in the countryside of the state, and the third one in number of MDR-TB notified in the state of *São Paulo* during this period. There were 43 notifications of MDR-TB and two cases of XDR-TB. In this analysis, three cases of patients with disease caused by poly-resistant *Mycobacterium tuberculosis*, including resistance to rifampicin or isoniazid plus an additional first-line drug were also included. All cases were reviewed and their characteristics are described in the present study.

At the reference center of HCRP the risk of death and the chance of cure showed a behavior similar to that observed for the state of *São Paulo*.

There was an increase, of 8 (2000 to 2003), to 15 (2004 and 2008), and to 25 (2009 to 2012) in the number of notified cases (Table 2). Regarding the outcomes, there was a mortality fall due to drug-resistant TB and an increase in the number of treatment completion and cure over these three periods: 25% (2000-2003); 66.7% (2004-2008) and 86.4% (2009-2012) (Pearson's chi-square test).

There was a significant reduction over time in the drug-resistant TB mortality among the patients seen at the reference service of HCRP. Comparison between Period 2 (2004-2008) and Period 1 (2000-2003) showed RR = 0.27 (0.66-1.07), $p = 0.131$ and comparison between Period 3 (2009-2012) and Period 1 showed RR = 0.18 (0.05-0.72), $p = 0.023$. The same behavior was observed regarding the chance of cure when Period 3 (2009-2012) was compared to Period 1 (2000-2003), with RR = 3.45 (1.61-7.40), $p = 0.003$ (two-tailed Fisher test). The chance of cure was not statistically significant when Period 2 (2004-2008) was compared to Period 1 (2000-2003), with RR 2.67 (0.95-7.48), $p = 0.089$.

Clinical, radiologic and microbiological findings in drug-resistant TB cases at HCRP

Half of the patients (24/48) seen at the HCRP center lived in the *Ribeirão Preto* region, with only four of them residing in the city of *Ribeirão Preto*. Twenty-three of the remaining 24 patients were from the northeastern region of the state of *São Paulo*, and one was from the state of *Mato Grosso* (Brazilian Midwest region). Two patients with XDR-TB were from the same region (northwestern part of *São Paulo* State), but they had no history of previous contact. The absolute majority of patients (81.2% N = 39) had a history of previous TB treatment (a minimum of one and a maximum of five previous treatments). Only three patients had confirmed HIV infection with lymphocyte T CD4+ counts lower than 100 cells/mm³. All the other TB diagnosed patients were tested and had a negative HIV serology.

Nine patients had no previous history of completed TB treatment and only three of them fulfilled the criterion for primary resistance of *M. tuberculosis*, less than 30 days of treatment when culture and drug susceptibility tests were performed. The other six patients were under regular TB treatment for four to five months when the sputum cultures

Table 1
Outcomes of MDR-TB cases notified in the state of *São Paulo* comparing three time periods

MDR-TB outcome SP State	P1: 2000-2003		P2: 2004-2008		P3: 2009-2012	
	n	%	n	%	n	%
Death due to MDR-TB*	80	30%	44	12%	36	8%
Death due to other causes	5	2%	11	3%	14	3%
Cure [#]	140	53%	238	66%	318	68%
Treatment failure	12	4%	27	8%	33	7%
Abandonment of treatment	23	9%	41	11%	64	13%
Others	6	2%	1	0	4	1%
Total	266	100%	362	100%	469	100%

*Relative risk of death due to MDR-TB: (P2 x P1) RR=0.40 (0.29-0.56); (P3 x P1) RR=0.25 (0.18-0.37) $p < 0.001$. [#]Chance of cure (P2 x P1) RR=1.25 (1.09-1.42); (P3 x P1) RR=1.29 (1.14-1.45) $p < 0.001$. Pearson's chi-square test.

Table 2
Outcomes of drug resistant TB cases at MDR-TB service of HCRP comparing three time periods

MDR-TB outcome HCRP	P1: 2000-2003		P2: 2004-2008		P3: 2009-2012	
	n	%	n	%	N	%
Death due to MDR-TB	4	50.0%	2	13.3%	2	8.0%
Cure	2	25.0%	10	66.7%	22	88.0%
Abandonment of treatment	1	12.5%	2	13.3%	0	0.0%
Others	1	12.5%	1	6.7%	1	4.0%
Total	8	100%	15	100%	25	100%

* Relative risk of death due to MDR-TB: (P2 x P1) RR=0.27 (0.66-1.07); (P3 x P1) RR=0.18 (0.05-0.72), $p < 0.05$. * Chance of cure (P2 x P1) RR=2.67 (0.95-7.48) $p = 0.089$ (not significant); (P3 x P1) RR=3.45 (1.61-7.40) $p = 0.003$ (two-tailed Fisher test).

were positive (Table 3). Until the end of 2009, the regular TB treatment in Brazil included rifampicin, isoniazid and pyrazinamide. After 2010, ethambutol was added to the scheme².

All patients had pulmonary disease and the chest X-ray or chest tomography revealed an apical opacification in 79.2% (38), bilateral opacification in 66.7% (32) and cavity images in 62.5% (30) of the notified cases in *Ribeirão Preto*.

Table 3

Demographic, clinical and radiological characterization of the 48 cases of drug resistant TB cases at the reference service of HCRP

Variables	Results
Demographic data	Mean age (min; max) 42 (21; 74) years
	Male sex 34 (70.8%)
	Alcoholism 30 (62.3%)
	Smoking 26 (54.2%)
History	HIV infection 6 (12.5%)
	Diabetes mellitus 2 (4.2%)
	Renal transplantation 1 (2.1%)
	Passage through the prison system 4 (8.4%)
	none 9 (18.8%)
Previous TB treatment	1 or 2 24 (50.0%)
	3 or 4 13 (27.1%)
	5 2 (4.1%)
Chest X-ray	Apical opacification 38 (79.2%)
	Bilateral involvement 32 (66.7%)
	Cavern 28 (58.3%)
	Pleural effusion 5 (10.4%)

Figure 1 shows the susceptibility profile of *M. tuberculosis* isolated from patients seen at HCRP. There was a high prevalence of resistance to rifampicin plus isoniazid, but various types of combined resistance to two and three drugs was also observed. We included in the analysis four

cases of polyresistance, i.e., resistance to rifampicin or isoniazid plus at least an additional first-line drug of the anti-TB scheme.

During this 13-year period, the predominant MDR-TB treatment scheme included a five-drug regimen with an injectable aminoglycoside (streptomycin), quinolones (ofloxacin or levofloxacin), ethambutol, pyrazinamide and terizidone. The most common side-effects observed with the use of the therapeutic schemes for MDR-TB were ototoxicity and vertigo induced by the aminoglycosides, arthralgia with the use of quinolones and pyrazinamide, diarrhea with the use of quinolones, optic neuritis with the use of etambutol, and depression associated with terizidone.

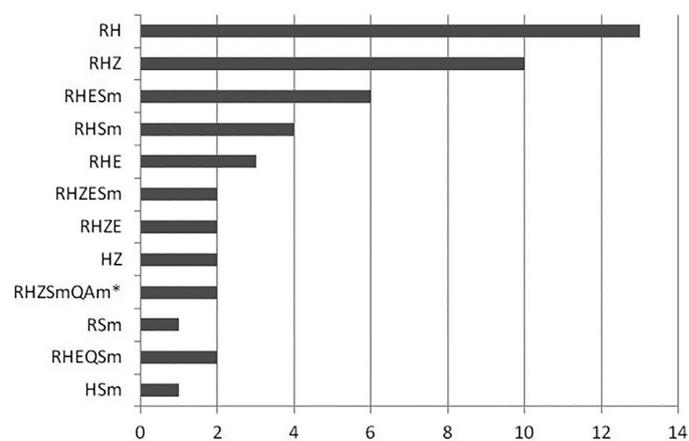


Fig. 1 - Profile of resistance to tuberculostatic drugs in the 48 cases studied at HCRP. R: rifampicin; H: isoniazide; Z: pyrazinamide; E: etambutol; Sm: streptomycin; Q: quinolone; Am: amikacin. * Two cases confirmed as TBXDR.

After the beginning of treatment, the time needed for *M. tuberculosis* culture conversion was less than six months in 35 patients (72.9%), seven to 12 months for 10 (20.8%) patients, and more than 12 months for three (6.3%) cases. In two patients there were no culture conversion, and complementary investigation confirmed XDR-TB. One of these patients died and the other was transferred for treatment to a hospital specialized in TB in the state, with a good evolution.

Another interesting finding observed during the follow-up of these

patients was the growth of 14 non-tuberculous Mycobacteria isolated from cultures of 10 patients who were notified from 2000 to 2012, at HCRP. The NTM species isolated by automated culture were: *M. avium* (1), *M. kansasii* (2), *M. fortuitum* (2), *M. chelonae* (1), *M. goodii* (1), and seven other species were not identified.

In addition, there were seven cases with TB and fungus co-infection. Three cases with pulmonary chronic aspergillosis had been confirmed by *Aspergillus fumigatus* culture and the detection of specific antibodies. There were also four patients with resistant TB who had high titers of antibodies against *Paracoccidioides brasiliensis* detected by counter immunoelectrophoresis.

DISCUSSION

Over the last two decades, the worldwide incidence of TB reached a peak in 2004, showing a slow decline thereafter in various regions of the globe. The goal established by WHO and followed by Brazil was to reduce the incidence of TB by 50% between 1995 and 2015 and has been consistently reached in terms of the reduction of number of cases of the disease^{1,2,3}. However, the results for TB associated with HIV, MDR-TB and XDR-TB are going in the opposite direction^{1,3,6}. The proportion of resistant cases in Brazil is still lower than what is observed in many regions of the world, with a similar epidemiological and social situation. However, due to a greater access to test drug susceptibility, an increase in the number of notified drug-resistant TB in the last decade^{6,7} has been observed worldwide. Our findings go in the same direction, showing that the number of MDR-TB notified in the state of São Paulo also increased in the past decade. Along with the increase in the drug-resistance, another reasonable explanation for these findings is the increased capacity to detect drug-resistant TB cases in the state¹⁷. The demographic characteristics of the cases in São Paulo State and for the reference center of HCRP were similar to those reported in other Brazilian studies regarding age and sex^{11,12,14}.

Despite the increase in the notified cases, the analysis of the outcomes of MDR-TB in the state of São Paulo revealed an expressive reduction of mortality and a significant increase in the cure rate and completed treatments among the MDR-TB cases. On the other extreme, the chance of cure was greater in the last two periods compared to the first one. Many factors may be involved in this improvement, but the creation of specialized services and training of multiprofessional teams for the diagnosis, follow-up and treatment are thought to be related to the better results observed along these 13 years. Porter and Teisberg described a positive correlation between the concentration of difficult and less common cases at few super-specialized services and the experience of the teams, resulting in better outcomes¹⁹.

Despite the increase in the cure rate and the reduction of deaths, it is interesting to note that the rate of abandonment of treatment has remained practically stable over the last 40 years. The mean rate of abandonment of TB treatment was 12% in the early 1980's, 14% in the 1990 decade, and even higher in the Brazilian capital cities^{20,21}. In this study, the abandonment of treatment for MDR-TB cases in the state of São Paulo, from 2000-2012, revealed a mean rate of 11.6%.

In an analysis of the first Brazilian surveillance from 1995 to 1998 data of the main MDR-TB service in the state of São Paulo, Fiuza de

Melo *et al.* observed that about 50% of the patients with TB and post-primary resistance had a history of previous treatment or abandonment of treatment²¹. In *Ribeirão Preto*, 39/48 (81.2%) of the notified patients had been submitted to a previous treatment for TB. Several studies have supported the relationship between the previous use of anti-TB drugs, especially when taken in an irregular manner, and the development of resistance¹¹⁻¹⁵.

A more detailed analysis of the 48 cases notified at the reference service of HCRP revealed the occurrence of bilateral opacification and the presence of apical caverns in chest imaging exams as the most frequent findings in this series, similar to the findings of Siqueira *et al.* who reported the presence of caverns in 36 (72%) of their 50 cases, in addition to bilateral involvement in 38 (76%) of the 50 cases studied in *Rio de Janeiro*¹⁴.

Most of the patients studied at HCRP (27.1%) were found to be resistant only to rifampicin and isoniazid (13/48), in disagreement with the cases of drug-resistant TB notified in *Rio de Janeiro* where resistance to rifampicin, isoniazid and two more first-line drugs was the most frequent drug susceptibility profile¹⁴.

Detected resistance to isoniazid is reported to be predictive to streptomycin resistance. In this study, 18 (38.3%) isolates out of 47, which were resistant to isoniazid showed a concomitant resistance to streptomycin. This association may occur in up to 50% of the times. However, as the clinical reliability of this test is not very high²², even if the drug susceptibility test indicates a susceptible isolate, amikacin, capreomycin and kanamycin should be considered as the injectable drugs to be used in the MDR-TB scheme. In Brazil, streptomycin is the first injectable drug of choice, except if the patient has been treated with this drug before¹⁰. All patients with history of previous use of streptomycin, in the *Ribeirão Preto* reference center, were treated with amikacin.

In the HCRP, there were two (9.1%) out of 44 patients with confirmed XDR-TB. One died and the other evolved to cure after a long treatment in a specialized hospital for drug-resistant TB in the State. Although it is still a rare occurrence, 77 countries worldwide had reported at least one case by the end of 2011. Information from countries with reliable data suggests that almost 10% of MDR TB cases worldwide are also XDR TB^{3,22,23}.

For the majority of patients (73%), negative culture results were observed six months after the beginning of the drug-resistant treatment. In contrast, negative culture results were observed between 15 and 60 days after the beginning of the standard treatment² for patients with susceptible TB. The culture continued to be positive for up to 12 months among 27% (13/48) of cases, showing the need to extend the use of the aminoglycoside or the total treatment time (from 18 to 24 months). In two of these cases negativity of cultures did not take place since they were XDR-TB cases.

The concomitant presence of positive cultures for non-tuberculous Mycobacteria in patients under drug-resistant TB treatment was not considered as a clinically relevant finding. These patients were not treated and it had no impact in the patients' outcomes. Pulmonary TB sequelae and residual fibrosis may be an appropriate environment for non-tuberculous Mycobacteria colonization²⁴.

The occurrence of active *Aspergillus* infection associated with active or sequelae TB residual cavern is a well described condition²⁵, even though cases of aspergillosis and drug-resistant TB^{26,27} are scarce. TB and paracoccidioidomycosis (PCM) co-infection is very common in South America, especially in the northeastern region of the *São Paulo* State, which is an endemic area. Around 8.3% of PCM cases used to be diagnosed associated with tuberculosis in high endemic areas²⁸.²⁹ However, there is only one published report of concomitant PCM and drug-resistant TB in a patient with acquired immunodeficiency syndrome and hepatitis C, from Peru³⁰. These fungi and drug-resistant TB association needs to be considered in endemic areas, whenever TB shows microbiological improvement (AFB sputum smear and negative culture) but there are persistent pulmonary symptoms.

Despite compiling important data about drug-resistant TB, this retrospective study has limitations because it deals with secondary data from a national surveillance databank as well as with patients' records. Along the 13-year study period, many significant changes happened in the country, in the population and in the health services (offer/demand and access) and in the drug-resistant TB treatment, which probably interfered in the observed outcomes.

CONCLUSION

In the studied period there was a clear trend towards the increase of drug-resistant TB diagnosis and cure rates, while a significant decrease in mortality in *São Paulo* State was observed.

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AUTHORS CONTRIBUTIONS

VRB: conceptualized, designed and wrote the paper; FGP, MJM: reviewed all the cases, created the data bank and prepared the tables and figures related to *Ribeirão Preto* cases; MA, MLVO: collected, reviewed and structured all the data from SITE-TB related to *São Paulo* State. All the authors reviewed and approved the final version of this paper.

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