*Tomasz Jagielski[1], Magdalena Klatt[2], Zofia Zwolska[2], Ewa Augustynowicz-Kopeć[2]

Spoligotype-defined population structure of drug-resistant *Mycobacterium tuberculosis* isolates in Eastern Poland

Struktura populacyjna lekoopornych szczepów *Mycobacterium tuberculosis* izolowanych z obszaru Polski Wschodniej, na podstawie typowania genetycznego metodą spoligotyping

[1]Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw
Head of Department: prof. Jacek Bielecki, PhD
[2]Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw
Head of Department: prof. Ewa Augustynowicz-Kopeć, MD, PhD

Key words

tuberculosis, *Mycobacterium tuberculosis*, drug resistance, Poland's "Eastern Wall", spoligotyping

Słowa kluczowe

gruźlica, *Mycobacterium tuberculosis*, lekooporność, Polska Wschodnia, spoligotyping

Summary

Introduction. The most detrimental impact on the epidemiology of tuberculosis (TB) in Poland may have the spread of tubercle bacilli from outside the country. This is because Poland is geographically situated in close proximity to the former Soviet Union countries, such as Latvia, Estonia, Ukraine, and Russia, where the incidence of TB, including drug-resistant (DR) TB is exceptionally high.

Aim. To describe the genetic diversity of DR *Mycobacterium tuberculosis* isolates, circulating in four provinces (Lublin, Podkarpacie, Podlasie, and Warmia-Masuria) constituting the so-called "Eastern Wall" of Poland.

Material and methods. A total of 44 DR *M. tuberculosis* isolates were spoligotyped. The results were compared with the international spoligotype database (SpolDB4). SpolDB4 and the web-based program SpotClust were used to classify isolates into phylogenetic clades.

Results. Spoligotyping resulted in 25 distinct patterns, of which five were not recorded in the SpolDB4. Of the remaining 20 types, 16 (80%) had already been reported in Poland. Most of the spoligotypes (56%) identified were found to be present in Poland's neighbors, such as Germany, the Czech Republic, Latvia, and Russia. At the phylogenetic level, the T and Haarlem families accommodated 80% of the isolates.

Conclusions. The *M. tuberculosis* population in Eastern Poland displayed features characteristic of a European country. Given the low frequency of imported genotypes and the absence of foreign-born patients, DR-TB in Eastern Poland appears to be due to the local transmission of *M. tuberculosis* strains that have been active in Poland for many years, rather than to the importation of strains from neighboring countries.

Streszczenie

Wstęp. Jednym z istotnych czynników wpływających na sytuację epidemiologiczną gruźlicy w Polsce jest transmisja prątków gruźlicy z krajów sąsiednich. Polska graniczy z byłymi postsowieckimi republikami (Łotwa, Ukraina, Rosja), gdzie wskaźniki zapadalności na gruźlicę, w tym gruźlicę lekooporną są szczególnie wysokie.

Cel pracy. Celem pracy było zdefiniowanie struktury genetycznej lekoopornych szczepów *Mycobacterium tuberculosis* izolowanych z obszaru Polski Wschodniej.

Materiał i metody. Przedmiotem badania była kolekcja 44 szczepów *M. tuberculosis* pochodzących od tyluż chorych na gruźlicę płuc z województw lubelskiego, podlaskiego, podkarpackiego i warmińsko-mazurskiego. Typowanie genetyczne szczepów przeprowadzono przy użyciu metody spoligotyping. Otrzymane wzory genetyczne porównywano w międzynarodowej bazie danych SpolDB4. W analizie filogenetycznej wykorzystano również program SpotClust.

Wyniki. Wśród 44 badanych szczepów wyróżniono 25 różnych wzorów genetycznych, z których 5 nie było zarejestrowanych w bazie SpolDB4. Z pozostałych 20 wzorów, 16 (80%) było wcześniej notowanych na terenie Polski. Większość (56%) wykrytych spoligotypów było charakterystycznych dla krajów graniczących z Polską, tj. Niemiec, Czech, Łotwy i Rosji. Blisko 80% wszystkich szczepów sklasyfikowano, na podstawie ich spoligotypów, w dwóch rodzinach molekularnych: T i Haarlem.

Address/adres:

*Tomasz Jagielski
Department of Applied Microbiology
Institute of Microbiology
Faculty of Biology
University of Warsaw
ul. I. Miecznikowa 1, 02-096 Warszawa
tel./fax +48 (22) 554-13-12
t.jagielski@biol.uw.edu.pl

Wnioski. Struktura populacyjna szczepów *M. tuberculosis* z obszaru Polski Wschodniej była typowa dla krajów europejskich. Niewielki udział genotypów o rodowodzie spoza Europy oraz brak cudzoziemców wśród chorych, od których pochodziły badane szczepy, wskazują na autochtoniczny charakter transmisji gruźlicy lekoopornej w województwach Polski Wschodniej.

INTRODUCTION

Tuberculosis (TB) still represents a significant global health problem, with an estimated 9 million new cases and almost 2 million deaths due to this disease per annum worldwide (1). One of the main reasons for the continuing TB prevalence is the emergence and spread of drug-resistant (DR), and more importantly multidrug-resistant (MDR) (defined as resistance to at least isoniazid [INH] and rifampicin [RMP]) *Mycobacterium tuberculosis* strains. Based on the most recent report by the World Health Organization (WHO), 20% of all TB cases are resistant to at least one first-line drug, and 5% are MDR cases (2). In Poland, in 2004, the number of DR- and MDR-TB cases was 246 (7.6%) and 51 (1.6%), respectively (2). These figures place Poland among the countries with low to moderate DR-TB rates in the world. The most detrimental impact on the epidemiology of TB in Poland may have the spread of DR-TB from outside the country. This is because Poland is geographically situated in close proximity to the former Soviet Union countries, such as Latvia, Estonia, Ukraine and Russia, where the incidence of DR-TB, including MDR-TB is particularly high (2).

With the advent of molecular biology tools, various genotyping methods have been developed for the differentiation of clinical isolates of *M. tuberculosis* (3). The use of molecular strain typing methods, in conjunction with traditional contact tracing approaches, has significantly improved our understanding of the epidemiology of TB. Among the genotyping methods available, those targeting different repetitive DNA elements and PCR-based are most widely used and preferred. One such a method is spoligotyping which detects polymorphisms in the *M. tuberculosis* complex direct repeat (DR) chromosomal locus. The DR locus contains multiple direct repeats, each of 36 bp, interspersed with non-repetitive, unique, spacer sequences (spacers) of 35-41 bp in length. The number of direct repeats as well as the presence or absence of specific spacers reflect the polymorphic structure of the DR region and thus demonstrate variations between the strains (4). Spoligotyping is a simple, rapid, robust, and highly reproducible method, whose results are expressed in an octal code format, so as they can be easily compared between different laboratories. Spoligotyping has successfully been applied for the detection of outbreaks (5, 6) and laboratory cross contaminations (7), as well as for the identification of mycobacterial species within the *M. tuberculosis* complex (4, 8) and recovery of genotypes of particular epidemiological importance, such as those of W-Beijing family (9).

A significant advantage of the method is the availability of the international spoligotype database, SpolDB4 (www.pasteur-guadeloupe.fr/tb/spoldb4; Institute Pasteur, Guadeloupe) (10). The SpolDB4 database contains a total of 1,939 distinct spoligotypes representing 35,925 strains of *M. tuberculosis* from 122 isolation countries. The organization of the spoligotype database offers a possibility to recognize the genetic structure of *M. tuberculosis* strains within particular region in relation to other geographical locations. This in turn provides clues about transmission of specific genotypes and/or genotype families (clades) in different human populations (11, 12).

AIM

The objective of this study was to explore the genetic diversity of drug-resistant *M. tuberculosis* isolates from Eastern Poland, by using spoligotyping, and to compare the data obtained with those from neighbouring countries[1] so that the dissemination of major phylogenetic clades of tubercle bacilli within the analysed region could be demonstrated.

MATERIAL AND METHODS

Patients

The population examined included 44 non-related, pulmonary TB patients residing in four provinces of Eastern Poland: Lublin (20 patients), Podkarpacie (13), Podlasie (10), and Warmia-Masuria (1) (fig. 1). The study group represented 90% of all culture-proven drug-resistant TB cases notified in those four Polish provinces in 2004 and 18% of all culture-proven drug-resistant TB cases notified in the whole country in the same year. Of the patients, 34 (77.3%) were males, and 10 (22.7%) were females. The patients were aged between 14 and 81 years. The median age for all the patients was 53 years.

This study was approved by the Ethics Committee of the National Tuberculosis and Lung Diseases Research Institute.

Bacterial strains

The analyzed 44 *M. tuberculosis* strains were collected in the National Tuberculosis Reference Laboratory (N.T.R.L.) throughout 2004. The diagnostic

[1]Throughout the article, the term “neighboring country” refers to a country that is in close geographical proximity to Poland, not necessarily sharing borders with Poland.

Fig. 1. A map of Poland and its geographical context. Lightly shaded is the so-called Poland's Eastern Wall, encompassing four provinces, i.e. Warmia-Masuria, Podlasie, Lublin, and Podkarpacie, from where the *M. tuberculosis* isolates were obtained.

specimens were: sputum (39), bronchoscopic material (4), and gastric washing (1). Primary isolation was performed using the Löwenstein-Jensen (L.-J.) medium and the BACTEC 460-TB system (Becton-Dickinson, Sparks, MD, USA), and the species identification was done by means of niacin test, gene probes (AccuProbe; GenProbe, San Diego, CA), and mycolic acid analysis by high-pressure liquid chromatography. Drug susceptibility testing was performed using the proportion method on L.-J. medium. The criterion used for drug resistance was growth of 1% or more of the bacterial population on critical concentrations of the drugs tested (i.e. 0.2 μg/ml for isoniazid [INH], 40 μg/ml for rifampicin [RMP], 4 μg/ml for streptomycin, and 2 μg/ml for ethambutol [EMB]) (13).

DNA isolation

Genomic DNA was obtained from L.-J. slants by the cetyl-trimethyl-ammonium bromide (CTAB) method, as previously reported (14).

Spoligotyping and spoligotype analysis

Spoligotyping was performed with a commercially available kit (Isogen Bioscience BV, Maarssen, The Neetherlands), according to the manufacturer's specifications and as described earlier (4).

Spoligotypes with 100% similarity were considered clusters, whereas non-clustered spoligotypes were referred to as unique. All spoligotypes obtained were compared to the world spoligotype database (SpolDB4) at the Pasteur Institute of Guadeloupe (www.pasteur-guadeloupe.fr/tb/spoldb4). In this database, SIT (Spoligotype International Type) designates spoligotype pattern shared by two or more patient isolates, as opposed to "orphan" which designates patterns reported for a single isolate.

The spoligotypes absent in SpolDB4, and thus identified for the first time, were designated either as new SITs (if two or more isolates harbored that spoligotype) or 'orphans' (if the spoligotype occurred in only one isolate). Major phylogenetic clades were assigned according to signatures provided in SpolDB4, which defined 62 genetic (sub-) lineages (10). Clade assignment of the spoligotypes not found in SpolDB4 (orphan types) was performed with SpotClust, an algorithm based on the SpolDB3 database, described previously15 and available online (http://cgi2.cs.rpi.edu/~bennek/SPOTCLUST.html).

The genotypic diversity index (GDI) was calculated by dividing the total number of spoligotypes identified by the total number of *M. tuberculosis* isolates tested.

Terms: (sub-)families and (sub-)clades were used interchangeably.

Statistical analysis

The Chi-square test or Fisher's exact test (if the cell count was less than five) were performed to define any association of drug resistance profiles with specific spoligotype families. A P value that was less than 0.05 was considered statistically significant.

RESULTS

Among the 44 isolates spoligotyped, 25 distinct patterns were observed (tab. 1). Half of the isolates were represented by a unique pattern, the other half were split into three clusters. Most of the clustered isolates (16; 72.7%) were allocated in a single large cluster belonging to SIT53. The two minor clusters SIT47 and SIT253 contained four and two isolates, respectively.

By comparison with the international database, only five (20%) spoligotypes designated 'orphan A-E', had not been previously recorded. Of the remaining 20 spoligotypes that matched pre-existing SIT designations, all but one (SIT442, found exclusively in the USA) were described in at least one European country, six (30%) were predominantly found in the European countries (SITs 280, 382, 511, 524, 891, and 1746) and 16 (80%) were already reported in Poland (the only SITs that had not previously been observed in Poland were SITs 73, 442, 524, and 1253). At the same time, most of the spoligotypes (14; 56% of all spoligotypes) found in this study were identified by SpolDB4 as being present in Poland's neighboring countries, such as Germany, the Czech Republic, Latvia, and Russia. In the last two countries, geographically the closest to the study area, the presence of 12 spoligotypes identified here had been reported earlier (11 spoligotypes reported in Russia, and five – in Latvia). However, within the population examined, only two spoligotypes (SIT1 and SIT280), represented by single isolates, were found whose activity prevails in Poland's eastern neighbors.

Upon phylogenetic analysis, 39 (88.6% of all isolates) isolates, with SIT labels, were classified into 5 major clades, with their frequencies being as follows:

Table 1. Description of *M. tuberculosis* spoligotypes found in this study and their comparison to the world spoligotype database (SpolDB4).

SIT[a]	Spoligotype Description	Clade[b]	Number of strains (%) in this study	% in this study as compared to database[c]	Distribution in neighboring countries[d]				
					Germany	Czech Republic	Latvia	Russia	Poland
Orphan A	□□□■■■■■□□■■■■■■■■■■■■□■■■■■■■■■■■■□□□□■■■■■■■■	U	1 (2.3)	100					
Orphan B	□■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■	U	1 (2.3)	100					
Orphan C	■■■□□□■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□■■	U	1 (2.3)	100					
Orphan D	■■■■■■■■■■■■□□□□■■□□□□□□□□□□□□■□■□□□□■■■□■■■	U	1 (2.3)	100					
Orphan E	■■■■■■■■■■■■■□■■■■■■■□□□□■■■■■■□■■□□□□■■■■■■■■	U	1 (2.3)	100					
1	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■	Beijing	1 (2.3)	0.03	19 (0.5)	14 (0.4)	76 (2.0)	426 (11.3)	7 (0.2)
3	□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■□■□□□□■■■■■■■	H3	1 (2.3)	1.5					1 (1.5)
37	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T3	1 (2.3)	0.6	4 (2.6)	8 (5.2)	1 (0.6)		4 (2.6)
42	■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■■	LAM9	1 (2.3)	0.08	6 (0.5)	4 (0.3)	19 (1.5)	68 (5.5)	6 (0.5)
46	■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□□□□□□	U (likely H)	1 (2.3)	1.0	2 (2.1)	13 (13.7)			6 (6.3)
47	■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■■■■■■	H1	4 (9.1)	0.5	24 (3.3)	38 (5.3)		6 (0.8)	16 (2.2)
50	■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■■	H3	1 (2.3)	0.07	49 (3.2)	83 (5.5)		10 (0.7)	19 (1.3)
52	■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	T2	1 (2.3)	0.2	7 (1.8)	17 (4.3)		5 (1.3)	6 (1.5)
53	■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T1	16 (36.4)	0.6	55 (2.2)	30 (1.2)	5 (0.2)	26 (1.0)	34 (1.4)
73	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	T2_T3	1 (2.3)	0.9					
253	■■■■■■■■■■■■■■■■■■■■□■■□□■■■■■■■■□□□□■■■■■■■■	T1	2 (4.5)	10.0				3 (15.8)	1 (5.3)
280	■■■■■■□□□□□□□□□□□□■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T1_RUS2	1 (2.3)	2.1	3 (6.5)		1 (2.2)	5 (10.9)	4 (8.7)
382	■■■■□■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■■■■■■	H1	1 (2.3)	11.1					1 (12.5)
442	■■■■■■■■■■■■□■■■■■■□■■■■■■■■■■■■■■■□□□□■■■■■■■■	T3	1 (2.3)	20.0					
511	■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□■□□□□■■■■■■■■	H3	1 (2.3)	5.0	2 (10.5)	4 (21.0)		1 (5.3)	3 (15.8)
524	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■□□■■	H3	1 (2.3)	14.3					
891	■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■□■■□□□□■■■■■■■■	LAM9	1 (2.3)	7.7				1 (8.3)	7 (58.3)
1214	■■■■■■■■■■■■□□□■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■	T1	1 (2.3)	8.3	1 (9.1)				1 (9.1)
1253	■■□■■■■■□□■■■■■■■■■■■■□■■■■■■■■■■■■□□□□■■■■■■■■	S	1 (2.3)	12.5	1 (14.3)			2 (28.6)	
1746	■□□■■■■■□□■■■■■■■■■■■■□■■■■■■■■■■■■□□□□■■■■■■■■	S	1 (2.3)	25.0					1 (33.3)

[a]Spoligotype International Type (SIT), designation of the spoligotype in the world spoligotype database (SpolDB4). A total of 25 STs were identified in this study.
[b]Clade assignment following SpolDB4 rules; U, unknown patterns within any of the major clades described in SpolDB4.
[c]Percentage of strains in SpolDB4 database after incorporation of the spoligotypes from the present study (n = 44).
[d]Distribution in neighboring countries according to data available in SpolDB4 database; in the column "Poland", only strains from previous studies were included.

Table 2. Orphan spoligotypes recognized in the study and their clade assignment.

	Spoligotype[a]		Clade[b]	Probability[c]	No. of isolates
	Binary description	Octal code			
A	□□□■■■■■□□■■■■■■■■■■■■□■■■■■■■■■■■□□□□■■■■■■■■	076377737760771	S	0.78	1
B	□■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□□■■■■■■	377777760000171	H1	0.99	1
C	■■■□□□■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□■■	707777777760711	T1	0.99	1
D	■■■■■■■■■■■■■□□□□■■□□□□□□□□□□□□□■□■□□□□■■■□■■■	777703000120731	LAM9	0.98	1
E	■■■■■■■■■■■■■■□■■■■■■■□□□□■■■■■■□■■□□□□■■■■■■■■	777757607660771	LAM9	0.99	1

[a]Spoligotype, A-E, arbitrary spoligotype designation
[b]SpotClust program-assigned clade
[c]Probability that the spoligotype pattern belongs to the clade

24 (54.5%) isolates belonged to the T clade, 10 (22.7%) belonged to the Haarlem (H) clade, two (4.5%) belonged to the Latin American-Mediterranean (LAM) clade, and another two (4.5%) belonged to the S clade. One isolate was classified as representing the Beijing family of strains. Based on the SpotClust analysis, of the five orphan isolates, two with spoligotypes designated D and E were assigned to the LAM clade, whereas isolates whose spoligotypes were designated A, B, and C were linked to the S, H and T clades, respectively (tab. 2). Overall, the genotypic family determination showed that the T and Haarlem families were most abundant, accounting for 36 (81.8%) of the isolates studied.

An analysis of drug resistance profiles showed that 30 (68.2%) isolates were resistant to only one drug, chiefly SM (16 isolates; 36.4% of all isolates) and INH (12; 27.3%) (tab. 3). Nine (20.4%) isolates were MDR, and five (11.4%) isolates were polyresistant but sensitive to RMP (non-MDR). While the vast majority (85.7%) of monoresistant-TB cases involved patients with no prior history of TB treatment (primary resistance), most of the polyresistant-TB cases (71.4%), including MDR-TB cases, referred to patients who had received TB therapy in the past (acquired resistance).

Table 3. Drug resistance patterns among *M. tuberculosis* isolates evaluated in this study.

Drug resistance pattern	Type of resistance		Total (n = 44) n (%)
	Primary (n = 28) n (%)	Acquired (n = 16) n (%)	
Monoresistance	24 (85.7)	6 (37.5)	30 (68.2)
INH	10 (35.7)	2 (12.5)	12 (27.3)
RMP	–	1 (6.25)	1 (2.3)
SM	14 (50)	2 (12.5)	16 (36.4)
EMB	–	1 (6.25)	1 (2.3)
Multidrug resistance	2 (7.15)	7 (43.75)	9 (20.4)
INH+RMP	–	2 (12.5)	2 (4.5)
INH+RMP+SM	2 (7.15)	4 (25.0)	6 (13.6)
INH+RMP+SM+EMB	–	1 (6.25)	1 (2.3)
Other patterns	2 (7.15)	3 (18.75)	5 (11.4)
INH+SM	2 (7.15)	2 (12.5)	4 (9.0)
INH+SM+EMB	–	1 (6.25)	1 (2.3)

INH – isoniazid; RMP – rifampicin; SM – streptomycin; EMB – ethambutol

No correlation between particular drug resistance profiles and spoligotype families was found. The only association that was statistically significant (P = 0.031) was that the MDR phenotype was more likely to be present in strains with other than T family genotypes (data not shown).

DISCUSSION

The present study provides a brief outline of the spoligotype-defined population structure of drug-resistant clinical isolates of *M. tuberculosis* in the so-called "Eastern Wall" region of Poland. The choice of the setting was motivated by two main reasons. First, it constitutes a complete eastern border of Poland, which is also the longest stretch of the eastern external border for the entire European Union. Hence, the analyzed region is particularly exposed to migration and refugee flows, mostly from the former Soviet Union member countries, where TB along with its drug-resistant forms are highly prevalent. Second, the Polish "Eastern Wall" region encompasses provinces that account for a fifth of the national burden of TB, with Lublin reporting the highest TB notification rates in Poland (36.0/100,000 population in 2004, compared to 24.9/100,000 for the whole country in the same year) (16).

This study was performed with the aim of determining the predominant spoligotypes and spoligotype families responsible for drug-resistant TB cases within the "Eastern Wall" of Poland. The most apparent finding was that the population structure of the examined *M. tuberculosis* was both relatively heterogeneous, with the genotypic diversity index of 56.5%, and compact, with only three SITs (SIT53, SIT47, and SIT253) accommodating half of all the isolates. The most predominant spoligotype was SIT53 which represented as much as 36.4% of total isolates. This spoligotype has a worldwide occurrence with an increased prevalence on the European continent (10). The importance of this genotype has been highlighted in all previous studies from Poland. It always has been the top ranked spoligotype, including 13.5% of drug-resistant *M. tuberculosis* strains retrieved from the whole country during 2000 (17), as well as 16.7% of drug-resistant strains isolated in 2004 in central Poland (Masovian province) (18), and 33.8% of INH-monoresistant isolates collected in 2004 throughout the country (19).

Noteworthy, SIT53 was the most commonly observed spoligotype in Bulgaria, Finland, Sweden, and the Czech Republic (in fact second only to SIT50), with the frequencies of isolation being 25.7, 12.9, 14.6, and 13.5%, respectively (20-23). All this indicates that SIT53 represents the currently most circulating and active *M. tuberculosis* clone not only in Poland, but in the whole Central and Northern Europe. Another important finding was the fact that most of the SITs found in this study have already been reported in Poland. Of the four SITs not recorded in the SpolDB4 as being present in Poland, two (SIT524 and SIT1253) have only recently been described in the country (18, 19). The remaining two types that had never been described in Poland before were SIT73 and SIT442. Whereas the former is widely prevalent in the USA and Italy, where it accounts for nearly 60% of its isolations in the European countries, the latter is exclusively confined to the USA. Consequently, those two genotypes are likely to represent clones that have spread to Poland from abroad.

Most of the SITs found in this study (70% of the SITs, and 56% of all spoligotypes) are, according to the SpolDB4, also present in Poland's neighbors (Germany, the Czech Republic, Latvia, and Russia). However, in these countries only few SITs are represented in high proportions. This is the case of SIT1 and SIT280, whose occurrence is important in Russia (Russia accounts for 11.3% and 10.9% of strains with those genotypes in the SpolDB4, respectively), as well as SIT46 and SIT511, that are highly represented in the Czech Republic (13.7% and 21% of strains with those genotypes in the SpolDB4, respectively). The higher prevalence of those genotypes in Poland's neighboring countries, than in Poland itself, may suggest that the genotypes were originally introduced to the country through its southern and eastern neighbors.

Finally, five (11.4%) patients were infected with *M. tuberculosis* isolates having a spoligotype that had not been described in the global database (types A-E). The finding of these new and unique spoligotypes, may suggest their specificity to the study setting.

At the phylogenetic level, 17 genotypes and 36 isolates (81.8% of all isolates) belonged to only two major clades, that is, the T and Haarlem clades. Whereas the T clade is a poorly defined and ubiquitous family of *M. tuberculosis*, the Haarlem clade has a European origin and is highly prevalent in Northern Europe (10, 24). In all studies performed so far on TB molecular epidemiology in Poland, those two families (T and Haarlem) formed the core of the population structure of tubercle bacilli (17-19). Interestingly, the dominant role of these families in shaping population genetic structure of *M. tuberculosis* has been recognized in many other countries of Central-Western Europe, including the Czech Republic (23), Sweden (25), Italy (26), Spain (27), and Portugal (28) (in the last three, a third co-dominant family was the LAM clade).

Of particular note is the near absence of the Beijing family strains in the analyzed *M. tuberculosis* population. (Only one TB case in this study was caused by this genotype). This is because *M. tuberculosis* isolates belonging to the Beijing genotype are frequently encountered in Poland's eastern neighbors. For example, in northwestern Russia, the proportion of Beijing strains was reported to be 47.1% (29), while in Estonia 29.2% (30). Furthermore, in these countries, as well as in other post-Soviet states, the Beijing genotype was responsible for a substantial percentage of MDR-TB cases, i.e. 57.8% – in Latvia (31), 76.7% – in Russia (32), and 87.5% in Estonia (30). In this context, it is to underline that the only Beijing isolate identified in this study showed an MDR phenotype. The importance of the Beijing genotype is not only its association with MDR, but also with high rates of transmission and enhanced virulence (33, 34). The paucity of Beijing genotype isolates in the studied collection may suggest that this genotype is not yet spreading efficiently in Poland. This idea is corroborated by previous results of Sajduda et al. (17), which showed the prevalence of the Beijing genotype similar to that observed in this study (2.3%) among Polish DR *M. tuberculosis* strains recovered in 2000 (2.8%). Nevertheless, the fact that four of the seven Beijing genotype isolates in the study of Sajduda et al., as well as that single Beijing isolate from the present study were obtained from patients born and living in Poland implies that transmission of the Beijing genotype does occur within the autochthonous population.

CONCLUSIONS

In conclusion, this study provides, for the first time, a snapshot of the *M. tuberculosis* strains circulating in Eastern Poland. The obtained results showed the population structure of *M. tuberculosis* typical of a European country, with the T and Haarlem families accommodating close to 80% of the isolates. In addition, the low frequency of imported genotypes along with the fact that all the patients were of Polish origin indicate that the occurrence of DR-TB in Eastern Poland is largely due to the local transmission of *M. tuberculosis* strains that have been active in Poland for many years, and only to a minor extent due to the importation of strains from neighboring countries.

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