ORIGINAL ARTICLE

Transmission of tuberculosis among people living in the border areas of Poland, the Czech Republic, and Slovakia

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KEY WORDS

genotyping, *Mycobacterium tuberculosis* complex, transmission, tuberculosis

ABSTRACT

INTRODUCTION In 2007, Poland, the Czech Republic, and Slovakia joined the Schengen Agreement, abolishing restrictions on people crossing the borders. Currently, these areas are places of population movements for economic, family, and touristic reasons. This favors the transmission of infectious diseases, including tuberculosis, and requires enhanced control over the spread of the source of infection in the population of patients living in the border areas.

OBJECTIVES The aim of this study was to investigate the genetic relatedness among *Mycobacterium tuberculosis* complex strains isolated from patients living in 3 border areas: Poland, the Czech Republic, and Slovakia.

PATIENTS AND METHODS The study group consisted of 209 patients with tuberculosis diagnosed and treated between 2007 and 2011 in health care facilities in the Silesia Province in Poland (121 patients [58%]), Žilina in Slovakia (57 [27%]), and the Moravian–Silesian Region in the Czech Republic (31 [15%]). Genotyping of strains was performed using spoligotyping and IS*6110*-Mtb1-Mtb2 polymerase chain reaction.

RESULTS Among 209 strains, 23 molecular families (clusters) were identified. Seventeen clusters were identified as national. Six international clusters consisted of 30 strains isolated from patients of various nationalities.

CONCLUSIONS We identified 6 potential outbreaks of tuberculosis transmission between patients of different nationalities. The circumstances favorable to potential contacts of patients included mainly travelling to the neighboring countries, hospital stays, and addictions. However, there was no evidence of an epidemiological link between these patients, so it may be assumed that if they had come in contact with one another, it was accidental. We observed that the greater incidence of tuberculosis on the Polish territory did not affect the incidence in the Czech Republic or Slovakia over the analysis period.

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Received: October 16, 2016.
Revision accepted: December 30, 2016.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2016; 126 (1-2): 32-40

INTRODUCTION Tuberculosis constitutes a significant health problem worldwide. The World Health Organization (WHO) estimates that approximately one-third of the global population is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) complex strains.[1,2] There are 8 to 9 million new cases recorded each year, and the number of deaths due to tuberculosis amounts to about 1 million people per year. Although high incidence rates affect mainly developing countries with poor social and economic conditions, tuberculosis is present in everyday medical practice. The reasons behind are the appearance of multidrug-resistant tuberculosis (MDR; defined as resistance to at least rifampicin and isoniazid among the first-line antitubercular drugs), pre-extensively drug-resistant tuberculosis (pre-XDR, defined as MDR resistance in addition to resistance to any fluoroquinolones or at least 1 injectable second-line drugs), and extensively drug-resistant tuberculosis (XDR; caused by MDR strains resistant to any fluoroquinolones and at least 1 injectable second-line drugs), as well as frequent coexistence

of tuberculosis and infection with human immunodeficiency virus (HIV).[3-5]

It has been proved that about 5% to 10% of infected persons who do not receive treatment for latent tuberculosis infection will develop tuberculosis disease at some time in their lives. For people whose immune systems are weak, especially those with HIV infection, the risk of developing tuberculosis is much higher than for people with normal immune system function.[6]

The development of tuberculosis shortly after infection is treated as the consequence of recent transmission, whereas cases of longer periods of mycobacteria latency are treated as reactivation.[7-9]

Molecular epidemiology is one of the main areas in tuberculosis research that is widely used to study the transmission epidemics and outbreaks of tubercle bacilli. It exploits the presence of various polymorphisms in the genome of the bacteria that can be widely used as genetic markers. Many DNA typing methods apply these genetic markers to differentiate various strains and to study the evolutionary relationships between them. The 3 widely used genotyping tools to differentiate *M. tuberculosis* strains are IS*6110* restriction fragment length polymorphism (RFLP), spacer oligotyping (spoligotyping), and mycobacterial interspersed repeat units–variable number of tandem repeats (MIRU-VNTR). A new prospect towards molecular epidemiology was introduced with the development of whole-genome sequencing and the next-generation sequencing methods, where the entire genome is sequenced, which not only helps in spotting minute differences between the various sequences but also saves time and cost. Next-generation sequencing has also proved to be useful in identifying single nucleotide polymorphisms, comparative genomics, and also various aspects of transmission dynamics. These techniques enable the identification of mycobacterial strains and facilitate the study of their phylogenetic and evolutionary traits.

While searching for the source of infection, molecular testing supplemented with epidemiological investigation is the only way to obtain reliable results. If an access to information about the circumstances of contact with a potential source of infection is not possible, such tests are frequently the only proof of transmission of tuberculosis in the human environment.[10,11]

Tuberculosis spreads easily because it is transmitted by droplet infection. Long-term studies on tuberculosis transmission in the setting of the infected individual have shown that the risk of infection in such conditions is very high and depends on the degree of infectivity of the index case, duration of exposure, proximity of contacts, and environmental factors.[12]

People that are the most exposed to infection and incidence of tuberculosis are those from the so called contacts strictly defined by the WHO.[13-15] Currently, when people from various regions of the world have vast possibilities of moving around and administrative borders of the states are of smaller significance, there is a potential threat of increased disease transmission among people of different nationalities.

The aim of the present analysis was to evaluate the transmission of tubercle bacilli between patients living in 3 neighboring countries: Poland, the Czech Republic, and Slovakia. In 2007, these countries acceded to the Schengen Agreement that abolished border checks. People are currently moving across these borders for economic, personal, and touristic reasons. In these areas, the lowest incidence rate of tuberculosis (4.22/100 000) was noted in the Moravian Region in the Czech Republic; slightly higher, in the Žilina Region in Slovakia (5.59/100000); and the highest, in the Silesia Province in Poland (28.00/100000).[16]

PATIENTS AND METHODS The study group included 209 patients with bacteriologically confirmed tuberculosis, who between the years 2007 and 2011 were diagnosed and treated in health care centers in cross-border areas of Poland (121 patients [58%]), Slovakia (57 [27%]), and the Czech Republic (31 [15%]). *M. tuberculosis* was cultured from the following clinical material: sputum (175 patients [84%]), bronchial washings (13 [6%]), gastric washings (9 [4%]), fluid from the pleural cavity (6 [3%]), laryngeal swab (3 [1.5%]), urine (2 [1%]), and forearm tissue punctate (1 [0.5%]).

Microbiological data about strains were obtained from the laboratory staff, and information about the patients—from the documentation collected by clinicians.

The strains were cultured on solid media: egg-based L-J medium, Ogawa and Sulova medium, or in automated systems: BBL MGIT, isotopic Bactec 460-Tb (Becton Dickinson, New Jersey, United States), and colorimetric MB/BacT (BioMerieux, Marcy l'Etoile, France). Identification of *M. tuberculosis* species was performed using the niacin test and molecular method (Hain Lifescience, Nehren, Germany). Drug-resistance of *M. tuberculosis* strains was determined on fluid and solid media in accordance with the methodology applied in laboratories for mycobacterium species.[17] Genotyping of strains was performed with the use of spoligotyping and IS*6110*-Mtb1--Mtb2 polymerase chain reaction (PCR).[18,19]

RESULTS **Epidemiological characteristic of patients** The study group comprised 148 men and 61 women. The age of patients ranged from 2 to 96 years (mean age, 58.1 years). Two age groups predominated: 45–64 years and older than 65 years. Among 205 patients (98%), pulmonary tuberculosis was diagnosed; in the remaining cases (4 [2%]), extrapulmonary type was observed: 2 subjects had tuberculosis of the urinary system, 1 patient—of soft tissues; and 1 patient—of the lymph nodes. In the population of patients with pulmonary tuberculosis, concomitant diseases were as follows: in 15 patients, specific pleuritis;

TABLE 1 Spoligotyping results for 157 *Mycobacterium tuberculosis* complex strains distributed among 25 spoligopatterns, examined by IS*6110*-Mtb1-Mtb2 polymerase chain reaction

Spoligotype by the SpolDB4 database		No. of strains
1.	T1 53	48
2.	H3 50	28
3.	H3 99	11
4.	T4_CEU1 39	8
5.	H1 47	9
6.	T5 68	5
7.	S 34	4
8.	U (likelyH3) 237	4
9.	T3 37	4
10.	T5 44	4
11.	T1 520	3
12.	LAM5 93	3
13.	BOV 820	2
14.	H2 2	2
15.	T1_RUS2 280	2
16.	U 90	2
17.	U (likelyH) 46	2
18.	T1 1278	2
19.	T2 52	2
20.	Beijing 1	2
21.	LAM9 42	2
22.	H4 35	2
23.	H3 511	2
24.	377777777000371 (not registered in SpolDB4)	2
25.	760007770000000 (not registered in SpolDB4)	2
total		**157**

in 4, specific peripheral lymph adenitis; in 1 patient, the larynx was involved; and in 1 patient, pneumoconiosis-tuberculosis was diagnosed.

Of the whole study group, 168 patients (80%) were diagnosed for the first time, 39 subjects (19%) had been treated earlier, and in the case of 2 patients (1%), the data were insufficient. There were 26 patients (12%) who came from the families with at least 1 person with a history of tuberculosis, while 180 patients (86%) did not report having such a history. In 3 patients (2%), such information has not been obtained.

Genotyping by spoligotyping At the initial stage of the molecular analysis, genomic DNA of 209 strains underwent spoligotyping. The obtained genetic patterns were compared with an international spoligotype database, SpoIDB4, and each pattern was determined. We identified 77 spoligotypes, and 177 strains (85%) were proved to be registered in the database. Thirty-two strains (15%) had new patterns, not recorded in the worldwide register. Unique (single) patterns were found in 52 strains (25%), and the remaining 157 strains (75%) were divided into 25 clusters including from 2 to 48 strains. The results of spoligotyping and the division of strains into specific molecular families are presented in TABLES 1 and 2.

The following spoligotypes were the most common: T1 53 was identified in 48 patients (23%); H3 50, in 28 patients (10%); H3 99, in 11 patients (5%); H1 47, in 9 patients (4%), and T4 CEU1 39, in 8 patients (4%). From 2 patients (1%), bovine mycobacteria, BOV 820, were cultured, and in 2 patients (1%), the Beijing family strains of *M. tuberculosis* were identified.

Genotyping by IS6110-Mtb1-Mtb2 polymerase chain reaction Further stages of the molecular analysis concentrated on 157 strains, which, as a result of spoligotyping, constituted 25 genetic families (clusters), whereas the strains with single genotypes were excluded.

A genetic relationship between strains within the families was examined using IS*6110*-Mtb1--Mtb2 PCR, by analyzing the value of the PCR products obtained in 2 independent reactions. The compatibility of spoligotypes and identity of DNA profiles of the strains compared in 2 amplifications decided about their belonging to 1 molecular cluster.

TABLE 2 Spoligotyping results for 52 *Mycobacterium tuberculosis* complex strains presenting single spoligopatterns, excluded from the IS*6110*-Mtb1-Mtb2 polymerase chain reaction analysis

Spoligotype by the SpolDB4 database		Spoligotypes not registered in the SpolDB4 database	
1.	T1 358	1.	376777760020771
2.	LAM3 and S /convergent 4	2.	677777776413771
3.	T1 120	3.	677777777360771
4.	T1 167	4.	757377777720771
5.	T2-T3 73	5.	757777743760771
6.	H3-T3 36	6.	770000403760771
7.	H3-S 914	7.	770002777760771
8.	T1 612	8.	771347777720671
9.	T1 (T4_CE1 ancestor?)	9.	775347777720661
10.	T1 31	10.	775347777760661
11.	U 1562	11.	777741007760471
12.	T1 205	12.	777741777720771
13.	T1 276	13.	777767757760671
14.	H3 49	14.	777777700000171
15.	LAM3 33	15.	777777777740171
16.	H1 151	16.	777771000000031
17.	T1 291	17.	777357774020771
18.	H3 1238	18.	677777737760731
19.	T1 628	19.	776177747760431
20.	H1 62	20.	777777607760771
21.	EAI5 617	21.	000000000000111
22.	H1 283	22.	777717000020711
23.	T1 285	23.	707777777760371
24.	H3 183	24.	777777400760771
		25.	777774037760771
		26.	740377774020771
		27.	777777774720731
		28.	777777400060771

Based on this criterion, the number of strains that formed clusters was reduced from 157 to 82, whereas the number of identified molecular families decreased from 25 to 23 (TABLE 3). At this stage of the analysis, molecular affinity of strains belonging to the following 11 spoligotypes was excluded: T3 37, T5 44 (in each 4 strains), Beijing 1, BOV 820, H2 2, T1_RUS2 280, LAM9 42, H4 35, H3 511, T1 1278, and T2 52 (in each 2 strains).

A subsequent stage of the study consisted in a detailed epidemiological analysis of 82 patients. The strains cultured from these patients were divided among 23 genetic clusters. The isolated strains were assigned to particular families. Thirty-nine (19%) strains were isolated from Polish patients, 10 strains (7%) from Czech patients, and 33 strains (23%) were cultured in Slovakia. Seventeen clusters were identified as national: 10 clusters were found among the strains cultured from Polish patients and 7 from Slovak patients.

Six clusters (A–F) included 30 strains isolated from patients of various nationalities: 14 Slovaks, 10 Czechs, and 6 Poles, and they were determined as international clusters (TABLE 4).

Among the population from the Czech Republic and Slovakia, 3 international clusters were identified: A/H3 50 (8 patients from Slovakia and 5 from the Czech Republic), D/T4_CEU1 39 (2 from Slovakia and 2 patients from the Czech Republic), and F/H1 47 (1 from Slovakia and 1 patient from the Czech Republic). The B/T1 53 cluster was common to the patients of all nationalities: the strains with such a DNA profile were isolated from 2 Poles, 3 Slovaks, and 1 Czech. C/T1 53 was typical of 3 patients from Poland and 1 patient from the Czech Republic. The strains with the genetic pattern E/S 34 were isolated from 1 female patient from Poland and 1 male patient from Slovakia.

DISCUSSION The analysis focused on the assessment of *M. tuberculosis* transmission between patients living in 3 adjoining countries: Poland, the Czech Republic, and Slovakia. Epidemiological data were supplemented by the results of molecular tests, on the basis of which potential epidemic foci were identified. Within these potential foci, the strains of identical DNA patterns were isolated. It was assumed that the separate groups of tuberculosis patients that expelled the strains belonging to the same cluster reflect possible transmission.[20]

TABLE 3 Results after spoligotyping and the combination of spoligotyping and the IS*6110*-Mtb1-Mtb2 polymerase chain reaction analysis

Genotyping methods	No. and percentage of strains in molecular clusters	No. and percentage of strains presenting single spoligotypes	No. of clusters	No. of strains in the individual cluster
spoligotyping (209 strains)	157 (75%)	52 (25%)	25	2–48
spoligotyping + IS6110-Mtb1-Mtb2 PCR (157 strains)	82 (39%)	127 (61%)	23	2–10

Abbreviations: PCR, polymerase chain reaction

TABLE 4 Selected epidemiological data about patients in international groups of tuberculosis transmission

Molecular cluster	No. of strains/patients	Nationality / No. of patients	Epidemiological data
A H3 50	13	Slovakia/8 Czech Republic/5	7 newly detected 6 previously treated 1 patient – tuberculosis in a household 2 patients – tourists 1 HIV-positive patient
B T1 53	5	Poland/2 Slovakia/2 Czech Republic/1	2 previously treated 3 newly detected 3 patients – tuberculosis in a household 2 patients – tourists
C T1 53	4	Poland/3 Czech Republic/1	3 Polish patients living close to each other
D T4_CEU1 39	4	Czech Republic/2 Slovakia/2	all patients previously treated 2 patients – tourists
E S 34	2	Poland/1 Slovakia/1	Polish woman – tuberculosis in household
F H1 47	2	Czech Republic/1 Slovakia/1	all newly detected tourists

Abbreviations: HIV, human immunodeficiency virus

The initial stage of the molecular analysis of the strains included spoligotyping. The technique is widely considered to be the screening method of choice in the molecular testing tuberculosis epidemiology. It allows to divide *M. tuberculosis* complex strains into 10 main genetic families (Beijing, Beijing-like, CAS, EAI, Haarlem, LAM, MANU, X, S, and T). The analysis conducted in 122 countries showed that the most frequent spoligotypes in European countries are H, LAM, and T.[21] A high proportion of strains of these genetic types was also observed in the present analysis: the most frequently isolated strains in the study population belonged to the family T (90 [43%]) and H (62 [3%]). It should be emphasized that within the T family, the most frequent was T1 53 (48 [23%]), which, as shown by Augustynowicz-Kopeć,[22] is the most common spoligotype in the population of tuberculosis patients in Poland.

In the study population, 2 patients (Poles) with Beijing tuberculosis were found. Although the cultured strain was susceptible to antimycobacterial drugs, the patient died despite treatment. In Poland, patients with tuberculosis of the Beijing genotype are registered each year and, although this genotype is typical of Asian countries, this form of tuberculosis affects more and more people worldwide.[23] It is believed that it is the most virulent and dangerous type, often incurable, and resulting in death. It has been proved that patients infected with Beijing mycobacteria more frequently and more rapidly develop active form of the disease, compared with patients infected with the strains of another genotype. Mycobacteria with this DNA profile are considered to be endemic to the region of China, but it has been proved that Beijing tuberculosis caused an epidemic also in other regions of the world, mainly in the countries of the former Soviet Union. Beijing tuberculosis constitutes approximately 50% of cases identified in East Asia and concerns at least 13% of all patients with tuberculosis worldwide.[24-27] In Poland, it has been identified for the first time in 2000, in population with MDR tuberculosis. Since then, significant changes in this group of patients have been observed. In the studies conducted by Kozińska et al[28] between 2000 and 2004, Beijing strains were isolated mainly from foreigners (69%

of strains). Probably because of legal and illegal crossing of the Polish eastern border, the influx of immigrants from Asia (Vietnam, Kazakhstan, and Chechnya), and failure to appropriately control the transmission of the disease, MDR Beijing tuberculosis started to spread among the Polish population and the proportion has been reversed: at present, 39% of strains are isolated from foreigners and 61%—from Poles.[28]

An interesting finding was the identification of another mycobacteria spoligotype untypical of Europe, namely, EAI, in 1 patient from Slovakia. It is the second most frequent type of tuberculosis, just after the Beijing family, identified in patients from Asia and the Far East (34%), Middle East, and Central Asia (24%) and Oceania (23%). The EAI strain was isolated from the patient coming from Asia; therefore, it may be presumed that the patient had been infected in his country of origin.

Bovine mycobacterial, BOV 820, were identified in 2 Poles. The molecular analysis excluded the genetic relationship between the strains, which implies that the patients were infected with this type of tuberculosis by 2 independent and unidentified sources. There have been numerous reports of tuberculosis transmission between animals and humans. It has been proved that the transmission of the source of infection may go in 2 directions: from humans to animals and, inversely, bovine tubercle bacilli may be transmitted from animals (mainly domestic and farm ones) to their keepers.[29,30]

In order to investigate the phenomenon of transmission between patients, we applied the 2-stage molecular analysis of the strains. The results of the first stage of the study again proved that spoligotyping is insufficient for investigating the routes of transmission of mycobacteria; however, it should be underlined that the method has numerous advantages, such as it is performed quickly, has high repeatability of the results, and big capacity (45 strains may be analyzed during 1 test). Furthermore, the method does not require large amounts of DNA and relies on genetic material isolated merely from several bacterial cells. Another important advantage of spoligotyping is the numeric record of molecular patterns of mycobacteria (binary and octagonal formats) and the possibility of cataloguing in the central database, SpolDB4.[31]

As mentioned above, spoligotyping identifies main molecular families among *M. tuberculosis*, but it is not an appropriate tool to examine relationships between mycobacteria belonging to 1 chain of infection transmission. In order to analyze the genome of the examined group of strains in detail, methods having greater ability to differentiate strains are used, usually MIRU-VNTR, IS*6110*-RFLP, and gene sequencing.[32] In the present study, spoligotyping was supplemented with IS*6110*-Mtb1-Mtb2 PCR, a method that has been already described as having similar ability to differentiate strains as MIRU-VNTR. The strength of this method is that it is fast, fairly robust, and easy to perform, but has significant technical limitations, including the need of high-quality DNA and therefore requiring prior culture of the isolates and the determination of results based on visual inspection of images of band patterns that are difficult to share between laboratories.[19,33,34]

Increasing differentiating power of the genetic methods used in the study correlated with the reduced number of strains constituting possible epidemiological groups. In spoligotyping, such groups were composed of 157 strains (75%), whereas in IS*6110*-Mtb1-Mtb2 PCR, the number of strains constituting potential epidemic groups was reduced to 82 (39%). The strains isolated from the remaining 127 patients (61%) had unique single, not repeated, DNA profiles.

The history of the patients allowed us to establish that the study group included 27 individuals with a history of close contact with the family member with tuberculosis. Hence, it may be presumed that the source of infection in case of these patients was their home environment. It is well known that a sputum-positive member of the family poses the biggest danger to the closest people. Such person is classified as belonging to the first epidemiological circle that requires special epidemiological surveillance. In their studies, Kozińska et al[35,36] have shown that transmission of tuberculosis occurred in 89% of the families that were under epidemiological investigation. High rates of transmission were also observed in numerous analyses conducted on other hermetic and small populations: the homeless, residents of social assistance homes, and hospital patients.

In the case of the remaining patients whose cultured strains had individual DNA profiles and who did not have a family history of tuberculosis, reactivation of the past infection or transmission of the strain "imported" to a given population from the outside should be considered. Also, it cannot be excluded that the presence of single genotypes may be caused by the changes in the genome of *M. tuberculosis* caused by DNA rearrangements that resulted from treatment, another host during transmission, or transformations connected with evolution.[37-39]

The main objective of the study was to show whether there is transmission of tuberculosis between the populations of 3 adjoining countries. It has to be emphasized that the study was conducted in the so called third epidemiological circle, which, according to the WHO definition, means the population of patients who contact accidentally (in bars, shops, during travel) and in whom the index case is difficult to identify.[40,41]

Six (A–F) potential foci of international transmission were analyzed. It included 30 patients: 14 Slovaks, 10 Czechs, and 6 Poles. Considering the circumstances of possible contacts that could have enabled the transmission of the infection, we first of all considered journeys to the neighboring countries, hospital stays, and addictions (alcoholism). There was no evidence of certain strictly

specified circumstances of contact between the patients; therefore, it may be assumed that they only could have met accidentally.

As already mentioned, the molecular relationship between strains could be the result of recent transmission, but it is necessary to examine other factors causing the clustering results, especially in situations where there is no epidemiological evidence on the link between patients.

The amount of recent transmission depends heavily on the genotyping system applied, and its discriminatory power determines the proportion of clustered isolates. Our results have shown the importance of a combined strategy for *M. tuberculosis* typing, in which clusters defined upon spoligotyping would be subject to further differentiation using methods with high discriminatory power.[33,42] Spoligotyping and IS*6110*-Mtb1-Mtb2 PCR produced clustering rates of 75% and 39%, respectively. The combination of these 2 methods provided certain and reliable evidence of the relationship of the tested strains.

However, clustering of *M. tuberculosis* isolates does not always represent recent transmission, and it can also reflect the persistence of well-conserved circulating endemic strains.[43] The absence of epidemiological data to confirm a clonal relationship among the isolates was an important limitation of this study.

Of note are significant differences in the incidence of tuberculosis in the analyzed area: the lowest incidence rate was observed in the Moravian Region in the Czech Republic; slightly higher, in the Žilina Region in Slovakia; and the highest, in the Silesia Province in Poland.[16]

Numerous authors suggest that the epidemiological situation in border areas is influenced by the situation of the country with a higher incidence of tuberculosis.[44,45] However, the results of the present study have not shown that a higher incidence in Poland impacted on the number of tuberculosis cases in the border regions in the Czech Republic or Slovakia.

Contribution statement JK and ZZ conceived the idea for the study. All authors contributed to the design of the research. JZ and JK were involved in data collection. MK and JZ analyzed the data. EA-K and JK coordinated funding for the project. All authors edited and approved the final version of the manuscript.

REFERENCES

1 Comstock GW. Frost revisited: the modern epidemiology of tuberculosis. Am J Epidemiol. 1975; 101: 363-382.

2 Getahun H, Matteelli A, Chaisson RE, et al. Latent Mycobacterium tuberculosis infection. N Engl J Med. 2015; 372: 2127-2135.

3 Davies PD. Risk factors for tuberculosis. Monaldi Arch Chest Dis. 2005; 63: 37-46.

4 Kolappan C, Gopi PG, Subramani R, Narayanan PR. Selected biological and behavioural risk factors associated with pulmonary tuberculosis. Int J Tuberc Lung Dis. 2007; 11: 999-1003.

5 Jain A, Dixit P. Multidrug-resistant to extensively drug resistant tuberculosis: what is next? J Biosci. 2008; 33: 605-616.

6 Centers for Disease Control and Prevention (CDC) Basic TB facts, risk factors. 2012.

7 Kaufmann SH. How can immunology contribute to the control of tuberculosis. Nat Rev Immunol. 2001; 1: 20-30.

8 Smith I. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Reviews. 2003; 16: 463-496.

9 Cardona PJ. New insights on the nature of latent tuberculosis infection and its treatment. Inflamm Allergy Drug Targets. 2007; 6: 7-39.

10 Desikan S, Narayanan S. Genetic markers, genotyping methods & next generation sequencing in Mycobacterium tuberculosis. Indian J Med Res. 2015; 141: 761-774.

11 Szopa M, Ludwig-Gałęzowska A, Radkowski P, et al. Genetic testing for monogenic diabetes using targeted next-generation sequencing in patients with maturity-onset diabetes of the young. Pol Arch Med Wewn. 2015; 125: 845-851.

12 Cardona PJ, Ruiz-Manzano J. On the nature of Mycobacterium tuberculosis-latent bacilli. Eur Respir J. 2004; 24: 1044-1051.

13 Becerra MC, Pachao-Torreblanca IF, Bayona J, et al. Expanding tuberculosis case detection by screening household contacts. Public Health Rep. 2005; 120: 271-277.

14 El-Sony A, Enarson D, Khamis A, et al. Relation of grading of sputum smears with clinical features of tuberculosis patients in routine practice in Sudan. Int J Tuberc Lung Dis. 2002; 6: 91-97.

15 Sepkowitz KA. How contagious is tuberculosis? Clin Infect Dis. 1996; 23: 954-962.

16 Global Tuberculosis Report. World Health Organization. 2012.

17 Pichula K. [Detection of acid-fast bacilli by culture. Microbiology of tuberculosis]. Warszawa, Poland: PZWL; 1977: 117-147. Polish.

18 Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol. 1997; 35: 907-914.

19 Kotłowski R, Shamputa IC, El Aila NA, et al. PCR-based genotyping of Mycobacterium tuberculosis with new GC-rich repeated sequences and IS6110 inverted repeats used as primers. J Clin Microbiol. 2004; 42: 372-377.

20 Gillespie SH, Dickens A, McHugh TD. False molecular clusters due to nonrandom association of IS6110 with Mycobacterium tuberculosis. J Clin Microbiol. 2000; 38: 2081-2086.

21 Brudey K, Driscoll JR, Rigouts L, et al. Mycobacterium tuberculosis complex genetic diversity: mining the fourthinternational spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol. 2006; 6: 23.

22 Augustynowicz-Kopeć E. [Drug resistant tuberculosis in Poland. Epidemiological, microbiological and genetic analysis]. Warszawa, Poland: Warszawski Uniwersytet Medyczny; 2007: 1-230. Polish.

23 Toungoussova OS, Caugant DA, Sandven P, et al. Impact of drug resistance on fitness of Mycobacterium tuberculosis strains of the W-Beijing genotype. FEMS Immunol Med Microbiol. 2004; 42: 281-290.

24 Park YK, Shin S, Ryu S, et al. Comparison of drug resistance genotypes between Beijing and non-Beijing family strains of Mycobacterium tuberculosis in Korea. J Microbiol Methods. 2005; 63: 165-172.

25 Glynn JR, Whiteley J, Bifani PJ, et al. Worldwide occurrence of Beijing/W strains of Mycobacterium tuberculosis: a systematic review. Emerg Infect Dis. 2002; 8: 843-849.

26 European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis. Beijing/W genotype Mycobacterium tuberculosis and drug resistance. Emerg Infect Dis. 2006; 12: 736-743.

27 Lasunskaia E, Ribeiro SC, Manicheva O, et al. Emerging multidrug resistant Mycobacterium tuberculosis strains of the Beijing genotype circulating in Russia express a pattern of biological properties associated with enhanced virulence. Microbes Infect. 2010; 12: 467-475.

28 Kozińska M, Augustynowicz-Kopeć E. Drug resistance and population structure of Mycobacterium tuberculosis Beijing strains isolated in Poland. Pol J Microbiol. 2015; 64: 399-401.

29 Zwolska Z, Krajewska M, Augustynowicz-Kopeć E. Mycobacterium bovis old and new problem in animal and men tuberculosis. Nowa Klinika. 2010; 17: 340-346.

30 Krajewska M, Kozińska M, Zwolska Z, et al. Human as a source of tuberculosis for cattle. First evidence of transmission in Poland. Vet Microbiol. 2012; 159: 269-271.

31 Augustynowicz-Kopeć E, Jagielski T, Kozińska M, et al. The significance of the spoligotyping method in epidemiological investigations of tuberculosis. Pneumonol Alergol Pol. 2007; 75: 22-31.

32 Cowan LS, Diem L, Monson T, et al. Evaluation of two-step approach for large-scale, prospective genotyping of Mycobacterium tuberculosis isolates in the United States. J Clin Microbiol. 2005; 43: 688-695.

33 Sajduda A, Dziadek J, Kotlowski R, Portaels F. Evaluation of multiple genetic markers for typing drug-resistant Mycobacterium tuberculosisstrains from Poland. Diagn Microbiol Infect Dis. 2006; 55: 59-64.

34 Jagielski T, Augustynowicz-Kopeć E, Pawlik K, et al. A two-step strategy for molecular typing of multidrug-resistant Mycobacterium tuberculosis clinical isolates from Poland. Pol J Microbiol. 2011; 60: 233-241.

35 Kozińska M, Brzostek A, Krawiecka D, et al. MDR, pre-XDR and XDR drug-resistant tuberculosis in Poland in 2000-2009. Pneumonol Alergol Pol. 2011; 79: 278-287.

36 Kozińska M. [Molecular epidemiological investigations among people closely related and evaluation of usefulness of typing methods]. Doctoral thesis. Warszawa, Poland: Instytut Gruźlicy i Chorób Płuc; 2011. Polish.

37 Warren RM, van der Spuy GD, Richardson M, et al. Evolution of the IS6110-based restriction fragment length polymorphism pattern during the transmission of Mycobacterium tuberculosis. J Clin Microbiol. 2002; 40: 1277-1282.

38 Bifani PJ, Plikaytis BB, Kapur V, et al. Origin and interstate spread of a New York City multidrug-resistant Mycobacterium tuberculosis clone family. JAMA. 1996; 275: 452-457.

39 van Soolingen D, Hermans PW, de Haas PE, et al. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol. 1991; 29: 2578-2586.

40 Augustynowicz-Kopeć E, Demkow U, Grzelewska-Rzymowska I. Guidelines of Polish Respiratory Society concerning diagnosis, treatment and prevention of tuberculosis in adults and in child. Pneumonol Alergol Pol. 2013; 81: 325-379.

41 Erkens CG, Kamphorst M, Abubakar I, et al. Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J. 2010; 36: 925-949.

42 Kremer K, van Soolingen D, Frothingham R, et al. 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. 1999; 37: 2607-2618.

43 Braden CR, Templeton GL, Cave MD, et al. Interpretation of restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from a state with a large rural population. J Infect Dis. 1997; 175: 1446-1452.

44 Dahle UR, Eldholm V, Winje BA, et al. Impact of immigration on the molecular epidemiology of Mycobacterium tuberculosis in a low-incidence country. Am J Respir Crit Care Med. 2007; 176: 930-935.

45 Kamper-Jørgensen Z, Andersen AB, Kok-Jensen A, et al. Clustered tuberculosis in a low-burden country: nationwide genotyping through 15 years. J Clin Microbiol. 2012; 50: 2660-2667.

ARTYKUŁ ORYGINALNY

Transmisja gruźlicy wśród mieszkańców terenów przygranicznych Polski, Czech i Słowacji

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SŁOWA KLUCZOWE

genotypowanie, gruźlica, *Mycobacterium tuberculosis complex*, transmisja

STRESZCZENIE

WPROWADZENIE W 2007 roku Polska, Czechy i Słowacja przystąpiły do układu z Schengen znoszącego kontrolę osób przekraczających granice. Aktualnie tereny te są miejscami przemieszczania się ludności z powodów ekonomicznych, rodzinnych i turystycznych. Fakt ten sprzyja transmisji chorób zakaźnych, m.in. gruźlicy, i wymaga wzmożonej kontroli rozprzestrzeniania się źródła zakażenia w populacji chorych zamieszkujących tereny przygraniczne.

CELE Celem pracy było zbadanie pokrewieństw genetycznych szczepów *Mycobacterium tuberculosis complex* wyhodowanych z materiałów klinicznych pobranych od chorych z terenów przygranicznych Polski, Czech i Słowacji.

PACJENCI I METODY Grupę badaną stanowiło 209 chorych na gruźlicę diagnozowanych i leczonych w latach 2007–2011 w placówkach opieki zdrowotnej na terenie województwa śląskiego w Polsce (121 chorych [58%]), żylińskiego na Słowacji (57 [27%]) i morawsko-śląskiego w Czechach (31 [15%]). Typowanie genetyczne szczepów wykonywano stosując *spoligotyping* i IS*6110*-Mtb1-Mtb2 PCR.

WYNIKI Wśród 209 szczepów zidentyfikowano 23 rodziny molekularne (klastery). Siedemnaście klasterów zidentyfikowano jako narodowe. Sześć klasterów międzynarodowych utworzyło 30 szczepów wyizolowanych od chorych różnych narodowości.

WNIOSKI W badanej populacji wskazano 6 potencjalnych ognisk transmisji gruźlicy między chorymi różnych narodowości. Mając na uwadze okoliczności kontaktu chorych, uwzględniano przede wszystkim podróże do krajów sąsiednich, pobyty w szpitalach i nałogi. Nie udowodniono jednak powiązania epidemiologicznego między chorymi, zatem można przypuszczać, że ich kontakt, jeśli wystąpił, mógł być przypadkowy. Nie wykazano, że większa zapadalność na gruźlicę w Polsce miała wpływ na liczbę zachorowań na terenie przygranicznym w Czechach czy Słowacji w czasie objętym analizą.

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Praca wpłynęła: 16.10.2015.
Przyjęta do druku: 30.12.2015.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2016; 126 (1-2): 32-40