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MOLECULAR ASPECTS

First insight into the molecular epidemiology of *Mycobacterium tuberculosis* in Santa Catarina, southern Brazil

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SUMMARY

Molecular epidemiology of *Mycobacterium tuberculosis* is useful for understanding disease transmission dynamics, and to establish strategic measures for TB control and prevention. The aim of this study was to analyze clinical, epidemiological and molecular characteristics of MTBC clinical isolates from Santa Catarina state, southern Brazil. During one-year period, 406 clinical isolates of MTBC were collected from Central Laboratory of Public Health and typed by spoligotyping. Demographic and clinical data were collected from the Brazilian National Mandatory Disease Reporting System. The majority of cases occurred in highest population densities regions and about 50% had some condition associated with TB. Among all isolates, 5.7% were MDR, which showed association with drug addiction. LAM was the most predominant lineage with 47.5%, followed by the T superfamily with 25.9% and Haarlem with 12.3%. The MST showed two major groups: the first was formed mainly by the LAM lineage and the second was mainly formed by the T and Haarlem lineages. Others lineages were distributed in peripheral positions. This study provides the first insight into the population structure of *M. tuberculosis* in SC State. Spoligotyping and other genotyping analyses are important to establish strategic measures for TB control and prevention.

1. Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* complex (MTBC), is a worldwide health problem, accounting for about 1.3 million deaths per year. According to the most recent Global TB Report by WHO, Brazil is one of the 22 high burden TB countries in the world with an estimated incidence of 46/100,000 population [1]. Santa Catarina State (SC) has one of the lowest TB incidence rates in Brazil (29.1/100,000). However, some cities like Itajaí (65.5/100,000 inhabitants) have higher than national average rates [2].

M. tuberculosis genotyping is useful for understanding the prevailing TB genotypes and their circulation within a certain population. The latter is important for understanding disease transmission dynamics, and to establish strategic measures for TB control and prevention. Moreover, the molecular characterization of *M. tuberculosis* in conjunction with a detailed analysis of clinical and epidemiological information is key to investigate the possible association of genotypic lineages with the clinical and epidemiological characteristics of the disease [3–5].

Since data about the molecular characterization of circulating *M. tuberculosis* strains in SC is scarce, we hereby provide a first insight into spoligotyping-based genetic diversity and the main genotypic lineages of MBTC clinical isolates circulating in SC, southern Brazil.

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Table 1
Comparison of the patients after treatment dropout and MDR-TB with HIV/AIDS, alcoholism and drug addiction status.

Comorbidity		TB history			Susceptibility		
		New case (n = 280)	Patients after treatment dropout (n = 36)	*p* Value	Susceptible TB (n = 303)	MDR-TB (n = 22)	*p* Value
HIV/AIDS	Yes	66 (23.6%)	10 (27.8%)	0.727	73 (24.1%)	8 (36.4%)	0.303
	No	214 (76.4%)	26 (72.2%)		230 (75.9%)	14 (63.6%)	
Alcoholism	Yes	57 (20.4%)	14 (38.9%)	0.022	64 (21.1%)	5 (22.7%)	0.792
	No	223 (79.6%)	22 (61.1%)		239 (78.9%)	17 (77.3%)	
Addiction drug	Yes	47 (16.8%)	17 (47.2%)	<0.001	55 (18.2%)	10 (45.5%)	0.005
	No	233 (83.2%)	19 (52.8%)		248 (81.8%)	12 (54.5%)	

Only the cases that had the HIV/AIDS, alcoholism and addiction drug status available in the National Information System for Notifiable Diseases were included in these analysis (n = 365 for TB history and n = 325 for susceptibility profile).

2. Materials and methods

2.1. Samples and drug susceptibility testing

This study included 406 MBTC isolates (one isolate per patient) received for routine culture, identification and DST at the Central Laboratory of Public Health of SC, from March/2010 to March/2011. The DST for isoniazid, rifampin, ethambutol and streptomycin was performed by the MGIT960 system (Becton Dickinson Diagnostic System, Sparks, MD) according to the manufacturer's instructions.

2.2. Demographic and clinical data

Demographic and clinical data were collected from the Brazilian National Mandatory Disease Reporting System (SINAN). Comorbidities were compared to TB history before actual diagnosis (new cases or retreatment after dropout), to the DST profile, death due to TB treatment dropout. TB death was compared to TB form (pulmonary and/or extrapulmonary) and susceptibility profile (MDR or pan-susceptible).

2.3. Spoligotyping and database comparison

Spoligotyping was performed by using commercially available membranes (Ocimum Biosolutions, Hyderabad, India) according to the protocol described previously [6]. Spoligotype patterns as octal codes were entered in the SITVIT2 proprietary database of the Institut Pasteur de la Guadeloupe which is an updated version of the previously released SITVITWEB database [7]. In July 2014, when the analyses were performed, the database contained about 112,000 MTBC genotypes of clinical isolates from 170 patient origin countries. In this database, Spoligotype International Type (SIT) designates patterns shared by two or more patient isolates, whereas “orphan” designates patterns reported for a single isolate. Genotypic lineages were assigned according to the new rules described in SITVIT2.

2.4. Data analysis

Nominal data were expressed as absolute or relative numbers and numeric data as mean and standard deviation. Comparisons between categorical variables were performed using the Fisher exact or Pearson chi-squared test, as appropriate. *P* values < 0.05 were considered statistically significant. The evolutionary relationships among all the observed spoligotypes were studied by drawing Minimum Spanning Trees (MSTs) with MLVA Compare (Ridom, Germany and Genoscreen, France). Spoligoforest trees were drawn using SpolTools software [8] and reshaped and colored using GraphViz software (AT&T Labs Research, USA).

2.5. Ethical approval

This study was approved by the Research Ethics Committee of Federal University of Santa Catarina (CEP/UFSC) by process number 168/07.

3. Results

3.1. Demographic and clinical data

Among all cases included in this study, 34.5% lived the Itajaí Valley, 33.0% in the Florianópolis Metropolitan Area (FMA) and 19.2% in northeastern region. About 50% (204/406) had some condition associated with TB, of which 22.2% were co-infected with HIV, 20.0% used excess alcohol and 18.7% used illicit drugs such as crack cocaine. More than 70% of TB/HIV cases were found in FMA (41.1%) and the Itajaí Valley (32.2%). Moreover, 92.5% (74/80) of the individuals who reported alcohol abuse and 71.3% (57/80) of those who reported drug addiction were male individuals (Table S1). Patients after treatment dropout showed an association with alcoholism ($p = 0.022$) and drug addiction when compared to new cases ($p < 0.001$) (Table 1).

The DST showed that 48 (11.8%) strains were resistant to one or more drugs and 23 (5.7%) were MDR. Comparing MDR-TB and TB

Table 2
Comparison of the TB death and TB dropout with HIV/AIDS, alcoholism and addiction drug status.

Comorbidity		Death			Dropout		
		Cure (n = 203)	Death (n = 15)	*p* Value	Cure (n = 203)	Dropout (n = 53)	*p* Value
HIV/AIDS	Yes	32 (15.8%)	8 (53.3%)	0.002	32 (15.8%)	22 (41.5%)	<0.001
	No	171 (84.2%)	7 (46.7%)		171 (84.2%)	31 (58.5%)	
Alcoholism	Yes	40 (19.7%)	4 (26.7%)	0.510	40 (19.7%)	21 (39.6%)	0.004
	No	163 (80.3%)	11 (73.3%)		163 (80.3%)	32 (60.4%)	
Addiction drug	Yes	29 (14.3%)	6 (40.0%)	0.019	29 (14.3%)	27 (50.9%)	<0.001
	No	174 (85.7%)	9 (60.0%)		174 (85.7%)	26 (49.1%)	

Only the cases reported as cure or TB death and that had the condition associated with TB available in the Brazilian National Mandatory Disease Reporting System (SINAN) were included in the analysis of TB death (n = 218) and only the cases reported as cure or treatment dropout that had the condition associated with TB available in the Brazilian National Mandatory Disease Reporting System (SINAN) were included in the analysis of TB outcome (n = 256).

history (Table 1), 3.6% (11/302) were new cases, 7.3% (3/41) were recurrent cases and 25% (9/36) were dropout cases. Moreover, MDR-TB showed an association with drug addiction ($p = 0.005$) (Table 1).

The TB outcome information was reported in 95.3% (387/406) of cases. TB death showed an association with MDR-TB ($p < 0.001$), since 3.8% (8/213) of the pansusceptible and 25.0% (2/8) of MDR-TB patients died. The percentage of death was higher among extrapulmonary cases (18.2% vs. 5.1% for pulmonary cases, $p = 0.039$). Regarding comorbidities, there was an association between death with HIV/AIDS ($p = 0.002$) and with drug addiction ($p = 0.019$). The treatment dropout group showed an association with HIV/AIDS ($p < 0.001$), alcoholism ($p = 0.004$) and drug addiction ($p < 0.001$) (Table 2).

3.2. Spoligotyping analysis

Spoligotyping of the 406 *M. tuberculosis* isolates yielded 110 different patterns, of which 48 were unique patterns and 62 were grouped in clusters (2–39 isolates per cluster). Ninety-six percent (390/406) belonged to 94 SITs and 3.9% (16/406) to orphan profiles. This study resulted in 11 new SITs in SITVIT2. Regarding genotypic lineages, Latin-American & Mediterranean (LAM) was the most predominant lineage with 47.5% (193/406), followed by the ill-defined T superfamily with 25.9% (105/406), Haarlem with 12.3% (50/406), European lineage X and East African–Indian (EAI) with 2.5% each (10/406), S lineage with 1.7% (7/406), Ural with 1.2% (5/406) and Manu and BOV with 0.25% each (1/406) (Figure 1). Interestingly, black or brown individuals were more associated with the X lineage in comparison to LAM lineage ($p = 0.045$).

Regarding the SITs, SIT42/LAM9 was predominant with 9.6% (39/406), followed by SIT73/T with 8.4% (34/406), SIT50/H3 with 7.1% (29/406), SIT64/LAM6 with 5.7% (23/406) and SIT216/LAM5 and SIT53/T1 sublineages with 4.9% (20/406) each. The other identified SITs showed one to eighteen isolates each (Table 3 and Table S2). In general, the lineages were equally distributed in SC, but some SITs were particularly predominant in some regions. In FMA, SIT64 (9.6%) was predominant, followed by SIT73 (8.1%) and SIT42 (6.7%). In the Itajaí Valley, the major SIT was SIT73 (13.7%), followed by SIT216 (8.6%) and SIT42 (7.9%); in the northeastern region, SIT50 was predominant (14.1%), followed by SIT53 (7.7%) and SIT42 (7.7%) (Figure 1). Figure 1 also shows divisions that may

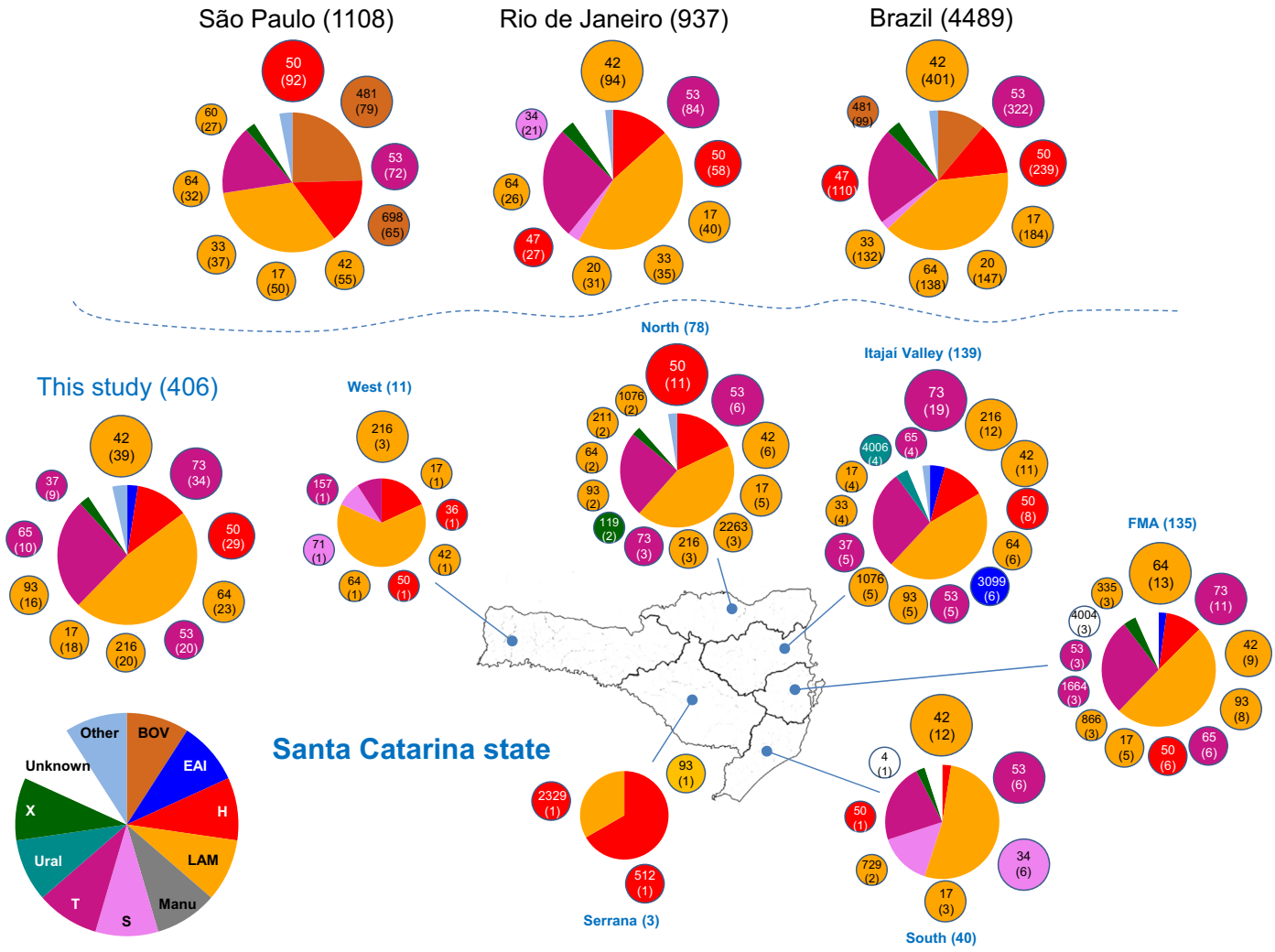


Figure 1. Distribution of lineages and main SITs (Spoligotypes International Types) in each mesoregion of Santa Catarina state (map), as well as in Sao Paulo and Rio de Janeiro states, and the whole Brazil country (without this study). Main pie charts indicate the lineage distribution and satellite spheres indicate the predominant SITs (represented by numbers) for each region. Numbers into brackets represent the number of strains. The size of satellite spheres is roughly proportional to the number of strains. For a better visibility, the 9 more predominant SITs have been taken into consideration for the whole study, Sao Paulo, Rio de Janeiro, and Brazil. However, concerning the mesoregions in our study, variable numbers of SITs were taken into account.

exist between mesoregions in SC and other Brazilian States (São Paulo and Rio de Janeiro).

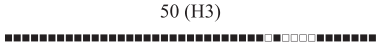
Seven sublineages and one unknown profile were identified among the 23 MDR strains. Identical MDR strains were found in different regions of SC. SIT42 and SIT106 were found in FMA, Itajaí Valley and northeastern region, SIT73 and SIT93 in FMA and Itajaí Valley and SIT50 and SIT2263 in FMA and northeastern region. The SIT2263 (four strains) was found only in MDR strains, and three of five SIT106 strains were MDR (Table 4).

The MST showed two major groups (Figure 2): the first group was formed mainly by the LAM lineage and was more scattered than the second one, which was mainly formed by the T and Haarlem lineages. As expected, orphan strains and unique strains with SITs were found in peripheral positions. For additional information, the reader is referred to the spoligoforest trees (Figure S1), as well as the MSTs related to drug resistance, ethnicity, mesoregions and gender in supplemental Figures S2–S5, respectively.

4. Discussion

This study provides the first insight into the distribution of MTB strains in SC using demographic, clinical and molecular analysis. SC has 6,248,436 inhabitants, of which 84.0% live in urban areas. It has the highest Gross State Product of the southern Brazil and ranks fourth in the country [9]. The SC population is predominantly made up of Portuguese, German and Italian descendants (88.1%) [10].

Table 3
Global distribution of predominant SITs (representing at least n = 4 strains) in this study as compared to SITVIT2 database.

SIT (Lineage) Spoligotype Description	Nb in study (%)	Nb in SITVIT2 before entering data	Distribution in Regions with >=3% of a given SITs *	Distribution in countries with >=3% of a given SITs **
42 (LAM9)	39 (9.61)	3275	AMER-S 31.02, AMER-N 11.35, EURO-S 10.84, EURO-W 9.06, AFRI-N 8.21, EURO-N 4.69, CARI 4.06, AMER-C 3.4, AFRI-E 3.4, AFRI-S 3.03	BRA 13.09, USA 11.35, COL 7.29, MAR 6.75, ITA 6.26, FXX 4.83, PER 3.55, ESP 3.2, VEN 3.17, ZAF 3.03
73 (T)	34 (8.37)	252	AMER-S 22.11, EURO-S 14.97, EURO-W 12.25, AMER-N 12.25, AFRI-S 9.52, AFRI-E 6.46, ASIA-E 4.08, AMER-C 3.4	BRA 17.35, ITA 12.59, USA 12.25, ZAF 9.52, FXX 6.8, CHN 4.08, MOZ 3.4
50 (H3)	29 (7.14)	3309	AMER-S 25.93, EURO-W 15.01, AMER-N 15.01, EURO-S 9.86, CARI 4.97, EURO-E 4.72, EURO-N 4.67, AFRI-N 3.63, AFRI-S 3.46, AFRI-M 3.23	USA 14.99, PER 13.67, BRA 7.16, FXX 5.88, AUT 5.2, ITA 4.64, ESP 4.64, ZAF 3.46, CMR 3.18, CZE 3.13
64 (LAM6) 	23 (5.67)	366	AMER-S 52.97, AMER-N 23.27, EURO-W 5.94, EURO-S 4.46	BRA 41.34, USA 23.27, GUF 5.94, PRT 3.47
53 (T1) 	20 (4.93)	6152	EURO-W 15.03, AMER-S 14.23, AMER-N 12.95, EURO-S 9.03, EURO-N 7.18, ASIA-W 7.02, AFRI-S 4.77, AFRI-E 4.46, ASIA-E 4.07, AFRI-N 3.38, EURO-E 3.13, CARI 3.1, AMER-C 3.1	USA 12.67, FXX 7.57, BRA 5.63, ITA 5.12, ZAF 4.66, PER 3.75, TUR 3.33, AUT 3.29
216 (LAM5)	20 (4.93)	17	AMER-S 82.5, AMER-N 12.5, EURO-S 5.0	BRA 70.0, USA 12.5, PER 7.5, ITA 5.0, VEN 2.5, ARG 2.5
17 (LAM2)	18 (4.43)	654	AMER-S 57.88, AMER-N 16.6, CARI 12.83, EURO-S 5.16	BRA 28.73, VEN 24.97, USA 16.6, HTI 7.67, ESP 4.05, GLP 3.07
93 (LAM5) 	16 (3.94)	354	AMER-S 52.17, CARI 16.45, AMER-N 16.23, EURO-S 6.93, EURO-W 3.9	VEN 20.35, USA 16.23, PER 14.72, BRA 12.99, HTI 10.39, ITA 4.55, GLP 4.33
65 (T1)	10 (2.46)	106	AMER-S 39.42, CARI 24.09, EURO-W 12.41, AMER-N 9.49, EURO-S 4.38	BRA 37.96, HTI 21.9, USA 9.49, FXX 6.57, ESP 3.65
37 (T3)	9 (2.22)	467	AFRI-E 19.45, EURO-N 12.06, EURO-W 11.67, ASIA-W 10.7,	ETH 16.93, USA 7.78, SWE 5.25, SAU 5.06, CHN 5.06, FXX 4.28, ITA 4.09,

			AMER-N 8.76, AMER-S 7.98, ASIA-E 6.23, EURO-S 5.84, EURO-E 4.47, ASIA-S 3.7, AFRI-S 3.31	BRA 3.7, ZAF 3.31, DNK 3.31
3099 (EAI5)	9 (2.22)	5	AMER-S 92.86, ASIA-E 7.14	BRA 92.86, CHN 7.14
33 (LAM3)	7 (1.72)	1113	AMER-S 34.41, AFRI-S 25.02, AMER-N 12.24, EURO-S 10.93, EURO-W 6.31, AMER-C 4.16	ZAF 25.02, PER 17.09, USA 12.24, BRA 11.01, ESP 6.85, ARG 4.39, FXX 4.08, ITA 3.54, HND 3.31
1076 (LAM1)	7 (1.72)	10	AMER-S 70.59, ASIA-N 11.77, ASIA-SE 5.88, AMER-N 5.88, AFRI-E 5.88	BRA 70.59, RUS 11.77, USA 5.88, MOZ 5.88, IDN 5.88
34 (S)	6 (1.48)	836	AMER-N 23.8, AFRI-S 17.62, EURO-S 14.53, AMER-S 11.79, EURO-W 9.15, AFRI-N 4.12, CARI 3.78, EURO-E 3.55, ASIA-W 3.43, EURO-N 3.2	ZAF 17.62, USA 15.22, ITA 12.13, CAN 8.47, BRA 6.98, FXX 4.12, BGR 3.55
866 (LAM9)	6 (1.48)	49	AMER-S 38.18, EURO-S 21.82, AMER-N 12.73, EURO-W 10.91	BRA 36.36, ITA 18.18, USA 12.73, MAR 10.91, FXX 10.91, ESP 3.64
106 (Unknown)	5 (1.23)	131	EURO-S 35.21, AMER-S 21.83, AMER-N 16.2, AMER-C 13.38, EURO-W 4.93, AFRI-N 4.93	ESP 33.8, USA 16.2, PAN 13.38, BRA 9.86, PER 3.52, FXX 3.52, COL 3.52, ARG 3.52
119 (X1)	5 (1.23)	1077	AMER-N 57.7, AFRI-S 12.95, AMER-C 9.69, AMER-S 5.16, EURO-N 3.44	USA 57.07, ZAF 12.95, MEX 9.42
1664 (T2)	5 (1.23)	3	AMER-S 100.0,	BRA 100.0,
47 (H1)	4 (0.99)	1486	EURO-W 19.09, AMER-S 16.81, AMER-N 14.59, EURO-S 12.67, EURO-N 9.84, EURO-E 6.78, ASIA-W 3.78, AFRI-N 3.42	USA 14.29, ITA 7.74, PER 7.56, AUT 7.56, BRA 7.14, FXX 6.24, FIN 5.64, CZE 3.54, ESP 3.36, SWE 3.18
729 (LAM1)	4 (0.99)	6	CARI 45.46, AMER-S 45.46, AFRI-S 9.09	BRA 45.46, HTI 36.36, ZAF 9.09, GLP 9.09
2263 (LAM9)	4 (0.99)	5	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
4006 (Ural-1)	4 (0.99)	0	AMER-S 100.0	BRA 100.0

* Worldwide distribution is reported for regions with more than 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), EURO (Europe), 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 TB genotypes circulating in Russia (a majority of Beijing genotypes) with those prevalent in Central, Eastern and South-Eastern Asia.
** The 3 letter country codes are according to http://en.wikipedia.org/wiki/ISO_3166-1_alpha-3; countrywide distribution is only shown for SITs with ≥3% of a given SITs as compared to their total number in the SITVIT2 database.

In this study, 87% of all TB cases were concentrated in the Itajaí Valley, FMA and northeastern region of SC, and about 70% of all TB/HIV cases were concentrated in the Itajaí Valley and FMA. These three regions have the highest population densities of SC. Moreover, the Itajaí Valley and FMA are among those regions with the highest incidence of AIDS in SC and the highest incidence of TB in Brazil [11].

Besides TB/HIV coinfection, several social problems were identified, such as alcoholism (20.0%) and illicit drug addiction (18.7%). Moreover, 11% of people with TB were institutionalized in prisons, what represents an important public health problem since prisons may serve as TB reservoirs for the general population [12–14]. Sixty-three individuals dropped out of treatment during this study;

Table 4
Distribution of MDR-TB strains in SC regions.

SIT	Number of strains	Subfamily	Distribution in SC regions
42	4	LAM9	FMA (1), Itajaí Valley (2), North (1)
2263	4	LAM9	FMA (1), North (3)
50	3	H3	FMA (2), North (1)
73	3	T	FMA (1), Itajaí Valley (3)
106	3	Unknown	FMA (1), Itajaí Valley (1), North (1)
93	2	LAM5	FMA (1), Itajaí Valley (1)
17	1	LAM2	Itajaí Valley (1)
150	1	LAM9	North (1)
216	1	LAM5	Itajaí Valley (1)
Orphan07	1	T2	FMA (1)

SIT: Spoligotyping International Type. FMA: Florianópolis Metropolitan Area.

dropout was associated with alcoholism ($p = 0.004$) and drug addiction ($p < 0.001$). Moreover, 25% of MDR-TB were cases of treatment dropout. The Brazil dropout rate varies from 10 to 18% in regional studies and 9–12% in a national study [15–21]. It is already well-known that alcoholism and illicit drug addiction are strong predictors of treatment dropout [22]. Moreover, MDR-TB is usually associated with poor patient treatment adherence [23]. These data show that alcoholism and drug addiction impair the TB treatment adherence in SC, which represents a risk for spread of MDR-TB and consequently a serious risk to TB control.

TB death also showed an association with drug addiction ($p = 0.019$) and MDR-TB ($p < 0.001$), as well HIV/AIDS ($p = 0.002$)

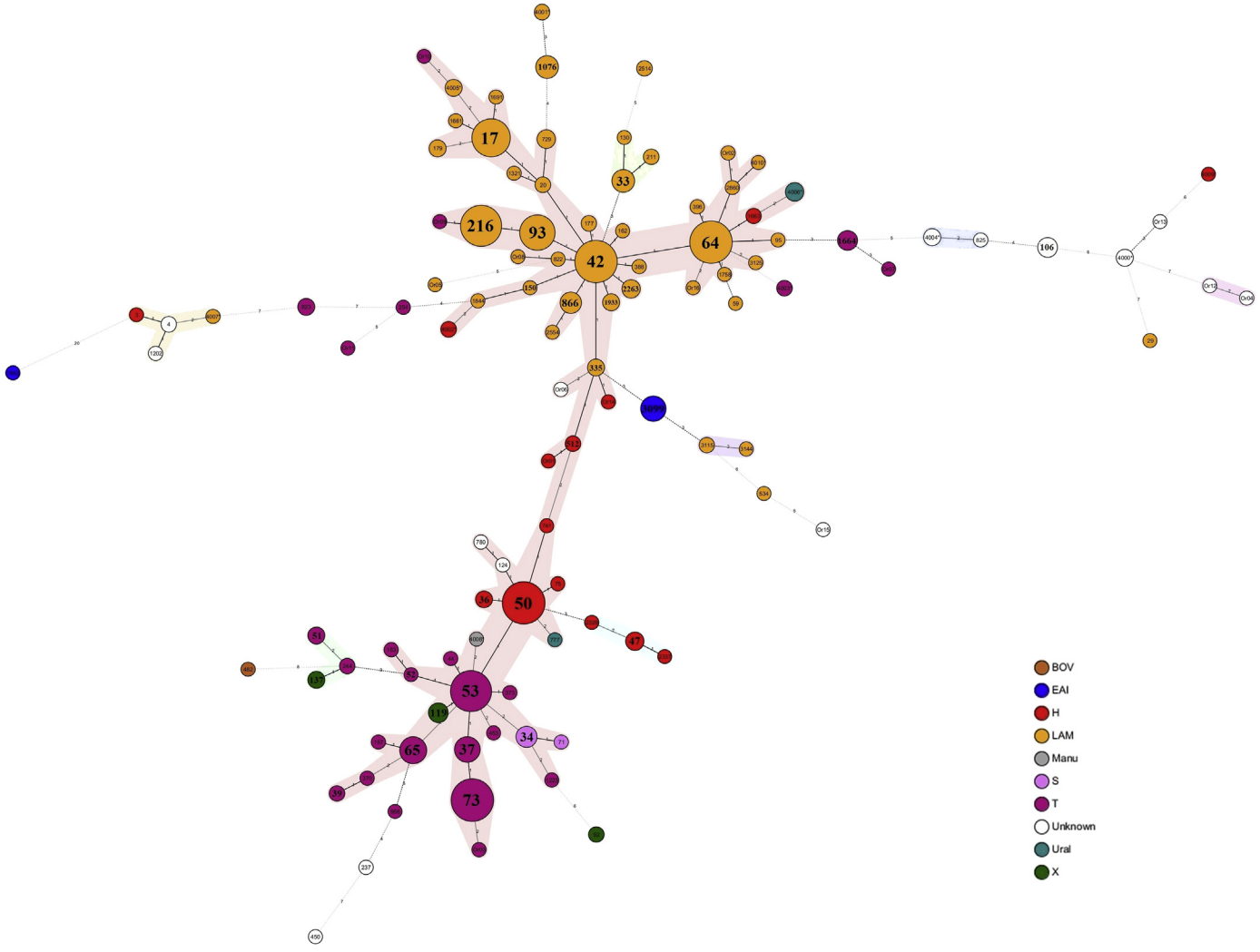


Figure 2. A minimum spanning tree (MST, drawn using MLVA Compare from GenoScreen and Ridom Bioinformatics) illustrating evolutionary relationships between the *M. tuberculosis* spoligotypes of the study (n = 406 patterns). The phylogenetic tree connects each genotype based on degree of changes required to go from one allele to another (the distance numbers are visible on each edge). Solid black line denotes one unique change between two patterns, while solid gray line denotes 2 changes, bold dashed line denotes 3 changes, and thin dotted line represents 4 or more changes. The size of the circle is proportional to the total number of isolates. The color of the circles indicates the phylogenetic lineage to which the specific pattern belongs (the corresponding colors of lineages are visible inside the figure). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and pulmonary/extrapulmonary TB ($p = 0.039$). Immunosuppression and socio-economic factors, including drug and alcohol abuse, contribute to the development of severe forms of TB, thereby increasing the mortality rate [24].

In this study, the LAM9 (16.5%) was the most frequent sublineage and SIT42/LAM9 (9.6%) was the most commonly identified spoligotype. These data are similar to those found in other studies performed in Brazil [7,25–29]. Moreover, SIT42/LAM9 is also the most prevalent profile in Portugal (19%). The T lineage (25.9%) was the second most frequent, followed by the H lineage (12.3%), which is found worldwide. Studies performed in other Brazilian southern cities have shown different frequencies of the H (11–31%) and T (7–27%) lineages [29,30]. Some predominant SITs were found in this study, such as SIT73/T (with 34 isolates) and SIT216/LAM5 (with 20 isolates). Both SITs are predominantly found in South America, but they were not common in other Brazilian states [7]. Additionally, Gomes et al. [24] found low percentages of SIT73/T and SIT216/LAM5 in isolates from 11 Brazilian states (not included SC state).

Economic, political and social practices in Brazil since colonization have resulted in several ethnic interactions. For centuries, Brazil, Portugal and Africa maintained close relations due to territorial discovery, colonization and slavery. Moreover, the 19th century was marked by an intense immigration of Europeans to Brazil, including German, Italian and Portuguese people, especially to SC [25,31]. Nowadays, SC receives nearly 4 million visitors (12% foreign visitors and 58% from other Brazilian states). Moreover, in comparison with other ports in the world, the port of Itajaí (located in the Itajaí Valley) is placed third when it comes to sea cargo handling [32]. It is likely that all these historic and economic facts are related to the genetic diversity of MTB strains in SC and could explain the similarity among the circulating MTB strains in SC and those circulating in Portugal and other European countries.

Despite a small number of strains, all four SIT2263/LAM9 strains were MDR. This SIT was found in a previous study with only MDR strains from SC and was associated to a specific mutation in gene *rpoB* (S531W) [33]. There were two SIT93-LAM5 isolates among MDR-TB strains. Interestingly these genotypes represented a big cluster in a previous study from the neighboring state Rio Grande do Sul [34]. It is important to highlight that the presence of identical MDR strains in different regions of SC suggests an inter-regional spread. MDR strain spread represents a potential risk to public health deserving special attention.

We drew the MST (Figure 2) for 406 strains based on spoligotyping to explore the evolutionary relationship in our study strains. As found by Gomes et al. (2012) [24], two major branches were formed, the first one mainly by the LAM lineage and the second one mainly by the T and Haarlem lineages. The central node of the first branch, formed mainly by LAM lineage strains, is SIT42/LAM9 and is surrounded by other bulky SITs from the LAM lineage (SIT64/LAM6, SIT93/LAM5, SIT216/LAM5 and SIT17/LAM2). The majority of unknown strains are linked to this group. Interestingly, SIT3099 was classified as EAI5 in our study; nevertheless, this profile was linked to SIT42/LAM9 in the MST (preceded by SIT335) and spoligoforest tree. Furthermore, SIT3099 is predominantly found in Brazil (only one copy of this genotype was found in China). The newly created SIT4006 was classified as belonging to the Ural-1 lineage [35] in this study; however, it was linked to SIT95/LAM6 in our spoligoforest (Figure S1) and to SIT1663/H3 in the MST. Interestingly, SIT1663 was found only in Brazil as SIT4006. Further genotyping analyses (such as MIRU-VNTRs typing) are needed to better identify the lineages.

5. Conclusion

This study provides the first insight into MTB population structure as well the current situation of TB in SC, along with its underlying social determinants. Although being in the Southern and in a more developed region of Brazil, TB/HIV coinfection, abuse of alcohol and illicit drugs were highly associated with TB as well with poor patient treatment adherence contributing to the development of MDR-TB. Identical MDR strains were identified in different regions, highlighting the potential risk to public health in SC. Further genotyping analyses should be conducted to better understand the dynamics of MTB transmission in SC and to establish strategic measures for TB control and prevention.

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Competing interests

None declared.

Ethical approval

This study was approved by the Research Ethics Committee of Federal University of Santa Catarina (CEP/UFSC) by process number 168/07.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2015.12.005.

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