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Rapid Detection of the *Mycobacterium tuberculosis* Beijing Genotype and Its Ancient and Modern Sublineages by IS*6110*-Based Inverse PCR

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The *Mycobacterium tuberculosis* Beijing genotype strains appear to be hypervirulent and associated with multidrug-resistant tuberculosis. Therefore, the development of a both rapid and simple method to detect the *M. tuberculosis* Beijing genotype is of clinical interest per se. Previously, we described a simple and fast approach to detect the Beijing genotype based on IS*6110* inverse-PCR typing. Here, we evaluated this method against a large, diverse, and recent collection of strains. The study sample included 866 *M. tuberculosis* strains representing but not limited to the regions in Russia, Europe, and East Asia where the Beijing genotype is endemic. Based on a spoligotyping method, 408 strains were identified as Beijing genotypes; they were additionally subdivided into ancient and modern sublineages based on the analysis of the NTF locus. All strains were further subjected to the IS*6110*-based inverse PCR. All of the Beijing genotype strains were found to have identical two-band (ancient sublineage) or three-band (modern sublineage) profiles that were easily recognizable and distinct from the profiles of the non-Beijing strains. Therefore, we suggest using IS*6110*-based inverse-PCR typing for the correct identification of the Beijing genotype and its major sublineages. The method is fast and inexpensive and does not require additional experiments but instead is implemented in the routine typing method of *M. tuberculosis*.

The Beijing genetic family is probably the most characterized phylogenetic lineage within *Mycobacterium tuberculosis*. This genotype was originally identified by using IS*6110*-restriction fragment length polymorphism (RFLP) and spacer oligonucleotide typing (spoligotyping) in strains collected in 1992 to 1994 in the Beijing area in China which coined the name (28). These strains are endemically prevalent in East Asia, South Africa, and Northern Eurasia (1, 9). Mokrousov et al. (19) hypothesized that primary dispersal of the Beijing genotype strains took place in China and had been driven by the Neolithic Proto-Sino-Tibetan farmers, whereas introduction of these strains into northern Eurasia was historically recent and might have been associated with expansion of the Mongol empire in the 13th to 15th centuries. The Beijing genotype is marked by genetic homogeneity and geographic omnipresence (1, 9). Taken together, these data likely reflect its global spread during the last century, if not in the last few decades. Recently, these strains have been found in countries as distant as Argentina (22), Malawi (8), and Australia (17), and new unexpected routes of their apparently secondary transmission are being uncovered (7). Currently, these strains attract great attention worldwide because they demonstrate high transmissibility (2), hypervirulent features (18, 29, 32), and an association with multiple-drug resistance (3). Therefore, the development of rapid and simple approach to detect the *M. tuberculosis* Beijing genotype is already of clinical interest.

Previously, we described a simple and fast approach based on IS*6110* inverse-PCR typing for the detection of the *M. tuberculosis* Beijing genotype strains in northwestern Russia (21). In the present study, we evaluated this method against the larger, diverse, and recently collected sample of strains representing but not limited to the regions in Russia, Europe, and East Asia where the Beijing genotype is endemic. In addition, this method was shown to be applicable to distinguish between major sublineages within the Beijing genotype.

MATERIALS AND METHODS

A total of 866 *M. tuberculosis* strains isolated between 2002 and 2005 were studied. These strains represented the following countries (regions or cities): Russia (n = 575; northwestern, northern, and central Russia, as well as Siberia), China (n = 122; Beijing), Vietnam (n = 117; Hanoi and Ho Chi Minh City), and Bulgaria (n = 51; Sofia, Plovdiv, Haskovo, and Veliko Tarnovo). The collections from China, Russia, and Vietnam were convenience samples, whereas Bulgarian strains were randomly collected.

The DNA of the studied strains was isolated according to the recommended method (27) and subjected to spoligotyping as described previously (13). A PCR approach was used to determine possible IS*6110* insertion(s) in the NTF region of the *M. tuberculosis* Beijing genotype strains as described by Plikaytis et al. (23).

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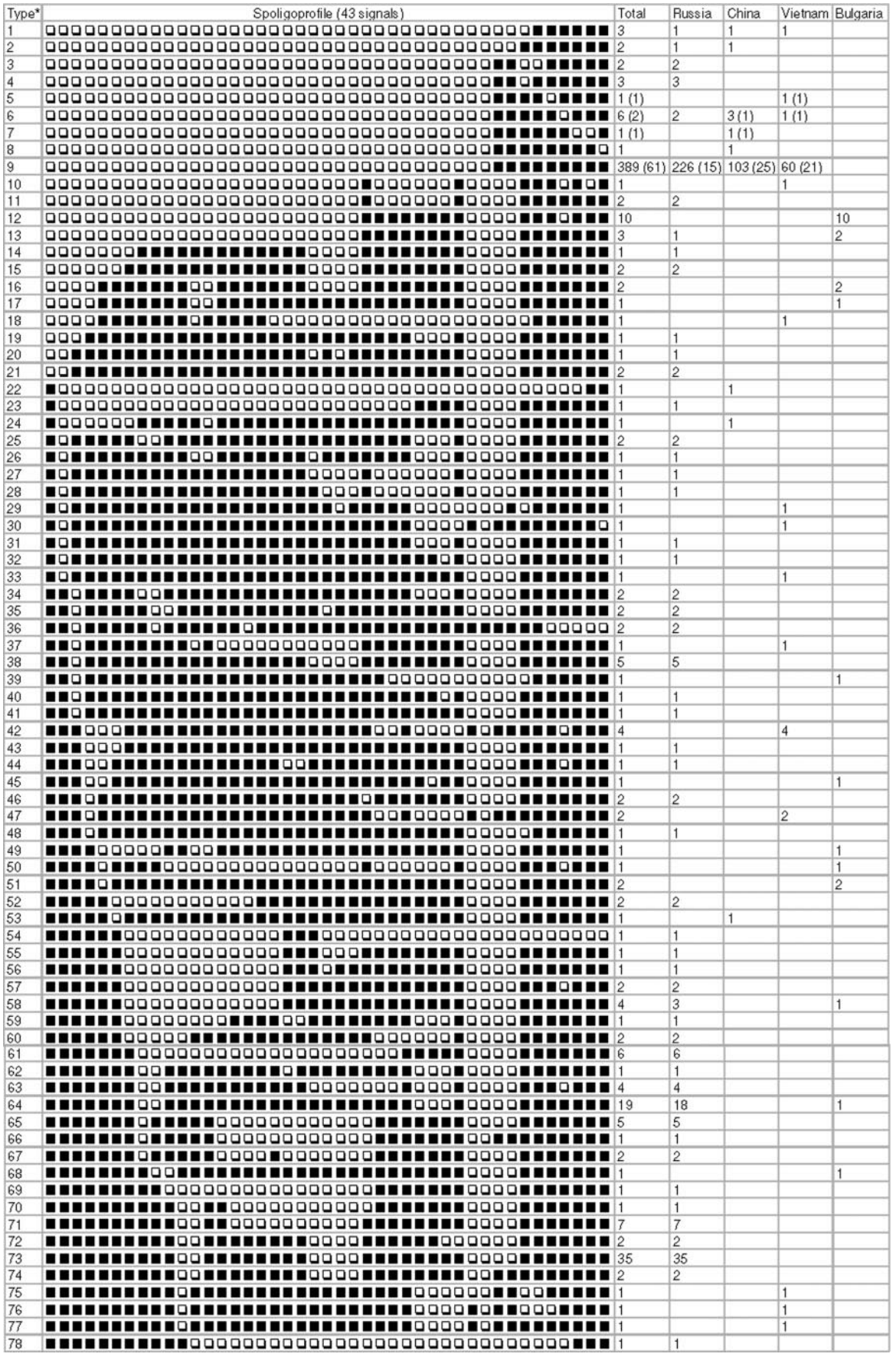


Type*	Spoligoprofile (43 signals)	Total	Russia	China	Vietnam	Bulgaria
1		3	1	1	1	
2		2	1	1		
3		2	2			
4		3	3			
5		1 (1)			1 (1)	
6		6 (2)	2	3 (1)	1 (1)	
7		1 (1)		1 (1)		
8		1		1		
9		389 (61)	226 (15)	103 (25)	60 (21)	
10		1			1	
11		2	2			
12		10				10
13		3	1			2
14		1	1			
15		2	2			
16		2				2
17		1				1
18		1			1	
19		1	1			
20		1	1			
21		2	2			
22		1		1		
23		1	1			
24		1		1		
25		2	2			
26		1	1			
27		1	1			
28		1	1			
29		1			1	
30		1			1	
31		1	1			
32		1	1			
33		1			1	
34		2	2			
35		2	2			
36		2	2			
37		1			1	
38		5	5			
39		1				1
40		1	1			
41		1	1			
42		4			4	
43		1	1			
44		1	1			
45		1				1
46		2	2			
47		2			2	
48		1	1			
49		1				1
50		1				1
51		2				2
52		2	2			
53		1		1		
54		1	1			
55		1	1			
56		1	1			
57		2	2			
58		4	3			1
59		1	1			
60		2	2			
61		6	6			
62		1	1			
63		4	4			
64		19	18			1
65		5	5			
66		1	1			
67		2	2			
68		1				1
69		1	1			
70		1	1			
71		7	7			
72		2	2			
73		35	35			
74		2	2			
75		1			1	
76		1			1	
77		1			1	
78		1	1			

FIG. 1. Schematic presentation of the *M. tuberculosis* spoligoprofiles. *, A local code used in the present study. The numbers of ancient Beijing strains (in case of the Beijing genotypes) are given in parentheses. An initial Excel file is available upon request.

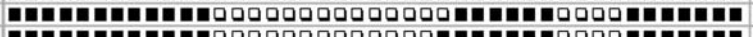
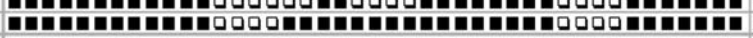
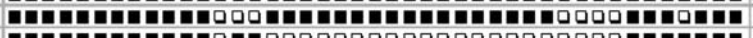
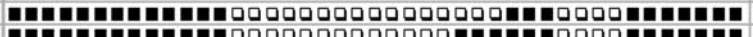
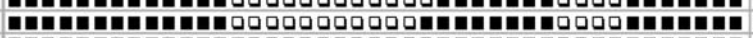
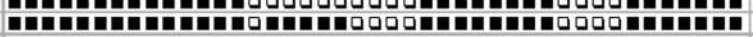
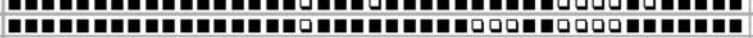
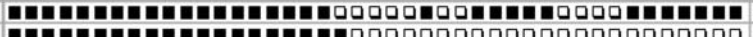
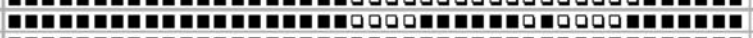
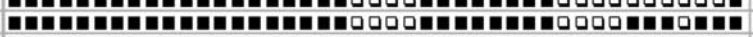
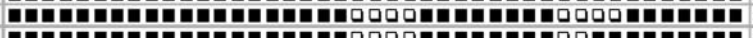
Type*	Spoligoprofile (43 signals)	Total	Russia	China	Vietnam	Bulgaria
79		1	1			
80		4	4			
81		1			1	
82		1			1	
83		3	3			
84		1	1			
85		1	1			
86		2	2			
87		1	1			
88		1	1			
89		1	1			
90		1	1			
91		1	1			
92		2	2			
93		1	1			
94		1			1	
95		1			1	
96		1	1			
97		7	7			
98		7	7			
99		1				1
100		1	1			
101		1	1			
102		7	7			
103		1		1		
104		1	1			
105		1	1			
106		26	26			
107		1	1			
108		1	1			
109		1			1	
110		2	2			
111		1	1			
112		1	1			
113		1			1	
114		1	1			
115		1	1			
116		5	4			1
117		4				4
118		1	1			
119		1	1			
120		1	1			
121		1	1			
122		1	1			
123		1	1			
124		2	1			1
125		55	54		1	
126		1	1			
127		3	3			
128		1			1	
129		1	1			
130		1				1
131		2	2			
132		1	1			
133		3			3	
134		1	1			
135		2	2			
136		3	1		1	1
137		1	1			
138		9	6			3
139		1			1	
140		1			1	
141		1	1			
142		1			1	
143		1			1	
144		1	1			
145		2	2			
146		1		1		
147		12	12			
148		1	1			
149		11	8		1	2
150		3	3			
151		1			1	
152		8	4	4		
153		1				1
154		43	25	1	5	12
155		2	1		1	
156		1			1	

FIG. 1.—*Continued*.

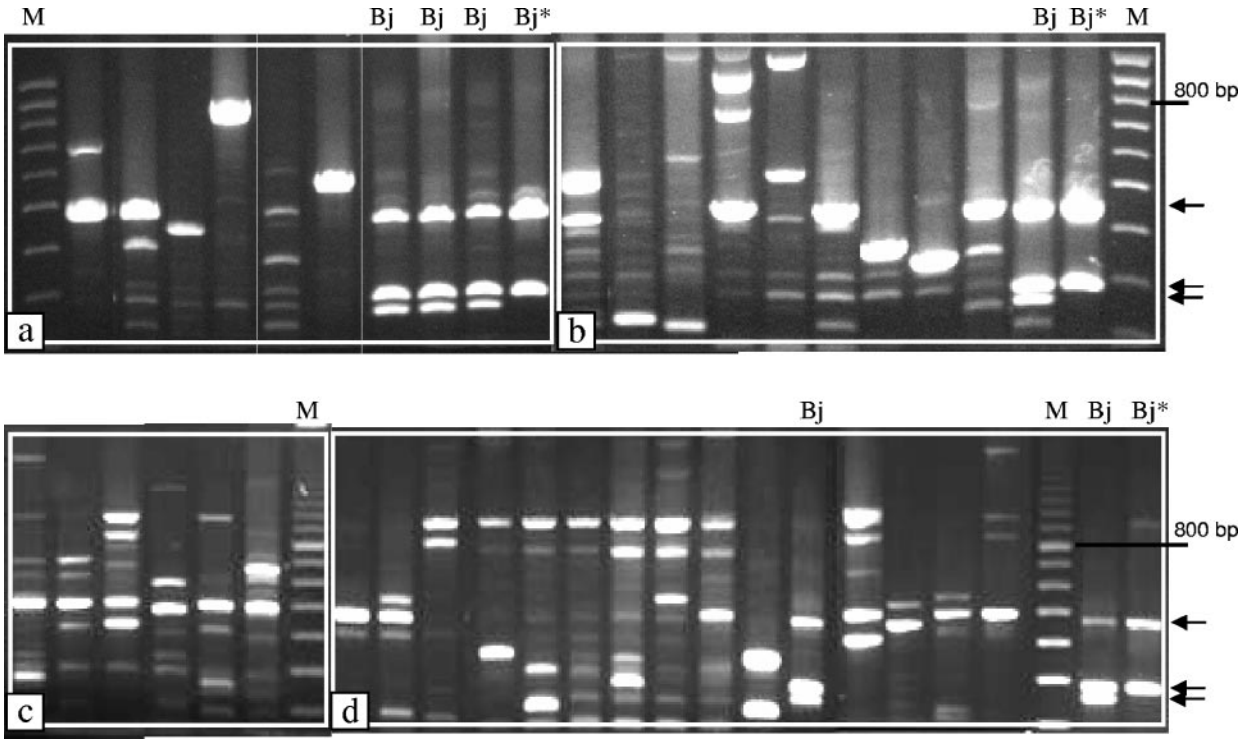


FIG. 2. Examples of IS*6110* inverse-PCR profiles of *M. tuberculosis* strains from China (a), Vietnam (b), Bulgaria (c), and Russia (d). Bj, Beijing genotype strains; ancient Beijing strains are also marked by an asterisk. M, 100-bp DNA ladder. Arrows indicate the PCR fragments specific for the Beijing genotypes (two- or three-band profile).

Genotyping by IS*6110*-based inverse PCR was performed with Ris1 and Ris2 primers (25) located outwardly at the 3′ and 5′ termini of IS*6110*. The T_m values for these primers were 54 and 55°C, respectively, as calculated with OligoDesign software (10). Purified DNA sample (0.1 to 0.5 μl) was added to the PCR mixture (final volume, 30 μl) that contained 30 pmol of each primer, 3.8 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Sileks, Russia; Amersham Biosciences, United Kingdom; or Promega, United States), and 200 μM concentrations of each deoxinucleoside triphosphate; the exact content of the PCR buffers is given in the *Taq* manufacturers' instructions. The reaction was performed in PTC-100 and PTC-200 thermal controllers (MJ Research, Inc.) as described previously (21) under the following conditions: an initial denaturation at 96°C for 3 min; 30 cycles of denaturation 95°C for 1 min, annealing 56°C for 1 min, and elongation 72°C for 1 min; and a final elongation 72°C for 4 min. The amplified fragments were electrophoresed in 1.3% agarose gels and visualized under UV light. In addition, we evaluated more stringent priming conditions, including increased annealing T (70°C) and reduced $MgCl_2$ concentration (1.5 mM).

Control for contamination during microbiological and genetic experiments was performed as recommended (5, 31). A control of possible contamination with previously amplified amplicons was performed by including a negative control sample (distilled water) in each PCR run; no contamination was detected.

The Hunter-Gaston index (HGI), an estimator of the discriminatory power of a typing method, was used to evaluate the diversity of the strain populations and was calculated as described previously (12).

RESULTS

A study sample included 866 strains recovered from the epidemiologically unlinked patients from geographically diverse locations. Spoligotyping subdivided these strains into a total of 156 spoligoprofiles, thus demonstrating a diversity of the studied collection of strains as a whole. For schematic presentation, spoligoprofiles were entered into a Microsoft Excel spreadsheet, rearranged by the sorting function, and assigned consecutive numbers (Fig. 1). Here, we defined the Beijing genotype strains by the characteristic spoligotype signature, i.e., the absence of the signals 1 to 34 and the presence of at least five of the nine remaining signals (35 to 43). By this approach, spoligotyping identified nine Beijing types in our sample, including three unique strains and six types shared by 2 to 389 strains (408 strains in total; types 1 to 9 [Fig. 1]). The non-Beijing types included 93 unique profiles and 54 types shared by 2 to 55 strains (458 strains in total). The high diversity of the non-Beijing strains was also manifested as a high HGI value of 0.96. The spoligotyping-based HGI was low for the Beijing sample (0.08), a finding that is not unexpected and is explained by the fact that most (389 of 407) Beijing family strains had identical nine-signal spoligoprofiles.

Additional rough subdivision within the Beijing genotype was performed by analysis of the NTF region for the presence, number, and orientation of IS*6110* insertions. It revealed that 61 strains had an intact NTF region; these strains were designated as "ancient" sublineage within the Beijing genotype, as previously proposed (19, 20). The remaining 328 Beijing strains had one IS*6110* direct insertion on the right side of the NTF region and were designated as the "modern" sublineage in the Beijing genotype (19, 20).

The sample of diverse strains of different genotypes was further evaluated by IS*6110*-based inverse PCR. This method generated multiband profiles in agarose gels for all studied strains (e.g., Fig. 2). Although the aim of the present study was not to compare the discriminatory power of the different typing schemes, we note that in some instances PCR typing further

differentiated within certain spoligotypes, such as, types 12, 138, 152, and 154 (Fig. 1, types ST125, ST47, ST52, and ST53; SpolDB4 [http://www.pasteur-guadeloupe.fr/tb/spoldb4]) representing likely evolutionarily stable variants of the DR locus. Nevertheless, a distinctive two- or three-band PCR profile in the agarose gel was generated for all Beijing strains, including those with abridged "Beijing-like" spoligoprofiles (types 1 to 8 in Fig. 1). In particular, a two-band (~290- and 470-bp) profile was generated for ancient Beijing strains, whereas a three-band (~260-, 290-, and 470-bp) profile was generated for modern Beijing strains (Fig. 2). When Beijing strains were run in the same gel with non-Beijing strains, their profiles were easily recognizable and clearly distinct from different profiles of the non-Beijing strains (Fig. 2).

It is known that PCR typing based on use of the IS*6110* outward primers alone or in combination with PGRS outward primers (6) lacks sufficient interlaboratory reproducibility (14), although this method shows better intralaboratory performance (4, 21). In the first publication of the method (25), it was demonstrated that a number of bands resulted from priming between one copy of IS*6110* and a nonspecific priming site near this copy of IS*6110*. Therefore, we evaluated this issue by repeating IS*6110* inverse PCR in different laboratories using different thermal cyclers and *Taq* DNA polymerases, as well as various stringent PCR conditions, such as increased annealing temperature and low $MgCl_2$ concentration, that may influence PCR and priming specificity. As a result, reproducible characteristic profiles of the two or three signature bands were consistently generated for Beijing genotype strains, suggesting specificity of the major fragments in their inverse-PCR profiles, although the best performance was achieved using the basic protocol (21).

DISCUSSION

Spoligotyping is by definition a "gold standard" method for detecting a Beijing strain. However, the present spoligotyping assay design allows (or, rather, urges) examination of up to 40 strains simultaneously, and stringent stripping of the membrane follows each hybridization before reuse of the membrane to test new strains (13). For this reason, this method is not always convenient in practice since it becomes rather expensive when smaller sets of strains or single isolates are to be analyzed at a time. Spoligotyping does not need purified DNA and hence is faster than IS*6110*-RFLP typing; however, it requires a special apparatus and reagents for hybridization and fluorescent signal detection. Several genotypic approaches have recently been published to detect the Beijing genotype strains. In particular, simple PCR or real-time PCR-based methods have been utilized to detect genomic regions specifically deleted in the Beijing strains (11, 26, 30). Their limitations are (i) a possibility (albeit rare) of false-negative results due to amplification inhibition when only one fragment is expected to be amplified (26) and (ii) more expensive equipment is required for the real-time PCR (11). The approaches to identify Beijing strains by IS*6110*-RFLP fingerprinting, followed by computer-assisted comparison with reference profiles (15) or by rehybridizing membrane with Beijing-specific probes (16), are comprehensive but appear to be even more laborious and time-consuming. Also, use of these methods implies an additional experiment or activity specifically aimed to detect only the Beijing genotype strains. A very recently published study suggesting a specific seven-copy signature of the MIRU-VNTR locus 26 in the Beijing genotype (24) lacks both sensitivity and specificity since, in fact, this locus is polymorphic in the Beijing genotype and, on the other hand, seven copies in this locus have been described in strains of other *M. tuberculosis* genotypes (reference 19 and references therein). For this reason, a simpler method based solely on a standard PCR and agarose gel electrophoresis (i.e., IS*6110* inverse PCR as proposed and evaluated here) to preliminarily detect a Beijing strain could become a useful complement to the IS*6110*-RFLP and spoligotyping schemes.

Although a detailed comparison with drug susceptibility data was beyond the scope of the present study, we noted a high rate of multidrug-resistant (MDR) strains in Russian and Bulgarian samples (39.2 and 21.6%, respectively). In the Russian sample, the MDR phenotype was found in 48.6% of the Beijing genotype strains versus 29.4% of the non-Beijing strains ($P < 0.0001$), suggesting that current transmission of MDR-TB in Russia is greatly influenced by the ongoing dissemination of the Beijing strains. In contrast, the Beijing genotype was not identified in the studied strains from Bulgaria, a country with close historical and recent links with Russia. Consequently, the current situation with MDR-TB in Bulgaria cannot be explained by the transmission of the Beijing genotype that apparently has not yet reached this country.

In conclusion, we believe that the IS*6110*-based inverse-PCR method can be used for correct identification of the Beijing genotype and its ancient and modern sublineages. The method is fast, straightforward, and inexpensive and does not require additional experiments but instead is implemented in the routine typing method for *M. tuberculosis*. It may be used in areas where the Beijing genotype is known to be endemically prevalent or is being frequently or sporadically imported. A limitation of our study was that strains from the two secondary foci of the Beijing genotype, South Africa and the United States, were not available; further studies are needed to evaluate the proposed method with *M. tuberculosis* strain collections from these areas.

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REFERENCES

1. **Bifani, P. J., B. Mathema, N. E. Kurepina, and B. N. Kreiswirth.** 2002. Global dissemination of the *Mycobacterium tuberculosis* W-Beijing family strains. Trends Microbiol. **10:**45–52.
2. **Caminero, J. A., M. J. Pena, M. I. Campos-Herrero, J. C. Rodriguez, I. Garcia, P. Cabrera, C. Lafoz, S. Samper, H. Takiff, O. Afonso, J. M. Pavon, M. J. Torres, D. van Soolingen, D. A. Enarson, and C. Martin.** 2001. Epidemiological evidence of the spread of a *Mycobacterium tuberculosis* strain of the Beijing genotype on Gran Canaria Island. Am. J. Respir. Crit. Care Med. **164:**1165–1170.
3. **Cox, H. S., T. Kubica, D. Doshetov, Y. Kebede, S. Rüsch-Gerdess, and S. Niemann.** 2005. The Beijing genotype and drug-resistant tuberculosis in the Aral Sea region of Central Asia. Respir. Res. **6:**134. [Online.] http://respiratory-research.com/content/6/1/134.
4. **Dela, A., A. Sajduda, I. Pawlowska, and J. Dziadek.** 2006. Molecular characterization of *Mycobacterium tuberculosis* isolates from Lodz, Poland: anal-

ysis by IS*6110* restriction fragment length polymorphism and double-repetitive-element PCR. J. Infect.

5. **Dragon, E. A., J. P. Spadoro, and R. Madej.** 1993. Quality control of polymerase chain reaction, p. 160–168. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
6. **Friedman, C. R., M. Stoeckle, W. D. Johnson, Jr., and L. W. Riley.** 1995. Double-repetitive-element PCR method for subtyping *Mycobacterium tuberculosis* clinical isolates. J. Clin. Microbiol. **33:**1383–1384.
7. **Garcia de Viedma, D., F. Chaves, J. Inigo, for the Tuberculosis molecular epidemiology study group.** 2006. New route of importation of *Mycobacterium tuberculosis* Beijing genotype. Emerg. Infect. Dis. **12:**169–170.
8. **Glynn, J. R., A. C. Crampin, H. Traore, M. D. Yates, F. D. Mwaungulu, B. M. Ngwira, S. D. Chaguluka, D. T. Mwafulirwa, S. Floyd, C. Murphy, F. A. Drobniewski, and P. E. Fine.** 2005. *Mycobacterium tuberculosis* Beijing genotype, northern Malawi. Emerg. Infect. Dis. **11:**150–153.
9. **Glynn, J. R., J. Whiteley, P. J. Bifani, K. Kremer, and D. van Soolingen.** 2002. Worldwide occurrence of Beijing/W strains of *Mycobacterium tuberculosis*: a systematic review. Emerg. Infect. Dis. **8:**843–849.
10. **Herold, K. E., and A. Rasooly.** 2003. Oligo Design: a computer program for development of probes for oligonucleotide microarrays. BioTechniques **35:** 1216–1221.
11. **Hillemann, D., R. Warren, T. Kubica, S. Rusch-Gerdes, and S. Niemann.** 2006. Rapid detection of *Mycobacterium tuberculosis* Beijing genotype strains by real-time PCR. J. Clin. Microbiol. **44:**302–306.
12. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
13. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. D. A. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
14. **Kremer, K., D. van Soolingen, R. Frothingham, W. H. Haas, P. W. 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.
15. **Kremer, K., J. R. Glynn, T. Lillebaek, S. Niemann, N. E. Kurepina, B. N. Kreiswirth, P. J. Bifani, and D. van Soolingen.** 2004. Definition of the Beijing/W lineage of *Mycobacterium tuberculosis* on the basis of genetic markers. J. Clin. Microbiol. **42:**4040–4049.
16. **Kurepina, N., E. Likhoshvay, E. Shashkina, B. Mathema, K. Kremer, D. van Soolingen, P. Bifani, and B. N. Kreiswirth.** 2005. Targeted hybridization of IS*6110* fingerprints identifies the W-Beijing *Mycobacterium tuberculosis* strains among clinical isolates. J. Clin. Microbiol. **43:**2148–2154.
17. **Lavender, C., M. Globan, A. Sievers, H. Billman-Jacobe, and J. Fyfe.** 2005. Molecular characterization of isoniazid-resistant *Mycobacterium tuberculosis* isolates collected in Australia. Antimicrob. Agents Chemother. **49:**4068–4074.
18. **Lopez, B., D. Aguilar, H. Orozco, M. Burger, C. Espitia, V. Ritacco, L. Barrera, K. Kremer, R. Hernandez-Pando, K. Huygen, and D. van Soolingen.** 2003. A marked difference in pathogenesis and immune response induced by different *Mycobacterium tuberculosis* genotypes. Clin. Exp. Immunol. **133:**30–37.
19. **Mokrousov, I., H. M. Ly, T. Otten, N. N. Lan, B. Vyshnevskyi, S. Hoffner, and O. Narvskaya.** 2005. Origin and primary dispersal of the *Mycobacterium tuberculosis* Beijing genotype: clues from human phylogeography. Genome Res. **15:**1357–1364.
20. **Mokrousov, I., O. Narvskaya, T. Otten, A. Vyazovaya, E. Limeschenko, L. Steklova, and B. Vyshnevskyi.** 2002. Phylogenetic reconstruction within *Mycobacterium tuberculosis* Beijing genotype in northwestern Russia. Res. Microbiol. **153:**629–637.
21. **Mokrousov, I., T. Otten, A. Vyazovaya, E. Limeschenko, M. L. Filipenko, C. Sola, N. Rastogi, L. Steklova, B. Vyshnevskiy, and O. Narvskaya.** 2003. PCR-based methodology for detecting multidrug-resistant strains of *Mycobacterium tuberculosis* Beijing family circulating in Russia. Eur. J. Clin. Microbiol. Infect. Dis. **22:**342–348.
22. **Morcillo, N., B. Di Giulio, C. Chirico, A. Kuriger, A. Dolmann, A. Alito, M. Zumarraga, D. van Soolingen, K. Kremer, and A. Cataldi.** 2005. First description of *Mycobacterium tuberculosis* Beijing genotype in Argentina. Rev. Argent. Microbiol. **37:**92–95.
23. **Plikaytis, B. B., J. L. Marden, J. Crawford, C. L. Woodley, W. R. Butler, and T. M. Shinnick.** 1994. Multiplex PCR assay specific for the multidrug resistant strain W of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **32:**1542–1546.
24. **Rao, K. R., N. Ahmed, S. Srinivas, L. A. Sechi, and S. Hasnain.** 2006. Rapid identification of *Mycobacterium tuberculosis* Beijing genotypes on the basis of the mycobacterial interspersed repetitive unit locus 26 signature. J. Clin. Microbiol. **44:**274–277.
25. **Ross, B. C., and B. Dwyer.** 1993. Rapid, simple method for typing isolates of *Mycobacterium tuberculosis* by using the polymerase chain reaction. J. Clin. Microbiol. **31:**329–334.
26. **Tsolaki, A. G., S. Gagneux, A. S. Pym, Y. O. Goguet de la Salmoniere, B. N. Kreiswirth, D. van Soolingen, and P. M. Small.** 2005. Genomic deletions classify the Beijing/W strains as a distinct genetic lineage of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **43:**3185–3191.
27. **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. Shinnik, and P. M. Small.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
28. **van Soolingen, D., L. Qian, P. E. W. de Haas, J. T. Douglas, H. Traore, F. Portaels, Z. Quing, D. Enkhasaikan, P. Nymadawa, and J. D. A. van Embden.** 1995. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. J. Clin. Microbiol. **33:**3234–3238.
29. **Vishnevskii, B. I., O. V. Narvskaia, S. N. Vasil'eva, N. V. Sapozhnikova, I. V. Mokrousov, and T. F. Otten.** 2002. Virulence of *Mycobacterium tuberculosis*. Probl. Tuberk. **10:**33–36. (In Russian.)
30. **Warren, R. M., T. C. Victor, E. M. Streicher, M. Richardson, N. Beyers, N. C. Gey van Pittius, and P. D. van Helden.** 2004. Patients with active tuberculosis often have different strains in the same sputum specimen. Am. J. Respir. Crit. Care Med. **169:**610–614.
31. **World Health Organization.** 1998. Laboratory services in tuberculosis control. III. Culture. World Health Organization, Geneva, Switzerland.
32. **Zhang, M., J. Cong, Z. Yang, B. Samten, and P. F. Barnes.** 1999. Enhanced capacity of a widespread strain of *Mycobacterium tuberculosis* to grow in human macrophages. J. Infect. Dis. **179:**1213–1217.