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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN PRAGUE AND SOUTH MORAVIA, CZECH REPUBLIC: GENETIC ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES BY IS*6110*-RFLP FINGERPRINTING AND SPOLIGOTYPING

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SUMMARY

Objectives: To genetically characterize and compare *Mycobacterium tuberculosis* isolates among culture-confirmed TB cases in two regions in the Czech Republic in 1998.

Methods: Consecutive *M. tuberculosis* isolates from 111 TB patients in Prague and 120 patients in the South Moravia region were genotyped using the standardized IS*6110* Southern blot hybridization method and by spoligotyping.

Results: Eighty of the Prague patients (72.1%) had isolates with unique RFLP patterns, while 31 (27.9%) had isolates which belonged to 10 clusters. Seventy-eight (64.7%) of the South Moravia strains displayed unique RFLP pattern and 42 (35.3%) were assigned into 15 clusters. The spoligotype profiles previously identified in the U.S. were found in 69 (33%) samples and newly identified Czech spoligotypes in 24 (11.4%) of the total number of examined strains.

Conclusions: The present population-based molecular epidemiological study performed in two regions of the Czech Republic in 1998 demonstrated the distribution of individual genotypes as well as clustered strains of M. tuberculosis isolated from TB patients, and confirmed the similarity between the Czech strain collection and the European Community TB Database, that includes countries with low TB rate. The sporadic import of TB cases from foreign countries and recent transmission events probably do not play significant roles in the epidemiological situation in the Czech Republic.

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INTRODUCTION

The achievements of molecular genetics have substantially enriched the traditional scope of the epidemiology of tuberculosis (TB) and have addressed most aspects of the natural history of mycobacterial infections (6, 21, 22). The standardized IS*6110* –based RFLP (restriction fragment length polymorphism) fingerprinting of *Mycobacterium tuberculosis* (*M. tuberculosis*) was introduced to the analysis of clinical samples isolated from TB patients in the Czech Republic in 1996, in a pilot study looking at transmission of tuberculosis among prisoners and between family members (6); subsequently, it was implemented in a point-prevalence analysis of cultures isolated from TB patients in Prague (9), in description of a multi-drug resistant TB microepidemic (7), and in the investigation of family outbreaks and transmission of TB in South Moravia (8, 11). The spoligotyping procedure was employed in a pilot study of selected Prague and South Moravian strains of *M. tuberculosis* (10), in a study on the molecular epidemiology of TB among prisoners (12) and in identification of the W-Beijing genotype in two Czech regions (13).

For the present community-based study, two geographically distant and epidemiologically disparate areas were chosen. The first was Prague, the capital of the Czech Republic, a metropolitan area with a population of more than 1.2 million people and the highest recorded TB incidence rate in the country (12.3 bacteriologically proven cases of TB of the respiratory system per 100,000 in 1998) (20). The second was South Moravia region, with a population of more than 1.9 million, and with the lowest incidence of pulmonary TB (5.5 bacteriologically verified cases per 100,000 in 1998). The aim of the study was to characterize the distribution of various genotypes of *M. tuberculosis* strains isolated in 1998 in Prague and South Moravia. Two standardized molecular strain typing techniques were used in this study: the IS*6110*-based RFLP genotyping, and the spacer-oligonucleotide (spoligotyping) analysis (2, 21).

MATERIALS AND METHODS

Clinical isolates of *M. tuberculosis*. Between January 1 and December 31, 1998, totals of 152 and 133 culture-confirmed TB cases were reported in Prague and the South Moravia region respectively. For genotyping purposes, 111 (73% of all culture-positive cases) isolates of *M. tuberculosis* were collected from TB patients in the Institute of Hygiene of the City of Prague, and 120 (90.2%) cultures from South Moravia were obtained from the patients in the Regional Institute of Hygiene in Brno. A single isolate from each patient was included in the analysis. Isolates from 41 (27%) Prague cases and 13 (9.8%) South Moravian patients were non-viable or not available. The isolates were subcultured on Lowenstein-Jensen media and transported to the Public Health Research Institute (PHRI) TB Center in Newark, NJ, U.S., for IS*6110*-based RFLP genotyping. Aliquots of 20 µl of the mycobacterial genomic DNA were then forwarded to the Wadsworth Center, New York State Department of Health, Albany, N.Y. for spoligotyping.

Patient demographic characteristics. Patient information, provided by the collaborating laboratories, was compared with data reported to the Republic Tuberculosis Surveillance Unit for compulsory notification. In some cases, it was also verified by reviewing records kept at the respective territorial TB and pneumonology departments. Information such as age, sex, country of birth and residence (in the Prague City quarters or in South Moravia districts) were recorded.

IS*6110* DNA fingerprinting. *M. tuberculosis* cells were harvested from Lowenstein-Jensen slants, and chromosomal DNA was isolated according to the standard procedures published elsewhere (21). Genomic DNA was digested with P*vuII*, transferred to a Hybond N+ membrane after separation by electrophoresis in 1% agarose gel, and hybridized with an IS*6110*-specific probe using ECL Direct Nucleic Acid Labelling and Detection System (Amersham Biosciences, Chalfont, St. Giles, UK). IS*6110* hybridization profiles were compared to the entire PHRI TB Center Database (over 17,000 patterns of clinical *M. tuberculosis* isolates from TB patients in U.S. and other countries) using a Sun Sparcs5 workstation and BioImage Whole Band Analyzer, version 3.4 (Genomic Solutions, Ann Arbor, MI). The nomenclature of strain clusters and families designation was described previously (14). Two or more strains with identical IS*6110* patterns isolated from different patients were defined as a cluster, and the strain family was considered to comprise clusters sharing > 60% of similarity. Unique ("orphan") strains that did not demonstrate 100% similarity to any pattern in the PHRI TB Center Database were designated as 001.

Spoligotyping procedure. Spoligotype analysis was performed according to a standard method (2). Spoligotype patterns were analyzed with BioImage Whole Band Analysis v. 3.4 software (Genomic Solutions, Ann Arbor, MI) on a Sun Ultra 10 workstation (Sun Microsystems Inc., Santa Clara, CA). Spoligotype patterns were assigned descriptive nomenclature according to the standard method (3).

RESULTS

Patients information. Among 111 patients (Table 1) whose *M. tuberculosis* isolates were genotyped (73% of all culture-positive cases collected in Prague between January 1 and December 31, 1998), there were 79 males (median age, 58.1 years, range 20–98 years) and 32 females (median age, 64.5 years, range 24–96 years). The patients came from all city quarters, and more than 60% resided in the four most densely inhabited districts P–4, P–8, P–9, and P–10. Three patients were homeless (Fig. 1, Table 1). In South Moravia, among 120 (90.2% of all culture-positive TB cases) patients with *M. tuberculosis* isolates genotyped, there were 87 males (median age 52.4 years, range 25–92 years) and 33 females (median age 66.4 years, range 27–91 years). The patients originated from all 14 districts, but nearly 60% of patients resided in the city and county of Brno (the regional center) and in three border districts: Zlín, Hodonín and Znojmo; one homeless person was also included in the study (Fig. 2, Table 1).

IS*6110* RFLP fingerprinting. The IS*6110* fingerprinting (Fig. 3) revealed a bimodal distribution of strains with the IS*6110* copy number ranging from 3 to 17 in Prague and from 6 to 22 copies in Moravian strains, peaking in the 8–9 copy-number range (Fig. 4). The IS*6110*-based RFLP analysis (Table 2, Fig. 3) showed that 80 (72.1%) of *M. tuberculosis* isolates from Prague and 78 (65%) isolates from South Moravia had unique (orphan) pattern, whereas 31 (27.9%) Prague strains had RFLP profiles that represented 10 different strain clusters, and 42 (35.3%) Moravian strains were assigned into 15 clusters. In Prague, four clusters contained isolates from two patients, two clusters contained isolates from three patients, three clusters had four strains, and the biggest cluster consisted of six strains. In South Moravia, eight clusters contained two strains, four clusters contained isolates from three patients, one contained four strains, and two clusters contained five strains. In Prague, the mean age of patients infected with any of the clustered strains was 62.2 years, compared to 59.1 years for patients with non-clustered strains. In South Moravia, the mean age for patients infected with any of the clustered strains was 53.5 years, compared to 57.7 years for patients infected with non-clustered strains (Table 2).

Geographical analysis showed that the majority of clustered cases in Prague (Fig. 1) were recorded in the four most densely inhabited city quarters: in Prague 8 (9 clustered cases among 13 totals), Prague 9 (5/18), Prague 10 (3/17) and Prague 4 (3/19). In South Moravia (Fig. 2), the majority of clustered strains originated from the regional capital Brno (16/33) and from the districts of Znojmo (8/12), Zlín (5/13), Jihlava (3/8), and Vyškov (3/10).

Spoligotyping analysis. Spoligotyping analysis was performed for 107 *M. tuberculosis* isolates from Prague and 102 isolates from South Moravia (Fig. 5). The strains represented 70.4% of the culture-positive TB cases reported in 1998 in Prague and 76.7% cases reported in South Moravia. In total, there were 151 males (mean age 53.3 years, range 20–98 years) and 58 females (mean age 64.8 years, range 24–91 years). Eleven individuals were foreign born and 198 were Czech Republic citizens. Three homeless persons from Prague and one from South Moravia were included in the study.

Among 209 *M. tuberculosis* isolates from Prague and South Moravia, prototype spoligotypes (according to the nomenclature of the Global Spoligo Database SpolDB3 (5)) were identified for

Table 1. *Demographic data of Czech patients in this study*

Characteristic	Prague	South Moravia	Total˝
Total patients	111 (100%)	120 (100%)	231 (100%)
Age range (years)	20 – 98	25 – 92	20 – 98
Age average (years)	59.4	56.2	57.7
Males, total	79 (71.2%)	87 (72.5%)	166 (71.9%)
Age range (years)	20 – 98	25 – 92	20 – 98
Age average (years)	58.1	52.4	55.1
Females, total	32 (28.8%)	33 (27.5%)	65 (28.1%)
Age range (years)	24 – 96	27 – 91	24 – 96
Age average (years)	64.5	66.4	65.5
Domicile (Prague quarter/South Moravia district)			
P1	3	B	33
P2	4	BL	2
P3	9	BV	8
P4	19	HO	12
P5	12	JI	8
P6	7	KM	6
P7	6	PR	3
P8	13	TR	2
P9	18	UH	5
P10	17	VY	10
Homeless	3	ZL	13
		ZN	12
		ZS	4
		Homeless	1

Districts of South Moravia: B – Brno City and County, BL – Blansko, BV – Břeclav, HO – Hodonín, JI – Jihlava, KM – Kroměříž, PR – Prostějov, TR – Třebíč, UH- Uherské Hradiště, VY – Vyškov, ZL – Zlín, ZN – Znojmo, ZS – Žďár n. S.

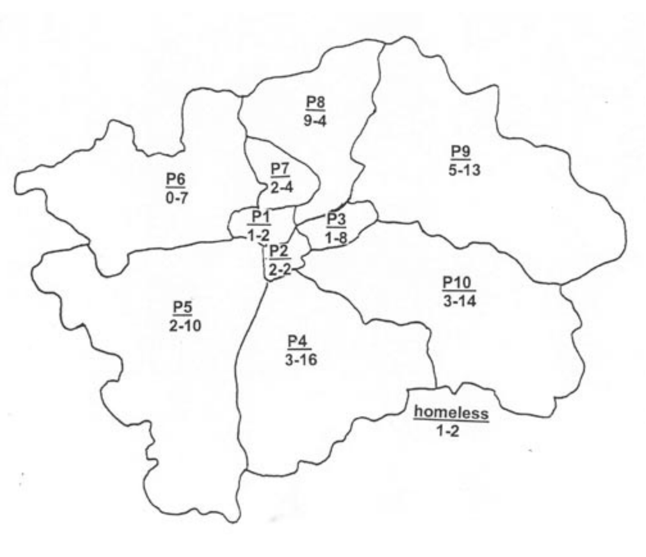

Fig. 1. *Distribution of patients with clustered and nonclustered RFLP profiles (n-n) of M. tuberculosis isolates in Prague quarters.*

116 (55.5%) strains (Table 3, Fig. 5). They were represented by the following prevalent families: Haarlem3 (18.1%), T1 (14.3%), Haarlem1 (8.1%), T2 (3.8%), Beijing (3.3%) and LAM10 (2.9%); the representation of the remaining five prototypes was low, and ranged between one and four cases. Being compared with Wadsworth Center Spoligotype Database, Czech collection revealed the spoligotype profiles previously identified in the U.S., in 70 (33.5%) Czech isolates, and newly identified Czech spoligotypes were found in 23 (11.0%) Czech samples. The frequency of prototype spoligos was slightly higher in the South Moravian strains (58.8% of the total) than in the Prague isolates (52.3%). On the other hand, US and newly identified Czech profiles were slightly more frequent among Prague strains (35.5 and 12.2% respectively) than among South Moravian isolates (30.4 and 10.8% respectively).

DISCUSSION

The Czech Republic, as a result of it’s geographical location and historical traditions, represents a buffer zone (17.6 TB cases per 100,000 in 1998) between the countries of the Western Europe

Table 2. *Summary of IS6110-based RFLP genotypes of M. tuberculosis strains isolated from TB patients in Prague and South Moravia*

Genotype	Prague	South Moravia
Individual genotypes	80 (72.1%)	77 (64.7%)
Age range	25 – 91	24 – 96
Age average	59.1	57.7
Clustered genotypes	31 (27.9%)	42 (35.3%)
Age range	29 – 97	26 – 85
Age average	62.2	53.5
Number of clusters	10	15
2 strains per cluster	4	8
3 strains per cluster	2	4
4 strains per cluster	3	1
5 strains per cluster	0	2
6 strains per cluster	1	0

reporting 13.2 cases per 100,000, and the Eastern Europe and the former Soviet Republics, where the annual notification rate reached 77.7 TB cases per 100,000 in 1998 (4). Classical epidemiological approaches cannot give a clear answer to the question whether the import of "eastern TB" is really a factor in the transmission of tuberculosis in the Czech Republic. However, traditional descriptive demographic information in the epidemiology of TB has been recently supplemented by data obtained by molecular typing of *M. tuberculosis*. At present, the genetic characteristics of *M. tuberculosis* populations have been described in various parts of the world including most Western European countries and a few territories in the Eastern Europe as well. Therefore, an analysis by molecular methods of *M. tuberculosis* cultures isolated from TB patients in the Czech Republic should contribute to the steadily growing body of knowledge on the geographical distribution of various *M. tuberculosis* genotypes circulating in Europe and elsewhere (5, 22).

The present study was aimed at establishing the point prevalence distribution of various RFLP genotypes and spoligotypes circulating in two communities, the capital city of Prague, with the highest incidence rate of TB in the Czech Republic, and the South Moravia, which reports the lowest incidence rates of TB (20). The samples in our study included 73% of all culture-positive pulmonary TB cases identified in Prague and 90% of those reported in South Moravia, and most likely are representative for the epidemiologic situation in the Czech Republic in 1998.

The IS*6110* RFLP genotyping of *M. tuberculosis* strains isolated in the present study in Prague yielded patterns that were relatively similar to those recorded there in 1995 (9). They are characterized by a polymorphic banding pattern, absence of isolates with a single copy of IS*6110*, a predominantly low number of IS*6110* bands ranging from 3 to 17 (5 to 15 in 1995) and a similar proportion of clustered profiles (29.3% in 1995 and 27.9% in 1998). In contrast to the absence of apparent family- or community-related contacts among patients infected with strains sharing identical RFLP patterns in 1995, the present study disclosed two cases suggesting a person-to-person transmission of TB: (a) identical RFLP patterns of the DF1 clone of *M. tuberculosis* (cluster P–4; Fig. 3) were found in a 44-year-old homeless man as the index person, and a 53-year-old laboratory technician and a 49-old-man (11, 18). The RFLP pattern of the NB clone (cluster P–8) suggested a probable transmission of TB from a 58-year--old man to his 47-year-old wife. It was proven that possibility of laboratory cross-contamination was ruled out, and identical RFLP profiles are the result of true transmission. The relatively high average age of Prague patients (59.4 years), the absence of TB in children and adolescents and the high proportion of individual genotypes (72.1% of typed strains) support our view expressed in a previously published paper (9), that endogenous reactivation, rather than exogenous transmission, is the major determinant of most cases. The occurrence of 10 distinct clusters comprising 27.9% of patients suggests that these cases probably developed the active disease from a recent exogenous transmission.

Unlike the Prague situation, there are no published data available to allow a comparative genotyping analysis of *M.*

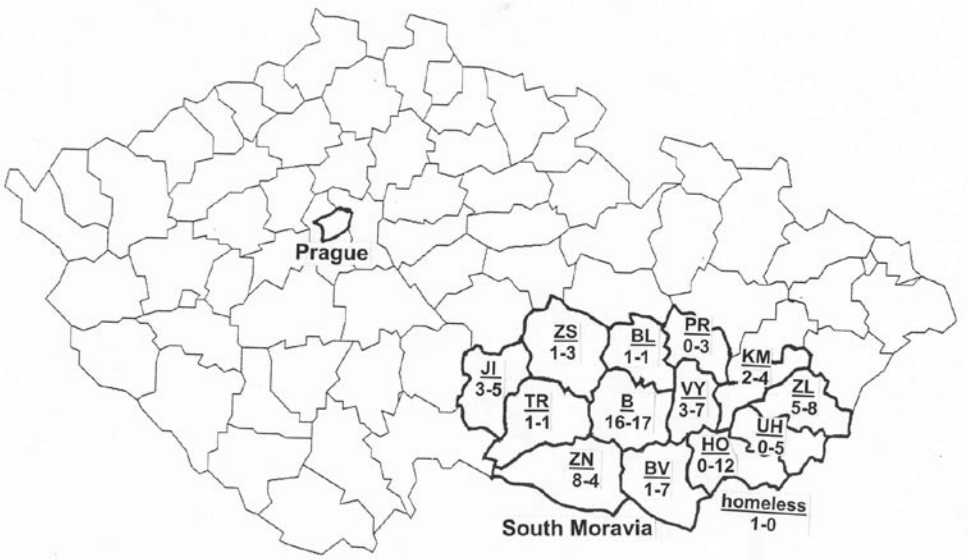

Fig. 2. *Distributions of patients with clustered and nonclustered RFLP profiles (n-n) of M. tuberculosis isolates in districts of South Moravia.*

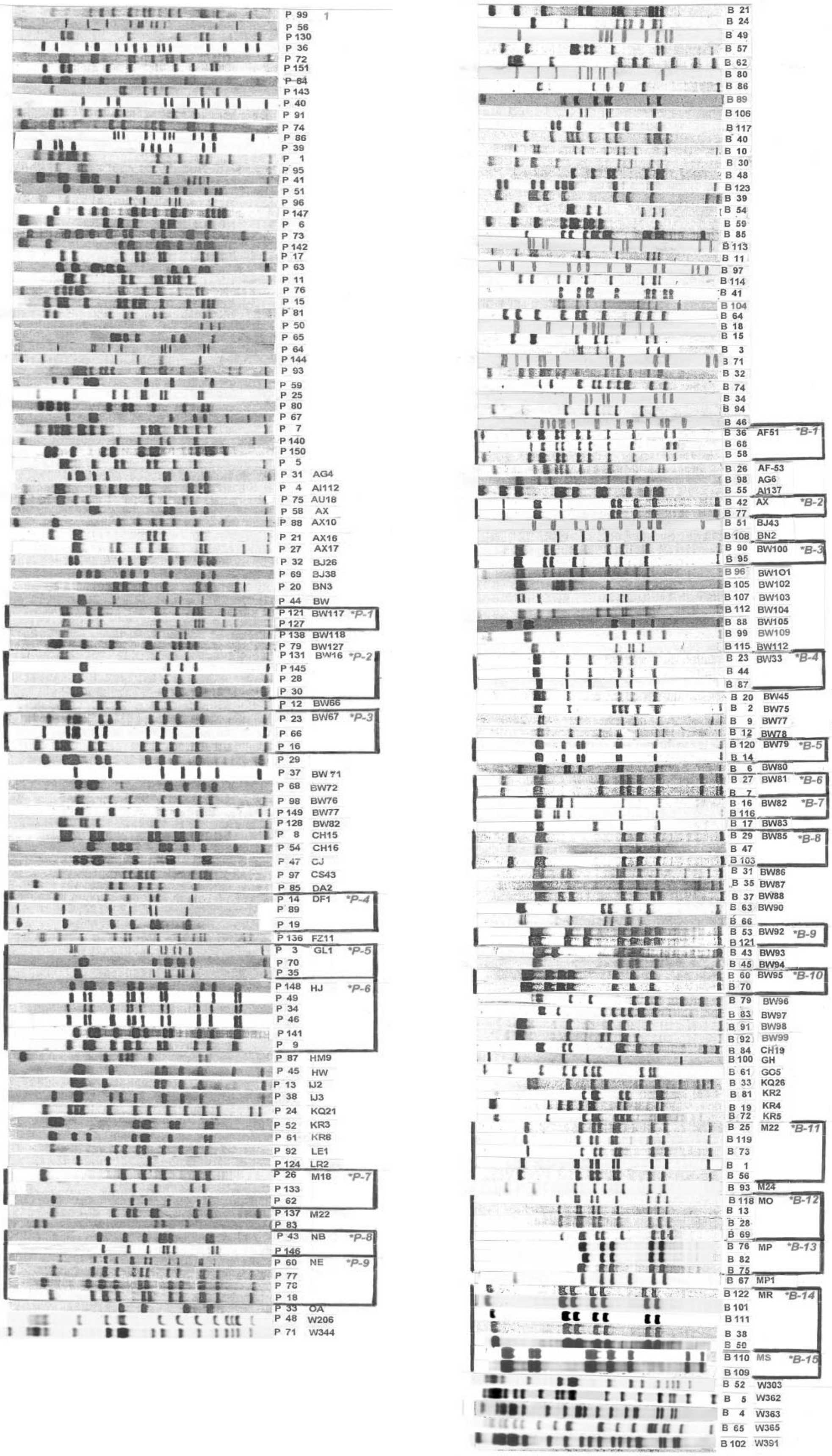

Fig. 3. *IS6110 RFLP patterns of M. tuberculosis isolates from Prague (designated P) and South Moravia (designated B). Clustered profiles are boxed.*

Table 3. *Spoligotypes recognized among Prague and South Moravian M. tuberculosis isolates*

Family/Origin	Prague South	Moravia	Total (%)
Prototype spoligotypes	56 (52.3%)	60 (58.8%)	116 (55.5)
Beijing	2	5	7 (3.3)
T1	16	14	30 (14.3)
T2	0	8	8 (3.8)
T3	2	2	4 (1.9)
Haarlem1	8	9	17 (8.1)
Haarlem2	2	1	3 (1.4)
Haarlem3	18	20	38 (18.1)
LAM2	1	0	1 (...)
LAM6	0	1	1 (...)
LAM7	1	0	1 (...)
LAM10	6	0	6 (2.9)
Wadsworth Center spoligotypes (US patterns)	38 (35.5%)	31 (30.4%)	69 (33.0)
Czech spoligotypes	13 (12.2%)	11 (10.8%)	24 (11.5)
Total	107 (100%)	102 (100%)	209 (100%)

tuberculosis isolates from South Moravia. Our findings for this region differ from our finding from Prague in several respects: the proportion of individual genotypes is lower in South Moravia (64.7% versus 72.1 in Prague). However, the South Moravian isolates show a higher proportion of clustered profiles (35.3%, in 15 clusters versus Prague's 27.9%, in 10 clusters). As in Prague, low-copy-number IS*6110* profiles weren't found, and the number of hybridization bands ranged from 6 to 22. Among others, these findings illustrate conspicuous differences in the epidemiological situation of TB between the two territories (5.5 bacteriologically verified cases of respiratory TB per 100,000 in South Moravia, against 12.3 in Prague).

In a previous paper (8), we described the use of IS*6110*-based fingerprinting to identify six TB outbreaks in four South Moravian districts, all among family members or relatives. In contrast, in the present study we could not establish reliable contacts among members of any of 15 identified clusters in this region, in part because the epidemiological investigation was not completed. Nevertheless, the slightly higher proportion of clustered strains in South Moravia (35.3% versus 27.9% in Prague) may be due to the fact, that migration into this area is rare, and stable communities, in which persons involved in chains of transmission from past decades still reside locally (16).

The comparative analysis of the Czech strain collection used in this study (231 samples) with the PHRI TB Center Database (more than 17,000 *M. tuberculosis* isolates from U.S. and the other countries including Russia, South Africa, Tanzania, India, Egypt, Germany, Singapore, Israel and Romania) revealed the matching of RFLP patterns isolated from TB patients in geographically distinct areas. The cluster HJ (14 IS*6110* copies) included strains isolated from 11 patients in the U.S. and eight patients in the Czech Republic (six in Prague and two in South Moravia). All strains assigned to this cluster had spoligotype NY_S00282 (octal code 777777743760771), previously identified in the U.S. The second cluster identified from three patients in South Moravia – BW33 – had also been isolated from 12 TB patients in the U.S. and four patients in Russia. It is interesting to note that the three Moravian strains have slightly different spoligotypes, variations of prototype Haarlem3, whereas the strains from the U.S. and Russia demonstrated the precise Harlem3 spoligotype (octal code 777777777720771). Some orphan strains, not clustered in our Czech data set, were found in the collections from the U.S. (cluster BW, 59 cases; cluster AX, 28 cases; cluster GH, nine cases; clus-

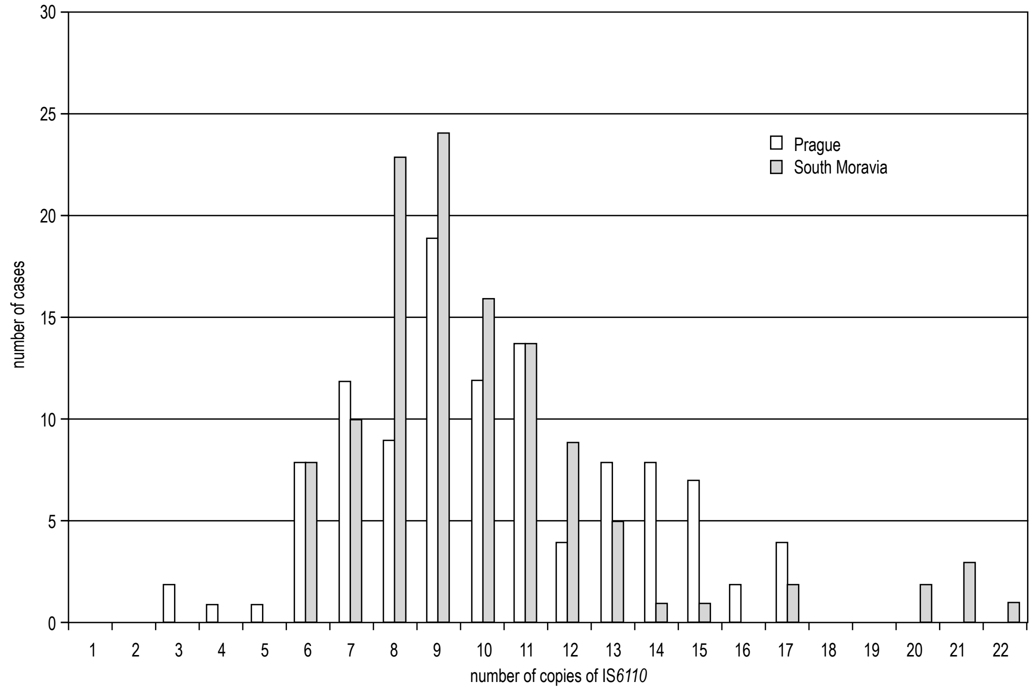

Fig. 4. *Distribution of M. tuberculosis strains isolated in Prague (light bars) and South Moravian (dark bars) by number of IS6110 copies.*

Family	Prague	S. Moravia	n°	Spoligotype hybdriziation pattern	Octal Code
T1	16	14	NY_S00002	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	777777777760771
Haarlem3	18	20	NY_S00005	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨●●●●●●●	777777777720771
Beijing	2	5	NY_S00034	▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●●●●●●●●●	000000000003771
T2	0	8	NY_S00035	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●▨●●●	777777777760731
LAM2	1	0	NY_S00074	●●▨●●●●●●●●●▨●●●●●●●▨▨▨▨●●●●●●●●▨▨▨▨●●●●●●●	677737607760771
USA	4	0	NY_S00079	●●●●●●●●●●●●●●●●●●●●●●▨●●●●●●●●●▨▨▨▨●●●●●●●	777777757760771
LAM6	0	1	NY_S00080	●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●▨●●●▨▨▨▨●●●●●●●	777777607560771
Global	1	1	NY_S00086	●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●●▨▨▨▨●●●●●●●	777777607760771
T3	2	2	NY_S00094	●●●●●●●●●●●●▨●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	777737777760771
Haarlem2	2	1	NY_S00182	▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	000000004020771
USA/Russia/Czech	1	1	NY_S00185	●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●▨●▨▨▨▨●●●●●●●	777777607720771
USA/India/Czech	3	4	NY_S00217	●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨	777777770000000
LAM7	1	0	NY_S00232	●●●●●●●●●●●●●●●●●●●▨▨▨▨▨●▨▨●●●●●▨▨▨▨●●●●●●●	777777404760771
Haarlem1	8	9	NY_S00241	●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	777777774020771
USA/Czech	1	0	NY_S00247	●●●▨▨▨▨●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨●●▨▨●●●●●	703777740003171
USA/Czech	0	1	NY_S00273	●●●●●●●●●●●●●●●●●▨●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨	777776770000000
USA/Czech/Russia	6	0	NY_S00282	●●●●●●●●●●●●●●●●●●●●●●▨▨▨●●●●●●●▨▨▨▨●●●●●●●	777777743760771
USA/Czech	0	1	NY_S00342	●●●●●●●●●▨●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨●●●●●●●	777377777720771
USA/Czech	1	0	NY_S00343	●●●●●●●●●●●●●●●●●●●●●▨▨●●●●●●●●●▨▨▨▨●●●▨●●●	777777717760731
Czech	1	0	NY_S00348	●●●●▨▨●●●●●●●●●▨●●●●●●●●●●●●▨▨●●▨▨▨▨●●●●●●●	747773777460771
Czech	1	0	NY_S00349	●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●▨▨▨▨●●●●●▨▨	777777774020760
Czech	3	0	NY_S00350	●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	740000004020771
USA/Czech	0	2	NY_S00372	●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●▨▨▨▨●●●▨●●●	777777774020731
USA/Czech/Russia	2	1	NY_S00388	●●●●●●●●●●●●●●●●●●▨●●●●●●●●●●●●●▨▨▨▨●●●●●●●	777777377760771
India/Czech	0	1	NY_S00500	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●▨▨●●	777777777760711
USA/Czech	0	2	NY_S00511	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨●▨●●●●●	777777777760571
USA/Czech	1	0	NY_S00516	●●●●▨●●●●●●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨●●●●●●●	757777777720771
USA/Czech	1	0	NY_S00521	●●●▨●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	737777774020771
Czech	1	0	NY_S00522	●●●●▨●●●●●●●●●●●●●●●●▨▨▨●●●●●●▨●▨▨▨▨●●▨●●●●	757777707720671
Czech	0	1	NY_S00523	●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●●●●●●▨●▨▨▨▨●●●●●●●	777777007720771
Czech	1	0	NY_S00524	▨●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨●●●●●●●	377777777720771
Czech	1	0	NY_S00525	●●●●●●●▨●●●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨●●●●▨●●	775777777720751
Czech	0	1	NY_S00526	●●●●●●●●▨●●●●●●●●●●▨●●●●●●●●●●▨●▨▨▨▨●●●●●●●	776777577720771
USA/Czech	1	0	NY_S00527	●●●●●●●●●●●●●●●●●●●●●▨▨▨●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	777777704020771
Czech	0	2	NY_S00528	●●●●●●●●●●●●●●▨●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨	777767760000000
USA/Czech	0	3	NY_S00529	●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨●▨▨▨▨●●●●●●●	777777700020771
Czech	0	1	NY_S00530	●●●●●▨●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨●●●●●●●	767777770000771
Czech	0	1	NY_S00532	●●●●●●●●●●●●●●●●●●▨▨●●▨▨●●●●●●▨●▨▨▨▨●●●●●●●	777777147720771
USA/Czech	1	0	NY_S00546	●●●●●●●●●●●●●●●●●●●●●●●▨●●●●●●●●▨▨▨▨●●●●●●●	777777767760771
USA/Czech	1	0	NY_S00547	●●●●●●●▨●●●●●●●●●●●●●●▨●●●●●●●●●▨▨▨▨●●●●●●●	775777757760771
USA/Czech/Russia	1	0	NY_S00550	●●●●●●●●●●●●▨●●●●●●●●●●●●●●●▨▨▨●▨▨▨▨●●●●●●●	777737777420771
Czech	4	0	NY_S00553	●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨●●●●●●	777777777000371
USA/Czech	2	4	NY_S00554	●●●●●●●▨▨▨▨▨▨●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●▨▨▨▨	774037777760700
Czech	0	1	NY_S00569	●●●●▨●●●●●●●▨●●●●●●●▨▨▨▨●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	757737604020771
USA/Czech	1	0	NY_S00571	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨●▨▨●●●●	777777777760471
Czech	0	1	NY_S00625	●●●●●●●●●●●●●●●●●●●●●●●▨●●●●●●▨●▨▨▨▨●●●●●●●	777777767720771
USA/Czech	0	1	NY_S00626	●●▨●●●●●▨●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●●●●●●●	676600000000771
USA/Czech	1	1	NY_S00660	●●●●●●●●●●●●●●▨●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	777767777760771
USA/Czech	1	0	NY_S00755	●●●●●●●●●●●●●●●●●●▨●●●●●●●●●●●▨●▨▨▨▨●●●●●●●	777777377720771
USA/Czech	1	0	NY_S00770	●●●●●●●▨▨●●●●●●●●●●●●●●●●●●●▨▨▨●▨▨▨▨●●●●●●●	774777777420771
USA/Czech	0	1	NY_S00828	●●●●●●●●●●●●●●●●●●●●●▨●●●●●●●●▨●▨▨▨▨●●●●●●●	777777737720771
USA/Czech	1	0	NY_S00853	●●●●●●●●●●●●●●●●●●●●▨●●●●●●●●●●●▨▨▨▨●●●●●●●	777777677760771
USA/Czech	1	0	NY_S00893	●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●●▨▨▨▨●●●●▨●●	777777607760751
Czech	1	0	NY_S00983	●●●●●●●●●●●●▨●●●●●●●●▨●●●●●●▨▨▨●▨▨▨▨●●●●●●●	777737737420771
Czech	0	1	NY_S00984	●●●●●●●▨▨▨▨▨▨●●●●●●●●●●●●●●▨●●●●▨▨▨▨●●●▨▨▨▨	774037777360700
Czech	1	0	NY_S00985	●●●●●●●●●●●●●●●●●●●▨▨▨●●●●●●●●●●▨▨▨▨●●●▨●●●	777777437760731
Czech	0	1	NY_S00986	●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨●●●●●●●▨▨▨▨●●●●●●●	777777403760771
Czech	0	1	NY_S00987	●●●●●●●●●●●●●●●●▨●●●●●●●●●●●●●●●▨▨▨▨●●●▨▨▨▨	777775777760700
Czech	0	1	NY_S00988	●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨●▨▨▨▨●●●●●●●	777777760020771
Czech	0	1	NY_S00989	●●●●●●▨●▨▨▨●●●●●▨▨●●▨▨▨▨●●●●●●●●▨▨▨▨●●●●●●●	772174607760771
Czech	1	0	NY_S00990	●●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨●▨●●●●	777777777000271
Czech	1	0	NY_S00991	▨●●●●●●●●●●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨●●●	377777777000031
Czech	0	2	NY_S00992	●●●●●●●●●▨▨▨▨▨▨▨▨▨●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	777000777760771
Czech	1	0	NY_S00993	▨▨▨▨●●●●●●●▨●●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	037677777760771
Czech	0	1	NY_S00994	●●●▨●●●●●●●●●●●●●●●●●●▨●●●●●●●●●▨▨▨▨●●●●●●●	737777757760771
Czech	0	1	NY_S00995	●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●▨▨▨▨▨▨●▨▨▨▨●●●▨▨●●	700000004020711
Czech	1	0	NY_S00996	●●●●●●●●●●●●●●●●●●●●▨●●●●▨▨▨▨▨▨●▨▨▨▨●●●●●●●	777777674020771
Czech	1	0	NY_S00997	●●●●●●●●●●●●●●●●●●●●●●●●●▨●●●●▨●▨▨▨▨●●●●●●●	777777775720771
Czech	0	1	NY_S00998	●●●●●●●●▨▨●●●●●●●●●●●●●●●●●●●●▨●▨▨▨▨▨▨▨●●●●	776377777720071
Czech	0	1	NY_S00999	●●●●●●▨●●●●●▨●●●●●●●●●●●●●●●●●●●▨▨▨▨●●●●●●●	773737777760771
Czech	1	0	NY_S01000	●●●●●●●●▨▨●●●●●●●●▨●●●●●●●●●●●●●▨▨▨▨●●●▨●●●	776377377760731
Czech	1	0	NY_S01001	●●●●●●●●●●●●▨●●●●●●●●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨	777737777700000
Czech	1	0	NY_S01003	●●●●●●●●●●●●▨●●●●●●●●●●▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●●▨●●	777737760000151
Czech	1	0	NY_S01004	▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨▨●●●●●●●●▨▨▨▨●●●●●●▨	000000007760770
Czech	1	0	NY_S01005	●●●●●●●●●●▨●▨●●●●●●●●●●●●●●●▨▨▨●▨▨▨▨●●●●●●●	777537777420771

Fig. 5. Spoligotypes identified in Prague and South Moravia, arranged according to the Jaccard similarity showing the prototype family or geographic origin, occurrence in Prague and South Moravia; Wadsworth Center designation, spoligotype hybridization pattern, and octal code.

ter BW90, six cases in the U.S. and one in South Africa; cluster HW, three cases), from India (cluster CH5, two cases) and Egypt (cluster DA2, one case). The clusters mentioned above belong, on the basis of spoligotyping results, to the group of Haarlem strains of *M. tuberculosis*, widely spread in European countries.

Comparing the *M. tuberculosis* RFLP results of studied populations, from Prague and South Moravia, we found they shared strains from only three clusters, including AX (one in Prague and one in South Moravia), BW22 (one and two, accordingly) and M22 (one in Prague and five in South Moravia). The small number of *M. tuberculosis* clusters shared between two communities suggests a low rate of recent transmission of TB in the Czech Republic.

On the other hand, W-Beijing strains isolated from Mongolian (W303) and Peruvian (W206) immigrants in the Czech Republic matched, in the PHRI TB Center collection, to the RFLP patterns of strains isolated from six (cluster W206) and three (cluster W303) Russian patients (Fig. 3).

Spoligotyping analysis also revealed the identical spoligopatterns of strains isolated in the Czech Republic, in the U.S. (21 patterns), Russia (five patterns) and India (two patterns). RFLP fingerprinting did not confirm the relatedness of those isolates and the similarity between strain profiles was lower than 60%.

Among the range of *M. tuberculosis* spoligotypes identified in our study, ubiquitous shared prototypes (55.5%) did not statistically dominate among spoligotype profiles. Among the 11 pro-

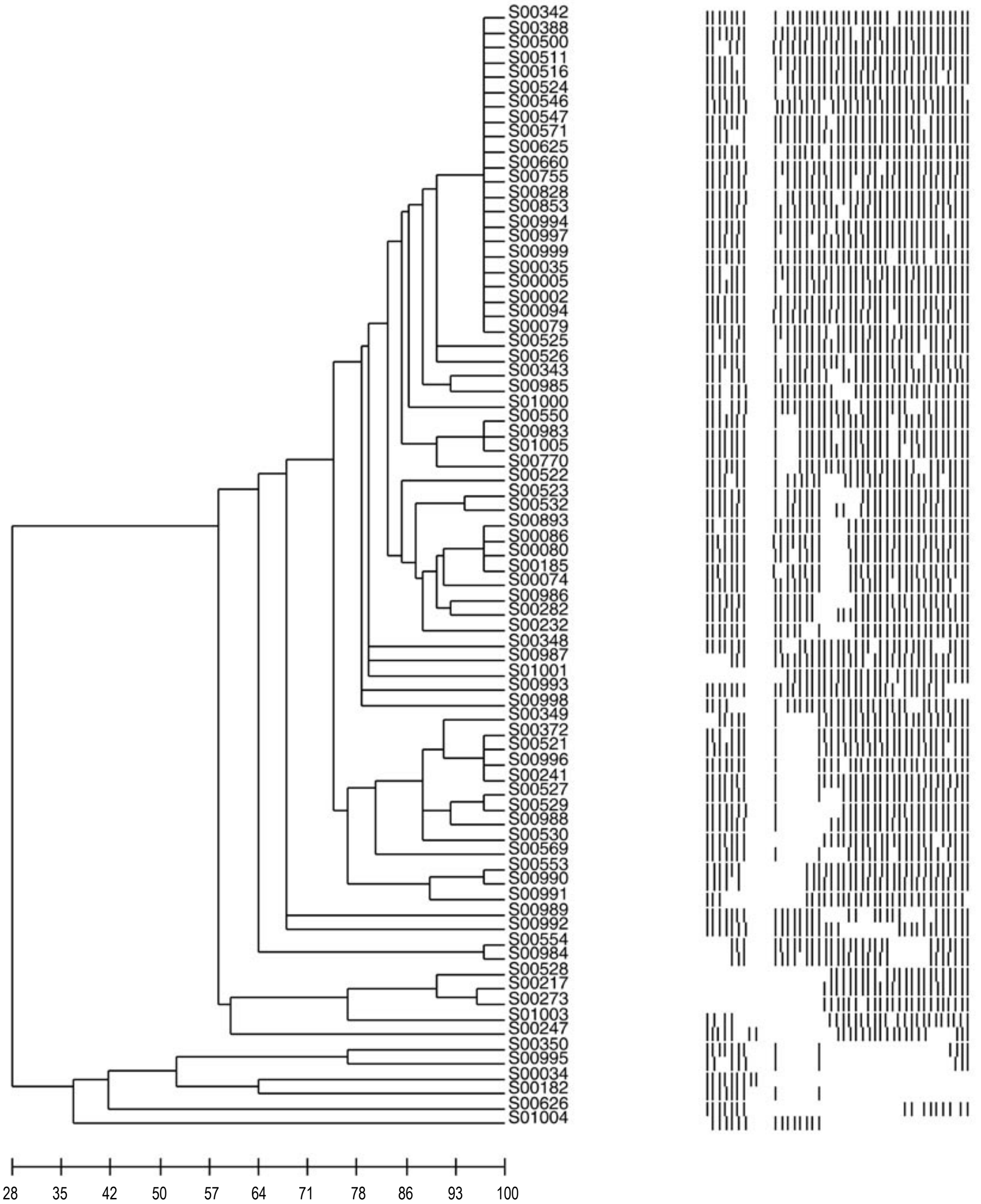

Fig. 6. Dendrogram of spoligotype patterns identified in Prague and South Moravia M. tuberculosis strains showing the percentage of similarity in the Jaccard coefficient, identified spoligotypes designated according to the nomenclature of Global Spoligo Database SpolDB3 (3, 5) and schematic RFLP hybridization patterns.

totype families found in Prague and in South Moravia, the types Haarlem3, T1 and Haarlem1 together prevailed and accounted for more than 73% of all isolates with prototype spoligotypes. The *M. tuberculosis* strains with the W-Beijing spoligotype were isolated from only seven patients (3.3%), in strong contrast to the much higher prevalence of W-Beijing strains observed in both the Asian endemic areas and in North America (1, 5). As was published previously, the W-Beijing strains were identified in the European Region of Russia in 44–52% of all culture-positive pulmonary TB cases (15, 17–19). Nevertheless, the low prevalence of W-Beijing isolates in the Czech Republic was comparable with the 4% prevalence of W-Beijing spoligotypes recorded among *M. tuberculosis* strains isolated in Western Europe (5). Other prototype spoligotypes known from the Global Spoligo Database SpolDB3 were rare or absent in both regional groups of *M. tuberculosis* isolates in our study. Notably, the East-African Indian EA and the *M. africanum*-associated Afri spoligotype families were not identified in our Czech strain collection.

The recognition of the W-Beijing spoligotype undoubtedly signifies a new phenomenon in the Czech Republic, reflective of the territorial expansion of this clear-cut genotype. Our previous observations revealed the importation of this genotype by a total of eight persons from four foreign countries: five were Mongolians working in various South Moravian districts, one was a Peruvian student residing temporarily in Prague, and two were foreign prisoners, one an Algerian from France and the other an Albanian war refugee (12, 13). Additionally, one Czech citizen with W-Beijing strain was a 47-year-old woman who contracted TB presumably from her Russian-born mother, and one was a Czech prisoner whose source of infection remains unknown.

Spoligotype analysis in this study also encompassed three patients described in our previous paper (11) on the transmission of TB from the index case, a 44-year-old homeless man to a 53-year-old laboratory technician and to a 49-year-old man. The link was confirmed by the identical seven-banded RFLP pattern DF1 found in all three subjects. Spoligotyping analysis disclosed the NY_S00553 pattern (octal code 777777777000371) in these strains and corroborated their genetic identity. Surprisingly, another patient, a 54-year-old man residing in a distant Prague quarter yielded the identical NY_S00553 spoligotype profile. However, the unique RFLP pattern (nine IS*6110* copies) of the strain isolated from this patient was different (patterns similarity with DF1 below 60%), and no contacts between him and the other three patients could be established. The NY_S00553 spoligotype belongs to a group previously identified in the U.S. strain collection (Fig. 5).

Twenty-four (11.5%) of the strains examined in this study against Wadsworth Spoligo Database, were classified as novel Czech spoligotypes (Fig. 5). Twenty-two showed unique patterns and one spoligotype (NY_S00992) was detected in two individuals residing in the same South Moravian district, although no apparent epidemiological link between these two patients could be identified; RFLP analysis showed patterns similarity < 60% (6 and 8 IS*6110* copies per genome). The proportion of unique spoligotypes in the present study (11.5%) is close to the prevalence of unique spoligotypes recorded in the global SpolDB3 database, in which 1300 (11.1%) orphan patterns among 11708 *M. tuberculosis* strains collected worldwide were detected (5). However, we presume that some of these Czech spoligotypes will match the known spoligotypes if they were incorporated into a comprehensive database. This view is based on the close relatedness of most Czech spoligotypes shown in the dendrogram in Figure 6, where they are generally interspersed between other profiles at a rather high similarity level. A few of them, showing a rather higher diversity in the dendrogram (e.g. patterns NY_S01004 and NY_S01003), may be considered as candidates for true orphan patterns (Fig. 6).

In conclusion, our population-based molecular epidemiological study, performed for two regions of the Czech Republic in 1998 using IS*6110* RFLP fingerprinting and spoligotyping techniques, has demonstrated the distribution of individual and clustered genotypes of *M. tuberculosis* strains isolated from TB patients, and has confirmed the similarity of the Czech strain mosaic to the epidemiological pattern of other European Community countries that have low TB transmission rates. The sporadic importation of TB by foreign-born individuals and recent transmission events probably do not play significant roles in the epidemiological situation of TB in the Czech Republic. At least in 1998, there were no registered cases that demonstrated transport of TB from the Eastern European countries or former Soviet Republics. A comprehensive study should be conducted to assess any recent changes in the epidemiological situation of pulmonary TB in Czech Republic.

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