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Short communication

Molecular epidemiology of *Mycobacterium tuberculosis* strains from prison populations in Santa Catarina, Southern Brazil

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ABSTRACT

The Tuberculosis (TB) notification rates are 5 to 81 times higher in prisons worldwide when compared to the general population. The state of Santa Catarina (SC) has few epidemiological data regarding TB in prisons. The aim of this study was to evaluate the molecular epidemiology of circulating strains in prisons of SC. The study comprised 95 clinical samples from six prisons. Among the cases included, all subjects were male, predominantly caucasians, and young adults, with low education level. The positive smear in the TB diagnosis comprised 62.0% of cases. About 50% of subjects had some condition associated with TB. The Spoligotyping results showed that the most frequent lineages were LAM (50.7%), T (22.2%) and S (11.6%). The 12-loci MIRU generated 62 different genotypes. The MSTs showed evolutionary relationships between *Mycobacterium tuberculosis* spoligotypes from SC and evolutionary relationships between the prison isolates and studied parameters. This first study on TB in prison units of SC highlighted the predominance of SIT216/LAM5, and SIT34/S. Interestingly, his profile was found to be different from that observed in a previous study performed with the state's general population. This data shows the need for continued surveillance of episodes of TB occurring among prison inmates in an emerging country like Brazil.

1. Introduction

Tuberculosis (TB) is one of the world's deadliest infectious diseases. TB notification rates are 5 to 81 times higher in prisons worldwide when compared to the general population (WHO, 2014). In Santa Catarina (SC), Southern Brazil, the incidence rate within the prison system in 2014 was 1295/100,000 inhabitants, 25 times higher when compared to the general population (Brasil, 2014; Brasil, 2016). The SC prison system's capacity is sufficient to accommodate only 60% of the total inmate population, demonstrating the rate of overcrowding in SC prisons (Brasil, 2014). The prolonged stay in enclosed and poorly ventilated spaces, overcrowding, lack of control measures for air-transmitted diseases, and high HIV infection rates among prisoners encourage the TB transmission (Dara et al., 2015).

Molecular epidemiology studies are useful tools in the identification of factors of TB distribution and transmission among the inmates. In

this study, Spoligotyping and Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeat (MIRU-VNTR) analyses were employed to determine the frequency of *Mycobacterium tuberculosis* (MTB) genotypes in clinical isolates from inmates of SC prisons. The genotypes were then related to clinical and epidemiological data, and compared with non-prison TB isolates in SC to verify any prison-related specificities.

2. Materials and methods

This study was approved by the Research Ethics Committee of the Federal University of Santa Catarina under number 03448612.9.0000.0121.

Ninety five MTB clinical isolates were obtained from the Central Laboratory of Public Health of SC (LACEN-SC), from January/2010 to December/2014. Samples were collected from 95 inmates from six

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prison units in SC: State Prison Complex - COPE (São Pedro de Alcântara), Male Prison of Florianópolis - PMF, Male Prison of Tubarão - PMT, Southern Penitentiary - PS (Criciúma), Santa Augusta Prison - PSA (Criciúma) and Prison Complex of the Vale do Itajaí - CPVI (Itajaí). Drug susceptibility testing (DST) was performed with the Bactec MGIT 960. Clinical-epidemiological data on the participants were obtained from the Notifiable Diseases Information System (SINAN).

MTB DNA was extracted using cetyltrimethylammonium bromide, as described by Van Soolingen et al. (1994), followed by Spoligotyping (Kamerbeek et al., 1997) and 12-loci MIRU-VNTRs (Supply et al., 2000). For genotyping analysis and comparison with an international database, spoligotype patterns were entered in the SITVIT2 proprietary database (Couvin and Rastogi, 2014, 2015). In this database, Spoligotype International Type (SIT) and a 12-loci MIRU International Type (12MIT) designate patterns shared by two or more isolates, whereas “orphan” designates patterns reported for a single isolate.

The Pearson Chi-Square test and Fisher's exact test were employed using the website “openepi.com” to compare the results between this and other studies. p Values < 0.05 were considered statistically significant. Data on prison isolates were compared with non-prison TB isolates in SC (Nogueira et al., 2016). Lastly, evolutionary relationships were studied through the elaboration of Minimum Spanning Trees (MSTs) using the BioNumerics Software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium).

3. Results and discussion

SC has a population of approximately 7,006,200 inhabitants, which corresponds to 3.4% of the Brazilian population (IBGE, 2017). The state's prison population corresponds to 0.25% of the SC general population and represents approximately 2.4% of the Brazilian prison population. It is estimated that over the past ten years the number of inmates in SC prisons increased > 40%, over twice the capacity of the state's prison units. Currently, the prison system's capacity is sufficient to accommodate only 60% of the total inmate population, demonstrating the rate of overcrowding that occurs in SC prisons (Brasil, 2014). SC has 49 prison units. The population of the six prisons included in this study represents 30% of the SC prison population (CNIJ, 2017).

3.1. Clinical-epidemiological and demographic characteristics

Clinical and epidemiological data for the 95 prison isolates are summarized in Supplemental Table S1. Cases distribution according to prison unit was as follows: 22.1% (21/95) were from COPE, 27.4% (26/95) from PMF, 10.5% (10/95) from PMT, 2.1% (2/95) from PS, 14.7% (14/95) from PSA and 23.2% (22/95) from CPVI. Of the 95 cases, 14.7% (14/95) had not been included in the notification system; therefore, the only available data on these cases came from LACEN-SC, including gender, age, prison unit, and DST results.

Results of the smear test at the time of diagnosis were positive in 62.1% (59/95). In addition, treatment follow-up smear testing showed that 62.0% of the cases remained positive after the second month of treatment. Moreover, in more than half of the prison units, smear test conversion occurred only in the fourth month of treatment. This demonstrated that the transmission chain of TB is maintained inside the prison for many months, a concerning fact, as the risk of transmission in institutionalized interactions is 10 times higher than in casual interactions (WHO, 2014). Approximately 64% (61/95) of the individuals were tested for HIV, and 32.8% (20/61) had positive results, rate higher than that found in other prisons throughout Brazil ($p < 0.001$) (Silveira et al., 2007; Kuhleis et al., 2012). DST results showed that 9.5% (9/95) of the isolates displayed some form of drug resistance and 3.2% (3/95) were MDR-TB. Similar results were obtained in the general population with TB in SC ($p > 0.050$) (Nogueira et al., 2016). Most cases were registered as new cases (76.6%; 62/81) and 17 (21.0%) as

relapse. Regarding the outcome, most cases were successfully treated (75.3%, 61/81), while 11 (13.6%) inmates abandoned treatment. Treatment default was observed mainly in younger individuals, who were HIV-positive, and had started the directly observed treatment (DOT) in prison. HIV-positive individuals have a lower chance of cure. Moreover, this default rate may be related to the interruption of treatment due to prison transfer or release. Treatment default was also related to TB/HIV coinfection ($p = 0.001$), being possibly due to the intensified adverse effects that occur with the concomitant treatment of both diseases.

3.2. Genotyping, database comparison and evolutionary relationships

Detailed information on Spoligotyping and 12-loci MIRU typing results in conjunction with demographic and epidemiological information is summarized in Supplementary Table S1. Briefly, spoligotyping identified 33 different profiles: 89 isolates were classified as belonging to 27 different SITs and six isolates were classified as orphans. There were 17 isolates with unique standards and 78 were grouped into clusters. This study resulted in five new SITs. The most frequently lineage was the Latin-American & Mediterranean (LAM), comprising 50.7% (48/95) of the isolates, followed by the T-superfamily with 22.2% (21/95), S lineage (11/95; 11.6%), East African-Indian (EAI) (4/95; 4.2%), Haarlem (3/95; 3.2%), Ural (1/95; 1.1%), and the European X lineage (1/95; 1.1%). The most frequent SITs were SIT216/LAM5 (13/95; 13.7%), SIT34/S (11/95; 11.6%) and SIT42/LAM9 (8/95; 8.4%). The other identified SITs comprised from one to six isolates each (Supplementary Table S1). The detailed analysis of spoligotyping defined clusters (representing 2 or more strains) and corresponding SITs among prison isolates in SC as compared to their global distribution in SITVIT2 database is in the Table 1. As can be easily seen, nearly all clusters corresponded to MTB Complex shared-types and lineages commonly found in South America, and specifically Brazil. The 12-loci MIRU analysis generated 62 different genotypes. Nineteen genetic groups were formed with 100% similarity, 14 of them comprising two isolates each (Supplementary Table S1), three with three isolates each (MIT34, MIT1757, MIT1761), one with seven isolates (MIT1758), and one with eight isolates (MIT1764).

To evaluate the evolutionary relationships among MTB spoligotypes in SC, MSTs were drawn for 481 isolates from inmates (data from this study) and non-inmates (Nogueira et al., 2016) (Fig. 1). A super representation of the S lineage was observed among the inmates (11.6%) when compared to the non-prison population (1.6%; $p < 0.01$). In the general population, isolates of the S lineage were observed only in the Southern region of SC (Nogueira et al., 2016), which explains its prevalence in the Criciúma prison unit, located in this region. However, the presence of SIT34/S was also verified in the unit of Great Florianópolis, demonstrating the possible dissemination of strains due to inmates transfer among SC prisons. A sub-representation of the Haarlem family was observed among the prison population (3.2%) compared to the non-prison population (12.7%; $p < 0.01$), this difference was not related to clinical-epidemiological data.

In global population of SC, EAI5 lineage representing 2.7% (SIT3099/EAI5, $n = 13/481$) of strains, split as 4.2% in prison ($n = 4/95$) vs. 2.3% ($n = 9/385$) in the non-prison population. In this context, it should be noted that in the global spoligotyping-based MST drawn for the 481 strains from SC, two major branches were formed, the first one mainly by the LAM lineage and the second one by the T and Haarlem lineages, corroborating earlier observations (Gomes et al., 2012). As mentioned recently (Nogueira et al., 2016), SIT3099 which was classified as EAI5 in our study; was closely linked to SIT42/LAM9 in the MST (Fig. 1B), surrounded by other major SITs from the LAM lineage (SIT64/LAM6, SIT93/LAM5, SIT216/LAM5 and SIT17/LAM2).

Considering the existing doubts about the EAI5 assignment to SIT3099 by the SITVIT2 database (Couvin and Rastogi, 2014, 2015), we decided to reevaluate its lineage based on combined spoligotyping and MIRU data

Table 1
Sporogony defined clusters and corresponding SITs among prison isolates of *M. tuberculosis* in Santa Catarina (representing 2 or more strains) as compared to their global distribution in SITVIT2 database.

SIT (lineage) sporogony description	Nb (%) in study	% in study vs. SITVIT2 ^a	Distribution in regions with > = 3% of a given SIT ^b	Distribution in countries with > = 3% of a given SIT ^c
SIT216 (LAM5)	13 (13.68)	23.64	AMER-S 87.27, AMER-N 9.09, EURO-S 3.64	BRA 83.64, USA 9.09, ITA 3.64
SIT34 (S)	11 (11.58)	1.22	AMER-N 23.11, AFRI-S 17.11, AMER-S 14.11, EURO-S 14.11, EURO-W 8.89, AFRI-N 4.0, CARIB 3.67, EURO-F 3.44, ASIA-W 3.33, EURO-N 3.11	ZAF 17.11, USA 14.89, ITA 11.78, CAN 8.22, BRA 8.0, FRA 4.0, BGR 3.44
SIT42 (LAM9)	8 (8.42)	0.21	AMER-S 35.06, AMER-N 10.62, EURO-S 10.14, EURO-W 8.48, AFRI-N 7.68, EURO-N 4.39, CARIB 3.8, AFRI-F 3.48, AMER-C 3.18	BRA 14.47, USA 10.62, COL 7.65, MAR 6.31, ITA 5.86, FRA 4.55, ARG 3.53, PER 3.32, ESP 3.0
SIT53 (T1)	6 (6.32)	0.09	AMER-S 17.09, EURO-W 14.15, AMER-N 12.19, EURO-S 8.51, ASIA-W 7.98, EURO-N 6.77, AFRI-E 4.65, AFRI-S 4.49, ASIA-E 3.86, AFRI-N 3.18	USA 11.96, FRA 7.14, BRA 5.87, ITA 4.82, TUR 4.51, ZAF 4.39, PER 4.29, AUT 3.1
SIT17 (LAM2)	5 (5.26)	0.67	AMER-S 59.97, AMER-N 15.93, CARIB 11.91, EURO-S 4.95	BRA 30.52, VEN 23.96, USA 15.93, HTI 7.36, ESP 3.88
SIT73 (T)	5 (5.26)	1.59	AMER-S 26.98, EURO-S 13.97, EURO-W 11.43, AMER-N 11.43, AFRI-S 8.89, AFRI-E 6.35, ASIA-E 3.81, AMER-C 3.17	BRA 18.41, ITA 11.75, USA 11.43, ZAF 8.89, FRA 6.35, ARG 4.44, CHN 3.81, MOZ 3.17
SIT60 (LAM4)	4 (4.21)	0.87	AFRI-W 35.36, AMER-S 26.9, EURO-W 6.72, AFRI-N 6.51, AFRI-W 5.86, EURO-S 5.21, AMER-N 4.12	ZAF 35.36, BRA 15.84, FRA 4.77, USA 4.12, MAR 4.12, GMB 3.69, ITA 3.47, VEN 3.25, PER 3.04
SIT65 (T1)	4 (4.21)	2.86	AMER-S 40.71, CARIB 23.57, EURO-W 12.14, AMER-N 9.29, EURO-S 4.29	BRA 39.29, HTI 21.43, USA 9.29, FRA 6.43, ESP 3.57
SIT3099 (EAI5)	4 (4.21)	22.22	AMER-S 94.44, ASIA-E 5.56	BRA 94.44, CHN 5.56
SIT64 (LAM6)	23 (5.67)	0.65	AMER-S 58.26, AMER-N 20.43, EURO-W 5.22, EURO-S 3.91	BRA 44.35, USA 20.43, GUF 5.0, PRT 3.04
SIT177 (LAM9)	3 (3.16)	2.33	AMER-S 68.99, EURO-S 10.85, EURO-W 7.75, AFRI-N, 3.88	BRA 51.16, ARG 9.3, FRA 7.75, ESP 6.98, ITA 3.88, PER 3.1
SIT613 (T1)	3 (3.16)	15.0	EURO-S 35.0, AMER-N 25.0, EURO-W 20.0, AMER-S 15.0, EURO-N 5.0	ALB 30.0, USA 25.0, BRA 15.0, AUT 15.0, ITA 5.0, SWE 5.0, FRA 5.0
4004 (Unknown)	3 (3.16)	100	AMER-S 100.0	BRA 100.0
93 (LAM5)	2 (2.11)	0.42	AMER-S 53.88, CARIB 15.72, AMER-N 15.72, EURO-S 6.71, EURO-W 3.77	VEN 19.71, USA 15.72, BRA 14.47, PER 13.42, HTI 10.06, ITA 4.4, GLP 3.98
729 (LAM1)	2 (2.11)	15.38	AMER-S 53.85, CARIB 38.46, AFRI-S 7.69	BRA 53.85, HTI 30.77, GLP 7.69, ZAF 7.69
2263 (LAM9)	2 (2.11)	16.67	AMER-S 50.0, AMER-N 20.0, EURO-S 10.0, EURO-N 10.0, ASIA-N 10.0	BRA 50.0, USA 20.0, RUS 10.0, ITA 10.0, FIN 10.0

^a Note that values above 12.5% (percentage in study vs. SITVIT2) are shown in **bold**.

^b Worldwide distribution is reported for regions with > 3% of a given SITs as compared to their total number in the SITVIT2 database. The definition of macro-geographical regions and sub-regions (<http://unstats.un.org/unsd/methods/m49/m49regin.htm>) is according to the United Nations; Regions: AFRI (Africa), AMER (Americas), ASIA (Asia), and OCE (Oceania), subdivided in: E (Eastern), M (Middle), C (Central), N (Northern), S (Southern), SE (South-Eastern), and W (Western). Furthermore, CARIB (Caribbean) belongs to Americas, while Oceania is subdivided in 4 sub-regions, AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Note that in our classification scheme, Russia has been attributed a new sub-region by itself (Northern Asia) instead of including it among rest of the Eastern Europe. It reflects its geographical localization as well as due to the similarity of specific TP genotypes circulating in Russia (a majority of Beijing genotypes) with those prevalent in Central, Eastern and South-Eastern Asia. Note that values for AMER-S are shown in **bold**.

^c The country codes are according to <http://www.worldatlas.com/aatlas/ctycodes.htm>; countrywide distribution is only shown for SITs with ≥ 3% of a given SITs as compared to their total number in the SITVIT2 database. Note that values for Brazil are shown in **bold**.

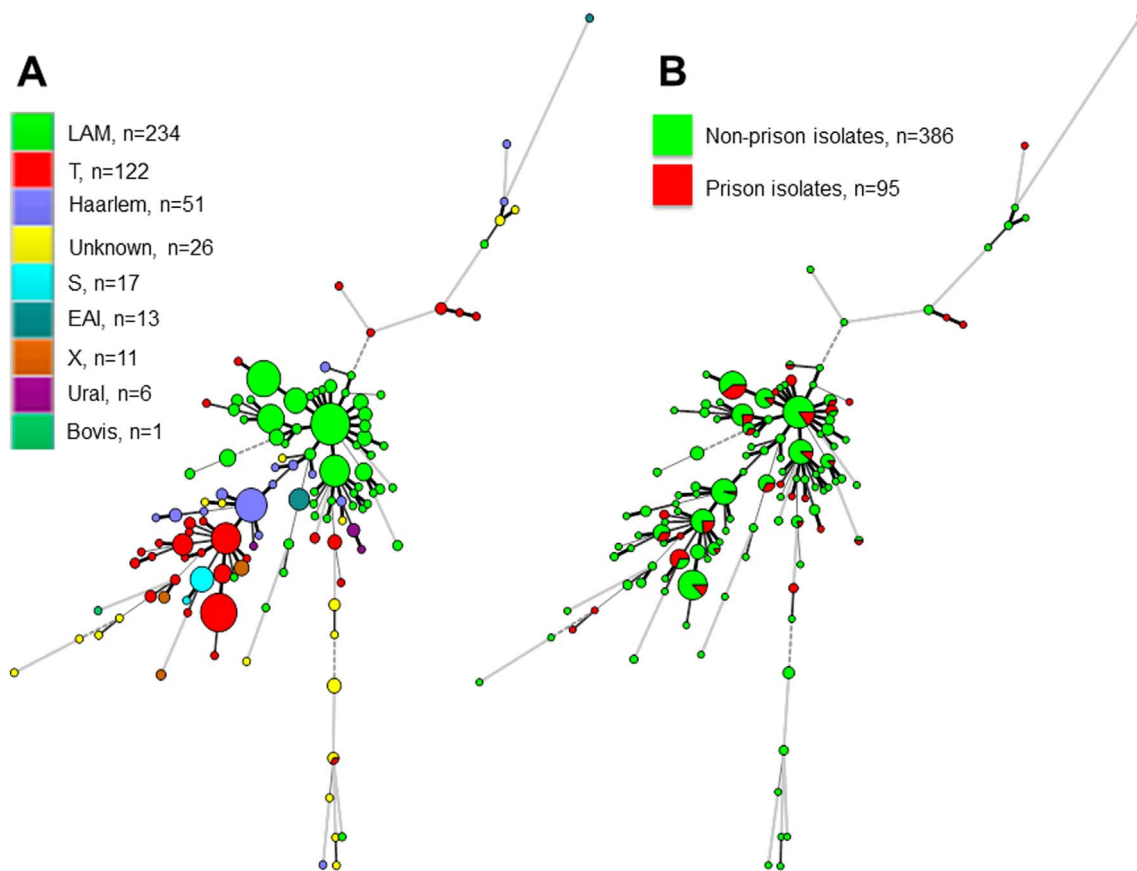


Fig. 1. Minimum spanning tree (MST) illustrating evolutionary relationships between *M. tuberculosis* spoligotypes from Santa Catarina, Brazil ($n = 481$ strains split as 385 non-prison vs. 95 prison isolates); (A). The MST with colors of the circles indicating the lineages to which each specific pattern belongs to; (B). Same MST showing the distribution of prison ($n = 95$) vs. non-prison ($n = 385$) isolates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

available and its geographical distribution using the in-house SITVI-TEXTEND database, and tools SPOTCLUST (http://tbinsight.cs.rpi.edu/run_spotclust.html) and TB-Lineage (http://tbinsight.cs.rpi.edu/run_tb_lineage.html). Depending on the algorithm chosen the SIT3099 binary pattern (111111111111111111110000111110000000011111) was classified as LAM9 with a probability of 0.998 (CBN or KBBN). Considering that in an independent investigation (results not shown), the SIT3099 strains were found to be associated with the RD-Rio deletion (which is specific of LAM strains; (Lazzarini et al., 2007)); we decided to revise SIT3099 from EAI5 to LAM9. Our decision was further corroborated by the values observed for MIRU locus 24 (MLVA 2687, highlighted in **bold**) for the 4 SIT3099 isolates in our study: 222427[¶]52332, 224126[¶]62631, 224425[¶]52633, and 222326[¶]41331; indeed a value of 1 for MIRU24 designates Euro-American Lineage of recent evolution (to which LAM belongs to), while a value above 1 is characteristic of ancestral isolates such as the EAI (Supply et al., 2006). Subsequently, this information was provided to SITVIT database curator, leading to the official change of SIT3099/EAI5 to SIT3099/LAM9 on June 12th, 2017 (<http://www.pasteur-guadeloupe.fr:8081/SpolSimilaritySearch/>; (Couvin et al., 2017)) (Fig. 2).

Aware of the advantages of the 15- or 24-loci MIRU formats over the 12-loci format, particularly when MIRU-VNTRs are used as the sole typing method (Jagielski et al., 2016). Nevertheless, a combination of spoligotyping and 12-loci MIRUs has been successfully used in resource-limited settings to decipher “potential” transmission chains (Couvin and Rastogi, 2014; Jagielski et al., 2016). Combining the 12-loci MIRUs with spoligotyping allowed to assess ongoing transmission, underlining a total of 12 clusters containing 35 isolates (Supplemental Table S1), which significant considering the relatively small sample size. In conjunction to the present cluster analysis, MST analysis further confirmed the unique nature of the large clusters comprised of LAM

(SIT216/LAM5/MIT1764 $n = 8$; SIT177/LAM9/MIT1756 $n = 3$; SIT60/LAM4/MIT1761 $n = 3$, followed by SIT216/LAM5/MIT1760 $n = 2$; note that a larger group contained isolates from all prison units while a smaller group corresponded to isolates from Itajaí); and S lineage clusters from the prison units of Criciúma, Florianópolis, and São Pedro de Alcântara (SIT34/S/MIT1758 $n = 7$, and S34/S/MIT1766 $n = 2$). The epidemiological variables, age group, HIV serology, case input registration and smear test results were proportionally distributed in various groups according to the general distribution; and these variables did not show any significant relationship with the phylogenetic groups within specific prison units. Nevertheless, all S lineage strains remained within the same MST group; hence considering that the combination of spoligotyping and 12-loci MIRUs has a fairly good discriminatory powers for genomic analysis of MTB (Pitondo-Silva et al., 2013), our results not only highlight the very high similarity between S lineage strains circulating in different prison units, but further reinforce the possibility of intra- and inter-institutional dissemination.

In conclusion, this first study on TB in prison units in the state of Santa Catarina highlighted the predominance of SIT216/LAM5 (almost exclusively in one of the prison units), and SIT34/S, which in the majority was from the southern region of the state, having been detected also in the region of Great Florianópolis. Interestingly, his profile was found to be different from that observed in a previous study performed with the state’s general population. This data is important for the development of specific policies and strategies for TB control in the prison population, and shows the need for continued surveillance of episodes of TB occurring among prison inmates in an emerging country like Brazil.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2017.12.010>.

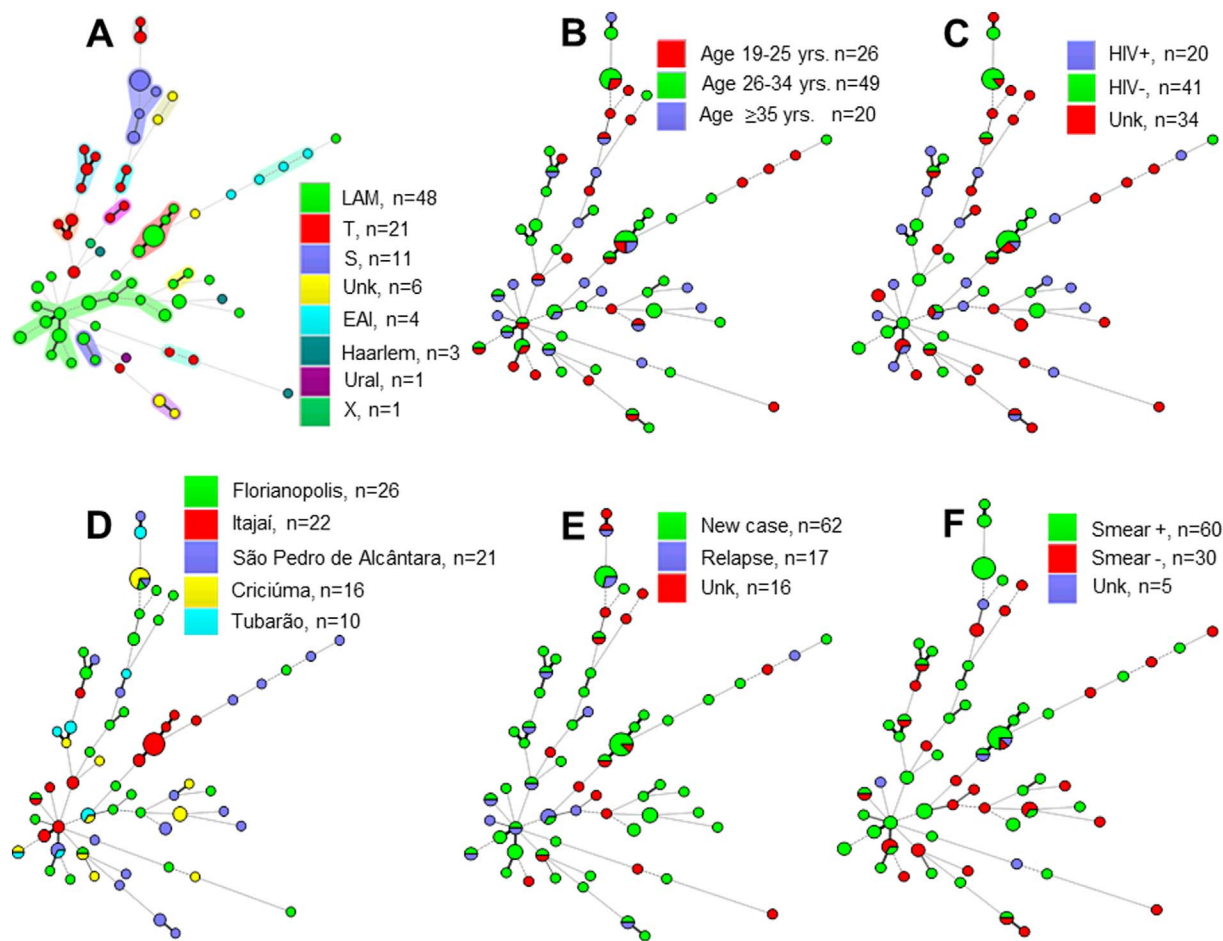


Fig. 2. Minimum spanning trees (MST) illustrating evolutionary relationships between the *M. tuberculosis* strains isolated from the prison inmates ($n = 95$ strains) genotyped using spoligotyping and 12-loci MIRUs. The individual trees show the relationships between the genotypes in function of studied parameters: (A) Genotypic lineages; (B) Age groups; (C) HIV serology; (D) City of origin; (E) New vs. relapse case; (F) Smear positive vs. negative samples. The structure of the tree represented by branches (continuous vs. dashed and dotted lines) and circles (representing each individual pattern) is the same as in Fig. 1.

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