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Characterization of Extensively Drug-Resistant Tuberculosis Cases from Valle del Cauca, Colombia

Extensively drug-resistant tuberculosis (XDR-TB), which is TB resistant to isoniazid and rifampin plus one fluoroquinolone and a second-line injectable drug, represents an obstacle for the treatment and control of TB. Previously, we reported four XDR-TB cases and a high proportion of the Beijing genotype among multidrug-resistant TB (MDR-TB) isolates in the state of Valle del Cauca, Colombia (3), where a MDR-TB hot spot had been identified (7). According to the information of the local TB program, to date 21 XDR-TB cases have been diagnosed in the country, 14 of which were from this state.

With the approval of the Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM) Review Board, we characterized the XDR-TB cases detected in Valle del Cauca in the period 2001 to 2009, including their clinical and epidemiological features.

Resistance profiles were identified using the proportion method on 7H10 agar (6) and confirmed by a supranational laboratory. Molecular characterization of the isolates was performed by spoligotyping (5) and 24-locus variable-number tandem-repeat (VNTR) typing (8). GenoType MTBDR*plus* and -*sl* assays (Hain Lifescience GmbH) were applied to identify mutations associated with resistance to first- and second-line anti-TB drugs. Shared types and lineages of *Myco-*

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TABLE 1 Phylogenetic and epidemiological data of XDR-TB isolates from Valle del Cauca, Colombia[m]

UPGMA Tree	ID	Spoligotype Lineage (SIT)[a]	MIRU-VNTR pattern (MIT)[b]	Year	Sex/ Age	Initial condition	BCG	HIV	Clinical outcome	Mutations			
										katG	*inhA*	*rpoB*	*gyrA*
	01	777777774020731 **H1 (SIT62)**	225325153323333554234423 **(MIT101)**	2001[e]	M/36	R	Yes	Neg	Died	S315T1[f]	WT2[h]	526-529[i]	D94N or D94Y
	02	777777774020731 H1 (SIT62)	225325153323333374234423 **(Orphan)**	2007	F/22	F	Yes	Neg	OT	S315T1[f]	WT	526-529[i]	D94N or D94Y and D94G
	03	776377777740731 **S (SIT3010)**	232325153324424352314234 **(Orphan)[c]**	2007	M/30	R	Yes	Neg	Died	WT	WT	S531L	D94G
	04	776377770000731 **Unk (SIT881)**	232325153322424352314234 **(Orphan)[c]**	2009	F/43	F	Yes	Neg	OT	S315T1[f]	WT	510-517[j]	D94G and D94A
	05	675737607760771 **LAM2 (SIT545)**	224226153321224382334133 **(Orphan)**	2009	M/46	F	Yes	Neg	OT	S315T2[g]	WT2[h]	S531L	D94G
	06	000000000003731 **Beijing (SIT190)**	223325171441524992264433 **(MIT46)[d]**	2009	M/44	R	Yes	Neg	OT	S315T1[f]	WT	S531L	A90V and D94G[k]
	07	000000000003731 Beijing (190)	223325171431524992264433 **(MIT45)[d]**	2009	M/30	N	Yes	Pos	Died	S315T1[f]	WT	S531L	D94G
	08[l]	000000000003731 Beijing (190)	223325171431524992264433 (MIT45)[d]	2007	F/24	N	NA	NA	Died	S315T1[f]	WT	S531L	D94G
	09[l]	000000000003731 Beijing (190)	223325171431524992264433 (MIT45)[d]	2007	F/16	N	NA	NA	Died	S315T1[f]	WT	S531L	D94G
	10	000000000003731 Beijing (190)	223325171421524992264433 **(Orphan)[d]**	2009	M/29	F	Yes	Neg	Died	S315T1[f]	WT	S531L	D94G

[a] Spoligotypes are shown in octal format; lineages are designated according to the SITVIT2 database.
[b] MIT numbers represent mycobacterial interspersed repetitive unit (MIRU) international types determined using 24 loci. The 8 unique profiles are defined as MIT101, -45, and -46 and five different orphan patterns.
[c] Strains 03 and 04, which differ at a single MIRU locus (4 and 2 copies for MIRU-40, respectively), showed related spoligotype binary patterns SIT3010 and SIT881; hence, it is possible that SIT881 evolved by loss of a single block of spacers 25 to 31 either directly from SIT3010 or from a linked ancestor.
[d] All the Beijing SIT190 strains (ID 06, 07, 08, 09, and 10) are highly related, since they represent a single locus variant in the 24-locus typing scheme (4, 3, 3, 3, and 2 copies for MIRU-39, respectively).
[e] Diagnosed in 2001 but classified as an XDR-TB case until 2007 according to the WHO definition.
[f] S315T1: base exchange at codon 315, AGC to ACC.
[g] S315T2: base exchange at codon 315, AGC to ACA.
[h] For this isolate, there was no hybridization either in the WT2 probe which analyzed nucleic acid in the position 8 or in either of the two mutations described.
[i] This gene region is defined by the absence of WT7 probe which could represent any of these mutations: H526R, H526P, H526Q, H526N, H526L, H526S, and H526C.
[j] Isolate with an absence in the hybridization probes WT2 and WT2/WT3 that could have represented any of these mutations: Q513L, Q513P, and del514–516.
[k] Heteroresistant strain.
[l] Epidemiologically linked cases.
[m] Abbreviations: BCG, *Mycobacterium bovis*; bacillus Calmette-Guérin vaccination SIT, spoligotype international type; ID, identification number; Unk, unknown; NA, data not available; M, male; F (in column 6), female; R, relapse; F (in column 7), failure of treatment with first-line drugs; N, new case; OT, on treatment; Neg, negative; Pos, positive; WT, wild type. Numbers in column 6 represent age in years.

TABLE 2 Worldwide distribution of the *M. tuberculosis* shared types (SIT) present among the 10 Colombian XDR-TB isolates as determined using the SITVIT2 database

SIT (clade) octal no. (SITVIT2 database no.)	Spoligotype	Distribution (%) in regions with ≥3% of a given SIT(s)[a]	Distribution (%) in countries with ≥3% of a given SIT(s)[b]
SIT62 (H1) 777777774020731 (497)		AMER-S 40.24, EURO-S 13.68, AMER-N 13.68, EURO-W 8.05, AFRI-E 4.02, AFRI-W 3.62	COL 37.63, USA 13.68, ITA 8.25, ESP 4.22, FXX 3.62, GMB 3.42
190 (Beijing) 000000000003731 (179)		ASIA-E 35.2, AMER-N 32.96, AMER-S 16.76, ASIA-SE 5.59	USA 32.96, CHN 24.58, COL 16.2, JPN 6.7, KOR 3.35
545 (LAM2) 675737607760771 (8)		AMER-S 50.0, EURO-W 25.0, AMER-N 25.0	COL 50.0, USA 25.0, FXX 25.0
881 (unknown) 776377770000731 (72)		AMER-S 76.0, AMER-N 16.0, EURO-S 8.0	COL 72.0, USA 16.0, VEN 4.0, ITA 4.0, ESP 4.0
3010 (S) 776377777740731 (9)		AMER-S 77.78, EURO-S 11.11, AMER-N 11.11	COL 66.67, USA 11.1, PER 11.1, ITA 11.1

[a] Worldwide distribution is reported for regions with SITs representing ≥3% of their total number in the SITVIT2 database. The definition of macrogeographical regions and subregions (http://unstats.un.org/unsd/methods/m49/m49regin.htm) is according to the United Nations. Regions: AFRI (Africa), AMER (Americas), ASIA (Asia), EURO (Europe), and OCE (Oceania), subdivided into E (eastern), M (middle), C (central), N (northern), S (southern), SE (southeastern), and W (western). Furthermore, CARIB (the Caribbean) belongs to the Americas, while Oceania is subdivided in 4 subregions, AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Note that in our classification scheme, Russia has been assigned a new subregion by itself (northern Asia) instead of including it among the rest of eastern Europe. That assignment reflects its geographical localization as well as due to the similarity of specific TB genotypes circulating in Russia (a majority of Beijing genotypes) to those prevalent in central, eastern, and southeastern Asia.

[b] The 3-letter country codes are according to http://en.wikipedia.org./wiki/ISO_3166−1_alpha-3; countrywide distribution is shown only for SITs representing ≥3% of their total number in the SITVIT2 database.

bacterium tuberculosis isolates were determined using the SITVIT2 database (property of Institute Pasteur de la Guadeloupe).

In total, 10 XDR-TB patients were identified. Their median age was 30 years, and two cases (representing friends) were epidemiologically linked. All patients suffered from either bilateral disease or compromised lung function, and one underwent pneumonectomy. Three patients were labeled as primary resistant cases as they had no history of TB, suggesting active transmission of XDR-TB. The remaining patients had been intermittently exposed to second-line drugs. Six patients had a fatal outcome, one of them being HIV positive (Table 1).

Shared types/lineages SIT190/Beijing, SIT62/H1, SIT881/unknown, SIT545/LAM2, and SIT3010/S were identified by spoligotyping. These genotypes, combined with the VNTR 24-locus results, yielded eight unique profiles, with MIRU-39 being the only locus that discriminated between the highly related Beijing/SIT190 strains (Table 1). The Beijing genotype was the most frequent (5/10), and one specific subtype, MIT45, was especially present among younger patients (≤30 years old), new TB cases, and fatal outcomes (Table 1).

Non-Beijing strains 03 and 04 differed only in a single locus (MIRU-40) and also revealed the highly related spoligopatterns SIT3010 and SIT881 (Table 1). It is conceivable either that SIT881 evolved directly from SIT3010 or that they both derived from a common ancestor.

The distribution of the 5 XDR-TB shared types and lineages identified by spoligotyping in this state compared to the worldwide distribution revealed their high geographical specificity for South America and for Colombia in particular (Table 2).

The most frequent mutations associated with resistance to isoniazid and rifampin were S315T1 (80%) and S531L (70%) in the *katG* and *rpoB* genes, respectively. These mutations were previously shown to be related to MDR-TB transmission in Europe (2). A1401G (100%) and D94G (90%) were the most common mutations in the *rrs* and *gyrA* genes, associated with resistance to aminoglycoside-cyclic peptides and fluoroquinolone, respectively (Table 1).

Most of the XDR-TB patients had been treated with moxifloxacin, and all isolates had mutations in the *gyrA* gene; however, nine were phenotypically susceptible to this drug; suggesting alternative mutations or other resistance mechanisms. Moreover, one isolate (ID 03) did not exhibit concordance between the phenotypic and genotypic susceptibility profiles for isoniazid, emphasizing the importance of phenotypic drug susceptibility testing as the basis for treatment.

The presence of the same mutations in the Beijing isolates, in addition to the highly similar VNTR patterns, suggests the clonal expansion of this particular Beijing lineage. Haarlem SIT62/H1 was the second most frequent (2/10) sublineage, as in previous observations in Colombia (Table 2) (3). Multiple mutational events and clonal expansion of one or more hypervirulent and resistant strains (e.g., Beijing and Haarlem) may be occurring in Valle del Cauca (1, 4).

The observed genetic relatedness between most of our XDR-TB isolates (Table 1), as well as their high geographical specificity (Table 2), highlights the emergence of XDR-TB strains within a pool of actively transmitted *M. tuberculosis* clinical isolates, which is highly alarming. Moreover, despite continuous efforts, a need remains to advance drug susceptibility testing and individualized treatment availability, especially since these patients have a prolonged infectious stage, resulting in a greater dissemination of these strains.

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