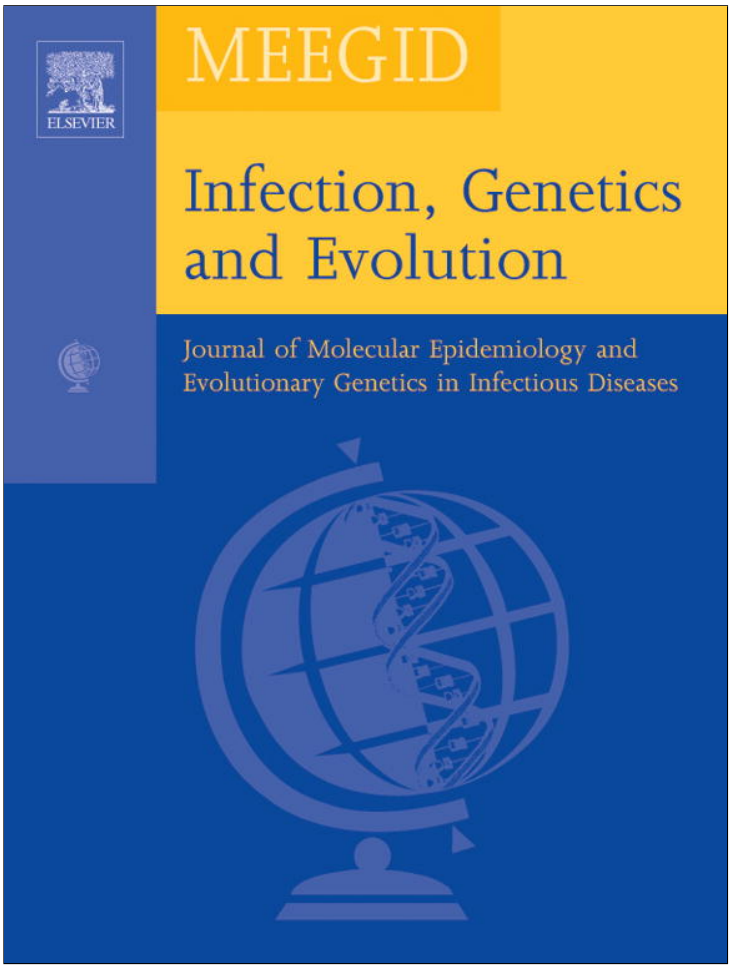

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Genotypes of *Mycobacterium tuberculosis* in patients at risk of drug resistance in Bolivia

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ABSTRACT

Bolivia ranks among the 10 Latin American countries with the highest rates of tuberculosis (TB) and multidrug resistant (MDR) TB. In view of this, and of the lacking information on the population structure of *Mycobacterium tuberculosis* in the country, we explored genotype associations with drug resistance and clustering by analyzing isolates collected in 2010 from 100 consecutive TB patients at risk of drug resistance in seven of the nine departments in which Bolivia is divided. Fourteen isolates were MDR, 29 had other drug resistance profiles, and 57 were pansusceptible. Spoligotype family distribution was: Haarlem 39.4%, LAM 26.3%, T 22.2%, S 2.0%, X 1.0%, orphan 9.1%, with very low intra-family diversity and absence of Beijing genotypes. We found 66 different MIRU-VNTR patterns; the most frequent corresponded to Multiple Locus Variable Analysis (MLVA) MtbC15 patterns 860, 372 and 873. Twelve clusters, each with identical MIRU-VNTR and spoligotypes, gathered 35 patients. We found no association of genotype with drug resistant or MDR-TB. Clustering associated with SIT 50 and the H3 subfamily to which it belongs ($p < 0.0001$). The largest cluster involved isolates from three departments and displayed a genotype (SIT 50/MLVA 860) previously identified in Bolivian migrants into Spain and Argentina suggesting that this genotype is widespread among Bolivian patients. Our study presents a first overview of *M. tuberculosis* genotypes at risk of drug resistance circulating in Bolivia. However, results should be taken cautiously because the sample is small and includes a particular subset of *M. tuberculosis* population.

1. Introduction

Tuberculosis (TB) is a worldwide spread infectious disease caused by the acid-fast bacillus (AFB) *Mycobacterium tuberculosis*. In the Americas there are an estimated 272,000 incident cases of TB, equivalent to 29 cases per 100,000 population. South America accounts for more than two thirds of the burden in the Region. Bolivia is a landlocked Andean country in South America with a population of 10 million inhabitants. It is bordered by Brazil to the North and East, Paraguay and Argentina to the South, Chile to the Southwest, and Peru to the West. Bolivia is one of the countries with the highest TB incidence rates in the Americas, and it also ranks among the top 10 countries regarding multidrug resistant (MDR) TB rate in the region. In 2010, TB notification was 135/100 000 and TB mortality 20/100 000 and MDR-TB accounted for 1.2% of new and 19% of previously treated TB cases (Pan American Health Organization, 2011; World Health Organization, 2011).

Since the early 1990s, genotyping of *M. tuberculosis* has been successfully used for epidemiologic research in a novel scientific field known today as molecular epidemiology. The obtained knowledge has high public health impact as it allows TB programs to determine risk factors for transmission at population-level, establish tailored public health strategies, and gauge the success of control measures (Kato-Maeda et al., 2011b). Strain genotyping has also proven useful for describing the global spread of different *M. tuberculosis* lineages (Brudey et al., 2006).

In those South American countries where the population structure of M. tuberculosis has been explored, the Euro-American lineage was found to be widely predominant (Abadia et al., 2009; Aristimuño et al., 2006; Candia et al., 2007; Cerezo et al., 2011; Dalla Costa et al., 2013; Ferro et al., 2011; Gomes et al., 2012; Guernier et al., 2008; Mendes et al., 2011; Taype et al., 2012). There is hardly any information on *M. tuberculosis* genetic diversity in Bolivia, and the recently launched SITVIT WEB database contains only two spoligopatterns from this country (Demay et al., 2012; <http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/>). Besides, Bolivia was not represented in a study aimed to determine MDR *M.*

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tuberculosis strain diversity in Ibero-America and to survey cross-border transmission of MDR-TB among Latin American countries and Spain (Ritacco et al., 2012a). Bolivia contributed only indirectly to that study with MDR *M. tuberculosis* genotypes of a modest number of immigrants to Argentina. In turn, Argentina is the only Latin American country where an extensive MDR-TB epidemic has been ongoing for almost two decades (Ritacco et al., 2012b). This is relevant to our study because many Bolivians who have settled in Argentina use to pay frequent visits to their homeland and, in turn, some Bolivian residents with TB travel to Buenos Aires, Argentina, seeking for higher quality medical assistance in organized, collective bus trips known as "sanitary tours".

In view of all that, the aim of this study was to identify the *M. tuberculosis* genotypes of TB patients at risk of drug resistance in Bolivia to obtain a preliminary assessment of the presence of any locally dominant drug resistant genotype(s) and shed light on the biodiversity of *M. tuberculosis* at risk of drug resistance in the country.

2. Materials and methods

2.1. Study population

In Bolivia, according to programmatic guidelines, diagnosis of pulmonary TB is mainly based on the presence of AFB on sputum smear examination. *M. tuberculosis* culture and drug susceptibility testing (DST) are performed in patients considered to be at risk for drug resistance, namely patients with TB treatment failure, default or relapse, exposed to a drug-resistant TB case, infected with HIV or presenting other immunosuppressing condition. In March–October 2010, a total of 134 AFB smear positive sputum specimens were submitted to culture and DST. The specimens were collected from the same number of consecutive adult patients (>18 years old) identified as being at risk of drug resistant TB in health centres throughout the country. Patient data regarding risk factor for drug resistant TB were collected at the departmental reference laboratories. This research was approved by the research review board of the Instituto Nacional de Laboratorios de Salud Ministerio de Salud y Deportes (INLASA), in La Paz, Bolivia.

2.2. Microbiological studies

Cultures grown on Löwenstein-Jensen slants were sent to the Mycobacteria Laboratory, INLASA. Reculture and biochemical species identification were performed according to standard procedures (Kent and Kubica, 1985). Upon reculture, 34 specimens were excluded from the study (12 were contaminated, 17 failed to grow, and 5 were identified as atypical mycobacteria). The remaining 100 mycobacterial isolates were confirmed to be *M. tuberculosis* by standard biochemical tests. The specimens were obtained in the following departments of the country: 43 in Santa Cruz, 21 in La Paz, 15 in Cochabamba, 8 in Tarija, 6 in Oruro, 5 in Chuquisaca, and 2 in Potosi. *M. tuberculosis* drug susceptibility testing (DST) to first line drugs rifampicin (RIF), isoniazid (INH), ethambutol (EMB), streptomycin (SM), and pyrazinamide (PZA) was performed at INLASA under supranational proficiency testing according to WHO standards (World Health Organization, 2009).

2.3. Genotyping

We performed spoligotyping according to the standard international protocol (Kamerbeek et al., 1997). An aliquot of each DNA sample was sent to Genoscreen (Lille, France) for 15-loci Mycobacterial Interpresed Repetitve Units (VNTR-MIRUs) typing (Supply et al., 2006).

We compared the spoligotypes in the SITVITWEB database available at the website of the Institute Pasteur Guadalupe (Demay et al., 2012, <http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/>), used the MIRU-VNTRplus database for MIRU-VNTR analysis (Allix-Béguec et al., 2008, <http://www.miru-vntrplus.org>), and for each MIRU-VNTR pattern a unique multi-locus variable number tandem repeat analysis (MLVA) MtbC15 code was assigned, when available, by using the MIRU-VNTRplus nomenclature. We applied the online tool Run TB-Lineage to predict the major *M. tuberculosis* genetic lineages (Aminian et al., 2010; Shabbeer et al., 2012; <http://tbinsight.cs.rpi.edu/run_tb_lineage.html>). The software Bionumerics v 5.1 (Applied Maths, St-Martens-Latem, Belgium) was used for MIRU-VNTR, and combined MIRU-VNTR/spoligotype clustering analysis. Clustering analysis was done using the unweighted pair group method with arithmetic averages (UPGMA). Categorical coefficients were used for MIRU-VNTR clustering analysis in which all MIRU-VNTR loci were weighted equally, and for combined analysis where MIRU-VNTR and spoligotyping were weighted equally. Isolates were considered to be in cluster when patterns showed 100% similarity in both MIRU-VNTR and spoligotype.

2.4. Statistical analyses

The Hunter–Gaston discriminatory index (HGDI) was estimated to investigate the discriminatory power of MIRU-VNTR loci (http://www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl). Genotype distributions were compared between groups of isolates (clustered and unique, drug resistant or MDR and susceptible, Santa Cruz and the rest of the country) by a chi square test, except when expected values were less than five, where a Fisher's exact test was used. A p value <0.05 was defined as statistically significant. The software MedCalc v12.2.1 was used for statistic calculations.

3. Results and discussion

3.1. Geographic distribution, risk factors and drug resistance profiles

The study sample included isolates originated from seven of the nine departments in which Bolivia is divided. Only the northernmost departments of Pando and Beni, which together account for 5% of the country population, were not represented because no TB patient at risk of drug resistance was identified there during the study period. Santa Cruz and La Paz, the most populated departments, held similar numbers of inhabitants in 2010; however, the former contributed to the study with the highest number of cases at risk of drug resistance ($n = 43$), doubling the number contributed by the latter ($n = 21$).

Risk factors for drug resistance were treatment failure in 45 cases, relapse in 26, treatment default in 19, exposure to a confirmed MDR-TB case in 3, HIV infection in 2, undetermined in 5. In our sample, Santa Cruz exhibited a higher proportion of relapses (46.5% vs 10.5%, $p < 0.001$) and a lower proportion of failures (23.3% vs 61.4%, $p < 0.001$) than all other departments whereas La Paz exhibited more failures (66.6% vs 39.2%, $p < 0.05$).

Of 43 isolates with resistance to any first line drug, 14 were MDR (i.e., resistant to at least INH and RIF). Frequencies of drug resistance patterns by geographic department are shown in Supplementary Table 1. We found no association of drug resistance or multidrug resistance with geographic origin.

3.2. Strain family distribution according to spoligotyping

The isolates displayed 42 different spoligotypes distributed as follows: 87 isolates matched 32 shared international types (SITs),

Table 1
Frequencies of 32 SITs, and 4 newly proposed SIT candidates, as identified amongst 99 *Mycobacterium tuberculosis* strains isolated in Bolivia, 2010.

SIT	Clade	Octal number	Strains in this study		Strains in SITVIT WEB		% in this study as compared to SITVIT WEB
			n	%	*n*	%	
50	H3	777777777720771	22	22.22	2188	3.76	1.0
33	LAM3	776177607760771	10	10.10	774	1.33	1.3
47	H1	777777774020771	5	5.05	1029	1.77	0.5
53	T1	777777777760771	5	5.05	3812	6.55	0.1
130	LAM3	776177607760731	5	5.05	69	0.12	7.3
521	T1	777777777760611	4	4.04	22	0.04	18.4
64	LAM6	777777607560771	3	3.03	245	0.42	1.2
373	T1	777777767760771	3	3.03	33	0.06	9.2
2	H2	000000004020771	2	2.02	337	0.58	0.6
42	LAM9	777777607760771	2	2.02	1952	3.35	0.1
49	H3	777777777720731	2	2.02	116	0.20	1.7
281	T1	777775777760771	2	2.02	22	0.04	9.2
1356	S	776377777760751	2	2.02	12	0.02	16.8
1685	LAM9	776077607760771	2	2.02	2	0.00	101.0
52	T2	777777777760731	1	1.01	526	0.90	0.2
60	LAM4	777777607760731	1	1.01	180	0.31	0.6
75	H3	777767777720771	1	1.01	41	0.07	2.5
78	T1	777777777760711	1	1.01	46	0.08	2.2
91	X3	700036777760571	1	1.01	194	0.33	0.52
93	LAM5	777737607760771	1	1.01	274	0.47	0.4
120	T1	777777577760771	1	1.01	5	0.01	20.2
180	H3	677777777720771	1	1.01	38	0.07	2.7
243	T1	777777777760600	1	1.01	17	0.03	5.9
334	T1	577777777760771	1	1.01	56	0.10	1.8
531	H1	737777774020771	1	1.01	6	0.01	16.8
791	H3	777777760020771	1	1.01	6	0.01	16.8
935	H3	775777777720771	1	1.01	10	0.02	10.1
1105	T1	777773777760771	1	1.01	10	0.02	10.1
1238	H3	777777776720771	1	1.01	4	0.01	25.3
1693	LAM5	737737607760771	1	1.01	3	0.01	33.7
2154	H1	777777434020771	1	1.01	5	0.01	20.2
2341	LAM5	777733607760771	1	1.01	2	0.00	50.5
Orphan* (MDG)	T1	777600001760771	2	2.02	1	0.002	202
Orphan* (USA)	H3	777777437720771	1	1.01	1	0.002	101
Orphan* (PRT)	nd	776177600000771	1	1.01	1	0.002	101
None**	nd	777603777760771	2	2.02	0	-	-

SIT: spoligo shared international type according to SITVIT WEB <http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/>.
* Spoligopatterns found in this study and represented by a single strain in the SITVIT WEB database, proposed as candidates for newly assigned SITs. Country codes: MDG Madagascar; USA United States of America; PRT Portugal.
** Spoligopattern absent from the SITVIT WEB database but found in >1 strain in this study, proposed as candidate for a newly assigned SIT.

12 isolates displayed patterns which did not match any SIT, and for one isolate the spoligotype was not available. In the first 32 rows of Table 1, SIT-matching spoligotypes are presented in decreasing order of frequency; the last four rows of Table 1 show four spoligotypes lacking SIT, which altogether include six isolates in our study; we propose these four spoligotypes as new candidates for SIT assignment because spoligotypes either matched orphan patterns in the SITVIT WEB database or between each other. Table 2 presents the characteristics of the six remaining isolates in our study which did not match either within each other in this study or in the SITVIT database and to our knowledge, have not been described elsewhere. Four of these six orphan strains had a LAM signature, as described by Brudey et al. (2006), suggesting that this family is rapidly evolving in Bolivia.

The frequencies of *M. tuberculosis* families among 99 isolates with spoligotype available were Haarlem (39.4%), Latin-American & Mediterranean (26.3%), ill-defined T family (22.2%), S (2.0%), X3 (1.0%). The family was not identified in 9.1% of the isolates. The most frequent subfamilies were: H3 (30.3%), LAM3 (15.2%), T1 (21.2%), and H1 (7.1%). SIT 50, found to be predominant in our

Table 2
Characteristics of 6 *M. tuberculosis* strains currently orphan of SIT, as identified in Bolivia, 2010, according to SITVIT WEB database (<http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/>).

Strain Identification	Octal number	15 loci MIRU-VNTR	MLVA MtbC15	Putativefamily*	Geographic Department
BO0113	777777400360771	254432342122217	nd	LAM	Santa Cruz
BO0162	037777700000611	263513233523344	nd	nd	La Paz
BO0236	776077703700171	233413442212337	6663	LAM3	Potosi
BO0941	776173607360731	233413442212249	nd	LAM3	Chuquisaca
BO0971	776137600360771	434433452212427	nd	LAM3	Chuquisaca
BO1591	777773775320771	253534233433325	nd	Haarlem	La Paz

SIT: spoligo shared international Type. MLVA: multiple locus variable analysis identification number according to 15 loci MIRU-VNTR 15 as designated in MIRU-VNTR*plus* database (<http://www.miru-vntrplus.org/MIRU/miruChooser.faces>). nd: not determined.
* Putative family assigned according to spoligo signatures as defined in (Brudey et al., 2006). The digit code under the 15 loci MIRU-VNTR column heading represents the numbers of repeats of each of 15 loci presented in the following order: 580, 2996, 802, 960, 1644, 3192, 424, 577, 2165, 2401, 3690, 4156, 2163b, 1955, and 4052.

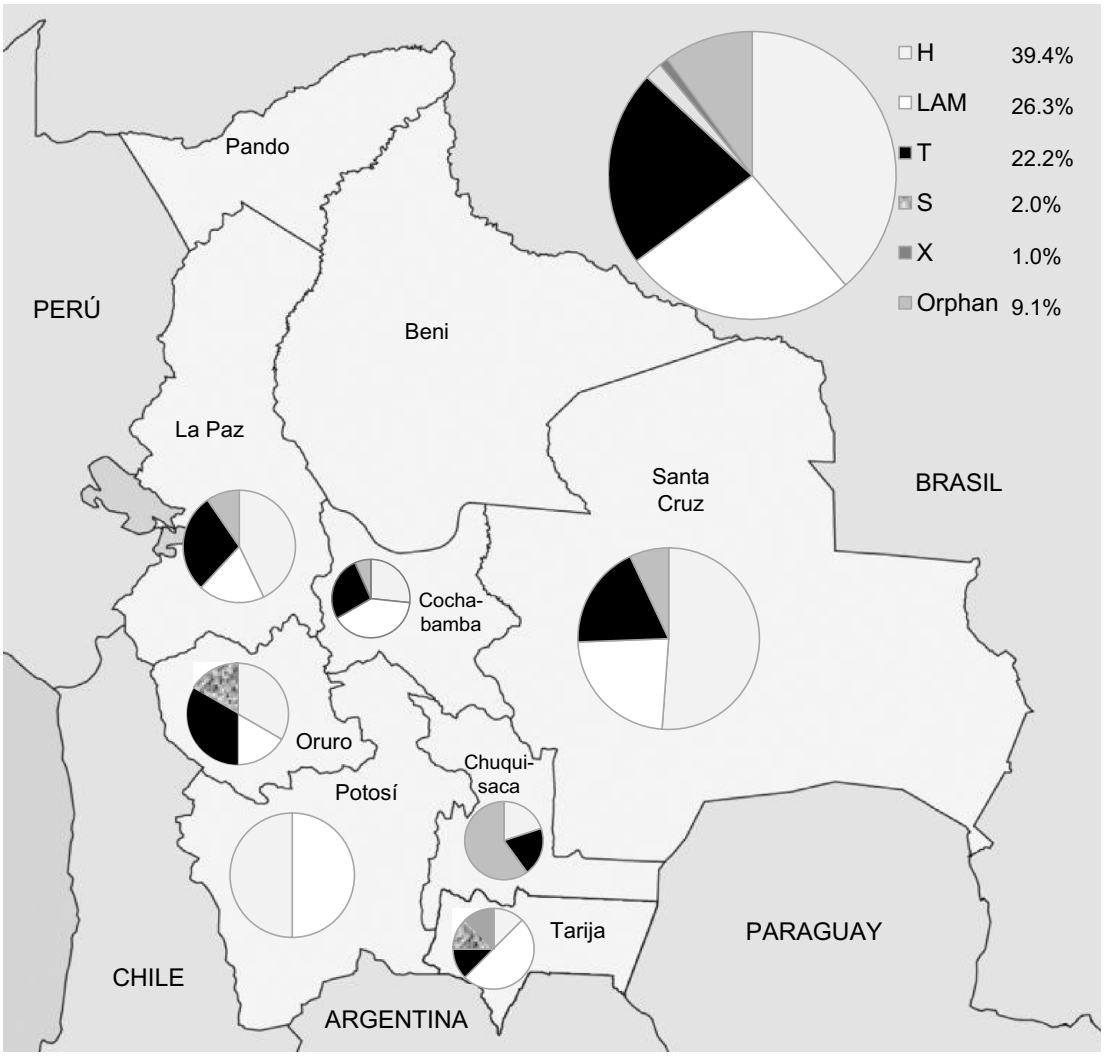

Fig. 1. Geographic distribution of spoligotype families in Bolivia, 2010.

study (22.2%), is the same SIT displayed by the two entries from Bolivia currently available in the SITVIT WEB database. The family distribution of *M. tuberculosis* by department is depicted in Fig. 1. We found no statistical difference in the distribution of families among drug susceptible and drug resistant isolates.

3.3. MIRU-VNTR strain diversity

We found 66 different MIRU-VNTR genotypes. A total of 48 isolates fitted into 14 MIRU-VNTR clusters and the remaining 52 had unique patterns. The most frequent patterns were MLVA MtbC15

Table 3
Frequencies of clustered MIRU-VNTR (15 loci) patterns among *Mycobacterium tuberculosis* isolates from 100 patients at risk of drug resistance, Bolivia, 2010.

Miru-VNTR type	MLVA MtbC15	Family (SIT)	Frequency	Department
253533233433527	860	H3, H1 (47, 50, 935, 2154)	12	Cochabamba, La Paz, Santa Cruz
2535 33233433427	372	H3 (49, 50)	4	Oruro, Santa Cruz
254333243232342	873	T1 (281, orphan)	4	Cochabamba, La Paz, Santa Cruz
233413442212437	7907	LAM3, none (33, 1985, orphan)	3	La Paz, Santa Cruz
253533233433327	2977	H3 (50)	3	Santa Cruz
263532232423139	nd	T1 (521)	3	Santa Cruz
233413542212347	nd	LAM3 (130)	3	Santa Cruz, Tarija
253533233433525	nd	H3 (50, 1238)	3	Cochabamba, Santa Cruz
143533233433527	nd	H1 (47)	3	Santa Cruz
253533232433527	1821	H3 (50, orphan)	2	Santa Cruz
256433342123236	4676	LAM5, LAM9 (42, 1693)	2	Cochabamba, Santa Cruz
256432342122237	nd	LAM5 (93, 2341)	2	Santa Cruz, Tarija
223413442212437	nd	LAM3 (33)	2	Cochabamba, La Paz
253333244232232	nd	T1 (53)	2	Oruro

MLVA: multiple locus variable analysis identification number according to 15 loci MIRU-VNTR as designated in MIRU-VNTR*plus* database (<http://www.miru-vntrplus.org/MIRU/miruChooser.faces>). SIT: spoligo shared international type. nd: not determined. The digit code under the 15 loci MIRU-VNTR column heading represents the numbers of repeats of each of 15 loci presented in the following order: 580, 2996, 802, 960, 1644, 3192, 424, 577, 2165, 2401, 3690, 4156, 2163b, 1955, and 4052.

860 and 372, both of the Haarlem family; and 873 of the T family. Table 3 presents the characteristics of the 14 MIRU-VNTR clustered patterns in decreasing order of frequency. As estimated by the Hunter-Gaston Diversity Index, the most discriminatory loci ($h > 6$) were 2163b, 4052, 960, 1955, 2996, 3690, and 2165. Six loci (VNTR 2401, MIRU 40, VNTR 577, VNTR 424, VNTR 4156, MIRU 16) showed moderately discrimination ($0.3 < h < 0.6$). The fact that only two loci had low discrimination power indicates that, within the narrow spectrum of families and sub-families shown by spoligotyping, there is enough genetic diversity in Bolivia to warrant the usefulness of MIRU-VNTR as a genotyping tool. It should be noted, however, that eight of the 14 isolates clustered by MIRU-VNTR were further split in different SITs by spoligotyping (Table 3). Therefore, for epidemiologic investigations, the combined use of both methods would substantially enhance strain discrimination in Bolivia. We found no association between MIRU-VNTR patterns or individual MIRU-VNTR alleles and drug resistance phenotype (Supplementary Table 2).

3.4. Combined analysis and phylogenetic insights

All 100 MIRU-VNTR patterns found in the study are presented in the Supplementary Figure, together with spoligopatterns and isolate characteristics in an UPGMA tree generated in BioNumerics from the MIRU-VNTR distance matrix obtained using categorical coefficients. Even though MIRU-VNTR and spoligotyping are based on markers driven by independent mechanism of variation, the branching based on MIRU-VNTR alone classified correctly virtually all the isolates of our sample within the main spoligotyping-defined families. The phylogenetic value of spoligotying has been questioned owing to the eventual occurrence of homoplasy in the DR region (Allix-Béguec et al., 2008; Comas et al., 2009). More recently, however, the accuracy of spoligotyping has been acknowledged (Kato-Maeda et al., 2011a) and proposed as the basis of a tool for lineage classification (Ozcaglar et al., 2012). The congruence found herein between MIRU-VNTR and spoligotyping further endorses the robustness of SITVIT assignation.

We determined the lineage of the orphan isolates in our study using the TB-lineage rule, a recently described online tool which predicts major genetic lineages of an isolate given its spoligotype and optionally MIRU locus 24 by applying Bayesian networks (Aminian et al., 2010; Shabbeer et al., 2012). As MIRU 24, the lineage defining locus was not present in the standard 15 loci VNTR-MIRU set used herein, our orphan isolates were predicted to belong to the Euro-American lineage on the basis of their spoligopatterns only. As for the isolate lacking spoligotype, we consulted its lineage in MIRU-VNTRplus by performing a similarity search and also by constructing a neighbour-joining tree together with the reference strains in the MIRU-VNTRplus database. Our isolate lacking spoligotype showed >0.4 similarity with the Euro-American strains in the database, and in the phylogenetic tree branched within the LAM family of this lineage.

Our results indicate that the family distribution of *M. tuberculosis* at risk of drug resistance is rather homogeneous in Bolivia, and characterized by the absolute dominion of genotypes acknowledged to belong to the modern Euro-American lineage. The ample predominance of Euro-American genotypes has been reported in South America (Brudey et al., 2006). In the published literature from other South American countries, LAM was often found to prevail (Abadia et al., 2009; Aristimuño et al., 2006; Candia et al., 2007; Cerezo et al., 2011; Dalla Costa et al., 2013; Ferro et al., 2011; Gomes et al., 2012; Guernier et al., 2008; Mendes et al., 2011), or proportions of the three main Euro-American families, namely LAM, Haarlem and T, were found to be even (Taype et al., 2012). Only two communications on genetic diversity in North Eastern Lima districts (Barletta Solari et al., 2012; Monteserin et al., 2011), and unpublished observations in Medellin (Jaime Robledo, personal communication) found Haarlem strains to predominate. It should be noted, however, that the predominance of Haarlem strains found in our study does not reflect the overall *M. tuberculosis* genotype diversity in Bolivia because the sample is

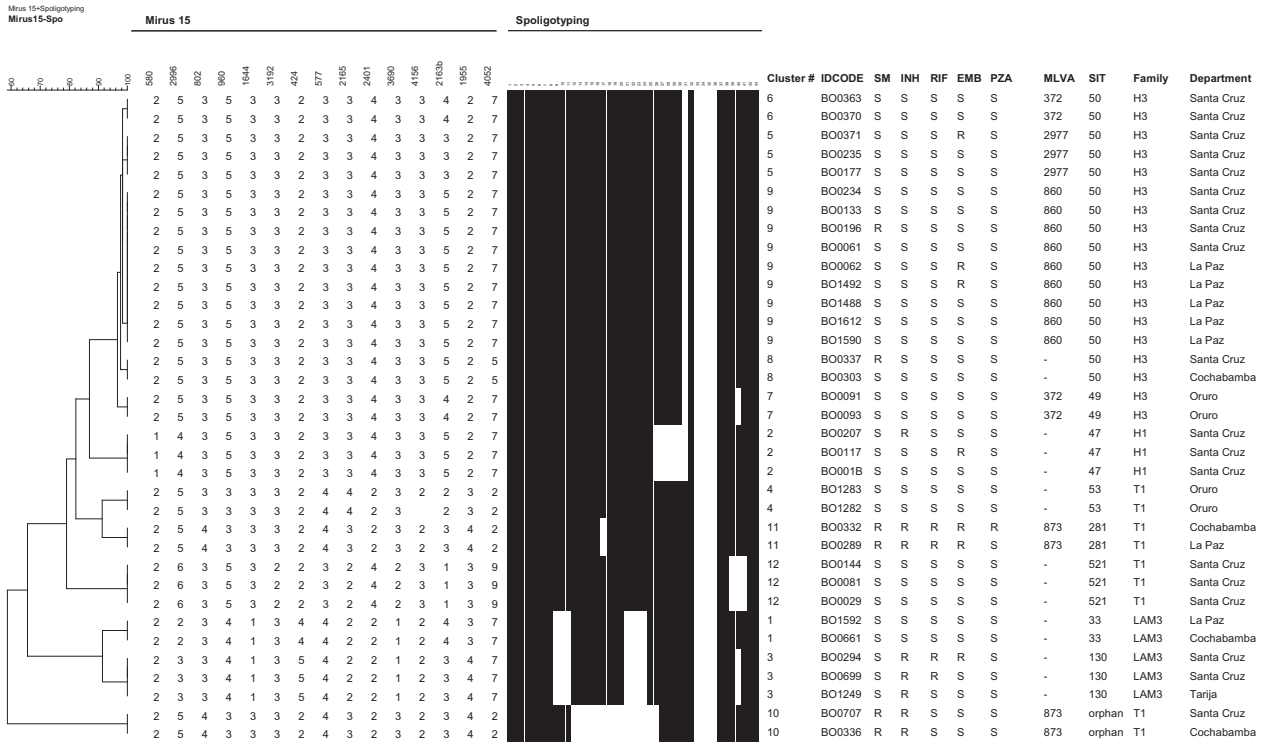

Fig. 2. Characteristics of 35 isolates involved in 12 clusters, each with identical MIRU-VNTR and spoligotype, as presented in a dendrogram constructed in BioNumerics by using the unweighted pair group method with arithmetic averages (UPGMA) from a matrix constructed using categorical coefficients of similarity.

small and involves a particular subset of *M. tuberculosis* population at risk of drug resistance. Still, the complete absence of the Beijing family in our study is noticeable considering that the neighbour country Peru is a main source of Beijing strains for other South American countries (Iwamoto et al., 2012; Ritacco et al., 2008), and that this genotype has been frequently associated with drug resistance (Parwati et al., 2010).

3.5. Clustering, epidemiological implications and limitations of the study

A total of 35 isolates were included in 12 clusters, each with perfect match of both MIRU-VNTR and spoligotype (Fig. 2). One cluster had 9 isolates and the remaining 11 had two or three cases each. We did not find association of clustering with risk factors for drug resistance, country department, or drug resistance profile. As our study was retrospective, based only on departmental laboratory records, and directed to a selected group, we did not aim to document or confirm transmission chains within genotypic clusters. We noted, however, that some clusters included isolates originated from a different department and/or displaying a different drug susceptibility profile. These isolates are unlikely to have direct epidemiologic links, and suggest the existence of certain successful genotypes more widespread than others in the country.

In particular, H3 SIT 50 was strongly associated with clustering ($p < 0.00001$) and included the largest cluster which was composed of isolates from 3 Bolivian departments (Fig. 2, cluster 9). The genotype of cluster 9 (SIT 50/MLVA 860) and that of its keen, cluster 8 (SIT 50/MLVA not determined), had been previously identified in Bolivian migrants into Spain and Argentina, and also found to share an uncharacteristic IS*6110* RFLP pattern with epidemiologically unrelated H3 strains (Ritacco et al., 2012a; Rodríguez et al., 2010). Altogether, these findings suggest that, within the otherwise globally widespread H3 SIT 50 genotype, this subset of Haarlem strains are particularly successful among Bolivian patients.

A single MDR isolate from Tarija harboring a genotype (H2 SIT2, MLVA MtbC15 1873) found to be unique in this study matched 100% the genotype of the M strain, a MDR *M. tuberculosis* strain which was responsible for a large and prolonged epidemic in Argentina (Ritacco et al., 2012b). Upon request of additional information on the MDR-TB patient from Tarija, we corroborated that he had relatives in Argentina and paid frequent visits to Buenos Aires. This finding reinforces the geographic specificity of the M strain for a narrow metropolitan hotspot area in Argentina, and its virtual absence elsewhere in South America. Apart from the patient in this study, the M strain was identified abroad only in two patients who had acquired the infection in the outbreak epicenter (Ritacco et al., 2012b).

A main limitation of our study is the small sample size, which represents only 1.1% of the total number of TB cases diagnosed in the country in the 8-month study period. For this reason, the low diversity of genotypes found herein cannot be extrapolated to the overall *M. tuberculosis* population. Also, results of statistical analysis should be taken cautiously because of the limited number of strains included in the work. In all, we consider this analysis of interest knowing that it is the first insight into genotypes of *M. tuberculosis* at risk of drug resistance in Bolivia.

4. Conclusions

The absolute predominance of modern Euro-American genotypes is in line with what is observed in other Latin American countries and might represent a heritage of the past Hispanic colonization. Haarlem strains, in particular of the H3 SIT50 genotype, were the most frequently found in this study on TB patients at risk of drug resistance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.04.010.

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