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Conspicuous multidrug-resistant *Mycobacterium tuberculosis* cluster strains do not trespass country borders in Latin America and Spain

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ABSTRACT

Multidrug-resistant *Mycobacterium tuberculosis* strain diversity in Ibero-America was examined by comparing extant genotype collections in national or state tuberculosis networks. To this end, genotypes from over 1000 patients with multidrug-resistant tuberculosis diagnosed from 2004 through 2008 in Argentina, Brazil, Chile, Colombia, Venezuela and Spain were compared in a database constructed ad hoc. Most of the 116 clusters identified by IS*6110* restriction fragment length polymorphism were small and restricted to individual countries. The three largest clusters, of 116, 49 and 25 patients, were found in Argentina and corresponded to previously documented locally-epidemic strains. Only 13 small clusters involved more than one country, altogether accounting for 41 patients, of whom 13 were, in turn, immigrants from Latin American countries different from those participating in the study (Peru, Ecuador and Bolivia). Most of these international clusters belonged either to the emerging RD^{Rio} LAM lineage or to the Haarlem family of *M. tuberculosis* and four were further split by country when analyzed with spoligotyping and rifampin resistance-conferring mutations, suggesting that they did not represent ongoing transnational transmission events. The Beijing genotype accounted for 1.3% and 10.2% of patients with multidrug-resistant tuberculosis in Latin America and Spain, respectively, including one international cluster of two cases. In brief, Euro-American genotypes were widely predominant among multidrug-resistant *M. tuberculosis* strains in Ibero-America, reflecting closely their predominance in the general *M. tuberculosis* population in the region, and no evidence was found of acknowledged outbreak strains trespassing country borders.

1. Introduction

In the past two decades multidrug-resistant tuberculosis (MDR TB), namely TB resistant at least to rifampin and isoniazid, has emerged worldwide with a transmission potential previously unsuspected (Devaux et al., 2009; Migliori et al., 2010; Schaaf et al., 2009). The prospect is grim because second line drugs needed to treat MDR TB are out of reach in vast areas of the world (WHO, 2010) and, even when properly treated, the prognosis of MDR TB is poor. As treatment success remains low, patients often

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remain smear-positive, i.e., contagious, for prolonged periods, thus increasing the chances of transmission. Besides, growing global mobility, in particular migration from lower- to higher-income areas is challenging TB control, even in well-resourced countries with low burden of the disease and efficient control programs.

In 2008, near 8400 new cases of MDR TB were estimated to occur in Latin America, with nine countries accounting for almost 90% of the cases. These countries are, in decreasing order of burden, Peru, Brazil, Ecuador, Mexico, Haiti, Dominican Republic, Argentina, Colombia, and Guatemala (WHO, 2010). In some places, the acquisition of MDR TB has been mainly ascribed to clinical and programmatic mismanagement (Escalante et al., 1998; Laserson et al., 2000; Telles et al., 2005). However, there is scarce information on MDR TB transmission within or between Latin American countries, and between these countries and Spain, which is a major destination for Latin American migrants. To our knowledge, there is only anecdotic or indirect evidence of across-border or transoceanic spread of MDR *Mycobacterium tuberculosis* strains within the Americas or to Europe (Candia et al., 2005; Codina et al., 1999; Long et al., 1999; Miramontes et al., 2007). However, such paucity of published evidence might rather be caused by insufficient international surveillance than by true lack of transmission.

In Ibero-America, large MDR TB outbreaks have been only reported in Argentina and Spain. In Argentina, certain MDR TB strains were initially transmitted among AIDS patients hospitalized in large urban health care centers (Aita et al., 1996; Morcillo et al., 1996; Ritacco et al., 1997) and later on also spread to HIV negative individuals (Palmero et al., 2003, 2005). As for Spain, a similarly large HIV-related outbreak was caused by a MDR *M. bovis* strain that happened to fit into the definition of extensive drug resistance, coined a decade later (Samper et al., 1997; Samper and Martín, 2007).

In this context, the Network for Molecular Epidemiology of Multidrug-Resistant Tuberculosis in Ibero-America (MULTITUB) was created with the support of the Latin American Science & Technology Development (CYTED) Program. Broad MULTITUB́s aims are the surveillance of transmission of MDR TB in Ibero-America, the detection of national or regional outbreaks, the identification of prevalent MDR TB strains in the region and, ultimately, the analysis of epidemiologic and molecular mechanisms facilitating transmission.

As a result of these efforts, the present work describes the diversity of *M. tuberculosis* genotypes causing MDR TB in Latin America and Spain, as determined by the merging and comparison of patterns existing in national or state databases.

2. Materials and methods

2.1. Data collection and management

Participant centers and countries were: (i) National Reference Laboratory for Tuberculosis ANLIS in Buenos Aires and Hospital Centrángolo in Vicente López, Argentina; (ii) Laboratório Central do Rio Grande do Sul in Porto Alegre and Instituto Adolfo Lutz in São Paulo, Brazil; (iii) Corporación de Investigaciones Biológicas in Medellín, Colombia; (iv) Instituto de Salud Pública de Chile in Santiago, Chile; (v) Instituto Pedro Kuri in Havana, Cuba; (vi) National Program of Tuberculosis and Instituto de Biomedicina in Caracas, Venezuela; and (vii) Instituto Aragonés de Ciencias de la Salud in Zaragoza, Spain. Participants were asked to provide available epidemiologic and genotyping data on all consecutive MDR TB isolates in the study period with available genotypes. Data from Argentina, Chile, Cuba and Spain encompassed all cases of MDR TB diagnosed in the study period. As for Venezuela, complete country coverage was available only for year 2004. For Brazil, the databases contained all patients diagnosed in Sao Paulo and Rio Grande do Sul States in the study period and the Colombian collection included all MDR TB patients detected in the area of Medellin in the period.

A total of 1346 *M. tuberculosis* genotypes from patients diagnosed with MDR TB in the participant countries from 2004 through 2008 were collected at the University of Zaragoza, together with basic microbiological, clinical and demographic data.

All personal identification was removed from the data sent to the University of Zaragoza for analysis. The study was approved by the ethics board of each participant institution and the Research Ethics Committee of Aragon, which authorized a waiver of informed consent from the human research subjects who provided the samples.

2.2. Genotype analysis

IS*6110* restriction fragment length polymorphism (RFLP) DNA fingerprinting (van Embden et al., 1993), spoligotyping (Kamerbeek et al., 1997) and VNTR-MIRUs-15 (Supply et al., 2006) were performed according to international standards. Causes of exclusion were: (i) genotype quality unsuitable for analysis, (ii) isolates in cluster with less than six RFLP bands lacking spoligotype, and (iii) second isolate of the same patient.

A dedicated genotype database was constructed with the aid of the software BioNumerics 5.1 (Applied Maths, St-Martens-Latem, Belgium) at the headquarters in the University of Zaragoza by merging bundles containing IS*6110* patterns and spoligotypes that had been generated in the different laboratories. Every IS*6110* RFLP bundle included at least one entry with the pattern of the reference strain Mt14323. Parameters were adjusted in such a way that Mt14323 patterns of all bundles matched 100% when compared with 1% tolerance by using the Dice coefficient for similarity analysis and the unweighted pair-group method with arithmetic averages for dendrogram construction (Heersma et al., 1998).

Strain family and spoligo-shared international types (SITs) were assigned according to SpolDB4, the international database at the Institute Pasteur of Guadeloupe (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/) (Brudey et al., 2006).

The following definitions were adopted: a cluster was a group of two or more patients whose genotypes had 100% identical IS*6110* RFLP patterns of six or more bands or 100% identical spoligotypes if identical IS*6110* RFLP patterns had less than six bands; a national cluster was that containing patients diagnosed in only one participant country; an international cluster was that containing patients diagnosed in two or more participant countries; a Latin American cluster (LAT-cluster) was an international cluster containing patients diagnosed in two or more participant Latin American countries; an Ibero-American cluster (IBA-cluster) was an international cluster containing patients diagnosed in Spain and one or more participant Latin American countries.

Isolates belonging to the Latin American and Mediterranean (LAM) family and grouped in international clusters were subjected to identification of RD^{Rio} lineage and characterization of mutations conferring resistance to rifampin. RD^{Rio} multiplex PCR was done following a recently published protocol (Gibson et al., 2008). Mutations conferring resistance to rifampin were determined by sequencing of the 91 bp "hot spot" region of the *rpoB* gene (Telenti et al., 1993).

Strains were classified as belonging to the Beijing genotype when their IS*6110* RFLP patterns showed more than 80% similarity to any strain in a panel of 19 selected Beijing reference patterns (kindly provided by Kristin Kremer, RIVM, the Netherlands) and confirmed by the characteristic spoligotype with absence of spacers 1–34 and hybridization to at least three of spacers 35–43 (Kremer et al., 2004).

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2.3. Statistical analysis

In order to identify risk factors for clustering, univariate analysis was performed using the *t*-test and *U* test for continuous variables and the Chi-square test for categorical variables. Variables identified by univariate analysis, and those identified in previous studies, were considered in a multivariate logistic regression model to identify with factors independently associated with clustering in MDR TB patients. Data were analyzed with SPSS version 14.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Demographic and clinical profile of patients

Of a total of 1346 registered entries, 87 did not meet inclusion criteria. MIRU-VNTR15 genotypes were only available for other 181 patients from three countries (Cuba, Venezuela and Spain); as no cluster was identified among these patients, the genotype method was excluded from further analysis. Altogether, five South American countries (Argentina, Brazil, Chile, Colombia, and Venezuela) and Spain delivered IS*6110* RFLP patterns of 1078 patients, referred hereafter as the study population. Spoligotype was available in 951 patients (Fig. 1).

Demographic and clinical profiles of the 1078 MDR TB patients in the study population are summarized in Table 1. The male to female ratio was 1.77. The mean age was 37.51 years (95% CI 36.55–38.48). Pulmonary disease was the predominant clinical presentation (502/531, 94.5%); 318/426 (75%) patients had smear-positive TB, and 133/170 (78%) reported a history of previous treatment for TB. The study included patients born in four continents: 971 (90.0%) were born in Latin America, 44 (4.1%) in Spain, 31 (2.8%) in other European country, 16 (1.5%) in Africa and 4 (0.4%) in Asia. A total of 155 (14.4%) patients were known to be immigrants to the country of isolate but the country of birth was not known for 12 (1.1%). Spain, with 82 immigrants, was the main destination followed by Argentina with 71. Altogether, migrants from European, African or Asian countries accounted for 51 entries and Spain was the country of destination for all but three of them. Latin American countries not participating in the study, but contributing with migrants, were Peru with 37 patients, Bolivia with 31, Ecuador with 10, Paraguay with three, Dominican Republic with two and Mexico with one. Only eight entries corresponded to migrants between countries participating in the study (Supplementary Table 1).

Table 1
Characteristics of clustered and unclustered patients with multidrug-resistant tuberculosis in five Latin American countries and Spain, 2004–2008, and their association with cluster status.

Characteristic	Nonclustered no. patients (%)	Clustered no. patients (%)
Gender (no. patients: 1043)		
Male	282 (63.2%)	384 (64.3%)
Female	164 (36.8%)	213 (35.7%)
Age (no. patients: 859)		
Median age, years	38.9	36.5
(IQR)	(37.3–40.4)	(35.2–37.7)
<15	11 (2.9%)	16 (3.3%)
15–44	241 (64.6%)	357 (73.5%)
45–64	101 (27.1%)	95 (19.5%)
⩾65	20 (5.4%)	18 (3.7%)
Origin (no. patients: 1066)		
Latin America	392 (86.5%)	579 (94.5%)
Spain	24 (5.3%)	20 (3.3%)
Other	37 (8.2%)	14 (2.3%)
Previous treatment (no. patients: 170)		
No	17 (18.7%)	20 (25.3%)
Yes	74 (81.3%)	59 (74.7%)
Site of disease no. patients: 531)		
Pulmonary	250 (94.0%)	252 (95.1%)
Other	16 (6.0%)	13 (4.9%)
Smear test result (no. patients: 426)		
Negative	41 (22.5%)	67 (27.5%)
Positive	141 (77.5%)	177 (72.5%)

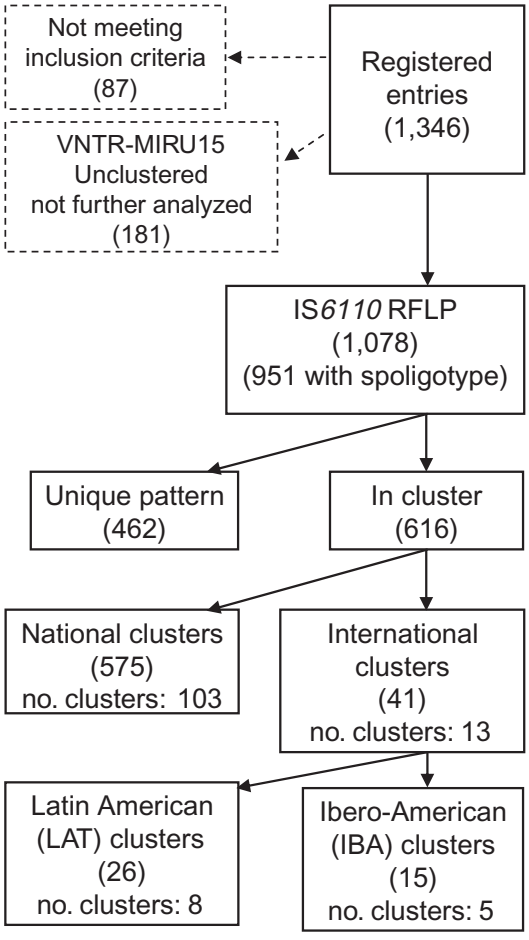

Fig. 1. Distribution of 1346 multidrug-resistant *Mycobacterium tuberculosis* genotype entries in the MULTITUB database, originated from six Latin American countries and Spain, 2004–2008. (Number within brackets indicate number of entries.)

3.2. Strain family distribution

Among 951 entries with spoligotype available, the predominant strain families were LAM (n: 354) and Haarlem (n: 275), followed by T (n: 166) (Fig. 2 and Supplementary Table 2). Undetermined and orphan patterns accounted for 91 patients. The less represented Euro-American genotypes were X (5 patients in Argentina, 8 in Brazil and 4 in Spain), S (7 patients in Argentina and 5 in Spain). The Beijing genotype family was identified by RFLP and confirmed by spoligotyping in 31 patients. Of these, 21 corresponded to immigrants to Spain (17 born in Asia or Eastern Europe, 3 born in Peru, and 1 born in Colombia). Ten patients with Beijing genotypes were detected in the participant Latin American countries: four in Argentina (1 Indonesian, 1 Peruvian, and 2 with undisclosed country of birth), three in Brazil (born in Brazil), two in Colombia (born in Colombia), one in Venezuela (born in Venezuela but known contact of a Peruvian MDR TB immigrant). The CAS family was represented by one Asian immigrant to Spain. *M. bovis*

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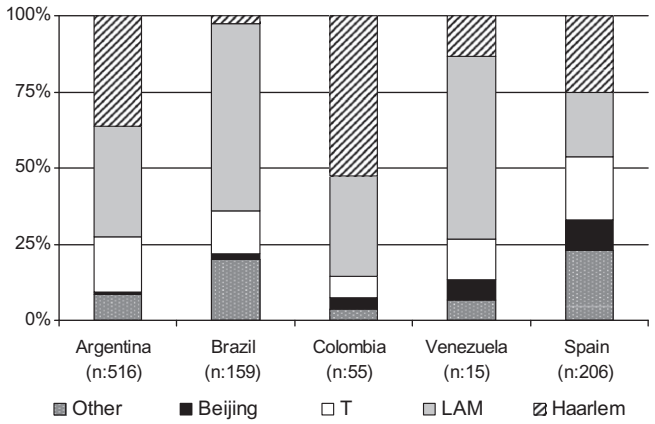

Fig. 2. Distribution of *Mycobacterium tuberculosis* families according to spoligotypes of 951 patients with multidrug-resistant tuberculosis from five Latin American countries and Spain, 2004–2008.

accounted for four entries (2 patients in Spain, 2 patients in Argentina).

3.3. IS6110 RFLP clustering

Genotyping showed 462 isolates (42.8%) with unique patterns and 616 isolates (57.1%) sharing patterns grouped into 616 clusters (Fig. 1). A total of 575 (53.3%) of 1078 patients were grouped in 103 national clusters, each containing 2–116 patients (Table 2). The percentage of patients in cluster ranged from 30% in Spain to 66% in Argentina. The three largest national clusters were found in Argentina, and corresponded to acknowledged outbreak strains: two variants of a strain of the Haarlem 2 SIT 2 genotype (116 and 25 cases, respectively) and a strain of the LAM3 SIT 33 genotype (49 cases). The fourth and fifth largest clusters were found in Brazil (23 cases) and Spain (9 cases), and belonged to the LAM2 and H1 strain family, respectively.

Only 13 clusters, consisting in 2–7 patients each, contained patients in more than one country.

Altogether, these international clusters included 41 (3.8%) patients, of whom 14 fitted into the Haarlem family and the same number into the LAM family (Table 3 and Supplementary figure). The only international cluster belonging to the Beijing family consisted in one Peruvian immigrant in Argentina and one Venezuelan patient whose source case was a Peruvian immigrant with MDR TB. Every participant country contributing with IS*6110* RFLP patterns was represented in at least one international cluster. All international clusters involved only two countries, except for cluster IBA-3, which involved three countries. Spoligotyping allowed to split four international clusters by country (LAT-2, LAT-4, IBA-3, and IBA-5), supporting their belonging to national, rather than international, clusters.

As described in Table 3, 26 patients were included in eight LAT-clusters and 15 patients were included in five IBA-clusters. LAT-2 was the largest international cluster and contained patients from Argentina and Colombia. This IS*6110* RFLP pattern, which belongs to the Haarlem family, was found to be prevalent among susceptible strains in databases of Argentina and Spain (data not shown). LAT-8 grouped five patients from Brazil and Colombia. Spain contributed to five IBA clusters with only one patient each. Only one of these five patients from Spain was a native Spaniard, another one was born in Equatorial Guinea and the remaining three were born in Latin America.

3.4. RD^{RIO} and resistance-conferring mutations in clustered LAM strains

All 14 LAM strains fitting into international clusters had the RD^{RIO} specific deletion, confirming their belonging to this lineage. Of these LAM RD^{RIO} clusters, LAT-4, IBA-3 and IBA-5 were disclosed by country when spoligotyping was applied. Likewise, patients from different countries included in LAT-4 and IBA-5 differed in the *rpoB* gene rifampin-resistance conferring mutations.

3.5. Association of clustering with demographic parameters

Characteristics significantly associated with clustering according to the univariate analysis (Table 1) were age and place of origin. Patients in clusters were significantly younger (median age 36.5 years; P_{25} to P_{75}, 37.3–40.4) than those not in clusters (median age 38.9 years; P_{25} to P_{75}, 37.3–40.4) ($P = 0.01$). In the multivariate analysis, only being Latin American origin (adjusted odds ratio [OR] 4.0, 95% confidence interval [CI] 2.0–8.1) and Spaniard (adjusted odds ratio 2.9, 95% CI 1.1–7.5) remained strongly associated with clustering (data not shown). Association of clustering with pulmonary disease, previous TB and sputum smear positivity was not investigated in view of the paucity of available information.

4. Discussion

The Euro-American lineage has been reported to be widely prevalent in South America (Brudey et al., 2006), the subcontinent contributing to our study with the majority of entries. Current knowledge indicates that, within the Euro-American lineage, three genotype families (Haarlem, LAM, and T) are the most prevalent in the region, with the LAM family accounting for 40–74% of the strains, Haarlem and T families competing for the second place, and all three families largely outnumbering families X and S (Aristimuño et al., 2006; Candia et al., 2007; Lazzarini et al., 2007). The frequency of the Beijing genotype in the general population was found to be below 1% in most of the surveyed South American countries (Aristimuño et al., 2006; Candia et al., 2007; Lazzarini et al., 2007; Morcillo et al., 2005; Telles et al., 2005), while it amounted to 5.9% in Lima, Peru and 11% in the port of

Table 2
Number of multidrug-resistant tuberculosis patients with genotypes available for analysis, as reported to the MULTITUB network project by five Latin American countries and Spain, 2004–2008, and number of patients in national and international clusters.

	No. patients (% of total patients in study)	No. patients (%) in national clusters	No. national clusters	Lowest-highest no. patients per national cluster	No. patients (%) in international clusters
Argentina	498 (46.2)	322 (64.6)	33	2–116	13 (2.6)
Brazil	350 (32.5)	175 (50.0)	46	2–23	14 (4.0)
Chile	13 (1.2)	5 (38.5)	2	2–3	1 (7.7)
Colombia	69 (6.4)	29 (42.0)	9	2–5	4 (5.8)
Venezuela	15 (1.4)	3 (20.0)	1	2–3	4 (26.7)
Spain	133 (12.3)	41 (30.8)	12	2–9	5 (3.8)
Total	1078 (100)	575 (53.3)	103	2–116	41 (3.8)

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Table 3
Shared international type (SIT), strain family and number of patients in 13 international IS*6110* RFLP clusters found among 1078 patients with multidrug-resistant tuberculosis, distributed by country of origin, in five Latin American countries and Spain, 2004–2008.

Cluster	SIT	Family	ARG	BRA	CHL	COL	VEN	SPA	Total
LAT-1	92	X3	1[a]	2					3
LAT-2	49, 50	H3	5[a]			2			7
LAT-3	50	H3	1		1				2
LAT-4	17, 20	LAM1, LAM2		1			1		2
LAT-5	1	Beijing	1[a]				1[b]		2
LAT-6	335	H3	1	1					2
LAT-7	53	T1		2		1			3
LAT-8	177	LAM9		4		1			5
IBA-1	47	H1	2[a]					1	3
IBA-2	91	X3	1					1[c]	2
IBA-3	891, 828, Or	LAM9, LAM4	1	3				1[d]	5
IBA-4	53	T1					2	1[b]	3
IBA-5	177, 828	LAM9, LAM4		1				1[e]	2
TOTAL			13	14	1	4	4	5	41

SIT: Shared International Type. ARG: Argentina, BRA: Brazil, CHL: Chile, COL: Colombia, VEN: Venezuela, SPA: Spain. Or: orphan. Country of origin of patients, when proven different from country of isolate is symbolized as follows.
[a] Peru.
[b] Contact of a Peruvian immigrant with MDR TB.
[c] Ecuador.
[d] Bolivia.
[e] Equatorial Guinea.

Buenaventura, Colombia (Laserson et al., 2000; Ritacco et al., 2008). Other main lineages known to be prevalent in East Africa, India, and Far East Asia were found to be sporadic in South America.

The overall distribution of MDR *M. tuberculosis* spoligotype families in the South American countries surveyed herein reflected closely the ample predominance of the Euro-American lineage in the general population of the region. LAM and Haarlem were the strain families most frequently found in our MDR TB sample of 951 spoligotype entries. LAM predominated largely over Haarlem in the participant Brazilian states and, to a lesser extent, in Venezuela. Haarlem strains prevailed in the surveyed area of Colombia, and both lineages were equally represented in Argentina. A much larger lineage diversity was observed in Spain, with a more even distribution. This is not surprising for a country with a marked cosmopolitan population, where MDR TB is a problem essentially linked to immigration from various continents, as shown by our finding of only one out of 44 native-born Spaniards involved in a potential transnational MDR TB transmission event. The Beijing family accounted for 10.2% (21/206) of MDR TB patients in Spain (3 born in Peru, 1 born in Colombia, 17 born in Eastern Europe or Asia). Altogether, Beijing strains had a much more modest presence among MDR TB patients in South America (1.3%, 10/745).

National clusters varied largely in size, with clustering being substantially higher in Latin American countries than in Spain. Again, this finding is consistent with the fact that Spain hosts immigrants from various different African, Latin American and Eastern European countries bearing heavy loads of TB. Still, the main cause of MDR TB clustering at the national level in Latin America was the ongoing transmission in Argentina of formerly documented outbreak MDR *M. tuberculosis* strains (Aita et al., 1996; Ritacco et al., 1997). Of these strains, the most conspicuous is the so-called strain M of the Haarlem 2 subfamily. Attention should be drawn to the fact that Argentina was the Latin American country most extensively sampled, which is not fortuitous: Argentina is the only country in the region where extensive and prolonged MDR TB transmission has been documented to date and, to survey such transmission, countrywide MDR TB genotyping has been systematically performed since 2003 in this country. Noticeably, apart from the Argentinean entries associated to this strain, Haarlem 2 was not represented elsewhere in the study, not even in the neighboring territories of Chile and Rio Grande do Sul in Brazil (Supplementary Table 2). Contrastingly, the aftermaths of a similarly large MDR TB outbreak produced by a *M. bovis* strain in Spain (Samper et al., 1997) are represented in this study by only two patients. Although both MDR outbreaks happened simultaneously, and the responsible strains are still circulating within their respective countries, the sharp difference in national cluster sizes of both strains denotes the much more efficient long-term containment of the infection achieved in Spain.

Trans-national clustering represented 9.0% (13/144) of all potential transmission events and involved 3.8% (41/1078) of patients with available RFLP. In a sense, our study mirrors the one performed on MDR TB in the European region (Devaux et al., 2009), although the conditions held in both studies are dissimilar. Ours includes a larger number of genotyped isolates (1078 versus 672), while the number of countries covered is much smaller (6 versus 19 in the European study). Even though both studies are not fully comparable, certain differences are worthy of mention. Differences are not particularly related to the number of IS*6110* RFLP clusters involving more than one country (18 clusters in Europe versus 13 Ibero American clusters). Instead, the main difference lays on the smaller size of international clusters in our study and, more specifically, on the absence of representation of strains causing large epidemics at the national level. Again, it should be stressed, however, that the country coverage of our study is incomplete, what, understandably, lowers the chances for detecting international transmission events.

There is no ground to assume that Latin American countries are undertaking more efficient infection control measures than European countries to preclude international transnational spreading of local outbreak strains. Autochthonous Latin American MDR *M. tuberculosis* strains seem to have a pattern of circulation that is restricted to particular areas by reasons that might be beyond public health interventions. As previously speculated, this geographical affinity might be rather related to individual strain treats, host ethnicity, and human-pathogen co-evolution (Gagneux et al., 2006).

M. tuberculosis strains of the Beijing family have contributed largely to the global spread of TB (Parwati et al., 2010). The Beijing genotype has been associated with increased incidence of TB and MDR TB in many parts of the world (Glynn et al., 2002). This seems not to be the case in Latin America where Beijing strains were found to be localized in certain areas (Diaz et al., 1998; Laserson et al., 2000; Murcia et al., 2010). Our results suggest that this

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genotype has little influence on the epidemiology of MDR TB in the region. In this sense, Peru has been pointed out as a possible gate of entry for Beijing strains into other South American countries and Spain (Codina et al., 1999; García de Viedma et al., 2006; Aristimuño et al., 2007; Ritacco et al., 2008). Our findings contributed, although very modestly, to this hypothesis, with only one transnational cluster of the Beijing genotype, which linked one Peruvian immigrant in Argentina and one contact of a Peruvian immigrant with MDR TB in Venezuela.

The lineage RD^{Rio} was first described in Brazil as afflicting 30% or more of the TB patients in the surveyed sites, and was also found to be significantly associated with severe pulmonary involvement (Lazzarini et al., 2007, 2008). This emerging lineage was identified in all four international clusters belonging to the LAM family in our study, all of them with a strong Brazilian representation. One of these clusters was IBA-3, the only one in the study that involved three countries, where the Spanish contribution happened to correspond to a Latin American immigrant. Spain contributed to IBER-5, another RD^{Rio}Cluster, with an immigrant born in Africa. This patient had been previously found to fit into a MDR TB cluster detected in Spain among African patients coming from the Gulf of Guinea (Gavín et al., 2009). It is tempting to speculate that this Afro-Latin American cluster might be related to past or present transoceanic trade between the Gulf of Guinea and Brazil.

Still, the largest international cluster (LAT-2) did not belong to the RD^{Rio} lineage but to the Haarlem strain family. This cluster included two Colombian patients and five Peruvian immigrants in Argentina. The 7-band IS*6110* RFLP pattern representative of this cluster had been previously ascribed to epidemiologic-unrelated isolates in five countries in Latin America and Spain and the genotype had been further split by country of origin, using spoligotype, VNTR-MIRUs, or both (Alonso Rodríguez et al., 2010). To this IS*6110* RFLP cluster belonged also the new Tacumbu strain described in Paraguay, which seems to be country-specific (Candia et al., 2007). Such ubiquity seems to be a feature of a few IS*6110* RFLP patterns belonging to the LAM and Haarlem families. These epidemiologically-equivocal RFLP patterns, represented in our study by clusters LAT-2, LAT-4, IBA-3 and IBA-5, could well accommodate a number of successfully emerging strains undergoing independent clonal expansion in different geographical settings (Devaux et al., 2009).

This work has several limitations. First, the number of Latin American countries included in the study is low and countries with the highest rates of TB, and especially of MDR TB, were not included. In fact, those Latin American countries are not represented because, at the time of the study, they had not yet established systems of *M. tuberculosis* genotype surveillance, either continuous or based on representative samples. Although a substantial input to the database consisted of immigrants from some of those countries, like Peru, Ecuador, Bolivia and Paraguay, the existence of further undetected transnational potential transmission events cannot be ruled out. Second, in some countries represented in the study, namely Brazil and Colombia, the geographic coverage of the sample was only subnational, and Venezuela provided data on patients identified in only one year. Third, clinical and epidemiologic information was largely incomplete and therefore statistical associations of clustering should be taken cautiously. All these limitations were caused by the retrospective design of our study, which used the information that was available in the participant laboratories. Although imperfect and not fully representative of the region under study, the data presented herein display the first supranational view of MDR TB genotypes circulating in Ibero American countries.

Unfortunately, Cuba could not be included in the analysis because VNTR-MIRUs, the only genotype patterns provided by this country, were only available, and in small numbers, from two other countries, thus precluding any meaningful inference. In this respect, the study uncovers the fact that, to date, most Latin American TB network laboratories have not yet incorporated VNTR-MIRUs genotyping for *M. tuberculosis* genotyping on a routine basis. These laboratories find it difficult to implement either the manual or the automated procedure and to further translate the RFLP data gathered in existing national databases into the MIRUs language.

It is concluded that: (i) the Euro-American lineage predominates among MDR *M. tuberculosis* strains circulating in Latin American countries and Spain, (ii) the detected potential MDR TB transnational transmission events are relatively small in size, although the existence of further undetected transnational clusters cannot be ruled out due to incomplete sampling, and (iii) there is no evidence of previously-documented outbreak MDR *M. tuberculosis* strains trespassing country borders. By launching a database and analyzing the extant genotype information, the MULTITUB network has accomplished its main objective of undertaking the surveillance of MDR TB transmission in Ibero-America. This knowledge is useful for national and international interventions aimed to a more stringent control of MDR TB in Ibero-America.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.06.006.

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