

## Molecular Genotyping and Epidemiology of *Mycobacterium tuberculosis* Isolates Obtained from Inmates of Correctional Institutions of Campinas, Southeast Brazil

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The objective of this study was to investigate the possible transmission of tuberculosis among 39 inmates with positive *Mycobacterium tuberculosis* smears in four correctional institutions located in Campinas City, SP, Brazil over a 19-month period. Fifty-one *M. tuberculosis* isolates from these inmates were characterized according to the number of IS6110 insertion elements present in their genomic DNA. The number of insertion elements in *M. tuberculosis* isolates varied from two to twelve. The dendrogram of similarity resulted in the grouping the isolates in six main clusters. These results, associated to epidemiological data, suggested the transmission of tuberculosis among inmates of the same and different institutions inmates. Univariate analysis of epidemiological data (total delay for beginning of treatment, previous treatment, and HIV status) and clustering occurrence showed that only "previous treatment" (OR = 7.65, p = 0.032) was associated with the possible transmission of tuberculosis in the studied prisons.

**Key-Words:** *Mycobacterium tuberculosis*, transmission, molecular genotyping, Campinas, SP, Brazil.

Tuberculosis (TB) became a major public health problem during the Industrial Revolution, when city overcrowding and very poor living conditions created the ideal circumstances for the spread of diseases. Although several public health measures and effective drug treatments have reduced the incidence of TB, about one-third of the world's population is still infected [1], with eight million new cases and two million fatalities per year [2,3].

In the last decades, the trend of incidence of TB in developed and underdeveloped countries has risen due to the deterioration of public healthcare facilities, poverty, and migration. This resurgence, including multidrug-resistant *Mycobacterium tuberculosis* strains has also occurred among high-risk population such as the HIV-infected citizens [3]. Brazil is badly affected, with large urban centers like São Paulo City with TB incidence rates as high as 45.9/100,000 inhabitants [4].

Prison populations are considered at high risk of TB infection due to their socio-economic characteristics and prison living conditions [5]. A study of 1,052 inmates in nine São Paulo municipality prisons confirmed the high prevalence rate of inmates with active TB (2,065 per 100,000 inmates). This prevalence is around 70 as high as that found among the general Brazilian population [6]. Additionally, the prison population has increased dramatically in Brazil in the last years, making correctional institutions ideal places for person-to-person transmission of infectious diseases, including TB and

human immunodeficiency virus (HIV), either alone or associated [7]. Besides the inmates' highest risk of contamination by *M. tuberculosis*, prison staff and visitors are also at risk. The community living in contact with at-risk people and ex-prisoners are also considered as being part of the high-risk population. These data indicate that urgent measures are needed to not only detect and treat TB-harboring inmates, but also trace the transmission routes in the community and in prisons [8]. The control of TB depends mainly on its rapid and accurate diagnosis, its effective treatment, and thorough contact tracing [9].

Before the introduction of molecular biology techniques in the diagnosis of *Mycobacterium* infection, direct microscopy using Ziehl-Nielsen smear of early-morning sputum was the only rapid diagnosis method. However, a positive smear requires the presence of about 10<sup>4</sup> acid-fast bacilli (AFB) per milliliter of sputum [9], which makes the diagnostic dubious at times.

To identify the TB route transmission, molecular biology tools such as polymerase chain reaction (PCR) [10], restriction fragment length polymorphism (RFLP) [11,12], and nucleic acid amplification (NAA) [13] have been used.

To the best of our knowledge, no molecular characterization of *M. tuberculosis* isolates from Brazilian male inmates has been carried out to date.

The objective of this study was to characterize TB isolates that were obtained from male inmates of different correctional institutions located in Campinas City, Southeast Brazil, and confirm TB transmission among same-cell and different institution inmates. We also studied the status of age, HIV infection, history of previous TB treatment, and treatment delays with disease transmission.

### Material and Methods

#### Study Setting and Patient Population

This retrospective study was conducted at four different correctional institutions located in Campinas City,

Received on 15 August 2008; revised 23 November 2008.

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The Brazilian Journal of Infectious Diseases 2008;12(6):487-493.  
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Southeast Brazil, from February 1997 to September 1998. Sputum examination of inmates with clinical signs of pulmonary TB and *M. tuberculosis* strain isolation and characterization were performed by Adolfo Lutz Institute, São Paulo, the national referral institute for infectious disease detection. Although a specific TB detection routine is not adopted in the correctional system in the Campinas region, all patients with clinical signs of TB confirmed by positive *M. tuberculosis* culture during the study period were included in this study. A total of 51 *M. tuberculosis* isolates from 39 inmates were evaluated. From some patients, more than one strain was isolated (Table 1). All strains had the insertion element IS6110 detected by polymerase chain reaction as described below.

This study was approved by the ethical committee of the Medical School of the State University of Campinas (UNICAMP).

**Table 1.** Strains isolated from the same inmate.

Patient/Strain	Date of strain isolation
14/19	03.24.97
14/20	08.07.97
14/21	11.18.97
14/22	01.12.98
14/23	07.02.98
20/30	11.18.97
20/31	12.03.97
18/27	09.16.97
18/28	10.24.97
12/16	08.28.97
12/17	09.08.97
29/40	04.07.98
29/41	05.28.98
11/13	07.04.97
11/14	09.04.97
11/15	09.08.97
8/8	06.27.97
8/9	07.23.97
8/10	08.28.97

#### Epidemiological Characteristics

Epidemiological characteristics such as age, total treatment delay (time between symptom onset and the beginning of treatment), history of previous TB treatment, and HIV infection were obtained directly from the inmate TB Notification Index Cards and Criminal Records.

#### Molecular Study

In this study, the insertion element IS6110 of *M. tuberculosis* strains [14,15] was used as a molecular marker for fingerprinting analysis throughout RFLP, as described below, and characterize the TB isolates that were obtained from inmates of different correctional institutions.

#### Bacterial DNA Extraction

Whole genomic DNA of *M. tuberculosis* was purified as previously described [16]. Briefly, *M. tuberculosis* colonies grown on Lowenstein-Jensen medium (LJ) were transferred to a micro centrifuge tube (2 mL) containing 1.5 mL of TE buffer (Sambrook et al. 1989) and heat-inactivated (80°C, 1 h). After spinning (12,000 g, 5 min) 50 µL of lysozyme (10 mg/mL) was added in and the tube was kept at 37 °C for 6 h. Next, 75 µL of 10% SDS/10 mg/mL proteinase K was added in and the suspension was vortexed. The tube was heated at 65°C for 10 min and 100 µL of 5 M NaCl was added in. After mixing, 100 µL of CTAB (1 g/10 mL)/NaCl (0.41 g/10 mL) was added to the tube under agitation and heated (65 °C, 10 min). DNA was extracted from the suspension using chloroform:isoamyl alcohol (24:1), precipitated with isopropyl alcohol, vacuum dried, suspended in 50 µL of TE buffer and freeze stored.

#### IS6110 Probe

Primers IS1(5'-TGGGTCCGCCAGGCCAGGTC-3') and IS3 (5'-GTAGGCGTCGGTGACAAAGGC-3') were synthesized using the IS6110 DNA sequence [14] and used to PCR amplify a 665pb DNA fragment located between bases 211 and 876 of this insertion element of a *M. tuberculosis* strain donated by Instituto Adolfo Lutz de São Paulo. The reactions were performed in a DNA thermal cycler (Perkin Elmer 480). The genomic DNA was denatured by heating at 94°C for 10 min, followed by 25 cycles at 94°C, 1 min; 63°C, 1 min; 72°C, 2 min. After DNA polymerization, the PCR product was purified using the Wizard DNA clean-up System (Promega), sequenced using the automated MegaBase sequencer (Amersham Biotech), and radioactively labeled [(α-<sup>32</sup>P)dCTP] using Oligolabelling Kit (Pharmacia LKB Biotechnology).

#### RFLP of *M. tuberculosis* Isolates Using the IS6110 Probe

Whole genomic *M. tuberculosis* DNA purified as previously described [16] was digested with restriction enzyme PstI (a enzyme that does not cut inside IS6110 element) and run in a 0.8% submersed agarose gel electrophoresis system. DNA transfer to nitrocellulose membranes and hybridization with the IS6110 probe under high stringency conditions were accomplished as described by Sambrook et al. [17].

#### Statistical Analysis

EPI-INFO 6.0 software [18] was used for statistical analysis of epidemiological data. Genomic diversity and similarity dendrograms were calculated by the UPGMA method [20] using the POPGENE software (Version 1.31) [19]. For the construction of the dendrogram, the presence of a given band (pattern) in a data matrix was coded as 1 and its absence, as 0.

#### Results

The 39 male inmates studied were aged between 21 and 63, mean age of 34 (standard deviation of 10.8 years). Thirteen (33.3%) patients did not have TB notification index card (Tables 2 and 3).

**Table 2.** Criminal Record Sector epidemiological data of inmates.

<b>Total treatment delay (weeks)</b>			
0 - 4	9	42.9	42.9
5 - 8	5	23.8	66.7
9 or more	7	33.3	100
Total	21	100	
Unknown	18		
<b>Previous treatment</b>			
New case	27	77.1	77.1
Retreatment	8	22.9	100
Total	35	100.0	
Unknown	4		
<b>HIV status</b>			
Positive	14	48.3	48.3
Negative	15	51.7	100
Total	29	100.0	
Unknown	10		

**Table 3.** Coincident incarceration periods.

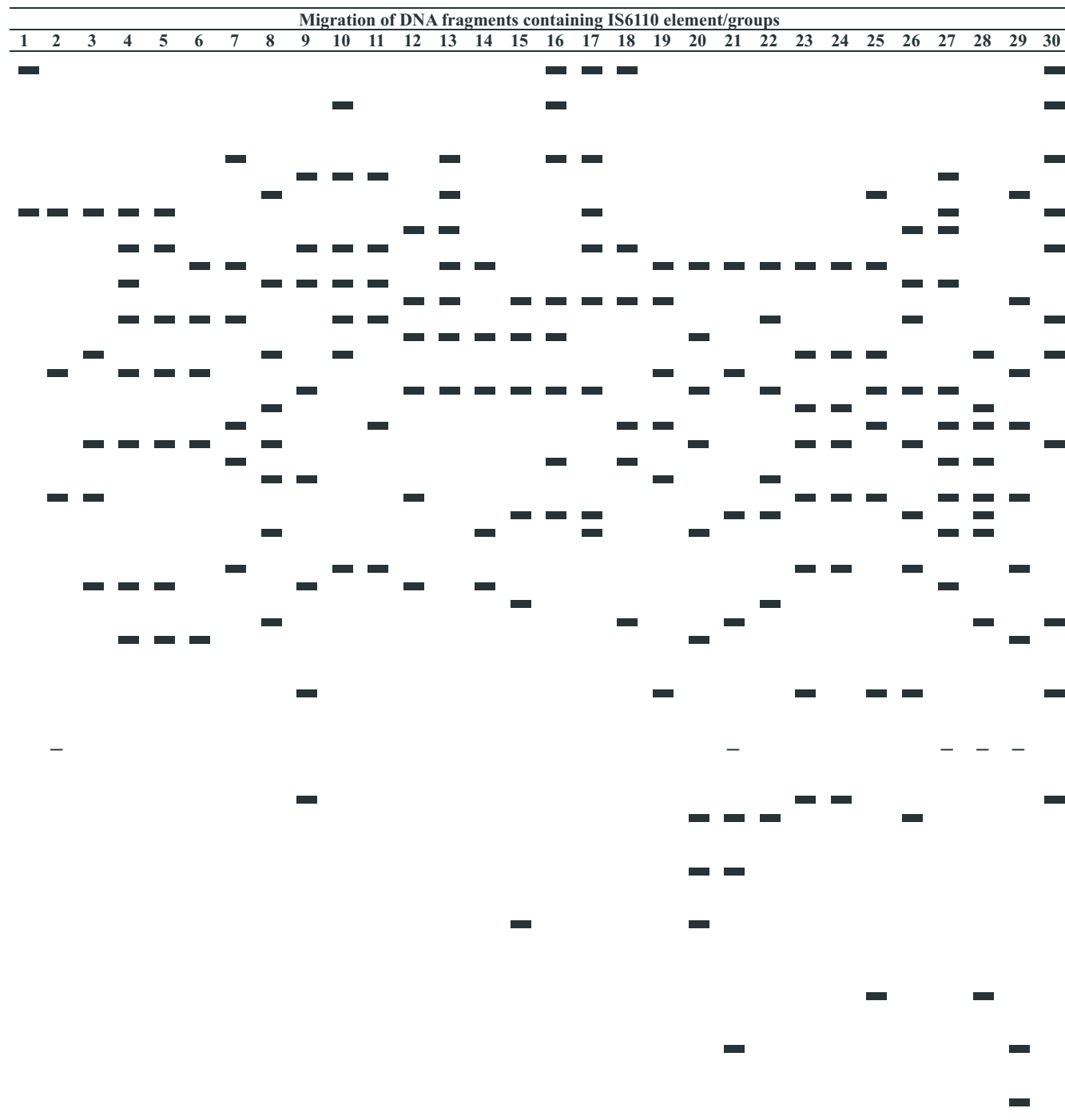
Cluster patterns	Patients	Coincident periods in prison (month/year)	Patients #: date of culture (month) (Prison)
I	22;36	06/97 to 04/99 - PI	22: 07/98 (PI) 36: 03/98 (PI)
V	16; 28	16 and 28: 01/97 to 12/97 - AN	16: 09/97 (AN) 28: 02/98 (AN)
X	19; 20	19 and 20: 01/94 to 12/94 CD and 06/92 to 01/93 - SB	19: 11/97 (CD) 20: 02/97 (AN)
XII	24;38	Without records for patient 38	24: 01/98 (CD) 38: 07/97
XIII	04; 17	coincident periods not found	04: 04/97(AN) 17: 09/97(CD)
XVIII	18; 25; 34	18 and 25: 02/98 to 07/98 - AN	18: 09/97(PI)
XX	01; 07	da Rocha, different times before the exam	01: 03/97(CD) 07: 04/97(PI)
XXVII	08; 13	Without records for patient 13	08: 06/07/08/98(PI) 13: 09/97

Detention Houses: Anatolia Nogueira, CD: Casa de Detenção, CDSP: Casa de Detenção Flaminio Favero (São Paulo), PI – Penitenciária I, SB: Cadeia Pública do São Bernardo.

The analysis of the number of *IS6110* insertion elements present in the genome of the 51 *M. tuberculosis* isolates demonstrated a copy number ranging from 2 to 12 with a mean number of 7 copies per isolate (Figure 1). These data allowed us the construction of a dendrogram of 30 *IS6110* patterns (I-XXX) comprising 6 main clusters (A-F) (Figures 1 and 2) with most of the strains (43/84.3%) in two clusters (A and B). Twelve patterns (40%) (I, IV, V, X, XII, XIII, XV, XVIII, XIX, XX, XXII, XXVII) were considered identical clones with 2 (6.7%), 5 (16.7%), 2 (6.7%), 3 (10%), 2 (6.7%), 2 (6.7%), 3 (10%), 4 (13.3%), 2 (6.7%), 2 (6.7%), 2 (6.7%), and 4 (13.3%) strains, respectively (Figure 2). Strains 8-10 (pattern XXVII), 13-15 (pattern XV), 16-17 (pattern XXII), 19-23 (pattern IV), 27-28 (pattern XVIII), 30-31 (pattern X), and 40-41 (pattern XXIX) were isolated from the same inmates.

Analysis of inmate epidemiological data from the Criminal Record Sector (Table 3) indicated delays in starting treatment (time between symptom onset and the beginning of treatment) of 0-4 weeks for 9 (42.9%), 5-8 weeks for 5 (23.8%) and of 9 or more weeks for 7 (33.3%) of the inmates. No data were available for 18 inmates. Twenty-seven (77.1%) of the inmates were new TB cases, while 8 (22.9%) had been previously treated. Data were not available on 4 cases. Additionally, 14 (48.3%) had confirmed HIV infection, 15 (51.7%) were HIV negative and 10 had unknown HIV status. Among them, some inmates had lived in the same correctional institutes at least once during their historical period of incarceration (Table 3); however, there was no information if they had shared the same cell. Inmates 19 (HIV<sup>+</sup>) and 20 (HIV<sup>-</sup>) lived in two different

**Figure 1.** Restriction fragment length polymorphism (RFLP) of DNA fragments containing IS6110 element/groups.

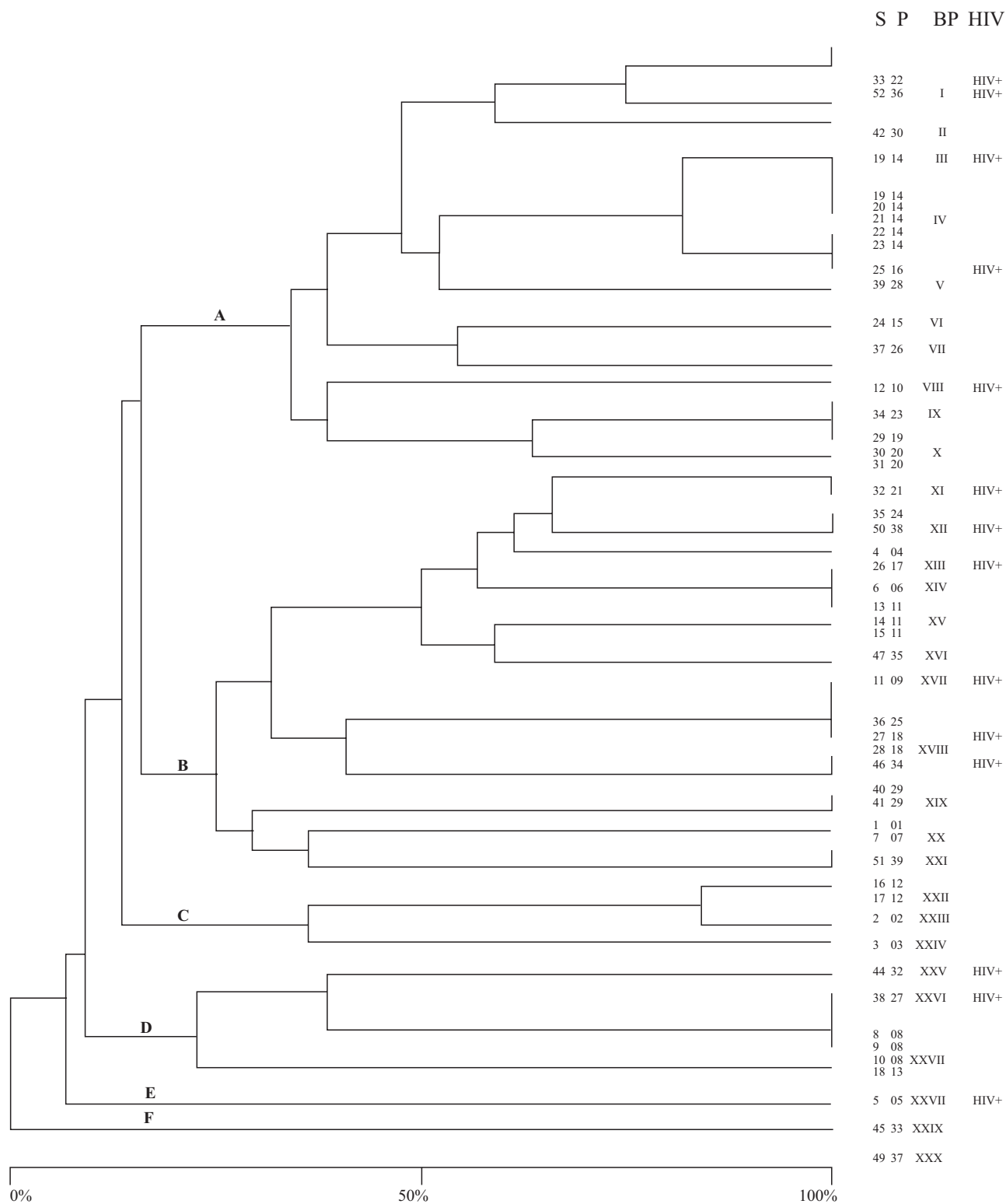


prisons during different periods of time (Table 2). The dendrogram of similarity (Figure 1) showed that inmates 22 (HIV<sup>+</sup>) and 36 (HIV<sup>+</sup>), 16 (HIV<sup>+</sup>) and 28 (HIV<sup>-</sup>), 19 (HIV<sup>?</sup>) and 20 (HIV<sup>-</sup>), 24 (HIV<sup>?</sup>) and 38 (HIV<sup>-</sup>), 04 (HIV<sup>-</sup>) and 17 (HIV<sup>+</sup>), 18 (HIV<sup>+</sup>), 25 (HIV<sup>-</sup>) and 34 (HIV<sup>+</sup>), 01 (HIV<sup>-</sup>) and 07 (HIV<sup>?</sup>), 08 (HIV) and 13 (HIV<sup>?</sup>) were colonized by *M.*

*tuberculosis* isolates with identical RFLP profiles (same clones).

The univariate analysis of the epidemiological data and clustering occurrence showed that only variable “previous treatment” (OR=7.65, p =0.032) was associated with possible recent TB transmission within prisons (Table 4).

**Figure 2.** Dendrogram of similarity (percent similarity) of *Mycobacterium tuberculosis* strains based on the RFLP of DNA fragments containing IS6110 element/groups.



S = strains, P = patients, BP = RFLP pattern, HIV+ patients with history of HIV infection.

**Table 4.** Univariate analysis of epidemiological data of the inmates and clustering occurrence.

Variables	Group I Cluster N (17)	Group II Non-Cluster N (22)	Odds ratio (OR)	P value
<b>Age</b>				
≥30	12	16	1.13	0.99
<30	4	6	1	
<b>Previous treatment</b>				
Retreatment	9	2	7.65	0.032*
New cases	10	17	1	
<b>HIV status</b>				
Positive	7	7	2.0	0.594
Negative	5	10	1	
<b>Treatment delay (weeks)</b>				
5 or more	7	5	1.75	0.669
0-4	4	5	1	

\* Significant value.

## Discussion

Molecular biology can provide the epidemiologist with powerful techniques to better discriminate between infectious agents with either extended generation time or difficult to characterize by the usual identification methods. *M. tuberculosis* is an infectious agent that presents both drawbacks. Among the molecular techniques used to characterize *Mycobacterium* strains, rRNA region polymorphism [21], the analysis of the number of IS6110 sequences [11,15,22] present in mycobacterium genome by either RFLP [22] or PCR [7,10], and DRE-PCR [7,23] are useful and practical tools to differentiate isolates.

In the last years, considerable efforts have been made to precise the epidemiology and characteristics of *M. tuberculosis* strains isolated in different regions of Brazil [7,24-31]. Some Brazilian authors [26,27,29,32] demonstrated the usefulness of the IS6110-restriction fragment length polymorphism for typing Brazilian *M. tuberculosis* strains, but Ferreira et al. [24] were the first to apply it to determine strain types in female inmate populations in São Paulo City, Brazil. Ferreira et al. [24] suggested that in 50% inmates infection was a reactivation process of a previous latent infection, while in the 50%, TB was transmitted within prison (exogenous infection). This indicates an epidemiologic picture with harsh consequences for inmates, their families, and the community.

In this study, we also used the characteristic insertion element IS6110 of *M. tuberculosis* [15,22] to typify the isolates obtained from different inmates incarcerated in different correctional institutions in Campinas area and to verify the transmission of TB between the same and different correctional institution inmates.

Our results agree with previous works [7,27,32] utilizing the detection of insertion element IS6110 in Brazilian *M. tuberculosis* isolates, which demonstrated that the number of this element per genome may vary (2-12 copies per genome). Our results also demonstrate that most of the isolates may be allocated to two main clusters. This denotes that just a few

strains may be responsible for the colonization of the hosts and prevail due to the close proximity between inmates, as corroborated by Ferreira et al. [24], who studied 350 female inmates and found only seven RFLP patterns for the studied *M. tuberculosis* strains.

Furthermore, 17/39 (43.5%) individual with identical IS6110 profiles lived at least once close together during their incarceration history and 7 (41.1%) of them were HIV positive (Table 3). These data altogether strongly suggest cross contamination of inmates living in the same and in different prisons. If this assertion is also true for HIV infection, the virus infecting the prisoners needs be further genotyped.

The results herein obtained also indicate an association among previous TB treatment, level of education, and recent transmission of TB. These results agree partially with those published by other Brazilian authors [7,27]. In our study, HIV infection was not associated with isolate clustering, although 48.3% of the patients were HIV positive. Under-notification or lacking inmate historical records may be a problem in epidemiological evaluations [33]. In this study, 33% of the inmates did not have disease notification index cards, which provide compulsory disease information to public health agencies. The lack of historical records for these inmates and the absence of clinical, demographic, and epidemiological data (Table 2) impaired an accurate analysis of association between TB and other diseases.

A remarkable epidemiological aspect of TB is the origin and spreading of multidrug resistant strains (MDR). Patients with MDR strains do not respond to treatment with commonly used drugs [7,28,34-36] and their treatment is long and expensive. Among the MDR screening features is the interruption of treatment and indiscriminate use of chemotherapy [7,28]. In a country like Brazil, with steadily rising incidence of criminality and high prison population turnover, it becomes necessary to follow up these patients and their contacts. Since this situation may be even worse in cities with bigger or a larger number of penitentiaries, our results suggest that treatment delay and inmate cross

contamination may contribute to the origin of strains with increased drug resistance. This urges for the adoption of public health measures by correctional officer to improve TB diagnosis and cure rates and avoid posing high risk to inmates, prison staff, and the general population.

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