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Targeted Hybridization of IS*6110* Fingerprints Identifies the W-Beijing *Mycobacterium tuberculosis* Strains among Clinical Isolates

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Targeted IS*6110*-based RFLP genotyping can be applied to rapidly identify specific groups of biomedically/epidemiologically relevant *Mycobacterium tuberculosis* clinical isolates. One such group is the W-Beijing strain family (also known as Beijing/W), implicated in significant nosocomial and community outbreaks worldwide. Using previously defined criteria, we developed a simple and accurate method to identify members of the W-Beijing family, based on rehybridization of Southern blot membranes used previously in routine IS*6110* DNA fingerprint analysis. The hybridization probe constructed ("W-Beijing polyprobe") contains the PCR-amplified fragments specific for three *M. tuberculosis* chromosomal loci used for the identification of W-Beijing strains. The targets include the *dnaA-dnaN* and NTF regions and the direct repeat locus. A total of 526 selected clinical isolates (representative of 253 different IS*6110*-defined strain types) were analyzed using the W-Beijing polyprobe. A total of 148 isolates from this collection were found to be members of the W-Beijing phylogenetic lineage, comprising 106 strains from the W-Beijing family (46 clusters) and 42 related isolates. Rehybridization results were confirmed by computer-assisted analysis. The sensitivity and specificity of this method were estimated at 98.7% and 99.7%, respectively. This study demonstrates that the W-Beijing polyprobe can accurately and reliably discriminate members of the W-Beijing phylogenetic lineage and the W-Beijing family of *M. tuberculosis* strains.

Over the last decade of *Mycobacterium tuberculosis* genotyping, one group of clinical isolates, the W-Beijing strain family, has received considerable attention. These strains have been shown to be highly prevalent throughout Asia and the countries of the former Soviet Union, and they have been associated with nosocomial and community outbreaks, global transmission, and drug resistance (3, 10, 28). The W-Beijing strains have been also associated with tuberculosis (TB) treatment failures and relapse cases (16). In vivo murine infection studies have suggested that W-Beijing strains fail to efficiently elicit a Th1-dependent protective immune response and have shown that they are more virulent (17, 18). As a result of the current epidemiology and laboratory studies associated with W-Beijing strains, public health research laboratories and TB control efforts would benefit from the ability to accurately identify these strains in any given population.

Independent genetic markers specific for W-Beijing strains have been described previously (3, 8, 15). These strains can be characterized on the basis of a unique IS*6110* insertion in the origin of replication (*oriC*) and a specific spacer oligotype (spoligotype; NY_S00034; type 1, SpolDB3) lacking spacers 1 through 34 in the direct repeat (DR) chromosomal locus. Strains bearing both these genetic characteristics have recently been classified into a large phylogenetic lineage (the W-Beijing lineage) defined by synonymous single-nucleotide polymorphism (sSNP) analysis as members of cluster II (12). W-Beijing strains with multiple (15 to 26) copies of IS*6110* that share similar restriction fragment length polymorphism (RFLP) genotyping patterns (>65%), and that have at least one specific IS*6110* insertion within the NTF locus, define the W-Beijing family of strains (3). A second IS*6110* insertion in the NTF locus defines the multidrug-resistant (MDR) W strains, recent descendants of the W-Beijing strain family that caused multiple institutional outbreaks in New York City (NYC) during the early 1990s and more than 500 active cases (5, 22, 24).

We have exploited the uniqueness of these genetic markers in establishing a single-step method that is based on the standardized IS*6110* Southern blot hybridization protocol. We report here the construction and utility of the "W-Beijing polyprobe," which includes three genetic markers, for rapid recognition of W-Beijing strains among a collection of *M. tuberculosis* clinical isolates by rehybridizing membranes used for routine IS*6110*-based RFLP genotyping.

MATERIALS AND METHODS

Clinical isolates. Since 1992 the TB Center at the Public Health Research Institute (PHRI) has been systematically subculturing, stocking, and genotyping clinical isolates of *M. tuberculosis*. The PHRI TB Center collection contains over 18,000 *M. tuberculosis* clinical isolates that have been catalogued on the basis of their IS*6110* RFLP patterns, which have been digitized, analyzed, and archived using a Sun Sparcs5 workstation and a BioImage Whole Band Analyzer (version 3.4; Genomic Solutions, Ann Arbor, Mich.). Clinical isolates originate primarily from New York City and New Jersey patients, but the collection also includes isolates from diverse global populations including Brazil, Chile, the Czech Republic, Egypt, Germany, Haiti, Honduras, India, Israel, Kenya, Mexico, Romania, Russia, Singapore, South Africa, Thailand, and Venezuela. From this collection, approximately 2,850 isolates have previously been assigned to the W-Beijing family according to the computer-assisted analysis and predefined

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TABLE 1. Primers used for PCR DNA amplification, their locations in the *M. tuberculosis* H37Rv genome, sizes of the PCR amplicons, and expected patterns after rehybridization of routinely IS*6110*-probed membranes with the W-Beijing polyprobe

Locus name, gene (Rv)	Primer designation	Primer sequence	Size of PCR-amplified fragment (bp)	Hybridization pattern in:		
				W-Beijing strains	W-Beijing ancestor strains	Non W-Beijing strains
Region A, *dnaA-dnaN* (*Rv0001–Rv0002*)	DnaA-F4	5′-CTGTGAGTGTCGCTGTGCAC-3′	450	3.36 kb; match with IS*6110*	3.36 kb; match with IS*6110*	10.85 kb; no match with IS*6110*
	DnaN-R	5′-CAACTCTTGTCGTAGCCGCG-3′				
DR, direct repeat (*Rv2813–Rv2816c*)	Sp66	5′-GCGTGGCTGTGGTGTGGCGGGCGA-3′	530	3.65 kb; no match with IS*6110*	3.65 kb; no match with IS*6110*	1-2-3 bands; match with IS*6110*
	Sp51	5′-TGAGCGCGAACTCGTCCACAGTCC-3′				
Region B, NTF (*Rv3129*)	B-F	5′-CGTGAGGCACCGAGGGTGTTTC-3′	380	a) 1.6 kb; no match with IS*6110*	1.4 kb; no match with IS*6110*	1.4 kb; no match with IS*6110*
	B-R	5′-CGGTAGTAACTGGGCGCGTTCG-3′		b) 1.1 kb; no match with IS*6110*		

criteria described elsewhere (3). Representative isolates from this collection were selected for the purpose of this study.

Selection of membranes. Thirty-two Southern blot nylon (Hybond-N+) membranes (526 isolates) were selected for rehybridization with the W-Beijing polyprobe among a collection of more than 1,000 membranes generated during routine IS*6110* RFLP genotyping. Twenty-two blots with at least one W-Beijing IS*6110* fingerprint pattern previously determined by computer-assisted analysis were selected, and 10 additional blots were randomly selected.

PCR amplification of desired fragments. Genomic loci used for PCR amplification have been described previously (14, 15, 24). Chromosomal DNA of the laboratory strain H37Rv, ATCC 35837, was used as a template for PCR amplification (http://genolist.pasteur.fr/TubercuList/) (6). Amplicons were designed to limit the number of hybridization bands. The primers used for PCR, their respective locations in the genome of *M. tuberculosis* H37Rv, and the sizes of the resulting amplicons are shown in Table 1 and Fig. 1. DNA amplification was conducted using thermocycler GenAmp PCR System 2700 (Applied Biosystems, Inc., Foster City, CA). A 100 μl reaction mixture with a final $MgCl_2$ concentration of 4 mM was incubated at 94°C for 2 min, followed by 35 cycles of 94°C for 20 s, 62°C for 10 s, and 72°C for 30 s, and a final 2 min at 72°C, with *Taq* DNA polymerase (Roche Diagnostics GmbH). PCR products can be purified if needed using the QIAquick PCR Purification kit (QIAGEN, Valencia, CA, catalog no. 28106).The proportion of the PCR-amplified fragments of the three W-Beijing polyprobe amplicons to be used for rehybridization of a single membrane is 1:2:2.5 [~5 μl (DR) + 10 μl (region A) + 12 μl (region B)]. Enhanced chemiluminescence labeling was performed as the manufacturer recommended.

Rehybridization procedure. *M. tuberculosis* chromosomal DNA was isolated, PvuII digested, electrophoresed, and transferred to a nylon membrane (Hybond-N+) as described elsewhere (26). Membranes previously hybridized with the 312-bp IS*6110* 3′-specific probe for RFLP genotyping were subjected to rehybridization with the W-Beijing polyprobe, prepared as a combination of PCR amplicons of three chromosomal loci. Single-locus-specific probes (Fig. 1) were also used for hybridization of 1,560 clinical *M. tuberculosis* isolates. Membranes were rehybridized with the W-Beijing polyprobe without additional washing, following daylight exposure for at least 20 h prior to secondary hybridization.

Composite blot of outlier isolates. In order to facilitate the recognition of exceptions among the W-Beijing isolates which do not display all the predicted hybridization patterns, a composite blot containing DNA from two controls (CDC1551 and H37Rv) and 15 W-Beijing clinical isolates with unusual hybridization patterns was assembled and rehybridized as a reference (Fig. 2A and B).

RESULTS

Rehybridization of Hybond-N+ membranes. The selected loci, primer sequences, and PCR amplicon sizes and the predicted hybridization patterns are shown in Table 1. The ultimate selection of the PCR amplicons proposed in this study is the result of the analysis of more than 1,560 strains hybridized independently with each single probe specific for the three designated chromosomal loci *dnaA-dnaN*, DR, and NTF, as shown in Fig. 1A, B, and C, respectively. These hybridization results identified probe sequences that cross-hybridized or failed to generate a robust signal and facilitated the classification of the strains (Table 1). Rehybridization with the W-Beijing polyprobe can be performed according to standard protocol on membranes previously used for IS*6110* RFLP genotyping and does not require an additional blot washing step (26). W-Beijing polyprobe rehybridization of membranes stored at room temperature yielded clear banding patterns regardless of the age of the membrane (up to 4 to 8 years). The rehybridization procedure might be limited to 4 h; hybridization buffer with resuspended enhanced chemiluminescence-labeled W-Beijing polyprobe can be reused for rehybridization of membranes at least twice if stored at −20°C.

Analysis of rehybridization results. An algorithm for interpretation of hybridization patterns is proposed (Fig. 3). Using the algorithm, each individual strain can be assigned to the W-Beijing phylogenetic lineage or the W-Beijing family, or classified as a non-W-Beijing strain.

Region A (*oriC*; *dnaA-dnaN* intergenic region). The A1 insertion of IS*6110* in the origin of replication (*dnaA-dnaN* intergenic region; *oriC*) results in the generation of two hybridizing bands (3.36 and 8.55 kb, instead of a single 10.85-kb band in wild-type *oriC*) when the full wild-type *dnaA-dnaN* fragment is used as a probe. This is due to the insertion of an additional PvuII restriction site brought in by transposition of an IS*6110* copy, as previously described (15). To simplify the hybridization patterns, a new pair of primers specific for the amplification of a 450-bp fragment was used (Table 1; Fig. 1). This probe demonstrated the presence of the A1 insertion by revealing a single 3.36-kb hybridization band (Fig. 2B, lanes 3 to 12 and 15 to 17). Comparison of two blots (Fig. 2A and B), generated by independently hybridizing and rehybridizing the same membrane with IS*6110*- and region A-specific probes, reveals matching 3.36-kb bands on both blots. *M. tuberculosis*

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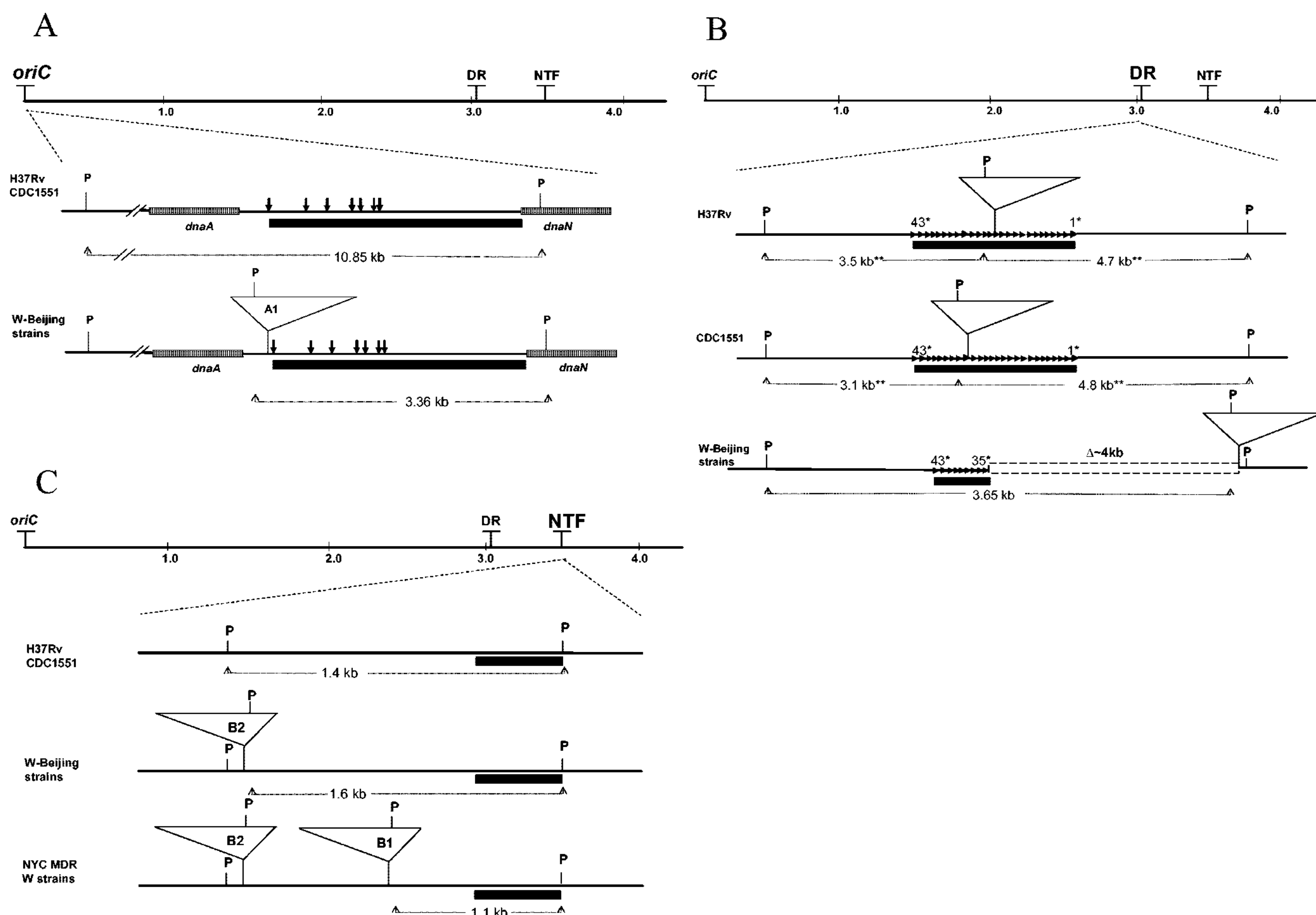

FIG. 1. Diagram of three chromosomal loci of the *M. tuberculosis* genome used for the W-Beijing polyprobe. (A) *dnaA-dnaN* (region A); (B) DR; (C) NTF (region B). Triangles indicate positions and orientations of IS*6110* in these three genomic loci. P denotes PvuII restriction sites. The molecular sizes of PvuII-PvuII DNA fragments, revealed by hybridization, are indicated below the fragments. The orientation of the chromosomal regions corresponds to their location in the genome of H37Rv. Vertical arrows indicate DnaA boxes in the origin of replication. Horizontal arrows in the DR indicate identical 36-bp sequences repeated; numbers 43, 35, and 1 indicate the presence of spacers, identified by the spoligotyping technique. A deletion of approximately 4 kb (Δ~4kb) in the DR region of W-Beijing strains is shown. *, spacers in the DR region determined by spoligotyping; **, sizes of DR-specific fragments in H37Rv and CDC1551 strains.

strains lacking an IS*6110* copy in *oriC* display a characteristic 10.85-kb band (Fig. 2B, lanes 1 and 2), which does not match any band in the corresponding IS*6110* image. A previous study has shown that the *dnaA-dnaN* intergenic region is a "hot spot" for IS*6110* transposition events; insertions in 11 different positions within this region have been identified in genetically distinct lineages of *M. tuberculosis* (15). With the exception of A1, as described here, IS*6110* insertions within the *dnaA-dnaN* intergenic region will result in hybridization patterns with one or two bands of different corresponding sizes.

DR region. W-Beijing isolates have a large deletion within the chromosomal DR locus (spacers 1 to 34 deleted). This results in a single 3.65-kb hybridization band on blots with PvuII-digested DNA from W-Beijing strains (Fig. 2B, lanes 3, 7 to 11, and 15 to 17), when they are rehybridized with the DR-specific probe (14). This band does not match that of any IS*6110*-specific pattern on an IS*6110* Southern hybridization blot. In contrast, non-W-Beijing strains show patterns with one, two, or three bands of variable sizes that correspond to the presence of 1 or 2 copies of IS*6110* in the DR (Fig. 2B, lanes 1 and 2). In these non W-Beijing isolates, some DR-specific bands may match IS*6110*-specific bands as long as such strains contain an IS*6110* insertion within the DR locus (25).

Region B (chromosomal locus NTF). All members of the W-Beijing family have at least one insertion within the NTF chromosomal locus, resulting in a 1.6-kb hybridizing band (Fig. 2B, lanes 9, 13 to 16). Additionally, some members of the W-Beijing family may have a second insertion within this locus. A specific second insertion in the same orientation 556 bp downstream has been identified in the New York City W-MDR strain, resulting in a unique 1.1-kb hybridization band which can be used to identify this specific strain (Fig. 2B, lane 17) (15, 24) but not other, unrelated W-Beijing-MDR isolates. However, most clinical isolates analyzed to date do not have an IS*6110* insertion within the NTF locus and show a 1.4-kb hybridization band that corresponds to the wild-type chromosome (Fig. 2B, lanes 1 and 2); H37Rv, CDC1551, and *Mycobacterium bovis* share this characteristic genotype. Likewise, some strains from the W-Beijing phylogenetic lineage ("ancestral" or "parental" strains) which probably diverged early dur-

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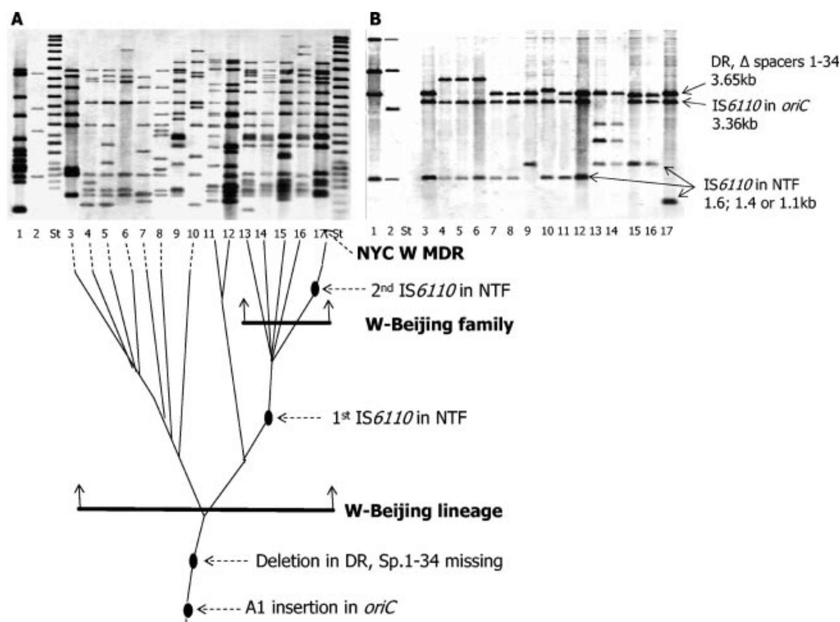

FIG. 2. Schematic phylogenetic tree of W-Beijing strains of *M. tuberculosis* hybridized with the IS*6110* probe (A) and rehybridized with the polyprobe (B). Lanes 1, H37Rv; lanes 2, CDC1551; lanes 3, HD3; lanes 4, AM; lanes 5, CI6; lanes 6, CI7; lanes 7, HD21; lanes 8, HD23; lanes 9, CK; lanes 10, KY; lanes 11, N2; lanes 12, N; lanes 13, W82; lanes 14, W99; lanes 15, W14; lanes 16, W148; lanes 17, NYC W MDR. Lanes 3 to 8 and 10 to 12 correspond to W-Beijing "ancestral" strains, and lanes 9 and 13 to 17 correspond to W-Beijing family strains (sharing >65% similarity of IS*6110*-based RFLP patterns). In lanes 4 to 6 and 10, the DR-specific hybridization band is shifted up due to the IS*6110* insertion in the PvuII fragment; in lanes 13 and 14, *oriC*-specific bands are shifted down due to a second copy of IS*6110* in the *dnaA-dnaN* region. Sp., spacers.

ing the course of evolution, prior to the IS*6110* transposition event in the NTF locus (P. Bifani, unpublished data), also have a wild-type genotype (Fig. 2B, lanes 3 to 8 and 10 to 12).

Evaluation of the polyprobe. Table 2 summarizes the rehybridization results using the W-Beijing polyprobe on membranes containing PvuII-digested DNA from *M. tuberculosis* clinical isolates. An algorithm for the interpretation of the results is shown in Fig. 3.

To evaluate this method, 526 clinical isolates (32 membranes) were rehybridized with the W-Beijing polyprobe and the banding patterns were analyzed blindly. Patterns were subdivided into three groups using the proposed algorithm. The first group consisted of 104 isolates with hybridization banding patterns of 3.65, 3.36, and 1.6 kb (corresponding to DR-, *dnaA-dnaN*-, and NTF-associated fragments) and 2 isolates with 3.65-, 3.36-, and 1.1-kb bands. These 106 strains were recognized as members of the W-Beijing family, as confirmed by previously performed computer-assisted comparative analysis

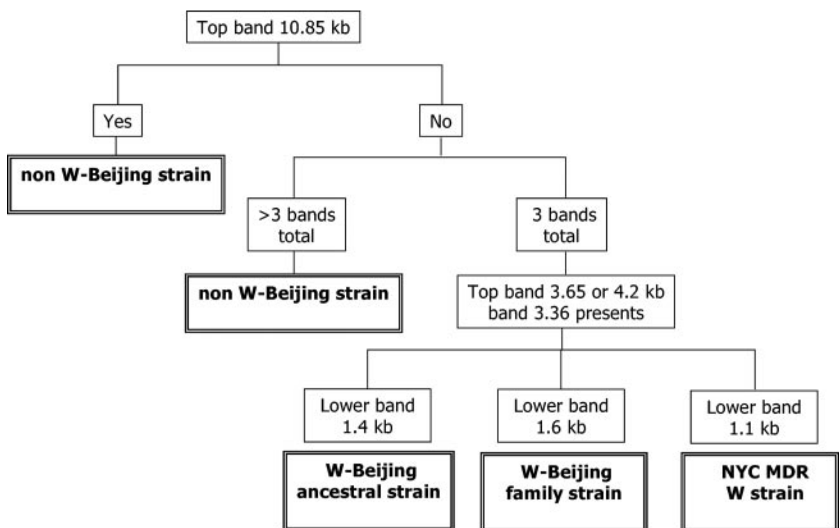

FIG. 3. Algorithm for identification of the W-Beijing strains by using the polyprobe for routine Southern blot rehybridization. This algorithm should be used for strains with more than 6 copies of IS*6110*.

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TABLE 2. Analysis of *M. tuberculosis* clinical isolates using the polyprobe

Parameter	Value
Total no. of *M. tuberculosis* clinical isolates analyzed	526
Total no. of isolates with characteristic IS*6110* W-Beijing pattern (W-Beijing family and ancestral strains)	149 (107 + 42)
Total no. of isolates with non-W-Beijing pattern by IS*6110* RFLP fingerprinting	377
No. of W-Beijing positive isolates confirmed by spoligotyping (spacers 1–34 missing)	64/64
No. of W-Beijing strains determined by hybridization with polyprobe	148 (149 − 2 + 1)
No. of non W-Beijing strains determined by using polyprobe	378 (377 − 1 + 2)
No. of false-positive isolates	1
No. of false-negative isolates	2
No. of undetermined isolates	0
Sensitivity of method	98.7%[a,c]
Specificity of method	99.7%[b,c]

[a] Sensitivity [(true positives)/(true positives + false negatives)], 147/149.
[b] Specificity [(true negatives)/(true negatives + false positives)], 377/378.
[c] Compared to IS6110 RFLP fingerprinting.

of highly similar IS*6110* RFLP banding patterns (>65% pattern similarity). Comparison with RFLP cluster analysis confirmed that they represented 46 independent clusters that differ from each other by at least 1 IS*6110* copy or by a shift in the banding pattern. A second group of 42 isolates with hybridization bands of 3.65, 3.36, and 1.4 kb corresponds to the members of the W-Beijing phylogenetic lineage (ancestral strains). These isolates were previously classified at the PHRI TB Center as members of N, HD, CI, KY, LB, and other arbitrarily designated families. Ancestral strains cannot be recognized by IS*6110* profiling given that they share less than 65% IS*6110* RFLP similarity with W-Beijing family isolates. These two groups together represent members of the W-Beijing phylogenetic lineage ($n = 148$). The third group consisted of 378 isolates which displayed one or two bands of variable sizes in addition to 10.85- and 1.4-kb hybridization bands. As expected, these 377 isolates were distinguished by the absence of IS*6110* insertions in *dnaA-dnaN* and NTF regions and at least 1 copy in the DR region and therefore did not belong to the W-Beijing phylogenetic lineage or family. With two false-negative identifications and one false-positive identification, the sensitivity and specificity of the W-Beijing polyprobe were determined ($n = 526$) to be 98.7% (147/149) and 99.7% (377/378) (Table 2), respectively. False-negative cases were represented by two W-family strains that had acquired a second IS*6110* copy in the *oriC* locus (Fig. 2, lanes 13 and 14); one non–W-Beijing–related strain with an IS*6110* insertion in a position other than A1 gave false-positive results.

DISCUSSION

An MDR *M. tuberculosis* strain associated with several nosocomial outbreaks and high mortality rates was identified in New York City in the early 1990s (5, 9, 21). This strain, arbitrarily designated strain W, has been the focus of several investigations. While strain W was identified in New York City, a concurrent study conducted in China reported on the predominance of a group of *M. tuberculosis* isolates characterized by a high copy number of IS*6110* elements (15 to 24 per genome) and related IS*6110* RFLP patterns which were named the Beijing strains (28). It is now recognized that W, W-related strains, and the Beijing strains are genetically related; they are now jointly referred to as the W-Beijing or Beijing/W strain family (3, 10). The genetic linkage between the W-Beijing strains has been determined by a combination of independent molecular markers, including IS*6110*-based RFLP, variable number of tandem repeats, deletion analysis, and spoligotyping (2–4, 15). Furthermore, recent work by Gutacker et al. showed that the W-Beijing strains are members of sSNP cluster II and part of a phylogenetic lineage (12). In Fig. 2 we show the schematic phylogenetic relationship of *M. tuberculosis* strains based on the genetic markers described in this report.

Recent reports have indicated that disease manifestations may be dictated in part by the interplay between strains of *M. tuberculosis* and the host immune system. One report showed that a particular strain, 210 (a member of the W-Beijing family), was able to replicate in cultured human macrophages at a four- to eightfold-higher rate than other, unrelated strains (29). Another study observed up to a 10-fold-higher bacillary load in the lungs and poorer survival of mice infected with a W-Beijing strain compared to other strains tested (18). These investigators note that these phenomena correlated with weak host immune response induced by W-Beijing strain infection. That is, W-Beijing strains were shown to elicit a poor cytokine-dependent Th1 protective host immune response, thereby delaying intracellular *M. tuberculosis* elimination by macrophages, which might in part explain the success of these strains. There have been numerous reports implicating the W-Beijing strains in outbreaks, extensive transmission, and high prevalence (3, 9, 23, 28). These attributes highlight the importance of this family of strains in TB control programs and necessitate their rapid identification within TB populations.

The goal of this study was to develop a fast, accurate, and low-cost method for *M. tuberculosis* strain identification, capable of distinguishing W-Beijing strains in any collection of clinical isolates where IS*6110* Southern blot hybridization genotyping has been previously performed. This method relies on the combination of three selected probes for rehybridization of existing membranes. The genetic probes target variable genomic loci unique to W-Beijing strains (Table 1); the proposed markers are well studied and result in effective and easy-to-interpret rehybridization pattern profiles.

The most definitive marker chosen includes the *dnaA-dnaN* intergenic region of the chromosomal origin of replication, the *oriC* locus. All W-Beijing strains have an IS*6110* insertion in this region in position A1 and a 3.36-kb hybridization band,

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accordingly; although no W-Beijing strains lacking the A1 insertion have yet been identified in our collection, some rare isolates which contain a second insertion within this locus have been described (15). These strains lack the 3.36-kb band (in routine IS*6110* RFLP patterns), and hybridization with the W-Beijing polyprobe demonstrates two additional bands of smaller size, in addition to the 3.65-kb (DR) and 1.6-kb (NTF) bands (Fig. 2B, lanes 13 and 14). It is important to recognize these exceptions, though they are rare, in order to avoid false-negative strain identification.

The second selected marker relies on a well-studied locus of the *M. tuberculosis* chromosome which includes a series of direct repeats, referred to as the DR region. The variability in the presence/absence of specific spacers in the DR forms the basis for spoligotyping (11). A search of the PHRI TB Center Spoligotype Database shows that most W-Beijing isolates have spoligotype NY_S00034 (octal code 000000000003771, type 1 in Spoldb3), lacking spacers 1 to 34, with positive hybridization to spacers 35 to 43; however, a few exceptions have been identified (Fig. 2, lanes 4 to 6 and 8) (2). As a result of IS*6110*-associated rearrangement, strains from the W-Beijing lineage have a characteristic deletion which removes part of the DR (spacers 1 through 34) and flanking chromosomal sequence (1, 27). These strains have a single 3.65-kb hybridization band that corresponds to the DR-specific fragment (Fig. 1).

The chromosomal NTF locus has been previously used as a target in multiplex PCR for the recognition of NYC W MDR strains (Fig. 2, lane 17) (24). In addition, the presence of at least 1 IS*6110* copy in the NTF locus provides a useful genetic marker for the identification of closely related clinical isolates which share a high similarity of RFLP profiles and were classified as members of the W family. The NTF-specific fragment used in the W-Beijing polyprobe can differentiate NYC W MDR strains (1.1-kb hybridization band) from W-Beijing family strains (1.6-kb band) and (in combination with region-A and region-B markers) the W-Beijing lineage strains from unrelated isolates.

Taken alone, each of the three chromosomal markers described above provides limited information for the identification of W-Beijing strains. However, when the markers are combined, the W-Beijing polyprobe yields high sensitivity and specificity for proper identification. An algorithm was proposed to interpret and identify the W-Beijing polyprobe banding patterns of strains that are members of the W-Beijing family and strains that belong to the W-Beijing phylogenetic lineage and to differentiate them from other, unrelated *M. tuberculosis* clinical isolates (Fig. 3).

Spoligotyping, considered the "gold standard" method for W-Beijing strain identification, combines into one group isolates with high similarity of RFLP images (>65% banding similarity) and phylogenetically related strains with much lower similarity of hybridization patterns. The latter group of strains does not share all the characteristics of W-Beijing family strains (RFLP pattern similarity, <65%), and its epidemiological significance is not clearly known, although several TB outbreaks have been associated with the various representatives of the W-Beijing phylogenetic lineage (7, 20).

Recently, a protocol to identify W-Beijing strains was proposed based on computer-assisted analysis of IS*6110*-based RFLP profile similarity of clinical isolates with a panel of 19 W-Beijing reference strains (13). In that study, clinical isolates that shared at least 80% IS*6110* banding similarity with any reference strain were determined to belong to the W-Beijing lineage. The W-Beijing polyprobe, described here, extends the recognition of isolates in at least two ways. First, the polyprobe increases the discriminatory power to identify W-Beijing strains. All 19 reference strains were positively recognized by the W-Beijing polyprobe and were further delineated as ancestral members of the W-Beijing phylogenetic lineage or as belonging to the W-Beijing family (data not shown). Second, the polyprobe, which relies not on IS*6110* patterns (and is not limited by the diversity of the reference panel) but rather on defined genetic characteristics, is more inclusive.

It must be noted that although the method described in this study (W-Beijing polyprobe) gives clear results that can be easily interpreted, each hybridization subgroup has exceptions which should be recognized in order to avoid inaccurate strain identification. As an example, some unrelated *M. tuberculosis* strains may also have IS*6110* insertions within the *dnaA-dnaN* region; however, they differ in the exact insertion site, and the orientation of IS*6110* differs from the characteristic A1 insertion (19).

The W-Beijing polyprobe can be used to identify rare *M. tuberculosis* isolates lacking the IS*6110* element (resulting in a non–W-Beijing hybridization profile). Additionally, the W-Beijing polyprobe is specific for members of the *M. tuberculosis* complex (*M. africanum*, *M. bovis*, *M. canetti*, *M. microti*, and *M. tuberculosis*) and does not hybridize with other mycobacterial species (e.g., *M. avium*, *M. fortuitum*, *M. xenopi*, *M. intracellulare*, *M. marinum*, *M. phlei*).

In conclusion, we have developed a rapid, effective, and low-cost method for W-Beijing strain identification. This approach highlights the possibility of using various genetic markers specific for different phylogenetic groups of *M. tuberculosis* strains. Currently, chromosomal markers such as SNP substitutions, deletions, or site-specific IS*6110* transpositions are being used to facilitate rapid identification of other major *M. tuberculosis* strain families (e.g., Haarlem or LAM strain families [8, 14]).

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