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# Population Dynamics of Tuberculous Bacilli in Cameroon as Assessed by Spoligotyping

**Francioli Koro Koro,[a,b] Yannick Kamdem Simo,[a] Félix Fotso Piam,[a] Jurgen Noeske,[c] Cristina Gutierrez,[d] Christopher Kuaban,[e] Sara Irène Eyangoh[a]**

Mycobacteriology Service, Reference Laboratory of NTP, Centre Pasteur of Cameroon, Cameroon-Pasteur Institute International Network, Yaoundé, Cameroon[a]; University of Yaoundé I, Faculty of Sciences, Department of Biochemistry, Yaoundé, Cameroon[b]; German International Cooperation (GIZ), Yaoundé, Cameroon[c]; Foundation for Innovative New Diagnostics, Geneva, Switzerland[d]; University of Yaoundé I, Faculty of Medicine and Biomedical Sciences/Jamot Hospital, Yaoundé, Cameroon[e]

**Genetic assessment by spoligotyping of 565 *Mycobacterium tuberculosis* complex strains collected from the Western Region of Cameroon between 2004 and 2005 has confirmed the establishment of the "Cameroon family" as the leading cause of tuberculosis in 45.9% of cases and evidenced the rapid quasi extinction of *Mycobacterium africanum*, isolated in 3.3% of tuberculosis cases.**

Tuberculosis (TB) remains a major cause of illness and death worldwide, especially in Africa and Asia (1). In Cameroon, the incidence of all clinical forms of tuberculosis is about 25,000 new cases per year (National Tuberculosis Programme [NTP] report no. 16, May 2011).

The understanding of tuberculosis transmission dynamics and epidemiology has been greatly enhanced by molecular epidemiologic studies using various DNA typing techniques in conjunction with classical epidemiological approaches (2). These techniques exploit various DNA elements as markers to assess the overall dissemination of strains. One such method is spoligotyping, which analyzes DNA polymorphism observed in the spacer sequences found in the direct repeat (DR) locus of the *Mycobacterium tuberculosis* complex (MTBC) genome. This technique is simple, rapid, and robust and has the advantage of enabling simultaneous distinction between MTBC isolates at subspecies level. It also measures overall diversity, thus providing information about major lineages. Furthermore, an international database has been established for the purpose of comparison of MTBC spoligotypes (3).

An initial retrospective study on the characterization of *M. tuberculosis* genotype strains collected in the Western Region of Cameroon was carried out between July 1997 and June 1998. This area was chosen by the NTP as a model to assess the impact of various interventions. The application of spoligotyping showed the striking regression of *Mycobacterium africanum* as an etiologic agent of tuberculosis from 50% in the 1970s to 9% in the 1990s (4). This study also showed the predominance of a group of strains named "Cameroon family" strains, representing 43% of tuberculosis cases and designated LAM-10 CAM in SpolDB4. Further analysis confirmed that the lack of spacers 22, 23, and 24 was the specific signature of this family due to the removal of IS*6110* in the DR region (5).

To further investigate the genetic diversity evolution and the dynamics of the dissemination of strains in the Western Region of Cameroon, spoligotyping was used to perform a retrospective analysis on a new collection of strains obtained during a 1-year survey (February 2004 to March 2005) to evaluate the impact of tuberculosis control on resistance to antituberculosis drugs, 7 years after the first study.

**Bacterial strains and spoligotyping.** Bacterial strains used in this study were isolated at the Mycobacteriology Reference Laboratory of the Centre Pasteur of Cameroon from pulmonary tuberculosis patients in the Western Region over a 1-year period (February 2004 to March 2005). All sputum smear-positive cases aged more than 15 years were included in the prospective survey. One sputum sample was collected from each patient in a transport medium solution (0.6% cetyl pyridinium bromide) and sent to the Mycobacteriology Reference Laboratory. Each sample was cultured on Löwenstein-Jensen (LJ) medium and LJ medium supplemented with 0.4% pyruvate. We isolated 622 MTBC strains identified by biochemical and phenotypical methods: 25 of *M. africanum*, 596 of *M. tuberculosis*, and one of *Mycobacterium bovis*. Of the 622 strains of MTBC isolated and kept frozen, 565 MTBC isolates were successfully subcultured on LJ medium and LJ medium supplemented with 0.4% pyruvate. The rest of the isolates could not be typed due to either contamination or the inability to revive them from subculture. DNA was extracted from each strain by using colonies grown on LJ medium. Spoligotyping to detect the 43 spacers was performed at the Mycobacteriology Reference Laboratory of the Centre Pasteur Cameroon using a commercially available kit (Isogen Biosciences BV, Maarsen, the Netherlands) as previously described (6). Results were entered into Microsoft Excel and compared with an international database, SITVIT2. Spoligotypes which could not be matched with those in the database were considered novel. For the other strains, the spoligotyping defined shared international types (SITs), and the corresponding family was designated. The Bionumerics program, version 5.10 (Applied Maths, Kortrijk, Belgium), was also used for comparison and dendrogram construction.

**Biodiversity analysis.** A total of 94 distinct spoligotypes were obtained from the set of 565 MTBC strains. Of the 94 spoligotypes,

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Address correspondence to Sara Irène Eyangoh, eyangoh@pasteur-yaounde.org.

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**FIG 1** Dendrogram and schematic representation of 94 spoligotypes identified from 565 *M. tuberculosis* complex strains isolated from positive pulmonary tuberculosis patients from West Cameroon. The degree of similarity of spoligotypes was calculated with the 1-Jaccard index, and the relationships between patterns were assessed by the unweighted-pair group method using average linkages. The spoligotype code, the shared international type (SIT), and lineage are listed. ND, not determined. CAM, Cameroonian spoligotype corresponding to a strain isolated in this study.

49 patterns matched those found in SpolDB4 and 45 were identified as novel (Fig. 1).

Of the total 565 MTBC strains, 19 were identified as *M. africanum*, representing 3.3% of MTBC strains. This result shows a real regression of *M. africanum* compared to the 9% described in the 1990s ($P = 0.0001$) (4). *M. africanum* appears to be really polymorphic; the 19 strains were divided into 13 spoligotypes, 7 of which were novel. The Afri-2 family previously reported as a majority in West African countries counted 15 strains (7).

The 546 strains identified as *M. tuberculosis* were split into 81 different spoligotypes. Of the 565 MTBC strains, a total of 260 strains belonged to the Cameroon family, representing 45.9% and 47.6%, respectively, of the *M. tuberculosis* isolates, compared with 43% and 47%, respectively, found in the late 1990s (4). The largest cluster of this family was formed by 186 isolates grouped in SIT 61. The other commonly described lineages were Haarlem (103 isolates), T1 (55 isolates), and T2 (48 isolates), which together showed an increasing trend. Figure 2 shows the evolution of the

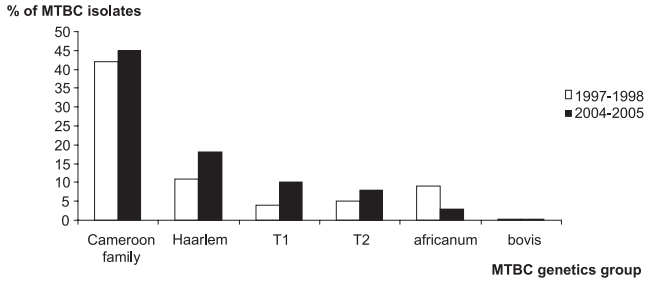


**FIG 2** Histogram showing the 7-year evolution of the MTBC population in West Cameroon.

MTBC population over a period of 7 years. As was the case in our previous study (4), none of the strains presented the spoligotype of the worldwide-expanded epidemic Beijing family; one strain was identified as *M. bovis*.

Analysis of genetic diversity of strains in the Western Region of Cameroon shows that the Cameroon family is well established and comprises nearly half of the isolates. This family belongs to the modern lineage group 2, including major epidemic strains (8). The Cameroon family, as suggested by different studies on the Beijing family (9), could also have some selective advantages over other *M. tuberculosis* genotypes present in Cameroon, including virulence, pathogenesis, and epidemiologic characteristics. However, geographic confinement could also explain the predominance of the Cameroon family. Data on the genetic diversity of MTBC in sub-Saharan Africa suggested that this family is mainly found in Central and West African and Caribbean countries (5). Other, larger studies should be carried out for bordering countries.

The analysis also shows that *M. africanum* seems to be disappearing in Cameroon, a Central African country, unlike in West African countries, which continue to report between 20% and 39% of cases attributable to this species (7, 10–13). Previous studies have raised potential limitations concerning the difficulties in isolating and identifying *M. africanum* due to metabolic constraints (14) and the bias that may have characterized studies that concluded that *M. africanum* was regressing in Africa (11). In our laboratory, we avoid these limitations by using for primoculture LJ medium supplemented with 0.4% pyruvate, the culture medium that has to date shown a better recovery rate for *M. africanum* than that in other media. Other studies using MGIT Bactec medium for primoculture found higher rates of *M. africanum* (15, 16). We have recently processed 1,920 respiratory specimens (from June 2011 to June 2012) using in parallel LJ medium, LJ-pyruvate medium, and the MGIT Bactec medium for primoculture, and we observed that the addition of MGIT Bactec has not increased the recovery of *M. africanum* in our setting (unpublished data; personal observation). These data are in favor of a real difference from West African countries and support the geographical pattern of *M. africanum* distribution found by de Jong et al. (7). Unless the recovery of *M. africanum* is difficult due to well-known metabolic constraints which could bias its prevalence, the noted regression in Cameroon seems genuine, since the results of 2 studies performed under the same conditions and in the same area were compared. In Cameroon, despite the high polymorphism of *M. africanum*, the mean age of *M. africanum*-infected people is 33 years (range, 19 to 69 years), and this is an indication of an ongoing transmission. Larger studies should be carried out under optimal conditions for *M. africanum* recovery in order to better define its distribution in terms of the geographical location of the country.

The dynamic approach of tracing changes in the MTBC population structure over years is not commonly applied, but it could contribute to highlighting clade-specific pathogenic characteristics (17).

Our study provides the first depiction of the molecular population dynamics of *M. tuberculosis* complex strains in Cameroon over a 7-year period. The reasons for the successful adaptation of the Cameroon family, the regression of *M. africanum*, and the absence of Beijing strains need to be explored in order to better understand the major forces driving the transmission dynamics within specific populations, which may significantly impact TB control and vaccine development strategies.

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S.I.E. and C.K. conceived and designed the experiments. S.I.E., F.K.K., Y.K.S., and F.F.P. performed the experiments. S.I.E., F.K.K., and C.G. analyzed the data. S.I.E. wrote the first draft of the paper. C.G. designed figures. All authors provided critical input.

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