JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2008, p. 4041–4044 Vol. 46, No. 12
0095-1137/08/$08.00+0 doi:10.1128/JCM.01315-08

Genetic Diversity of Isoniazid-Resistant *Mycobacterium tuberculosis* Isolates Collected in Poland and Assessed by Spoligotyping▽

Ewa Augustynowicz-Kopeć, Tomasz Jagielski, and Zofia Zwolska*

Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland

Received 11 July 2008/Returned for modification 2 September 2008/Accepted 23 September 2008

The genetic compositions of 71 isoniazid-resistant *Mycobacterium tuberculosis* strains from Poland were determined by spoligotyping. Nearly 80% of the isolates belonged to either the T or the Haarlem family. The genotypic diversity was largely due to variation within those families. The scarcity of imported genotypes suggested that the *M. tuberculosis* population studied has an endemic nature.

Isoniazid (INH) is one of the most important drugs for both the therapy and the prophylaxis of tuberculosis (TB). However, strains of *Mycobacterium tuberculosis*, the causative agent of TB, resistant to INH have increasingly been isolated throughout the world. Globally, the burden of INH resistance is estimated to be 13.3% (25). In Poland, in 2000, the overall rate of resistance to INH reached 6%, or 1.5 times higher than that in 1997 (2). In 2004, 5% of all cases of *M. tuberculosis* infection were reported to be INH resistant. INH monoresistance is the most prevalent type of *M. tuberculosis* resistance in Poland, with the median rates of primary and acquired resistance being 2.4% and 3.3%, respectively (25).

In recent years, molecular typing methods have been successfully employed to define the genetic relationships between *M. tuberculosis* strains and to delineate the patterns of transmission of TB in human populations (16). Currently, one of the methods most frequently used to differentiate clinical isolates of *M. tuberculosis* is spoligotyping, which detects polymorphisms in the *M. tuberculosis* complex direct repeat (DR) chromosomal locus, which contains a series of 36-bp DRs interspersed with 35- to 41-bp unique spacer sequences (14).

The aim of this study was to use spoligotyping to assess the genetic diversity of *M. tuberculosis* strains with the INH-monoresistant phenotype in Poland in 2004.

(Part of the results included in this study were presented in abstract form [abstr. D13] at the 8th International Meeting on Microbial Epidemiological Markers, Zakopane, Poland, 14 to 17 May 2008.)

The study included 71 *M. tuberculosis* isolates from 71 nonrelated, adult TB patients with pulmonary TB (52 men and 19 women; age range, 14 to 85 years; median age, 48 years) residing in 13 different regions (voivodeships) of Poland. The population analyzed represented 87% of all culture-proven INH-resistant TB cases for which notification was provided in Poland in 2004 and close to 30% of the total number of drug-resistant pulmonary TB cases recorded in Poland throughout 2004.

Primary isolation was performed with Löwenstein-Jensen (LJ) medium and the Bactec 460-TB system (Becton Dickinson, Sparks, MD); and species identification was done by the niacin test, the use of gene probes (AccuProbe; GenProbe, San Diego, CA), and mycolic acid analysis by high-pressure liquid chromatography. Drug susceptibility testing was performed by the proportion method on LJ medium. The criterion used for the determination of drug resistance was the growth of 1% or more of the bacterial population on critical concentrations of the drugs tested (i.e., 0.2 μg/ml for INH, 40 μg/ml for rifampin [rifampicin]), 4 μg/ml for streptomycin, and 2 μg/ml for ethambutol) (2). Bacterial DNA was extracted from the LJ medium slants by the cetyltrimethylammonium bromide (CTAB) method (22). Spoligotyping was performed with a commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands), according to the manufacturer's instructions and as described earlier (14). Spoligotypes with 100% similarity were considered clusters, whereas nonclustered spoligotypes were referred to as unique. All spoligotypes obtained were compared to those in the world spoligotyping database (SpolDB4) at the Pasteur Institute of Guadeloupe (www.pasteur-guadeloupe.fr/tb/spoldb4). The isolates whose spoligotype patterns were already recorded in SpolDB4 were assigned shared types (STs), whereas isolates whose spoligotypes were identified for the first time were designated either new STs (if two or more isolates had that spoligotype) or orphans (if the spoligotype occurred in only one isolate). Clade assignment of the spoligotypes not found in SpolDB4 (orphan types) was done with the SpotClust program, an algorithm based on the SpolDB3 database whose principle was described previously (23) and that is available online (http://cgi2.cs.rpi.edu/~bennek/SPOTCLUST.html).

A total of 30 different genetic profiles were identified among the 71 *M. tuberculosis* isolates, resulting in an overall diversity (the number of spoligotypes divided by the number of isolates) of 42%. Twenty-one (30%) isolates exhibited unique patterns, and the remaining 50 (70%) isolates were grouped into nine clusters, with 2 to 24 isolates per cluster. A comparison of the profiles obtained with the SpolDB4 international spoligotyping database allowed the attribution of the spoligotype ST designations. Of the 21 unique patterns, 12 (57%) were already described in SpolDB4, while the remaining 9 (43%) were previously unreported orphan types. Among the nine clusters, five containing 3 or more isolates each were considered major spoligotypes and represented 42 (59%) of the isolates. Almost

* Corresponding author. Mailing address: National Tuberculosis and Lung Diseases Research Institute, Department of Microbiology, Płocka 26, Warsaw 01-138, Poland. Phone: 48 22 43 12 382. Fax: 48 22 43 12 182. E-mail: z.zwolska@igichp.edu.pl.

▽ Published ahead of print on 1 October 2008.

TABLE 1. Spoligotypes identified for *M. tuberculosis* isolates evaluated in this study and their clustering

Spoligotype	Clade	ST[a]	No. of isolates	Geographic distribution[b]
Clustered	T1	53	24	Ubiquitous
	H3	50	7	Ubiquitous
	H1	47	5	Ubiquitous
	T1	1278	3	AUT, CZE, ESP, ITA, POL, USA
	ND[c]	A	3	POL
	T1	253	2	ARG, DNK, FIN, FXX, IDN, NLD, POL, RUS, USA, VEN
	T1_RUS2	280	2	AUS, AUT, DEU, EST, FIN, GEO, LVA, POL, RUS, SWE, TUR, USA
	S	1253	2	ARG, DEU, FXX, RUS, TUR
	ND	B	2	POL
Unique	T3	37	1	Ubiquitous
	T4	40	1	AUT, BEL, CZE, DEU, DNK, ESP, ETH, FIN, FXX, GBR, GEO, GLP, IRN, ITA, LBY, MDG, NLD, POL, RUS, USA
	T5	44	1	Ubiquitous
	H4	262	1	POL, RUS, SWE, TUR, USA
	H1	382	1	AUT, EST, FXX, POL, USA
	T1	498	1	AUT, CZE, DEU, GBR, USA
	U	602	1	AUT, BEL, BRA, DEU, FIN, GEO, IDN, IRN, ITA, NLD, NZL, RUS, SWE, USA, VNM, ZAF
	T1	612	1	ARG, FXX, GBR, USA
	U	775	1	AUT, CZE, HUN, NLD, SWE, USA
	U	1410	1	AUT, BGD, BRA, POL, PRT, SAU
	U	1498	1	MYS, USA
	H3	1640	1	FXX, NLD
	ND	C to K	1	POL
Total			71	

[a] ST designation of the spoligotype in the world spoligotype database (SpolDB4). There were a total of 30 STs.
[b] Ubiquitous, spoligotype found in all eight geographic regions (AFR, Africa; CAM, Central America; EUR, Europe; FEA, Far-East Asia; MECA, Middle-East and Central Asia; NAM, North America; OCE, Oceania; SAM, South America); ARG, Argentina; AUS, Australia; AUT, Austria; BEL, Belgium; BGD, Bangladesh; BRA, Brazil; CZE, Czech Republic; DEU, Germany; DNK, Denmark; ESP, Spain; EST, Estonia; ETH, Ethiopia; FIN, Finland; FXX, metropolitan France; GBR, United Kingdom; GEO, Georgia; GLP, Guadeloupe; HUN, Hungary; IDN, Indonesia; IRN, Iran; ITA, Italy; LBY, Libya; LVA, Latvia; MDG, Madagascar; MYS, Malaysia; NLD, Netherlands; NZL, New Zealand; POL, Poland; PRT, Portugal; RUS, Russia; SAU, Saudi Arabia; SWE, Sweden; TUR, Turkey; USA, United States; VEN, Venezuela; VNM, Viet Nam; ZAF, South Africa. Country codes are according to the ISO 3166 specifications (ftp://ftp.ripe.net/iso3166-countrycodes.txt).
[c] ND, not defined in the SpolDB4 world spoligotype database.

half of the clustered isolates ($n = 24$; 34% of all isolates) belonged to ST53, which is the most prevalent genotype in Europe. All strains that were labeled with an ST number fell into four spoligotype-defined clades: T (STs 37, 40, 44, 53, 253, 280, 498, 612, and 1278), Haarlem (STs 47, 50, 262, 382, and 1640), U (STs 602, 775, 1410, and 1498), and S (ST1253), comprising 36 (51%), 15 (21%), 4 (6%), and 2 (3%) isolates, respectively. Of the 30 spoligotypes found in this study, 12 (40%) had previously been reported in Poland (Table 1). Eleven spoligotypes (37%) with 14 (20%) isolates were new and had not been found elsewhere in the world. In order to assign these spoligotypes to the existing clades, they were subjected to analysis with the SpotClust algorithm. Thus, the spoligotypes designated D, E, F, G, and K were linked to the T clade, whereas the spoligotypes designated B, H, and I were linked to the Latin American and Mediterranean (LAM) family. Three isolates harbored spoligotypes that were closely related to that of strain H37Rv. Finally, two spoligotypes represented by one isolate each were shown to belong to the Haarlem and Central Asian (CAS) clades (Table 2).

Overall, the family assignment demonstrated that a major proportion of the strains analyzed belonged to either the T (58%) or the Haarlem (22%) family.

The spoligotyping method has been widely accepted as a valuable tool for epidemiological studies of TB. It has proven useful for the tracking of outbreaks (10, 19) and laboratory cross contamination (6) and for description of the global spread of TB (7). Spoligotyping has considerable advantages in that it is simple, robust, and highly reproducible. Since the technique is PCR based, it can be applied directly to clinical samples, thus allowing fingerprinting of a large number of isolates to be performed in a very short time (14). An important aspect of the method is the binary result format, which makes the data generated easily interpretable, computerizable, and comparable between laboratories (5). The establishment and development of an international spoligotyping database (SpolDB) have contributed tremendously to the gaining of a better understanding of the genetic structure of the global *M. tuberculosis* population and its evolution. Recently, an updated version of SpolDB4 that includes 1,939 different spoligotypes (STs) identified worldwide and that is separated into phylogenetic clades (families) has been launched (4). Moreover, a web-based program, SpotClust, has been devised to assign spoligotypes (especially those not found in the SpolDB4 database) to ST families (23). Altogether, spoligotyping combined with bioinformatic analyses provides an efficient approach for determination of the diversity of circulating *M. tuberculosis* strains and assessment of the extent and the dynamics of TB transmission.

In the present study we have employed spoligotyping to

TABLE 2. Spoligotypes specific to Poland and their clade assignments

Spoligotype			Clade[a]	Probability[b]	No. of isolates
Arbitrary designation	Binary designation	Octal designation			
A	■■■■■■■■■■■■■□□□□■□□□■■■■■■■■■■■□□□□■■■■■■■	777741077760771	H37Rv	0.98	3
B	■■■■■■■■■■■■■□□□□■□□□□□□■■■■■■■□□□□■■■■■■■	777741003760771	LAM9	0.94	2
C	□□□□□□□□□□□□□□□■■■■■□□□□□□□□□□□□□■■■■■■■■	000017600003771	CAS	0.99	1
D	■■■□□□■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□■■	707777777760711	T1	0.99	1
E	■■■■□□□□□□■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□□□□	740777777760700	T2	0.98	1
F	■■■■■■□□□□□□□□□□□□■■■■■■■■■■□■■■■□□□□■■■■■■■	770000777360771	T3	0.99	1
G	■■■■■■■■■□■■■□□■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777347777760771	T1	0.99	1
H	■■■■■■■■■■■■□■■■■■■□□□□■■■■□□□□■□□□□■■■■■■■	777737607420771	LAM9	0.99	1
I	■■■■■■■■■■■■■□□□■□□□□□□■■■■■■■■□□□□■■■□■■■	777761007760731	LAM9	0.97	1
J	■■■■■■■■■■■■■■□□■□■■■■■■□□□□□□■□□□□■■■■■■■	777771374020771	H1	0.99	1
K	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□■■■	777777777740031	T1	0.99	1

[a] SpotClust program-assigned clade.
[b] Probability that the spoligotype pattern belongs to the clade.

investigate the genetic diversity of INH-resistant *M. tuberculosis* isolates in Poland.

Among the 71 isolates tested, 30 different spoligotype patterns were identified, indicating the significant heterogeneity of the population studied. However, upon phylogenetic analysis, the vast majority of the genotypes were allocated to only two major clades, namely, the T and Haarlem clades, which covered 41 (58%) and 16 (22%) of the *M. tuberculosis* strains analyzed, respectively. Both these families are highly prevalent in Europe. According to the SpolDB4 database, the ill-defined T family encompasses spoligotypes which likely represent relatively old genotypes prevalent in Europe, whereas the Haarlem lineage has a European origin and is essentially localized in northern European countries (7, 8). Our results have clearly shown that the T and Haarlem families make up the backbone of the genetic structure of the *M. tuberculosis* population studied. The observed propensity of specific mycobacterial lineages to spread within particular geographical areas has recently been linked to the adaptation of the pathogen to different human host populations (9).

The three most prevalent spoligotypes (ST53, ST50, and ST47) accounted for more than half of the isolates responsible for TB cases, suggesting an important role of these genotypes in local TB transmission in Poland. This could be further supported by the fact that the same three types were found to be dominant in two previous spoligotyping studies in Poland (1, 17). This observation indicates the continuing activity of these genotypes in the country. Conversely, 22 (31%) of INH-resistant *M. tuberculosis* strains yielded spoligotypes previously unreported in Poland. These spoligotypes either had already been defined in the global database or were identified for the first time. Whereas the finding of the former may be indicative of the transmission of imported *M. tuberculosis* strains, the presence of the latter suggests Polish phylogeographic specificity. Interestingly, one of the Poland-specific spoligotypes has been demonstrated to belong to the CAS clade, which is very poorly represented in Europe. A possible explanation for this may relate to phylogenetic convergence, which relies upon the independent acquisition of two similar structures without common ancestors (24).

Finally, clustering of the genotypes obtained by spoligotyping may suggest the active transmission of INH-resistant *M. tuberculosis* isolates in Poland. However, whereas unique spoligotypes can confidently be assumed to represent different strains and thus reflect the reactivation of a remotely acquired infection, spoligotype clustering as a proxy for recent or ongoing transmission must be interpreted with caution. This is because spoligotyping is substantially less discriminatory than the IS*6110*-based restriction fragment length polymorphism (RFLP) typing method, which is referred to as a "gold standard" for the molecular epidemiology of *M. tuberculosis* (15, 22). Several studies have shown that spoligotyping overestimates the number of isolates with identical DNA fingerprints compared to the number obtained by the IS*6110*-RFLP method (12, 13, 15) and that this overestimation can be as large as 50% (11). Nevertheless, a combination of spoligotyping with other PCR-based techniques, such as double-repetitive-element PCR (20) or mycobacterial interspersed repetitive-unit–variable-number tandem-repeat typing (21), has been demonstrated to afford a resolution capacity close to that of IS*6110*-RFLP. It must be emphasized, however, that none of the currently used genetic markers provides an accurate and sufficiently discriminatory system for the genotyping of *M. tuberculosis*. Such a system could reliably estimate the extent of recent transmission. Moreover, the clustering of *M. tuberculosis* isolates, as measured by DNA clonality, might not be attributed to recently transmitted infections but to the coincident reactivations of strains endemic to the area or independent evolutionary convergence (3). Thus, genotypic clustering is not always consistent with the epidemiological relatedness of the cases analyzed, and it is of great importance to interpret genotyping results in conjunction with those of the conventional contact tracing methodology.

Altogether, the results of spoligotyping, as the sole typing method, are not conclusive in terms of determining the amount of recent transmission in population-based studies (12, 18). Although it is useful as an initial screening test, spoligotyping must be followed by other fingerprinting methods with higher discrimination abilities. Consequently, evaluation of the actual rate of ongoing INH-resistant *M. tuberculosis* transmission in Poland requires the use of additional genetic markers, preferably coupled with conventional contact tracing data.

Nonetheless, spoligotyping alone remains a highly informative genotyping method that provides insight into the genetic

structure, evolution, and spreading dynamics of the global *M. tuberculosis* population.

In conclusion, our spoligotyping has revealed the diversity of the *M. tuberculosis* isolates in Poland and has shown that the isolates are similar to those in a typical European country, with a predominance of isolates of the T and Haarlem families, both of which accounted for nearly 80% of the isolates from the TB cases studied. The diversity of the genotypes was largely due to variations within those families. At the same time, several new, previously unreported genotypes have been identified. A scarcity of cases harboring imported genotypes may be indicative of the endemic nature of the *M. tuberculosis* population studied.

We are deeply indebted to Vitali Sintchenko (University of Sydney, Sydney, Australia) for critical revision of the manuscript.

The study was supported by a grant from the Ministry of Science and Higher Education, Poland (R13 021 03).

REFERENCES

1. **Augustynowicz-Kopeć, E., T. Jagielski, M. Kozińska, A. Zabost, M. Klatt, and Z. Zwolska.** 2008. Molecular analysis of drug-resistant *Mycobacterium tuberculosis* isolates collected in central Poland. Clin. Microbiol. Infect. **14:** 605–607.
2. **Augustynowicz-Kopeć, E., Z. Zwolska, A. Jaworski, E. Kostrzewa, and M. Klatt.** 2003. Drug-resistant tuberculosis in Poland in 2000: second national survey and comparison with the 1997 survey. Int. J. Tuberc. Lung Dis. **7:**645–651.
3. **Braden, C. R., G. L. Templeton, M. D. Cave, S. Valway, I. M. Onorato, K. G. Castro, D. Moers, Z. Yang, W. W. Stead, and J. H. Bates.** 1997. Interpretation of restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates from a state with a large rural population. J. Infect. Dis. **175:**1446–1452.
4. **Brudey, K., J. R. Driscoll, L. Rigouts, W. M. Prodinger, A. Gori, S. A. Al-Hajoj, C. Allix, L. Aristimuño, J. Arora, V. Baumanis, L. Binder, P. Cafrune, A. Cataldi, S. Cheong, R. Diel, C. Ellermeier, J. T. Evans, M. Fauville-Dufaux, S. Ferdinand, D. Garcia de Viedma, C. Garzelli, L. Gazzola, H. M. Gomes, M. C. Guttierez, P. M. Hawkey, P. D. van Helden, G. V. Kadival, B. N. Kreiswirth, K. Kremer, M. Kubin, S. P. Kulkarni, B. Liens, T. Lillebaek, M. L. Ho, C. Martin, I. Mokrousov, O. Narvskaïa, Y. F. Ngeow, L. Naumann, S. Niemann, I. Parwati, Z. Rahim, V. Rasolofo-Razanamparany, T. Rasolonavalona, M. L. Rossetti, S. Rüsch-Gerdes, A. Sajduda, S. Samper, I. G. Shemyakin, U. B. Singh, A. Somoskovi, R. A. Skuce, D. van Soolingen, E. M. Streicher, P. N. Suffys, E. Tortoli, T. Tracevska, V. Vincent, T. C. Victor, R. M. Warren, S. F. Yap, K. Zaman, F. Portaels, N. Rastogi, and C. Sola.** 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol. **6:**23.
5. **Dale, J. W., D. Brittain, A. A. Cataldi, D. Cousins, J. T. Crawford, J. Driscoll, H. Heersma, T. Lillebaek, T. Quitugua, N. Rastogi, R. A. Skuce, C. Sola, D. van Soolingen, and V. Vincent.** 2001. Spacer oligonucleotide typing of bacteria of the *Mycobacterium tuberculosis* complex: recommendations for standardized nomenclature. Int. J. Tuberc. Lung Dis. **5:**216–219.
6. **de C. Ramos, M., H. Soini, G. C. Roscanni, M. Jaques, M. C. Villares, and J. M. Musser.** 1999. Extensive cross-contamination of specimens with *Mycobacterium tuberculosis* in a reference laboratory. J. Clin. Microbiol. **37:**916–919.
7. **Filliol, I., J. R. Driscoll, D. van Soolingen, B. N. Kreiswirth, K. Kremer, G. Valetudie, D. Duc Anh, R. Barlow, D. Banerjee, P. J. Bifani, K. Brudey, A. Cataldi, R. C. Cooksey, D. V. Cousins, J. W. Dale, O. A. Dellagostin, F. Drobniewski, G. Engelmann, S. Ferdinand, D. Gascoyne-Binzi, M. Gordon, C. Gutierrez, W. H. Haas, H. Heersma, E. Kassa-Kelembho, H. Minh Ly, A. Makristathis, C. Mammina, G. Martin, P. Mostrom, I. Morkousov, V. Narbonne, O. Narvskaya, A. Nastasi, S. Ngo Niobe-Eyangoh, J. W. Pape, V. Rasolfo-Razanamparany, M. Ridell, M. Lucia Rossetti, F. Stauffer, P. N. Suffys, H. Takiff, J. Texier-Maugein, V. Vincent, J. H. de Waard, C. Sola, and N. Ragosti.** 2003. Snapshot of moving and expanding clones of *Mycobacterium tuberculosis* and their global distribution assessed by spoligotyping in an international study. J. Clin. Microbiol. **41:**1963–1970.
8. **Filliol, I., J. R. Driscoll, D. van Soolingen, B. N. Kreiswirth, K. Kremer, G. Valétudie, D. D. Anh, R. Barlow, D. Banerjee, P. J. Bifani, K. Brudey, A. Cataldi, R. C. Cooksey, D. V. Cousins, J. W. Dale, O. A. Dellagostin, F. Drobniewski, G. Engelmann, S. Ferdinand, D. Gascoyne-Binzi, M. Gordon, M. C. Gutierrez, W. H. Haas, H. Heersma, G. Källenius, E. Kassa-Kelembho, T. Koivula, H. M. Ly, A. Makristathis, C. Mammina, G. Martin, P. Moström, I. Mokrousov, V. Narbonne, O. Narvskaya, A. Nastasi, S. N. Niobe-Eyangoh, J. W. Pape, V. Rasolofo-Razanamparany, M. Ridell, M. L. Rossetti, F. Stauffer, P. N. Suffys, H. Takiff, J. Texier-Maugein, V. Vincent, J. H. de Waard, C. Sola, and N. Rastogi.** 2002. Global distribution of *Mycobacterium tuberculosis* spoligotypes. Emerg. Infect. Dis. **8:**1347–1349.
9. **Gagneux, S., K. DeRiemer, T. Van, M. Kato-Maeda, B. C. de Jong, S. Narayanan, M. Nicol, S. Niemann, K. Kremer, M. C. Gutierrez, M. Hilty, P. C. Hopewell, and P. M. Small.** 2006. Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. USA **103:**2869–2873.
10. **Goguet de la Salmonière, Y. O., H. M. Li, G. Torrea, A. Bunschoten, J. D. A. van Embden, and B. Gicquel.** 1997. Evaluation of spoligotyping in a study of the transmission of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **35:**2210–2214.
11. **Gori, A., A. Bandera, G. Marchetti, A. Degli Esposti, L. Catozzi, G. P. Nardi, L. Gazzola, G. Ferrario, J. D. A. van Embden, D. van Soolingen, M. Moroni, and F. Franzetti.** 2005. Spoligotyping and *Mycobacterium tuberculosis*. Emerg. Infect. Dis. **11:**1242–1248.
12. **Gori, A., A. D. Esposti, A. Bandera, M. Mezzetti, C. Sola, G. Marchetti, G. Ferrario, F. Salerno, M. Goyal, R. Diaz, L. Gazzola, L. Codecasa, V. Penati, N. Rastogi, M. Moroni, and F. Franzetti.** 2005. Comparison between spoligotyping and IS*6110* restriction fragment length polymorphisms in molecular genotyping analysis of *Mycobacterium tuberculosis* strains. Mol. Cell. Probes **19:**236–244.
13. **Goyal, M., N. A. Saunders, J. D. A. van Embden, D. B. Young, and R. J. Shaw.** 1997. Differentiation of *Mycobacterium tuberculosis* isolates by spoligotyping and IS*6110* restriction fragment length polymorphism. J. Clin. Microbiol. **35:**647–651.
14. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
15. **Kremer, K., D. van Soolingen, R. Frothingham, W. H. Haas, P. W. M. Hermans, C. Martin, P. Palittapongarnpim, B. B. Plikaytis, L. W. Riley, M. A. Yakrus, J. M. Musser, and J. D. A. van Embden.** 1999. Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. J. Clin. Microbiol. **37:**2607–2618.
16. **Mathema, B., N. E. Kurepina, P. J. Bifani, and B. N. Kreiswirth.** 2006. Molecular epidemiology of tuberculosis: current insights. Clin. Microbiol. Rev. **19:**658–685.
17. **Sajduda, A., A. Brzostek, M. Popławska, N. Rastogi, C. Sola, E. Augustynowicz-Kopeć, Z. Zwolska, J. Dziadek, and F. Portaels.** 2004. Molecular epidemiology of drug-resistant *Mycobacterium tuberculosis* strains isolated from patients with pulmonary tuberculosis in Poland: a 1-year study. Int. J. Tuberc. Lung Dis. **8:**1448–1457.
18. **Scott, A. N., D. Menzies, T.-N. Tannenbaum, L. Thibert, R. Kozak, L. Joseph, K. Schwartzman, and M. A. Behr.** 2005. Sensitivities and specificities of spoligotyping and mycobacterial interspersed repetitive unit–variable-number tandem repeat typing methods for studying molecular epidemiology of tuberculosis. J. Clin. Microbiol. **43:**89–94.
19. **Sola, C., A. Devallois, L. Horgen, J. Maïsetti, I. Filliol, E. Legrand, and N. Rastogi.** 1999. Tuberculosis in the Caribbean: using spacer oligonucleotide typing to understand strain origin and transmission. Emerg. Infect. Dis. **5:**404–414.
20. **Sola, C., L. Horgen, J. Maïsetti, A. Devallois, K. S. Goh, and N. Rastogi.** 1998. Spoligotyping followed by double repetitive element PCR is a rapid alternative methodology to IS*6110*-fingerprinting for epidemiological studies of tuberculosis. J. Clin. Microbiol. **36:**1122–1124.
21. **Supply, P., S. Lesjean, E. Savine, K. Kremer, D. van Soolingen, and C. Locht.** 2001. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. J. Clin. Microbiol. **39:**3563–3571.
22. **van Embden, J. D. A., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, T. M. Shinnick, and P. M. Small.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
23. **Vitol, I., J. Driscoll, B. Kreiswirth, N. Kurepina, and K. P. Bennett.** 2006. Identifying *Mycobacterium tuberculosis* complex strain families using spoligotypes. Infect. Genet. Evol. **6:**491–504.
24. **Warren, R. M., E. M. Streicher, S. L. Sampson, G. D. van der Spuy, M. Richardson, D. Nguyen, M. A. Behr, T. C. Victor, and P. D. van Helden.** 2002. Microevolution of the direct repeat region of *Mycobacterium tuberculosis*: implications for interpretation of spoligotyping data. J. Clin. Microbiol. **40:**4457–4465.
25. **WHO and IUATLD.** 2008. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 2002–2007: anti-tuberculosis drug resistance in the world: 4th global report. WHO, Geneva, Switzerland.