**ABSTRACT**

We report on *M. tuberculosis* detection among inmates in detention facilities in the region of São José do Rio Preto, São Paulo State, Brazil. From 2003 to 2006, 1,070 inmates from three prisons with suspicion of tuberculosis were evaluated. *Mycobacterium* sp. infection was tested using acid-fast bacilli and/or culture. Statistical analysis was performed using EPI INFO and BioEstat. There were no significant differences in the frequencies of positive results between the three correctional facilities. Tuberculosis infection was identified in 6.9% of all prisoners and the drug susceptibility profile showed that 4.2% of the prisoners had isoniazid-resistant isolates while 6.2% had rifampicin-resistant bacteria. Isoniazid and rifampicin resistant isolates were obtained from inmates with positive results by acid-fast bacilli, pointing to the possibility of intra-institutional transmission. These data will be useful for future studies to establish the level of tuberculosis in the São Paulo state penitentiary system and the drug resistance profile.


**RESUMO**

Detecção do *Mycobacterium tuberculosis* no sistema penitenciário

Descrevemos retrospectivamente a detecção de *Mycobacterium tuberculosis* (MT) e o seu perfil de susceptibilidade entre detentos de três presídios da região de São José do Rio Preto, São Paulo, Brazil.
Entre 2003 e 2006 foram avaliados 1.070 detentos com suspeita de tuberculose (TB). A positividade do *Mycobacterium* sp. foi avaliada pela baciloscopia e cultura. A análise estatística foi realizada utilizando o Epi Info e BioEstat. Não houve diferença significante nos resultados positivos entre as três unidades penitenciárias. Foi encontrado um percentual de 6,9% de positividade para MT e o perfil de sensibilidade mostrou que 4,2% dos detentos apresentaram isolados resistentes a isoniazida, enquanto 6,2% foram resistentes à rifampicina. Todos os isolados resistentes foram obtidos de presos bacilíferos, apontando para a possibilidade de transmissão intra-institucional. Nossos dados abrem portas para o entendimento da magnitude da tuberculose e o perfil de resistência às drogas do MT no sistema penitenciário do Estado de São Paulo.


INTRODUCTION

Tuberculosis (TB) is a significant health problem in penitentiary systems throughout the world. Prisons are considered important environments for TB transmission due to the characteristic airborne dissemination of *Mycobacterium tuberculosis* (5, 23). Indeed, prison inmates are at a higher risk of infection due to the favorable conditions that include overcrowding, low air circulation, poor hygiene and sanitary conditions, nutritional deficiency, high-risk behavior (e.g. alcohol abuse, illicit drug consumption), and contact with other infectious diseases (14, 16, 23).

From the epidemiological point of view, this situation is a threat to TB control as contamination of the relatives of inmates and other visitors, and prisoners released back into the community increases the probability that infected individuals will enter into contact with the general population (2, 4, 9). Another important issue is related to the fact that this is a mobile population, moving from one prison to another and eventually reentering the community. *M. tuberculosis* positive prisoners, whether treated or not, can live in two or even more prisons where they establish contact with other prisoners and prison staff (15, 28).

Data of the Brazilian Ministry of Justice show that 496,251 individuals were incarcerated in prisons in 2010, with an incidence of 259/100,000 inhabitants. In 2010, in the State of São Paulo, there were 170,916 inmates with an incidence of 413/100,000 inhabitants (http://portal.mj.gov.br/data/pages) (18). According to a publication of the São Paulo State Department of Health, the incidence of tuberculosis in the prison population in the state in 2006 was 800 cases per 100,000 inmates, a rate several times higher than that of 43 per 100,000 in the general population (29).

The distinctive features of prisons and of prisoners require specific measures to control tuberculosis that differ from those used for the general population (9). Additionally, as each detention facility may present a unique epidemiological and sociodemographic profile depending upon the area and the population, as has already been described for the city of Rio de Janeiro (25), knowledge of what makes control different in each setting is crucial to establish effective individual TB control programs. This may be even more important in countries with high prevalence of TB where prevention is imperative (9).
Appropriate TB treatment and prevention against transmission depend on rapid and reliable laboratory results. It is vital that laboratories detect *M. tuberculosis* in specimens and identify resistance to specific antibiotics (22).

The aim of this work is to report on *M. tuberculosis* detection among inmates in detention facilities in the region de São José do Rio Preto, São Paulo State, Brazil and on the susceptibility of the bacteria to antimycobacterial drugs in this setting.

**MATERIAL AND METHODS**

This is a cross-sectional descriptive retrospective study. Sao Jose do Rio Preto is located in the northwestern region of Sao Paulo State, bordering the states of Minas Gerais and Mato Grosso do Sul. The 15th Regional Department of Health (XV DRS) in São José do Rio Preto covers 101 municipalities belonging to the XXIX (66 municipalities) and XXX (35 municipalities) Epidemiological Surveillance Groups with a population of 1,480,128 inhabitants (19). The area covered by the XV DRS includes four prisons of the State Penitentiary Administration Secretariat (SAP) (26).

The Adolfo Lutz Institute in São José do Rio Preto (IAL-SJRP) is a referral laboratory for all towns covered by the XV DRS. All samples collected for clinical diagnosis of tuberculosis in the region are forwarded to this laboratory, including those from the aforementioned correctional centers (three centers for males and one for females). The capacity of these four units is 2,818 prisoners, however in 2010 the prison population reached 3,863 thus exceeding the capacity by 37% (26). For this study, the women’s prison was excluded as no samples were sent to the laboratory throughout the entire study period.

Samples collected from January 2003 to December 2006 were evaluated. Prisons A and C are closed pavilions and in 2006, when both participated in a rebellion involving São Paulo state prisons, they had mean populations of 2,105 and 1,324 inmates with overcrowding of 46.4% and 25.2% individuals, respectively (10). Prison B is a semi-open facility with a mean population of 774 prisoners in 2006 (Data obtained in a memorandum 131/2009-GC/CROESTE from the Penitentiary Administration Secretariat, West Region Prison Units, Coordinator Office, Presidente Venceslau, São Paulo).

Although the study was retrospective, data were obtained from laboratory records as all samples obtained from patients with suspected tuberculosis in prisons in the region during the period were sent and analyzed by the IAL-SJRP.

Sputum samples were obtained by medical staff at each prison whenever there was suspicion of active TB or the possibility of contact with infected prisoners accordingly to standard procedures of the Brazilian Ministry of Health. Suspicion was aroused whenever an inmate presented productive cough for at least two weeks, had previous history of the disease and/or history of cell sharing with a TB carrier.
TB was diagnosed by a positive smear for acid-fast bacilli and/or by a culture of *Mycobacterium sp.*, both tests are routinely performed (13) by the staff of the regional referral center.

The smears were prepared according to the procedure recommended by the Ministry of Health. The investigation of acid-fast bacillus (AFB) after Ziehl-Neelsen staining was performed by semiquantitative counting with classification by crosses: negative (AFB was not found in 100 fields), rare (1 to 9 AFB in 100 fields), 1+ (less than 1 AFB/field in 100 fields), 2+ (1-10 AFB/field in 50 fields) and 3+ (more than 10 AFB/field in 20 fields), as recommended by the WHO (20).

Cultures in Ogawa Kudoh (OK) medium were decontaminated using a swab impregnated with sputum sample. The impregnated swab was placed in a sterile tube containing 3 mL of 4% NaOH for two minutes and then seeded using circular movements in OK medium. The culture was incubated at 37°C for up to 60 days (20) with weekly readings; at the end of this period, if the culture did not exhibit growth, it was classified as negative. The time to identify positive samples or the presence of contamination was recorded.

Positive cultures were evaluated by gross appearance (morphology and color of the colonies) and microscopy after Ziehl-Neelsen staining. Additionally, the *M. tuberculosis* isolates were sent to the Central Laboratory of the Adolfo Lutz Institute in São Paulo (IAL-São Paulo) to investigate the drug susceptibility profile for four drugs, isoniazid (INH), rifampicin (RMP), streptomycin (SM) and ethambutol (ETH), using the BACTEC MGIT 960 system (Becton & Dickinson). The pyrazinamide test was performed using the pyrazinamidase method (8). Identification of the *Mycobacterium* sp. species was achieved using the PRA-hsp65 molecular method (7).

Data storage and statistical analysis was performed using the Epi-Info (version 3.5.1) and BioEstat (version 5.0) computer programs. This study was carried out according to a protocol approved by the Research Ethics Board of the Medicine School in São José do Rio Preto (CEP-FAMERP nº 391/2007).

RESULTS

The numbers of inmates in Prisons A, B and C in the years 2003 to 2006 are presented in Table 1.

From 2003 to 2006, samples of 1,070 (1,070/15,792 - 6.8%) inmates with potential TB (according to the Brazilian Ministry of Health criteria) of three correctional centers were evaluated by smear or culture for *Mycobacterium* sp (Table 2).

A total of 1,070 inmates (538 - 50.2% from Prison A, 266 - 24.9% from prison B, and 266 - 24.9% from Prison C) were tested. The distribution of positive smears and cultures and the identification of the strain and drug susceptibility testing in Prisons A, B and, C are shown in Table 3.
Table 1. Mean annual number of inmates from 2003 to 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Prison A</th>
<th>Prison B</th>
<th>Prison C</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>2031</td>
<td>781</td>
<td>969</td>
<td>3781</td>
</tr>
<tr>
<td>2004</td>
<td>1770</td>
<td>674</td>
<td>1049</td>
<td>3493</td>
</tr>
<tr>
<td>2005</td>
<td>2442</td>
<td>674</td>
<td>1216</td>
<td>4332</td>
</tr>
<tr>
<td>2006</td>
<td>2105</td>
<td>757</td>
<td>1324</td>
<td>4186</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8348</td>
<td>2886</td>
<td>4558</td>
<td>15792</td>
</tr>
</tbody>
</table>

Source: memorandum 131/2009-GC/CROESTE from the Penitentiary Administration Secretariat, West Region Prison Units, Coordinator Office, Presidente Venceslau, São Paulo State

Table 2. Tests for *Mycobacterium* sp. of prisoners in three prisons in 2003 to 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Sputum collected n (%)</th>
<th>Positive smear and/or culture n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>261 (24.4)</td>
<td>7 (2.7)</td>
</tr>
<tr>
<td>2004</td>
<td>127 (11.9)</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>2005</td>
<td>429 (40.1)</td>
<td>24 (5.6)</td>
</tr>
<tr>
<td>2006</td>
<td>253 (23.6)</td>
<td>17 (6.7)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>1070 (100)</td>
<td>52 (4.8)</td>
</tr>
</tbody>
</table>

Table 3. Sputum positive smears/cultures, identification of mycobacterial strains and drug susceptibility testing of prisoners held in the three prisons from 2003 to 2006

<table>
<thead>
<tr>
<th>Sputum smears and cultures</th>
<th>N</th>
<th>Prison A n (%)</th>
<th>Prison B n (%)</th>
<th>Prison C n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive smear and positive culture</td>
<td>35</td>
<td>18 (34.6)</td>
<td>7 (13.5)</td>
<td>10 (28.6)</td>
</tr>
<tr>
<td>Negative smear and positive culture</td>
<td>17</td>
<td>8 (34.6)</td>
<td>4 (7.7)</td>
<td>5 (9.6)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>52</td>
<td>26 (50)</td>
<td>11 (21.2)</td>
<td>15 (28.8)</td>
</tr>
</tbody>
</table>

Identification

<table>
<thead>
<tr>
<th>Identification</th>
<th>N</th>
<th>Prison A n (%)</th>
<th>Prison B n (%)</th>
<th>Prison C n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>49</td>
<td>24 (46.2)</td>
<td>10 (19.2)</td>
<td>15 (28.8)</td>
</tr>
<tr>
<td><em>Mycobacterium fortuitum</em></td>
<td>1</td>
<td>-</td>
<td>1 (1.9)</td>
<td>-</td>
</tr>
<tr>
<td>NTM*</td>
<td>2</td>
<td>2 (3.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>52</td>
<td>26 (50)</td>
<td>11 (21.2)</td>
<td>15 (28.8)</td>
</tr>
</tbody>
</table>

Drug susceptibility

<table>
<thead>
<tr>
<th>Drug susceptibility</th>
<th>N</th>
<th>Prison A n (%)</th>
<th>Prison B n (%)</th>
<th>Prison C n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>43</td>
<td>21 (43.7)</td>
<td>8 (16.7)</td>
<td>14 (29.2)</td>
</tr>
<tr>
<td>Resistant to isoniazid</td>
<td>2</td>
<td>-</td>
<td>2 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>Resistant to rifampicin</td>
<td>3</td>
<td>3 (6.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>48</td>
<td>3 (6.2)</td>
<td>2 (4.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

NTM = Nontuberculous mycobacteria – unidentified species;

There was no significant difference in the frequencies of positive results between the three penitentiary units [Prison A (26/538) 4.8%; Prison B (11/266) 4.1% and Prison C (15/266) 5.6%; p-value = 0.45].

*Mycobacterium tuberculosis* complex isolates were identified from the sputum of 94.2% of the prisoners tested. Only one inmate (1.9%) had a positive
culture for *Mycobacterium fortuitum* whereas two others (3.8%) had positive cultures for nontuberculous mycobacteria (NTM) the species of which remain unidentified.

The sensitivities of strains from 48 individuals were tested. The *M. tuberculosis* drug susceptibility profile showed that the isolates of two prisoners (2/48 - 4.2%) were isoniazid-resistant (Prison B) while another three inmates (3/48 - 6.2%) had rifampicin-resistant bacteria (Prison A). All resistant bacteria were isolated from individuals tested positive by the acid-fast bacillus test and no multi-drug resistant (MDR) strain was identified.

Both tests, sputum smears and cultures, were performed for only 70.5% (754/1,070) of suspected cases. Thirty-five positive smears had also positive cultures, while 17 negative smears turned out to be positive cultures, increasing the number of *Mycobacterium* sp. positive individuals by 17 (2.4%).

On the other hand, 700 negative smears had also negative cultures (McNemar’s test; p-value = 0.0007; Fisher Exact test; P-value < 0.0001) giving a total positivity among suspected cases of 6.9%.

**DISCUSSION**

Undoubtedly, the persistency of TB among prisoners represents a threat to the control of this serious public health issue. Previous studies have evaluated this situation in Brazil, mainly in the Southeastern region of the country (1, 21, 24, 25, 29) and in other countries (2, 6, 11, 30, 31). To our knowledge, this is the first survey of TB prevalence in prison units in the northwestern region of São Paulo State.

Overall, our study showed similar figures for TB diagnosis by both positive smears (4.5%) and cultures (2.4%) compared to a one-year prospective study conducted in a population of inmates from nine prisons in the western sector of São Paulo city (1). However, another Brazilian study showed higher numbers in three Rio de Janeiro city facilities, reporting 32.4% confirmed laboratory cases among prisoners suspected of having TB after X-ray examinations (25). Similarly, Oliveira & Cardoso (21) reported higher smear positivity (70.3%) in an 8-year study (1993-2000) in Campinas city, SP (366 Km from SJRP). On the other hand, these study designs differ in many aspects making comparisons very difficult.

Based on the *Mycobacterium tuberculosis* detection described herein, our data differ from previous studies (1, 21, 25) carried out in other Brazilian regions. In respect to the study conducted in the western region of São Paulo city (1), these differences may be because the authors included all prisoners, independently of whether they had clinical indication of infection; they found 0.8% positive results for smears and 5.3% for cultures. In relation to the Rio de Janeiro study, again different to the findings of this study, the authors used X-rays of all prisoners to detect pulmonary TB. In this case the difference was probably due to the fact that lung lesions are not synonymous of TB. The X-Ray examination is occasionally
useful for defining uncertain findings, however this technique has not been shown to have a conclusive impact on patient management (17). Regarding the results from Campinas city, the authors detected higher infection levels than ours when they evaluated a database with all notified TB cases.

Nontuberculous mycobacteria were previously found in a high percentage of isolates (33 out of 54 strains) by Abrahão et al. (1) with a predominance of \textit{M. fortuitum} and a frequency of 7.4\% of unidentified species, which can be explained by the investigative design of this study that included all prisoners independently of whether they had symptoms of TB or not. Interestingly, all bacteria were isolated from inmates who had been in contact with soil and underground water during attempts to escape. These findings show that this group of microorganisms may be important in this population and their evaluation should be considered in future prison-based studies as mycobacteriosis caused by these species may respond differently to TB-directed treatment (1, 3, 27).

The antimycobacterial susceptibility profile in the current study showed single drug resistance only, which differs from the 2.5\% and 14.3\% of multidrug resistance (MDR) detected in the cities of Rio de Janeiro (25) and São Paulo (1), respectively and from several international publications (2, 11, 12, 31). However, our data are comparable to the profile of resistance for isoniazid and rifampicin reported in the city of Rio de Janeiro (25). It is worth to mention that the isoniazid and rifampicin resistant strains were only obtained with samples from prisons A and B and all from inmates who were positive in the acid-fast bacillus test, which suggests local transmission.

In this study, increases in the number of cases of TB were chiefly observed in 2005 and 2006 coinciding with the overcrowding in prisons and may be explained by the intense investigations during this period.

CONCLUSION

The observed progressive increase in the percentage of TB in prisoners, in the northwestern region of São Paulo state throughout this 4-year retrospective study highlights the need for TB control measures in this penitentiary population.

The \textit{M. tuberculosis} drug susceptibility profile showed that 4.2\% of the isolates were isoniazid-resistant and 6.2\% of infected prisoners had rifampicin-resistant bacteria. All resistant bacteria were isolated from individuals tested positive by the acid-fast bacillus test.

Our data will be useful for future studies on clinical evaluation and other diagnostic approaches to establish the level of TB infections and the status of \textit{M. tuberculosis} drug resistance in São Paulo state prisons as well as to encourage the inclusion of rapid molecular diagnostic tests for TB. This is an urgent necessity with the announcement of 44 new prison units in São Paulo state.
ACKNOWLEDGMENTS

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PRÓXIMOS EVENTOS NA ÁREA DE PATOLOGIA TROPICAL E SAÚDE PÚBLICA
MEETINGS TO BE HELD ON THE AREA OF TROPICAL PATHOLOGY AND PUBLIC HEALTH

XXVI Congresso Brasileiro de Microbiologia, 02 a 06 de outubro de 2011, Foz do Iguaçu, Paraná. Informações: http://www.sbmicrobiologia.org.br/26cbm

27a Reunião de Pesquisa Aplicada em doença de Chagas e 15a Reunião de Pesquisa Aplicada em Leishmaniose, Uberaba, MG, 26 a 28 de outubro de 2011.


60th Annual meeting of the American Society of Tropical Medicine and Hygiene, Philadelphia, USA, 4rd to 8th December, 2011. Information: www.astmh.org/meetings

The ASEAN Congress of Tropical Medicine and Parasitology, de 15 a 17 de maio de 2012, Manila/Filipinas. Informações: Dr. Lydia R. Leonardo. Chair, Organizing Committee of Fifth ACTMP. email address: 5thactmp@gmail.com

64ª. Reunião Anual SBPC, de 22 a 27 de julho de 2012, São Luiz-MA. Informações: http://www.sbpcnet.org.br/saoluis/home/


XXI Congresso Latino-Americano de Microbiologia - CLAM 2012, de 28/10 a 01/11 de 2012, Santos-SP. Informações: http://www.sbmicrobiologia.org.br/Latino/