

Short Communication

Usefulness of *Mycobacterium tuberculosis* Molecular Typing in a Tuberculosis Low-Endemic Agro-Industrial Setting of Brazil

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SUMMARY: To highlight the transmission and major phylogenetic clades of *Mycobacterium tuberculosis*, a retrospective study was carried out at two health facilities in a small agro-industrial area in São Paulo, Brazil, that has a low tuberculosis incidence rate. IS6110-RFLP and spoligotyping were performed on the isolates, with the former revealing that 31.3% (35/112) of strains were clustered. Epidemiological links were found in 16 of the 35 clustered patients and were associated with transmission among patients living in public housing. Spoligotyping grouped 62.8% of the strains. The T genetic family predominated among the isolates. Of interest is that five strains had a pattern characteristic of African or Asian origin (ST535), and two others were of the rare localized type ST1888 (BRA, VEN). In addition, three new types—1889, 1890, and 1891—were identified. Spoligotyping showed that some ST may be circulating to or from Brazil, and RFLP revealed ongoing transmission in inadequately ventilated public-housing buildings. This may point to a failure in tuberculosis control policy.

Among all countries in the Americas, Brazil reports the second-highest tuberculosis (TB) mortality and morbidity, comprising an incidence rate of 62/100,000 (1). Brazil is divided into five geographic regions, among which the distribution of TB cases varies greatly. The highest number of cases is concentrated in the southeast region (39,836), where São Paulo State (SP) accounts for approximately half of all cases per year, with the highest concentrations in the state's big cities (2). However, in small cities in central SP, such as Araraquara, TB transmission is almost unknown (3). Araraquara is an agro-industrial city 272 km from São Paulo city, with a population of 182,471 and a low TB incidence rate (26/100,000). It is a preferential location of Brazilian immigrants who work in sugarcane and citric agro-industrial activities. To provide initial information about the genetic diversity of *Mycobacterium tuberculosis* strains, a retrospective study was carried out. From a total of 341 cases of pulmonary TB registered between 1998 and 2001, *M. tuberculosis*-positive cultures were available for 112 (33%) patients, of whom 84 were outpatients and 28 were inpatients; 28 were female and 84 male; 15 were HIV-positive; 16 had prior treatment for TB; 80 were susceptible to antimycobacterial drugs; 13 were multidrug-resistant; and 8 showed resistance to one or two drugs. Most of the 112 patients were illiterate or had not completed primary school (80/112).

Genotyping was performed by IS6110-based restriction fragment length polymorphism (RFLP) (4,5) and spoligotyping (6), the patterns of which were designated as shared type (ST)

according to the international SpolDB4 (7).

The RFLP of 112 strains yielded 35 strains (31.3%) distributed among 14 clusters comprising 2 to 4 patients each. Epidemiological links were found in 16/35 (45.7%) strains, belonging to 6 of the 14 clusters (42.9%) (Table 1). It is noteworthy that half of the clustered patients contracted TB through extra-domiciliary transmission (8/16) and that 5 of these 8 were individuals living in public housing, which was composed of 908 poorly ventilated small apartments with 3,500 residents. Although our study may contain bias because of the small number of strains typed, it reveals that some situation is going on in the region and that further evaluation is needed to improve intervention. Thus, molecular characterization based on IS6110-RFLP is an important tool with which to study *M. tuberculosis* diversity and to analyze epidemiological links.

Spoligotyping was performed in 70 strains isolated from 42 outpatients and 28 inpatients randomly selected from the patient population in 1998-1999, and yielded 38 different patterns. Forty-four strains (62.8%) were grouped into 12 clusters comprising 2 to 12 strains each. Spoligotyping overestimated the number of clustered isolates. A similar result was found previously in the higher TB incidence area (74.9/100,000) in south Brazil (8), but those authors' suggestion that the high clonal diversity would be attributable to the high incidence of TB in the region may not fully explain the wide diversity. Neither study included all TB cases diagnosed in the selected period, and this may have biased the results or may simply reflect the lower discriminatory power of the spoligotyping method. In our study, only one spoligotyping cluster, of two strains, was correctly identified as belonging to the same RFLP cluster. This finding is similar to that of a previous study, which compared spoligotype- and RFLP-

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Table 1. RFLP clustered patients from Araraquara, Brazil, with epidemiologic link

Strain no.	RFLP (Spol) cluster	Isolation period	HIV status	Drug susceptibility profile	Epidemiologic finding
074		2000-01	P	S	Drug addict living in same house state condominium (HSC)
112		2000-01	N	S	
051	A (ND)	2000-01	P	S	
A060		1998-99	N	ND	
025		2000-01	N	Primary SM ^R	Father
033	A ₁ (ND)	2000-01	N	Acquire SM ^R	Daughter
024		2000-01	N	ND	–
014		2000-01	N	Primary PZA ^R	Father
017	A ₂ (ND)	2000-01	N	Primary PZA ^R	Mother
030		2000-01	N	ND	Son
114	P (ND)	2000-01	N	S	Neighbors from the same buildings (HSC)
023		2000-01	P	S	
A035	IV (ST42) ¹⁾	1998-99	P	S	Live in the same neighborhood in close street, both cab-drivers
A040		1998-99	P	S	
A09	II	1998-99	P	SM ^R	Drug addict
A026		1998-99	P	ND	

¹⁾: The only cluster defined by both genotypic methods.

HIV, human immunodeficiency virus serology; P, positive; N, negative; S, susceptible to all drugs tested; SM^R and PZA^R, streptomycin and pyrazinamide resistant; UK, unknown; ND, not done.

Table 2. Major shared spoligotypes (ST) in Araraquara, São Paulo, Brazil¹⁾

ST	Geographic distribution	Phylogenetic clade	Araraquara no. (%)
53	Ubiquitous common	T ₁	12 (17.1)
535	BRA, CMR, FIN, GEO, GNB, MYS, PRT, RUS, USA, VEN	T ₁ (Asian or African)	5 (7.1)
240	AUT, BRA, CUB, DEU, GUP, ITA, USA (Ubiquitous raro)	U	5 (7.1)
47	Ubiquitous common	H1	4 (5.7)
51	Ubiquitous recurrent	Like-PGG3 ²⁾	3 (4.3)
1891	BRA	Like-H ₃	3 (4.3)
42	Ubiquitous common	LAM ₉	2 (2.8)
92	Ubiquitous recurrent	X ₃	2 (2.8)
153	AUT, BRA, CUB, FIN, ITA, NDL, SWE, USA (Ubiquitous epidemic)	T ₂	2 (2.8)
1888	BRA, VEN (Localized rare)	T ₁ (ST 535 offspring)	2 (2.8)
1889	BRA	T ₁ (ST 535 offspring)	2 (2.8)
1890	BRA	T ₂ (ST 535 offspring)	2 (2.8)

¹⁾: Based on ref. 7.

²⁾: Likely principal genetic group 3 (T). H, Haarlem lineage; LAM, Latin American Mediterranean lineage.

AUT, Australia; BRA, Brazil; CMR, Cameroon; CUB, Cuba; DEU, Deutschland; FIN, Finland; GUP, Guadeloupe; GEO, Georgia; GNB, Guinea Bissau; ITA, Italy; MYS, Malaysia; NDL, The Netherlands; PRT, Portugal; RUS, Russia; SWE, Sweden; USA, United State of America; VEN, Venezuela.

clustered patterns and showed low probability of correspondence between them. Thus, spoligotyping may have little acceptability as an operating parameter for wide epidemiological studies (8,9).

Table 2 shows the major ST identified. Modern TB strains, including prototypes T₁ and T₂, were the most prevalent lineage shared by the clustered strains (56.8%, 25/44). According to the SpolDB4, Brazil accounts for large percentages of ST535 and ST240 cases (59 and 36%, respectively) (7). ST535 seems to have an Asian or African origin, as does its offspring ST1888. It is not surprising to find these patterns in our community; southeastern and northeastern Brazil have a strong African influence because of the slave trade, which was abolished only in the 19th century. After that (mid-1880s),

the southeast region (especially SP) became a destination of European immigrants, including Portuguese, Germans, Spaniards, Jews, and Italians; in addition, non-European immigrants, such as Syrians, Lebanese, and Japanese made major contributions to the ethnic composition of the Brazilian people. This diversity may explain, for example, the presence of the localized ST153, which is found predominantly in Italy. Although the Italian influence in southern Brazil was important, a previous investigation did not describe this pattern (8), but that study and our own studies shared similar prevalences of the ubiquitous types 53 (T₁ clade) and Haarlem 1 and 3 families.

ST53 is the second most frequent strain in the database, suggesting it is easily transmitted and possibly more adapted

to human beings. However, further investigation is needed to elucidate these points. We described the localized rare ST 1888, which has been identified only in Venezuela; it may be an offspring of ST535, as are types 1889 and 1890 which, together with 1981 (likely-H₃), were described as a new, not previously identified type. Further investigation may clarify whether or not they are circulating predominantly in South America. The ST92 (X family) is represented in our study but not in the south Brazil study. According to the SpolDB4, Brazil occupies the 13th position in the number of cases of X₃ subfamily infection. We found this strain with low IS6110 copy numbers, a characteristic that was reported for the X family (7,10).

Together with ST53, four other types are ubiquitous—ST42, ST47, ST92, and ST51—which respectively occupy the 2nd, 7th, 8th, 13th, and 37th positions among the most frequent types identified in the database (8). Out of a total of 38 different spoligotypes, 14 (36.8%) were true orphans, meaning they did not correspond to any family patterns recognized and classified in SpolDB4. Thus, spoligotyping is useful for acquiring information about the evolutionary phylo-geographic diversity in a restricted setting, and here showed that some ST may be circulating to or from Brazil.

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