

Global Distribution of *Mycobacterium tuberculosis* Spoligotypes

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We present a short summary of recent observations on the global distribution of the major clades of the *Mycobacterium tuberculosis* complex, the causative agent of tuberculosis. This global distribution was defined by data-mining of an international spoligotyping database, SpolDB3. This database contains 11,708 patterns from as many clinical isolates originating from more than 90 countries. The 11,708 spoligotypes were clustered into 813 shared types. A total of 1,300 orphan patterns (clinical isolates showing a unique spoligotype) were also detected.

Since the publication of the second version of our spoligotypes database on *Mycobacterium tuberculosis* (1), the causative agent of tuberculosis (TB), the proportion of clustered isolates (shared types [STs]) increased from 84% (2,779/3,319) to 90% (11,708/13,008). Fifty percent of the clustered isolates were found in only 20 STs. Three of these isolates are *M. bovis*, including *M. bovis* BCG (ST 481, 482, and 683). The

addition of the next 30 most frequent STs increased the total proportion of clustered isolates (65% instead of 50% initially).

A total of 36 potential subfamilies or subclades of *M. tuberculosis* complex have been tentatively identified, leading to the definition of major and minor visual recognition rules (Table). The ancestral East-African Indian family (EAI) is made up of at least five main subclades, whereas at least three major spoligotyping patterns are found within the Haarlem family (2). Two families found in central and Middle Eastern Asia (CAS1 and CAS2) are newly defined. The X family (3) is also currently split into at least three well-defined subclades. However, the subdivision of family T (T1–T4, likely to represent relatively old genotypes), which differs from the classic ST 53 (all spacers present except 33–36), remains poorly defined. Similarly, the Latino-American and Mediterranean family (LAM) is tentatively split into subclades LAM1–LAM10 (4). Spoligotyping used alone is not well suited for studying the phylogeny of these two clades (T and LAM). Such study will require results from other genotyping methods such as IS6110-restriction fragment length polymorphism (5) or mycobacterial interspersed repetitive units–variable number of DNA tandem repeats (6). Among well-characterized major clades of tubercle bacilli, four families represent 35% of 11,708 clustered isolates (Beijing 11%, LAM 9.3%, Haarlem 7.5%, and the X clade 7%).

The global distribution of the most frequently observed spoligotypes by continent in SpolDB3 is as follows. Among the patterns originating in North America (n= 4,276, 32% of the total number of isolates in the database), 16% of the strains are of the Beijing type, 14% belong to ST 137 or ST 119 (X family), and 8% are unique (results not shown). In Central America (n=587, 4.5%), 8% of the strains belong to the ubiquitous ST 53, 7% are ST 50, and 6% are ST 2; the last two STs are part of the Haarlem family. In South America (n=861, 6.6%), the distribution of ST 53 and ST 50 accounts for 10% and 9%, respectively, of the spoligotypes, whereas ST 42 accounts for as much as 9% of the total isolates. The origin of ST 42 remains to be established. In Africa (n=1,432, 11%), ST 59 and ST 53 account for 9% of all isolates studied thus far; however, the values obtained for ST 59 are biased because strains from Zimbabwe are overrepresented. We also observed that *M. africanum* ST 181 accounts for as much as 6% of all spoligotypes from Africa in our sample.

In Europe (n=4,360, 33.5%), ST 53 represents as much as 9% of the spoligotypes, ST 50 and 47 (Haarlem family) repre-

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sent 8% of the cases, and the Beijing family accounts for 4% of the spoligotypes. In the Middle Eastern and central Asian region, where the number of samples obtained is still very low (n=351, 2.7%), a high diversity of strains within the EAI and CAS families has been observed, and no single pattern currently exceeds 5%. Further studies of isolates from these regions are needed, e.g., in India, where our sampling is still anecdotal (n=44 isolates). Notwithstanding the scarcity of available data from this region, the observed diversity suggests that this region might be of great interest for further study of the genetic variation of tubercle bacilli. Contrary to what we observed for the Middle East and central Asia, the Far East Asian region (n=801, 6.1%) is characterized by the prevalence of a single genotype, the Beijing type family, a family linked to emerging multiresistance (7). One out of two strains in the Far East is a Beijing type. In Oceania (n=340, 2.6%), ST 19 and Beijing account for 15% and 13%, respectively, of clustered isolates. Thus, this preliminary analysis of the spoligotype distribution of SpolDB3 clearly shows major differences in the population structure of tubercle bacilli within the eight subcontinents studied (Africa; Europe; North America; Central America; South America; Middle East and Central Asia; Far East Asia; and Oceania).

At present, SpolDB 3 is an experimental tool that has yet to prove its usefulness in tracking epidemics. Nevertheless, the facility with which matches between spoligotypes can be detected suggests that this tool may be a good screening mechanism for population-based studies on recent TB transmission. Indeed, the detection of a rarely found ST in SpolDB3 may be a catalyst that signals researchers to look for the clonality of the isolates and to study their epidemiologic relatedness.

Data-exchange protocols through inter-networking will also be implemented in the near future. Working groups such as the European Network for Exchange of Molecular Typing Information (available from: URL: www.rivm.nl/enemti) are coordinating such initiatives. The expanded use of the Bionumerics software (third upgrade; Applied Maths, St. Martens-Latem, Belgium) may also foster this research field. SpolDB3 will also be instrumental in facilitating better understanding of the driving forces that shape tubercle bacilli evolution. Further research should now emphasize the use of data-mining methods, in combination with experts' knowledge, to tackle the complex dynamics of the population's genetics of tubercle bacilli and TB transmission (3). Our sample represents the compilation of many national studies and, as such, should be considered as an ongoing population-based project aimed at studying global TB genetic diversity. Nevertheless, obtaining a more precise and representative snapshot of the genetic variability of *M. tuberculosis* complex will require a larger sampling. Although only partially representative of worldwide spoligotypes of *M. tuberculosis* complex, SpolDB3 contains a reservoir of genetic information that has already proved useful for defining the phylogenetic links that exist within the TB

genomes and for constructing theoretical models of genome evolution. Much remains to be done to evaluate the potential of global genetic databases to better characterize casual contacts (that could lead to identification of sporadic cases) in TB epidemiology. An improved version of our database, which will focus on areas with a high prevalence of TB, is currently in development; as of August 26, 2002, it had 20,000 isolates and 3,000 alleles. Ongoing population-based genotyping projects will likely help shed light on contemporary and ancient tubercle bacilli's evolutionary history.

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