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.

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

<table>
<thead>
<tr>
<th>UPGMA Tree</th>
<th>ID</th>
<th>Spoligotype Lineage (SI)</th>
<th>MIRU-VNTR pattern (MIT)</th>
<th>Year</th>
<th>Sex/ Age</th>
<th>Initial condition</th>
<th>BCG</th>
<th>HIV</th>
<th>Clinical outcome</th>
<th>Mutations</th>
<th>katG</th>
<th>inhA</th>
<th>rpoB</th>
<th>gyrA</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>2235251613323333594234423 (MIT101)</td>
<td>2001</td>
<td>M/36</td>
<td>R</td>
<td>Yes</td>
<td>Neg</td>
<td>Died</td>
<td>S315T1</td>
<td>WT2</td>
<td>526-529</td>
<td>D94N or D94Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>223525161332333394234423 (Orphan)</td>
<td>2007</td>
<td>F/22</td>
<td>F</td>
<td>Yes</td>
<td>Neg</td>
<td>OT</td>
<td>S315T1</td>
<td>WT</td>
<td>526-529</td>
<td>D94N or D94Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>22352516133241542314234 (Orphan)</td>
<td>2007</td>
<td>M/30</td>
<td>R</td>
<td>Yes</td>
<td>Neg</td>
<td>Died</td>
<td>WT</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>22352516133241542314234 (Orphan)</td>
<td>2009</td>
<td>F/43</td>
<td>F</td>
<td>Yes</td>
<td>Neg</td>
<td>OT</td>
<td>S315T1</td>
<td>WT</td>
<td>510-517</td>
<td>D94G and D94A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>22352516133222438234143 (Orphan)</td>
<td>2009</td>
<td>M/44</td>
<td>R</td>
<td>Yes</td>
<td>Neg</td>
<td>OT</td>
<td>S315T1</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>223525161332415422464433 (MIT46)</td>
<td>2009</td>
<td>M/30</td>
<td>N</td>
<td>Yes</td>
<td>Pos</td>
<td>Died</td>
<td>S315T1</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>223525161332415422464433 (MIT46)</td>
<td>2007</td>
<td>F/24</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>Died</td>
<td>S315T1</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>223525161332415422464433 (MIT46)</td>
<td>2007</td>
<td>F/16</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>Died</td>
<td>S315T1</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>223525161332415422464433 (Orphan)</td>
<td>2009</td>
<td>M/29</td>
<td>F</td>
<td>Yes</td>
<td>Neg</td>
<td>Died</td>
<td>S315T1</td>
<td>WT</td>
<td>SS51L</td>
<td>D94G</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Spoligotypes are shown in octal format; lineages are designated according to the SITVIT2 database.
- 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.
- 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.
- 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).
- Diagnosed in 2001 but classified as an XDR-TB case until 2007 according to the WHO definition.
- S315T1: base exchange at codon 315, AGC to ACC.
- S315T2: base exchange at codon 315, AGC to ACA.
- D94G: base exchange at codon 510, GAG to GAG.
- 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.
- 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.
- Isolate with an absence in the hybridization probes WT2 and WT2/WT3 that could have represented any of these mutations: H526R, H526P, H526Q, H526N, H526L, H526S, and H526C.
- Heteroresistant strain.
- Epidemiologically linked cases.
- Abbreviations: BCG, Mycobacterium bovis; bacillus Calmette-Guérin vaccine; 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.
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).

<table>
<thead>
<tr>
<th>SIT (clade) octal no. (SITVIT2 database no.)</th>
<th>Spoligotype</th>
<th>Distribution (%) in regions with ≥3% of a given SIT(s)</th>
<th>Distribution (%) in countries with ≥3% of a given SIT(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIT62 (H1) 777777774020731 (497)</td>
<td><img src="image" alt="Spoligotype" /></td>
<td>AMER-S 40.24, EURO-S 13.68, AMER-N 13.68, EURO-W 8.05, AFRI-E 4.02, AFRI-W 3.62</td>
<td>COL 37.63, USA 13.68, ITA 8.25, ESP 4.22, FXX 3.62, GMB 3.42</td>
</tr>
<tr>
<td>190 (Beijing)</td>
<td><img src="image" alt="Spoligotype" /></td>
<td>ASIA-E 35.2, AMER-N 32.96, AMER-S 16.76, ASIA-SE 5.59</td>
<td>USA 32.96, CHN 24.58, COL 16.2, JPN 6.7, KOR 3.35</td>
</tr>
<tr>
<td>545 (LAM2) 6757360707671 (8)</td>
<td><img src="image" alt="Spoligotype" /></td>
<td>AMER-S 50.0, EURO-W 25.0, AMER-N 25.0</td>
<td>COL 50.0, USA 25.0, FXX 25.0</td>
</tr>
<tr>
<td>881 (unknown)</td>
<td><img src="image" alt="Spoligotype" /></td>
<td>AMER-S 76.0, AMER-N 16.0, EURO-S 8.0</td>
<td>COL 72.0, USA 16.0, VEN 4.0, ITA 4.0, ESP 4.0</td>
</tr>
<tr>
<td>3010 (S) 776377777740731 (9)</td>
<td><img src="image" alt="Spoligotype" /></td>
<td>AMER-S 77.78, EURO-S 11.11, AMER-N 11.11</td>
<td>COL 66.67, USA 11.1, PER 11.1, ITA 11.1</td>
</tr>
</tbody>
</table>

4 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) consists of several 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) consists of several subregions.

5 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.

6 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.

**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.

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) lineage, 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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We declare no potential conflict of interest relevant to this article.

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Luisa Maria Nieto
Beatriz E. Ferro
Sonia L. Villegas
Centro Internacional de Entrenamiento e Investigaciones Médicas—CIDEIM
Cali, Colombia

Carolina Mehaffy
Colorado State University
Fort Collins, Colorado, USA

Liliana Forero
Secretaría Departamental de Salud del Valle
Cali, Colombia

Cesar Moreira
Secretaría de Salud Pública Municipal
Buenaventura, Colombia

Nalin Rastogi
WHO Supranational TB Reference Laboratory
Institute Pasteur de la Guadeloupe, Abymes
Guadeloupe, France

Dick van Soolingen
Tuberculosis Reference Laboratory
National Institute for Public Health and the Environment (RIVM)
Bilthoven, The Netherlands

Department of Pulmonary Diseases/Department of Clinical Microbiology
Radboud University Nijmegen Medical Centre
Nijmegen, The Netherlands