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Mycobacterium tuberculosis of the RD^{Rio} Genotype Is the Predominant Cause of Tuberculosis and Associated with Multidrug Resistance in Porto Alegre City, South Brazil

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Spoligotyping has shown *Mycobacterium tuberculosis* strains to be composed of different lineages, and some of them are not just geographically restricted but also affect specific ethnic populations and are associated with outbreaks and drug resistance. We recently described a particular subtype within the Latin American-Mediterranean (LAM) family, called RD^{Rio}, widespread in Brazil. Moreover, recent data also indicate that RD^{Rio} is present in many countries on all continents and is associated with cavitary disease and multidrug resistance (MDR). To further explore the relationship between RD^{Rio} and MDR, we conducted a study in a tuberculosis (TB) reference center responsible for the care of MDR patients in Rio Grande do Sul, the southernmost Brazilian state. From a collection of 237 clinical isolates, RD^{Rio} alone was responsible for one-half of all MDR cases, including one large group composed of strains with identical IS*6110*-restriction fragment length polymorphism (RFLP) and having the LAM5 signature. We additionally had complete data records for 96 patients and could make comparisons between the presence and absence of RD^{Rio}. No difference in clinical, radiological or laboratory features was observed, but a significantly greater number of cases with MDR were described in patients infected with an RD^{Rio} strain ($P = 0.0015$). Altogether, RD^{Rio} was responsible for 38% of all TB cases. These data support and confirmed previous findings that RD^{Rio} is the main agent responsible for TB in Brazil and is associated with drug resistance. Considering that RD^{Rio} is a globally distributed genotype, such findings raise concern about the increase in MDR in certain human populations.

Despite increasing efforts, tuberculosis (TB) continues to be a significant cause of morbidity and mortality around the globe, particularly in developing countries, where the increase in the number of multidrug-resistant (MDR) strains and the emergence of extensively drug-resistant (XDR) strains make the situation even more dramatic, posing a significant threat to the WHO goals of reducing the prevalence and deaths due to TB by 50% by 2015 and the elimination of TB as a public health problem by 2050 (http://www.who.int/tb/strategy/stop_tb_strategy/en/index.html). There are several factors responsible for this problem, most of them related to economic and social issues such as poverty, drug addiction, HIV-positive status, and difficult access to anti-TB drugs, among others. Although these factors are unquestionable, others directly related to the microorganism itself are also probably significant but less studied and understood.

The deciphering of the complete genome of *Mycobacterium tuberculosis* H37Rv in 1998 (1) led to a better understanding of its biology, facilitating the analysis and further expanding the use of molecular tools for interrogation of the genome, such as IS*6110*-restriction fragment length polymorphism (RFLP), spoligotyping, and mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR) typing (2–4). Among these methods, spoligotyping proved to be a particularly useful tool. It is a standardized amplification method targeting the polymorphic Mtb complex-specific direct repeat (DR) locus and is visualized as a binary 43-digit pattern or reduced to an octal form of 15 digits (2). Compared to IS*6110*-RFLP and MIRU-VNTR typing, spoligotyping is unable to achieve the same level of discrimination; however, the assay is rapid, inexpensive, and robust, and the data can be easily exchanged between laboratories. Therefore, spoligotyping is often used as a first-line genotyping method and is the basis of the definition of the major genotype families of *M. tuberculosis* such as the Beijing, Haarlem, T, X, S, East African-Indian (EAI), Latin American-Mediterranean (LAM), and Central Asian (CAS) families, among others. Each of these patterns is included in the SpolDB4, an international database developed and maintained by the Pasteur Institute of Guadeloupe, which includes spoligotypes submitted by investigators from all over the world (5) and is available online (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo). Some of the spoligotype families (also called spoligofamilies) are preferentially distributed in particular geographic regions, indicating that they are either spreading locally or better adapted to certain human populations (5–7). Importantly, some spoligofamilies have been associated with outbreaks and multidrug resistance, the Beijing (and Beijing-like) spoligofamily being the best-known example (8–11). Although

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predominant in far-east Asia, the Beijing spoligofamily is much less frequently found in the Americas, Europe, and Africa, where LAM, Haarlem, T, and S families predominate (5). The LAM family, a member of single nucleotide polymorphism (SNP) cluster VI (12), appears to be the most prevalent *M. tuberculosis* lineage globally, accounting for ~15% of the global TB burden (5). Despite its success, little is yet known about the epidemiology, biological behavior, and clinical attributes of disease caused by strains of the LAM family. Similar to Beijing family strains, members of the LAM family have also been associated with drug resistance and outbreaks (13–16), including the recent description of a LAM strain as the leading cause of XDR-TB in South Africa (17).

An additional reason for studying the features of the spoligofamilies was the recent discovery of RD^{Rio}, a subfamily inside the larger LAM spoligofamily, which seems to be prevalent in several places in Brazil (18–23) but also in many other countries, particularly in Central and South America, Europe, and Africa (19). Analysis of TB isolates and registry data from New York, NY, between 2001 and 2005 showed that RD^{Rio} was responsible for 8% of all TB cases (24), a remarkably high rate considering the population diversity in that city. Moreover, RD^{Rio} was associated with cavitary disease in the Brazilian population (22) and a tendency (not statistically significant) to cause cavitary disease and increased transmission efficiency among U.S.-born blacks and Hispanics and persons of Latin American or Caribbean Heritage (24) as well as with drug resistance (24). To confirm and expand these findings, the present study was undertaken in Rio Grande do Sul (RS), the southernmost Brazilian state, for which no data on RD^{Rio} status and characteristics exist. In contrast to the other parts of the country, South Brazil received a high influx of Europeans (particularly Germans and Italians) in the beginning of the last century. This study was approved by the ethical committee of the School of Public Health of Rio Grande do Sul.

MATERIALS AND METHODS

Study setting. According to the World Health Organization, Brazil is on the list of high-burden TB countries and among the 22 nations in which 80% of the world's new TB cases occur. In Brazil, 81,946 new TB cases were reported in 2010, and this was translated to incidence, prevalence, and mortality rates of, respectively, 43, 47, and 2.6 per 100,000 inhabitants (25). Isolates from this study were obtained from cases diagnosed at the TB clinic of the "Sanatório Partenon Hospital" (HSP), a regional TB reference center located in Porto Alegre City, the capital of Rio Grande do Sul State, Brazil. The center is the only institution in the state to care for MDR patients. Porto Alegre City has a population of 1.44 million inhabitants and a TB incidence of 93 per 100,000 habitants, the highest in RS, with 1,506 new cases in 2008 (Instituto Brasileiro de Geografia e Estatística [IBGE]; http://www.saude.rs.gov.br/dados/1293727576139Situa%E7%E3o_TB_RS_2.pdf). Overall, Rio Grande do Sul State has a TB incidence of 47 per 100,000 habitants.

Mycobacterial strains. The present study comprises 237 *M. tuberculosis* isolates, each collected from different TB patients between 2004 and 2006. Testing of the isolates' drug susceptibility to isoniazid, rifampin, ethambutol, and streptomycin was performed at the Central Laboratory (LACEN) of Rio Grande do Sul State. MDR was defined as drug resistance to at least isoniazid and rifampin. The MDR isolates constitute the entire MDR collection available in the state at that time. The sensitive strains were a convenience sample chosen to match the MDR in terms of patient population and compliance with treatment. All strains were identified to the species level by morphological and biochemical analysis (26), and the tests for drug susceptibility were performed by the proportion method (27), using *M. tuberculosis* H37Rv ATCC 27294 as a susceptible control strain. Isolates with single-drug resistance were not included. The MDR isolates were additionally submitted to sequence analyses for polymorphisms in the *katG* and *rpoB* genes, and part of the results were published elsewhere (28).

Nucleic acid extraction. Chromosomal DNA was extracted from cultures on Löwenstein-Jensen medium, using the cetyltrimethylammonium bromide (CTAB) method as previously described (29).

Spoligotyping. Spoligotyping was performed using a commercial kit, according to the manufacturer's instructions (Isogen Biosciences B.V., Netherlands). The spoligotype patterns were compared with the updated SITVIT database, which provides information on the shared type distributions of *M. tuberculosis* spoligotypes worldwide.

Detection of RD^{Rio}. A multiplex PCR was performed to differentiate RD^{Rio} from wild-type (WT) strains as previously described (19, 21, 22). Briefly, two sets of primer pairs were used to target either the IS*1561* locus (positive only in WT strains and corresponding to a band size of 530 bp) or the region flanking the RD^{Rio} locus (positive only in RD^{Rio} strains and corresponding to a band size of 1,175 bp); the presence of both RD^{Rio} and WT strains in a single sample is indicated by the presence of both bands (22).

Clinical, radiological, and laboratory data. Complete data records were available for 103 patients, but for 7 of them both RD^{Rio} and WT bands were identified in the same clinical isolate, indicating either mixed infection or laboratory contamination. These seven cases were excluded, leaving 96 patients for analysis, including 25 drug-susceptible and 71 drug-resistant cases. The MDR group was from secondary drug resistance cases, with multiple treatments. To better match this group, the drug-sensitive cases were also from patients with irregular treatment but who had not developed drug resistance. Clinical data included gender, race, presence of hemoptysis, weight loss, fever, alcoholism, and HIV status. Radiological data included the presence of lung cavitation, homogenous infiltrate, miliary pattern, pleural effusion, and mediastinal adenopathy. Bacteriological data included smear staining for acid-fast bacilli (AFB), culture, and sensitivity to anti-TB drugs.

Statistical analysis. Continuous variables were compared by the *t* test and categorical variables by the X2 or Z-test (proportions), as appropriate. The comparison relied on logistic regression. Results for continuous variables were means ± standard deviations (SD). The two-sided 0.05 threshold was used for statistical significance. Analysis was performed using the statistical software XLSTAT (Addinsoft, France).

RESULTS

Among the 237 *M. tuberculosis* isolates, 122 (51%) were sensitive to all drugs and 115 (49%) were resistant to at least both isoniazid and rifampin, and among the latter set, 18 strains were also resistant to streptomycin and another 3 strains were resistant to ethambutol. A single strain was resistant to all four drugs, but at that time no further tests were performed to evaluate whether this strain was XDR. All isolates were spoligotyped and classified according to the spoligopatterns available in the SITVIT website. All spoligotyping patterns and genotypes (RD^{Rio} or WT) for each *M. tuberculosis* isolate are listed in Table 1.

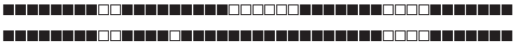
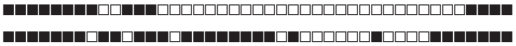
In total, LAM strains account for 53% of the MTB collection (RD^{Rio} alone accounts for 38%), whereas T and Haarlem families account for 21% and 7.6%, respectively. The other families are represented in <5% of the strains. Among the 122 susceptible isolates, 53 were LAM (43%), 29 belonged to the T family (24%), 13 were Haarlem strains (11%), 3 were of the S family, and another 3 were of the X family (2% each). In addition, nine strains were classified as U or unknown (7%) and 14 isolates (11%) had no described spoligotype and were classified as orphans. In total, the susceptible strains bunched into 9 different clusters. RD^{Rio} was exclusively responsible for 64% (34/53) of the susceptible LAM strains, 28% (34/122) of all susceptible strains, and 48% (56/115)

TABLE 1 Number of samples, MDR profile, RD^{Rio} genotype, and spoligotyping pattern for each *M. tuberculosis* patient strain from Porto Alegre City[a]

n	MDR	Genotype	Spoligotyping pattern	Spoligofamily	SIT
10	No	RD^{Rio}	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	42
5	Yes	RD^{Rio}	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	42
3	Yes	RD^{Rio}	□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	177
2	No	RD^{Rio}	■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM1	20
2	Yes	RD^{Rio}	■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM1	20
7	No	RD^{Rio}	■■□■□■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM1	729
6	Yes	RD^{Rio}	■■□■□■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM1	729
1	No	RD^{Rio}	■■□■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■□□■■	LAM2	179
1	Yes	RD^{Rio}	■■□■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■□□■■	LAM2	179
9	No	RD^{Rio}	■■□■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM2	17
10	Yes	RD^{Rio}	■■□■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM2	17
1	Yes	RD^{Rio}	■■□■■■■■■■■■□■■■■■□■□□□□■■■■■■■■□□□□■■■■■■■■	LAM2	826
1	Yes	RD^{Rio}	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	LAM4	60
23	Yes	RD^{Rio}	■■■■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM5	93
1	Yes	RD^{Rio}	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■□■■■□□□□■■■■■■■■	LAM6	64
2	Yes	RD^{Rio}	■■■■■■■■□□□■■■■■■■■□□□□□□□□□□□□□□□□□□□□■■■■■	U (likely H3)	106
1	Yes	RD^{Rio}	□□□□□□□□□□□□□□□□□□□□□□□□■■■■□□□□□□□□□□□□□□□□	U	863
1	No	RD^{Rio}	■■□■□■■□■■□□■■■■■■■■□□□□■■■■□■■■□□□□■■■■■■■□	Orphan	
1	No	RD^{Rio}	□■■■■■■■■■■■■■□■■■■■■□□□□■■■■■■■■□□□□■■□■■□■■	Orphan	
1	No	RD^{Rio}	■■■■■■■■■■■■■□□□□■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	Orphan	
1	No	RD^{Rio}	■■■□■■■■■■■■□■■□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	Orphan	
1	No	RD^{Rio}	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■□■■■□□□□■■■■■■■□	Orphan	
6	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	T1	53
7	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T1	53
1	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□□□□	T1	51
3	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□□□□■	T1	244
1	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□□□□■	T1	244
10	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	T1	65
2	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	T1	65
1	No	WT	■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■□□□□□■■■■■■■■	T1	1241
1	Yes	WT	■■■□■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T1	205
3	No	WT	□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	T2	853
1	No	WT	■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□■■■□□□□■■■□■■■	T2	392
1	No	WT	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■□■■■	T2-T3	73
1	Yes	WT	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■□■■■	T2-T3	73
1	No	WT	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T3	37
1	No	WT	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■□□□□■	T3	158
1	Yes	WT	■■■■■■■■■■■■■■■■■■■■□■■□■■■■■■■■□□□□■■■■■■■■	T5	58
7	Yes	WT	■■■■■■■■■■■■■■■■■■■■□■■□■■■■■■■■□□□□■■■■■■■■	T5-MAD2	58
1	No	WT	■■■■■■■■■■■■■■■■■■■■□■■□■■■■■■■■□□□□■■■■■■■■	T5-MAD2	58
3	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	H1	47
1	No	WT	□□□□□□□□□□□□□□□□□□□□□□□□□□□□■□□□□□■□□□□■■■■■■■■	H2	2
1	Yes	WT	□□□□□□□□□□□□□□□□□□□□□□□□□□□□■□□□□□■□□□□■■■■■■■■	H2	2
6	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■■	H3	50
1	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■■	H3	50
2	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■□	H3	615
2	No	WT	■■■■□■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■■	H3	99
1	No	WT	■■■■□□□□□□□□□□□□□□□□□□□□□■□□□□□□■□□□□■■■■■■■■	H3	487
1	No	WT	□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■□■□□□□■■■■■■■■	H3	3
2	No	WT	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□	U (likely H3)	237
6	Yes	WT	■■■■■■■■□□□■■■■■■■■□□□□□□□□□□□□□□□□□□□□■■■■■■	U (likely H3)	106
1	Yes	WT	■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■□■■■□□□□□■■■■■■■	U	396
1	No	WT	■■■■■■■■■■■■■■■■■□■■■■■■□□□□□□□□□□□□□□□□□□□□	U	450
1	No	WT	■■■■■■■■■■■■■□□□□■■■■■■□□□□□□□□□□□□□□□□□□□□□□	U	1659
1	Yes	WT	■■■■■■■■■■■■■□□□□■■■■■■□□□□□□□□□□□□□□□□□□□□□□	U	1659
5	No	WT	□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□□□□□□□□□□□□□□□□	U	863
1	Yes	WT	□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□□□□□□□□□□□□□□□□	U	863
7	No	WT	■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	42
6	Yes	WT	■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	42
1	Yes	WT	■□■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	866
3	No	WT	■■■■■■■■□□□■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM3	33

(Continued on following page)

TABLE 1 (Continued)

n	MDR	Genotype	Spoligotyping pattern	Spoligofamily	SIT
1	No	WT		LAM3	1354
4	Yes	WT		LAM3	33
1	Yes	WT		LAM3	211
1	No	WT		LAM4	391
2	Yes	WT		LAM4	60
1	Yes	WT		LAM5	93
1	No	WT		LAM5	176
6	No	WT		LAM6	64
3	Yes	WT		LAM6	64
1	No	WT		X1	119
1	Yes	WT		X2	137
2	No	WT		X3	92
1	Yes	WT		X3	92
1	No	WT		S	34
1	No	WT		S	401
1	No	WT		S	831
1	No	WT		Orphan	
1	No	WT		Orphan	
1	Yes	WT		Orphan	
1	No	WT		Orphan	
1	No	WT		Orphan	
1	No	WT		Orphan	
1	No	WT		Orphan	
1	Yes	WT		Orphan	
1	Yes	WT		Orphan	
1	Yes	WT		Orphan	
1	No	WT		Orphan	
1	Yes	WT		Orphan	
1	No	WT		Orphan	
1	No	WT		Orphan	

[a] Data from the SpolDB4 and the SITVIT website. Orphan, pattern not yet described in the SpolDB4 and the SITVIT website. SIT, shared international type number.

of the total resistance strains. This was statistically significant ($P = 0.002$). The 115 MDR isolates were classified as follows: 74 LAM strains (64%), 20 T family strains (17%), 5 Haarlem strains (4%), 2 S family strains (1.7%), 11 Unknown strains (9%), and 5 orphans (4%). RD^{Rio} alone was responsible for around one-half of all MDR strains (56/115), making up the largest family responsible for MDR strains in this study. In contrast, T and Haarlem families were responsible for 17% and 4%, respectively, of all MDR strains. IS*6110*-RFLP analysis was performed in all MDR LAM strains (data not shown). Noteworthy, a large cluster was found, composed of 22 isolates belonging to LAM5 (SIT 93), which presented three RFLP patterns: one with 20 isolates and two others belonging to a single pattern each. All were classified as RD^{Rio}. They had the same 315 AGC-ACC S-T mutation on the *katG* gene and the 531 TCG-TTG S-L mutation on the *rpoB* gene. This result identified a significant ongoing outbreak of RD^{Rio} strains.

Clinical, bacteriological, and radiological data and the presence of RD^{Rio} were analyzed for 96 patients with complete data records. The site of TB was pulmonary in 95% of the cases. A history of TB was described for 30% of the patients infected with susceptible *M. tuberculosis* strains and for 90% of those infected with MDR strains. In total, RD^{Rio} was found in 38% of all TB patients and in 34 of 71 MDR patients, constituting almost one half of all MDR strains ($P = 0.0015$) (Table 2). The other half was distributed among several spoligofamilies. The presence of RD^{Rio} was statistically associated with MDR even after logistic regression analysis. No other epidemiological, clinical, or radiological results were associated with TB caused by RD^{Rio} (Table 2).

TABLE 2 Sociodemographic, clinical, radiological, and bacteriological data for pulmonary TB patients at the time of diagnosis

Characteristic	RD^{Rio} ($n = 37$)	WT ($n = 59$)	*P* value
Gender (M/F)	28/9	37/22	0.0683
Race (white/not white)	22/15	35/24	0.5804
Weight loss	4	10	0.2177
Fever	18	23	0.8736
Hemoptysis	17	23	0.8186
Alcohol use	18	27	0.7063
Illicit drug use	14	14	0.1465
HIV positive	13	16	0.5819
Cavity or CXR	17	25	0.3658
Miliary infiltrate	4	7	0.4367
Pleural effusion	4	2	0.0934
Mediastinal adenopathy	2	1	0.8185
MDR	34	37	0.0015

DISCUSSION

There is increasing evidence that specific *M. tuberculosis* strains possess unique genetic traits and virulence phenotypes. As examples, we mention strains within the W/Beijing spoligotype that produce a modified phenoglycolipid associated with an insertion in the *pks15/1* gene and dampened induction of interleukin-6 (IL-6), IL-12, and tumor necrosis factor (TNF) (30, 31) and the CDC1551 strain that is associated with inducing a higher proinflammatory response (32). One of the mechanisms of *Mycobacterium* that could underlie the evasion from the host immune response is alteration of expression of the genes belonging to the PPE/PE_PGRS family, encoding surface proteins associated with mycobacterial virulence and host immune response (1, 33, 34). We previously speculated that in *M. tuberculosis* strains with the RD^{Rio} deletion, the loss of two PPE genes (PPE55 and PPE56) could minimize host immune recognition, leading to enhanced virulence and/or transmissibility (21). Indeed, both PPE55 and PPE56 have been shown to be expressed *in vivo* and upon entry into interferon-activated macrophages and are immunogenic in humans (35, 36). In addition, polymorphisms in PPE55 and/or PPE56 have been noted in several clinical strains of *M. tuberculosis* and *M. tuberculosis* complex (MTC) species (26, 37).

Independent of the possible mechanisms of associated virulence modification, it is clear that RD^{Rio} is presently causing TB in many parts of the world (19). The high transmissibility of RD^{Rio} strains is also suggested by studies showing an elevated secondary case rate in San Francisco, CA (38), and New York, NY (24), while a higher active-TB case rate in persons infected by RD^{Rio} strains was described in The Gambia (30). In Brazil, strains carrying RD^{Rio} have been isolated from one-third of the TB cases from two geographically distant metropolises (21, 22), a rate similar to that observed in the present study. Notably, in all of them RD^{Rio} was the main agent responsible for causing TB cases among all other spoligofamilies.

Though these data strongly suggest that RD^{Rio} may have some biological advantage, there is no proof that the clinical picture or outcome of patients infected by these strains is characteristic or even more severe than that caused by other lineages. One possible strategy used by RD^{Rio} is to induce more cavitation, as was previously demonstrated (22), which is a known risk factor for transmissibility. In the current study, however, we were unable to show an association with cavitation, and this is probably related to population selection, as the former study involved TB patients with suspected drug resistance or comorbidities. In contrast, the current study involved known MDR and drug-sensitive tuberculosis cases, where both groups included patients with multiples treatments and advanced cases, with high rates of cavitary disease, which is probably the reason for the absence of statistical difference in the presence of cavitary disease. It is interesting that preliminary data suggest that close contacts of patients infected by LAM strains (no data are available regarding the RD^{Rio} status) have more positive tuberculin skin test (TST) reactions than patients infected by other spoligofamilies (our unpublished data). As RD^{Rio} is the main component of the LAM family in Brazil, it is possible that this finding reflects a feature of the RD^{Rio}; but this needs to be further confirmed with new studies.

Our present results demonstrate that about one-half of all MDR cases were caused by RD^{Rio} strains, including a large cluster of LAM5 MDR strains as proved by IS*6110*-RFLP analysis. It should be noted that the number of patients infected by this cluster influenced the statistical result. To our knowledge, this is the first description of an outbreak of RD^{Rio} MDR strains. Additional data from other groups also indicate that RD^{Rio} could be associated with drug resistance. Gavín et al. described MDR strains of *M. tuberculosis* RD^{Rio} sublineage (LAM9 subfamily) in Spain from Equatorial Guinean patients (39), and Brown et al. showed that LAM1 (a marker for RD^{Rio}) was associated with resistance to both pyrazinamide and streptomycin in MTB cases from London, United Kingdom (33), while the study performed in New York, NY, demonstrated that RD^{Rio} was associated with resistance to isoniazid (24). Although this is a matter of speculation, it is interesting that a strain from LAM4, a subfamily that contains both WT and RD^{Rio} strains, is the major cause of XDR-TB in South Africa (17) and that both LAM9 (predominantly RD^{Rio} but also containing WT) and LAM1 (exclusively RD^{Rio}) subfamilies were major contributors to drug resistance in Russia (14, 16). Unfortunately, the status of RD^{Rio} in these strains is unknown, and the prevalence of RD^{Rio} in these places is yet to be defined. Our hypothesis is that RD^{Rio} strains are able to transmit more efficiently in certain ethnic populations such as U.S.-born blacks and Hispanics and persons who were born in Latin American and Caribbean countries as was previously demonstrated (24).

In summary, our data confirm that RD^{Rio} *M. tuberculosis* is also a major cause of TB in Southern Brazil. The mechanisms for such an impressive presence compared with other prevalent spoligofamilies are still undefined, and no particular clinical features have been associated with this genotype so far. Our hypothesis is that RD^{Rio} strains are able to transmit more efficiently in certain ethnic populations. In this context, the development of drug resistance in this successful lineage might bring additional obstacles to the control of tuberculosis, both in Brazil and in several other countries. Prospective cohort studies are needed to analyze the real contribution of the RD^{Rio} genotype to outbreaks, its role in the global TB burden, and the risk of significant development of MDR outbreaks caused by this lineage.

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