Original Article

Tuberculosis Transmission in the Population of Patients from the Krakow Region (Poland) Based on the Epidemiological and Molecular Methods

Katarzyna Kruczak[1], Ewa Augustynowicz-Kopeć[2], Monika Kozińska[2], Grażyna Passak-Stańda[3], Ewa Niżankowska-Mogilnicka[1], Krzysztof Sładek[1]

[1]Department of Pulmonology, Jagiellonian University Medical College, [3]John Poul II Hospital, Mycobacterium Tuberculosis Reference Laboratory, Krakow, [2]Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland

Abstract

Background: The transmission of tuberculosis may affect the incidence rate of the disease in Poland. Genetic methods are of assistance in tracing the infection transmission, identifying its sources, determining the risk groups, and focusing on the preventive actions. **Objectives:** The objectives of this study lie in an assessment of tuberculosis transmission by genetic methods with the assistance of the standard epidemiologic interview. **Methods:** The genome DNA of 275 *Mycobacterium tuberculosis* (Mtb) strains from tuberculosis patients, inhabitants of the city of Krakow, was subjected to a genetic analysis via the spoligotyping method and the IS6110-Mtb1–Mtb2 polymerase chain reaction (PCR) method. If the DNA profiles were identical in both of the PCRs, they were considered identical and classified within one molecular family. **Results:** Among 275 strains, 104 genetic patterns (spoligotypes) were identified. Two hundred and three strains were divided into 66 molecular families (clusters) and analyzed with the IS6110- Mtb1–Mtb2 PCR method. Eighteen clusters were separated. In the Mtb1–Mtb2 clusters, 21 patients were in the risk groups (the homeless, prisoners, and nursing home residents). We did not confirm any direct or temporary contacts between the patients constituting the Mtb1–Mtb2 clusters (apart from the risk groups). However, the patients in these clusters often lived in the same parts of Krakow. **Conclusions:** The standard epidemiologic interview in tuberculosis patients should be combined with genetic methods. Active transmission of tuberculosis occurs largely among the individuals maintaining probably periodic contacts. The patients who are in the risk groups may play an important role in the transmission of tuberculosis.

Keywords: Cluster, risk groups, spoligotyping, transmission, tuberculosis

Introduction

Tuberculosis is an airborne infectious disease. The following factors support its occurrence: deterioration of social/living conditions, homelessness, alcoholism, HIV infection, chronic kidney diseases, hematological diseases, cytoreduction, and immunosuppressive treatment.[1,2] During 2007–2011, in Krakow city, we observed that the decrease of the tuberculosis incidence rate has been inhibited in Poland. In 2007 and 2011, tuberculosis incidence rate in Poland was 22.5 and 22.2 (actually in 2017 – 15.1) respectively; in Krakow region, tuberculosis incidence rate was 18.6 in 2007, 16.5 in 2011, and 13.5 in 2017.[2,3] A considerably high number of individuals suffering from tuberculosis constituted the homeless; the disease was increasingly more often observed in foreigners, including the multidrug-resistant variant caused by the Beijing strains. The question of what conditions favor such a high incidence rate in this region remains unanswered. Are there any particular sources of infection, for example, the homeless? Do the individuals with tuberculosis infect their household members, their colleagues from work? What percentage among the new cases are exogenous infections, what percentage are the reactivations of "old" lesions in the lungs, and under the influence of which factors do they occur? The

Access this article online

Quick Response Code:

Website: www.ijmyco.org

DOI: 10.4103/ijmy.ijmy_11_19

Address for correspondence: Dr. Katarzyna Kruczak, Department of Pulmonology, Jagiellonian University Medical College, Skawińska 8, 31-066 Krakow, Poland.
E-mail: krucza@gmail.com

ORCID:
https://orcid.org/0000-0002-2468-3921

How to cite this article: Kruczak K, Augustynowicz-Kopeć E, Kozińska M, Passak-Stańda G, Niżankowska-Mogilnicka E, Sładek K. Tuberculosis transmission in the population of patients from the Krakow region (Poland) based on the epidemiological and molecular methods. Int J Mycobacteriol 2019;8:60-9.

fully developed illness leads to destruction of the pulmonary tissue, multiplication of a large number of mycobacteria, and a high degree of infectiveness for the surrounding individuals. Determining the source and routes of spreading the infection is an important element of fighting the disease.

New molecular biology methods have come to the assistance of modern epidemiologic determinations. Genotyping (genetic typing) leads to determining the individual genetic profiles (fingerprints) for the analyzed *Mycobacterium tuberculosis* (Mtb) strains, owing to which it is possible to determine their mutual relationship. This is of significance when carrying out epidemiologic research in tuberculosis. By applying these methods, one may probably identifies the disease transmission outbreak in various population centers, identifies laboratory cross infection,[4-7] and differentiates an endogenous reinfection from an exogenous one. The analysis of restriction fragment length polymorphism (RFLP) which contains the insertional sequence IS6110 is among the molecular methods that are widely applied in tuberculosis epidemiology. Among the methods of genetic typing in large groups, there are methods based on the amplification of nucleic acids. Of these methods, one that deserves particular attention is the spoligotyping (spacer oligonucleotide typing) method, which, apart from IS6110 RFLP, is the most often applied in molecular epidemiological tuberculosis research.[4-11] The spoligotyping method applies the polymorphism of the chromosomal direct repeat (DR) region, occurring among the mycobacteria belonging to the Mtb complex (Mtbc). The number and position of the separating sequences in the DR regions is the criterion of strain differentiation.[9,11] The subsequent stage of the molecular analysis may be the analysis of the strains belonging to the same spoligo family employing the IS6110-Mtb1–Mtb2 polymerase chain reaction (PCR).[8] If the genetic profiles are the same – as verified by both methods – the strains are considered to be probably identical and classified as one molecular family.[9,12] Strains from one molecular family form clusters (groups with an identical genetical pattern). Patients whose strains are from the same cluster are epidemiologically related – we may suppose that there is transmission of tuberculosis between them.[5-8,10,13] Finding and identifying the clusters may probably lead to determining the sources of infection and the ways of tuberculosis spreading, which also has a significant preventive effect. The spoligotyping method should be applied as a screening method during the initial stage of the epidemiological research of Mtb. The subsequent step should be employing a method with a high differentiating potential; for example, IS6110 RFLP or IS6110-Mtb1–Mtb2 PCR.[13-16]

Methods

Before proceeding with the examination, the patients were given a form presenting information and the informed consent form for participation in the study. They also discussed with a physician who clarified any doubts associated with the participation in the study. Following the granting of the consent for participation in the investigation, the study patients were asked to sign the so-called "statement" form, in which they gave permission to use their personal data only for scientific purposes (pursuant to the Personal Data Protection Act dated August 28, 1997). Permission was also obtained from the Bioethical Committee, dated November 30, 2009.

In the years 2009–2011, the genome DNA was isolated from the Mtbc strains from 275 tuberculosis patients from Krakow, who were ill in the years 2007–2011, and the results were subjected to the molecular analysis employing the spoligotyping and IS1610Mtb1–Mtb2 methods.

The study group consisted of 211 men (77%) and 64 women (23%). One cultured tuberculosis strain from each individual was examined. The predominant group included individuals between 41 and 60 years of age (40%) with a pulmonary form of tuberculosis (94%) and patients who were sputum positive – with a dominance of positive sputum smear positive – acid-fast bacilli positive (AFB+) (63%), with predominant sensitivity to the administered antimycobacterial drugs. In the investigated group, there were 55 patients (20%) belonging to the risk groups (the homeless, prisoners, and nursing home residents) [Table 1].

Table 1: The profile of patients participating in the investigation

Characteristics of patients	Number
Quantity of patients	275
Sex	
Male	211
Female	64
Age	
0-40	76
41-60	110
>60	89
Pulmonary/extrapulmonary form	
P	258
EP	17
AFB	
AFB+	163
AFB−	102
Sensitivity to drug	
Streptomycin	
Sensitive	237
Resistant	38
Isoniazid	
Sensitive	247
Resistant	28
Rifampicin	
Sensitive	264
Resistant	11
Ethambutol	
Sensitive	269
Resistant	6
Belonging to risk groups (the homeless, prisoners, and nursing home residents)	55

AFB: Acid-fast bacilli, AFB+: Acid-fast bacilli positive, AFB−: Acid-fast bacilli negative, EP: Extrapulmonary form, P: Pulmonary form

The strains cultured in the years 2007–2011 from the tuberculosis patients (*Mycobacterium tuberculosis* Reference Laboratory in Krakow) were referred to the Microbiology Department of the Institute of Tuberculosis and Lung Diseases in Warsaw. Applying the spoligotyping method, groups of strains with identical genetic patterns (clusters) were obtained, which were subsequently submitted for another analysis employing the IS6110-Mtb1–Mtb2 PCR method.

The bacterial strains from the obtained cultures were stored in the form of a suspension in a buffer for freezing in the temperature of −70°C. Subsequently, the cultures were revived in the Löwenstein–Jensen medium and used for all marking and chromosomal DNA isolation. The strain being of a given kind was confirmed with the niacin test and the ProbeTec (Becton-Dickinson, Franklin Lakes, New Jersey, US) genetic probe. The drug sensitiveness tests were conducted employing the standard methods.

Subsequently, DNA was isolated from the mycobacteria from the culture. The isolation was performed via thermal inactivation of the cultured mycobacteria, lysis with lysozyme, and sodium dodecyl sulfate-proteinase K. Subsequently, we extracted mycobacterial DNA with cetyltrimethylammonium bromide-NaCl solution, purified it using a chloroform-isoamyl alcohol mixture, precipitated, centrifuged, rinsed the residue in 70% ethanol, dried, and suspended in 20 μL of water.

Subsequently, the genetic analysis of the Mtb strains with the spoligotyping method was conducted. The method is based on polymorphism of the DR region which is characteristic for the Mtbc.[5,9] The region contains repeating sequences comprised of 36 base pairs which are separated by unique, short sections containing 35-41 base pairs. The number of DR sequence copies is a characteristic feature of a given strain. The method identifies the presence or absence, within the DR region, of a specific sequence separated by the hybridization of PCR products to 43 oligonucleotides with a covalent bond with a nylon membrane. Specific starters are used during the reaction.

The IS6110-Mtb1–Mtb2 PCR method is based on the IS6110 sequence characteristic for the Mtbc and is supplemented with the analysis of the polymorphic areas of the genome abundant in GC pairs. The method is based on two PCR reactions varying in the starters (IS1+ IS2+ Mtb1 and IS1+ IS2+ Mtb2). The specific clonal configuration of the DNA fragments in the agarose gel attests to the polymorphic character of the strains. The mycobacteria genome DNA was affected by the Pvull restriction enzyme, and the PCR reaction was performed. Specific starters were used. Both reactions were conducted with the application of traditional sets.[5,9]

The hybridization patterns obtained via the spoligotyping methods were analyzed by two individuals, presented in the form of a binary and an octagonal notation, and compared with the patterns recorded in the international SpolDB4 database (www.pasteur-guadelupe.fr/tb/spoldb4).[15] The analysis of the genetic profiles obtained using the IS6110-Mtb1–Mtb2 PCR method was carried out employing the LabWorks 4.5 software SCI Technologies, Inc., Huntsville, Alabama, US. Identical strains had identical mycobacterial configuration.

Results

One hundred and four genetic patterns (spoligotypes) were identified among the 275 strains. Sixty-six patterns have been recorded in the international spoligo SpolDB4 base, whereas 38 have not been recorded [Table 2].

Two hundred and three strains were divided into 66 molecular families (clusters), separated on the basis of an identical spoligotype. Twenty-five of these families have been recorded in the SpolDB4 base and seven of the families have not been recorded in the base [Table 3].

The most numerous patient group was in the T153 family (26%), followed by the H350 (12%), T252 (5%), and 177777677760771 families that contained 11 strains from 11 patients, similarly as the 777737777700771 family (5% of the patients). The H147 family included ten patients (5%).

Four patients were among the Beijing family (2%) [Table 2].

The patients from the spoligo families constituted 74% (203/275) of all the individuals participating in the investigation.

In the spoligo clusters, the predominant group were male (80%), patients aged between 41 and 60 years (46%), those suffering from the pulmonary form of tuberculosis (94%), those presenting with tuberculosis with a positive AFB+ direct smear (63%), and those with strains that were mostly sensitive to the antimycobacterial medication of the first choice. Forty-four individuals were in the risk groups (22%) [Table 4].

The subsequent stage was the analysis of 203 strains creating 32 spoligo families employing the IS6110Mtb1–Mtb2 CRP method [Table 5]. Among the researched spoligo families, the Mtb1–Mtb2 families were distinguished, which demonstrated identical genetic patterns when using the second method. They constituted 35% of all the patients participating in the investigation (96/275) and in spoligo group they constituted 47% (96/203).

The spoligo families, which had identical genetic patterns detected employing the PCR Mtb1–Mtb2 method, formed Mtb1–Mtb2 families, were identical, and closely related genetically. Tuberculosis transmission between these strains was strongly suspected [Table 5].

In the Mtb1–Mtb2 clusters, there was a male predominance (78/96 – 81%); individuals were aged 41–60 years (43%); and patients had a pulmonary form of tuberculosis (96%), had sputum smear positivity (56%), and had a predominant sensitivity to the employed antimycobacterial medication; 21%–22% were among the risk groups.

The highest number of strains constituting the Mtb1–Mtb2 families was noted among the T153 family (21%), followed

Table 2: The spoligotypes registered and not registered in the SpolDB4 base

The spoligotypes registered in the spoligo base						The spoligotypes not registered in the spoligo base			
1	T1 53	23	U (likelyH3) 237	45	CAS1_DELHI 26	1	177777677760771	23	777775377760771
2	H3 50	24	T1 191	46	T1 1558	2	777737777700771	24	777777577320771
3	T2 52	25	H3 748	47	T4_CEU1 39	3	777777770000000	25	777777401760771
4	H1 47	26	T2-T3 73	48	T1_RUS2 1103	4	000002004020771	26	5777777777360770
5	H2 2	27	H3-T3 36	49	T1 1051	5	577777777720711	27	000000007740771
6	H3 746	28	T1_RUS2 280	50	T5 68	6	777777774020711	28	777777774020611
7	T3 37	29	H3 1282	51	H3 3	7	657777777720771	29	777773740060771
8	Beijing 1	30	H4 762	52	EAI4_VNM	8	777737777420371	30	777777770000371
9	H1 382	31	U 786	53	H3 741	9	777777345760471	31	777357777700771
10	H3 511	32	LAM10_CAM 61	54	H4 262	10	777777777760511	32	777741077760771
11	U 1928	33	H3 99	55	U 402	11	777763774020771	33	777637777420711
12	H4 35	34	T1 1233	56	U 511	12	777773037760771	34	777307777420661
13	LAM3 and S/convergent 4	35	U 1184	57	X2 347	13	777627777720771	35	757777774020771
14	T4 40	36	T1 122	58	S 34	14	000002000000000	36	777347777760661
15	T5 44	37	T1 535	59	T1 520	15	757777776000731	37	577777777760771
16	U (likely H) 46	38	T1 628	60	T1 370	16	777402007760771	38	776017777720771
17	H3 37	39	T5 1913	61	H1 1807	17	757777717720771		
18	H3 925	40	H1 218	62	LAM3 33	18	777777730060771		
19	LAM9 42	41	T2-T3 832	63	T1 131	19	776377777720071		
20	LAM9 891	42	T1 462	64	T1 243	20	403377777760771		
21	T1 771	43	H3-LAM9 67	65	T1 801	21	776737607760731		
22	U 775	44	LAM11_ZWE 812	66	U 124	22	777737700000171		

Table 3: The spoligo families

n	Spoligotype	Quantity of strains	Numbers of strains	*n*	Spoligotype	Quantity of strains	Numbers of strains
1	T1 53	57	809, 906, 1014, 70, 905, 627, 1187, 981, 1241, 1221, 1942, 2337, 2622, 1513, 445, 846, 1, 2392, 35, 4293, 150, 989, 2795, 717, 9, 3016, 748, 2098, 532, 2704, 2112, 2134, 2668, 2851, 4762, 162, 421, 173, 473, 325, 101/745, 475, 1072, 1202, 1569, 224, 457, 601, 369, 901, 1910, 2096, 1939, 1723, 1968, 2161, 1797	17	T4 40	3	1043, 1585, 1750
2	H3 50	24	1324, 807, 1104, 1186, 1266, 1157, 1140, 1323, 859, 2373, 2779, 2600, 1128, 388, 1245, 1028, 553, 2241, 4361, 345, 115, 831, 1678, 192	18	T5 44	3	1751, 1826, 1513
3	T2 52	12	904, 893, 1122, 881, 2423, 1511, 675, 1738, 591, 2493, 1109, 325	19	U (likely H) 46	3	772, 1220, 456
4	177777677760771	11	1119, 963, 1322, 978, 760, 1253, 489, 924, 554, 167, 1634	20	000002004020771	2	1138, 924
5	777737777700771	11	1088, 1608, 1581, 959, 2757, 1062, 654, 2910, 1300, 293, 541	21	577777777720711	2	3145, 1261
6	H1 47	10	1089, 1062, 1052, 2474, 2442, 3166, 4005, 357, 378, 3231	22	777777774020711	2	632, 1054
7	H2 2	7	1351, 3026, 747, 182, 139, 1542, 716	23	H3 37	2	1195, 228
8	H3 746	6	1267, 944, 971, 1465, 2437, 408	24	H3 925	2	1272, 1444
9	T3 37	5	972, 1345, 872, 22, 1800	25	LAM9 42	2	880, 969
10	Beijing 1	4	3729, 3463, 764, 774	26	LAM9 891	2	919, 961
11	H1 382	4	838, 969, 406, 2427	27	T1 771	2	2160, 807
12	H3 511	4	939, 1328, 2680, 844	28	U 775	2	970, 1050
13	U 1928	4	935, 1001, 1433, 4782	29	U (likelyH3) 237	2	1160, 31
14	777777770000000	3	2908, 2558, 2583	30	657777777720771	2	837, 568
15	H4 35	3	946, 482, 111	31	T1 191	2	1847, 3068
16	LAM3 and S/convergent 4	3	3831, 1857, 2016	32	H3 748	2	2645, 1164

Table 4: The profile of the participants in the investigation constituting the spoligo families

	Profile of patients belonging to spoligo groups	Profile of patients not belonging to spoligo groups
Quantity of patients	203	72
Sex		
Male	162	49
Female	41	23
Age		
0-40	56	20
41-60	94	16
>60	53	36
Pulmonary/extrapulmonary form		
P	191	67
EP	12	5
AFB		
AFB+	127	36
AFB−	76	26
Sensitivity to drug		
Streptomycin		
Sensitive	171	66
Resistant	32	6
Isoniazid		
Sensitive	181	66
Resistant	22	6
Rifampicin		
Sensitive	196	68
Resistant	7	4
Ethambutol		
Sensitive	198	71
Resistant	5	1
Belonging to risk groups (the homeless, prisoners, and nursing home residents)	44	11

AFB: Acid-fast bacilli, AFB+: Acid-fast bacilli positive, AFB−: Acid-fast bacilli negative, EP: Extrapulmonary form, P: Pulmonary form

by the H350 (15%), 777737777700771 (10%), 177777677760771 (8%), and H147 family (7%) [Table 5].

In a few of the Mtb1–Mtb2 families, the predominant group of patients was in the 0–40 years' age group (H350, 777737777700771, H147) [Table 6]. The Mtb1–Mtb2 families included a total of 21 patients who were in the so-called risk groups (the homeless, prisoners, and nursing home residents); they contributed to 11 clusters [Table 7]. Two homeless persons were among the following families: H147, H337, T544, and U1928. Two prisoners were in the H350 family and two nursing home residents were in the H22 family [Table 7]. In the remaining five Mtb1–Mtb2 families, there were 2–3 patients who were in various risk groups [Table 7].

Having conducted the standard epidemiological interview, we concluded that, among the patients belonging to the aforementioned risk groups within the Mtb1–Mtb2 families, there might have been close or periodic contacts, and thus the transmission of tuberculosis. Among the other patients within the Mtb1–Mtb2 families, we did not confirm any close (including family) or periodic contacts; these patients did not know each other, but often lived in the same districts of Krakow and were using the same public utility institutions [Figure 1].

Discussion

An important step in the epidemiological research of tuberculosis was the introduction of molecular methods based on polymorphisms in the Mtb genome. The sources of these polymorphisms are the repeated DNA sequences, the genetic changeability, and the number of repeated DNA sequences which is characteristic for a given strain (fingerprint). An important element of typing is the subsequent comparison of strains with identical genetic patterns – they form groups of the so-called clusters. Their source may be the same, and they may be actively transmitted between one another. The genetic comparison of the strains may be valuable for the differentiation of exogenous and endogenous infections in hospitals and of cross infections.[13-16]

An initial analysis may be based on the spoligotyping method – it is a screening method and is quite frequently used in epidemiological research addressing tuberculosis. Spoligotyping has allowed for separating ten main molecular families (T, Haarlem, Beijing, LAM, MANU, X, S, CAS i EAI). Numerous investigations focusing on tuberculosis strains have allowed for determining that the T, H, and LAM families are predominant in Europe.[17]

Table 5: The *Mycobacterium tuberculosis* 1-*Mycobacterium tuberculosis* 2 families among the spoligo families

n	Spoligotype	Quantity of strains	Quantity and number of strains with the identical molecular pattern Mtb1-Mtb2
1	T1 53	57	20 (1187, 1241, 1513, 846, 1, 2392, 35, 4293, 989, 2795, 9, 748, 532, 2704, 2668, 173, 473, 2096, 1968, 1797)
2	H3 50	24	14 (807, 1104, 1266, 1157, 1140, 1323, 2779, 2600, 1128, 1245, 553, 2241, 345, 115)
3	777737777700771	11	10 (1608, 1581, 959, 2757, 1062, 654, 2910, 1300, 293, 541)
4	177777677760771	11	8 (1119, 963, 1322, 978, 760, 924, 554, 167)
5	H1 47	10	7 (1062, 1052, 2474, 2442, 3166, 378, 3231)
6	T2 52	12	6 (904, 893, 1511, 1738, 591, 2493)
7	H2 2	7	6 (3026, 747, 182, 139, 1542, 716)
8	H3 746	6	3 (1267, 944, 971)
9	LAM3 and S/ convergent 4	3	3 (3831, 1857, 2016)
11	T5 44	3	3 (1751, 1826, 1513)
12	Beijing 1	4	2 (3463, 764)
13	H1 382	4	2 (838, 969)
14	U 1928	4	2 (1433, 4782)
15	777777770000000	3	2 (2908, 2558)
16	H4 35	3	2 (482, 111)
17	000002004020771	2	2 (1138, 924)
18	577777777720711	2	2 (3145, 1261)
19	H3 37	2	2 (1195, 228)

Mtb: *Mycobacterium tuberculosis*

In the present study, we have analyzed, via the spoligotyping method, 275 strains from 275 tuberculosis patients residing in Krakow. There were 203 patients constituting spoligo families – which was 74% of all the examined individuals. Males in the age range of 41–60 years who were sputum positive were predominant in this group. The dominant families were T153 (26%), T252 (5%), H350 (12%), and H147 (5%); 5% of the patients were classified as belonging to the spoligo families not recorded in the base (orphans). Similar data were presented in the study conducted by Augustynowicz-Kopeć and Kozińska in the tuberculosis patient population in Poland.[18,19] It is interesting to note that 33 patients have been classified in families not yet recorded in the SpolDB4 base, which perhaps suggests the presence of new strains transmitted from outside or reactivated from a latent infection acquired in the past [Table 3].

In the examined group of 275 patients, we have found four Beijing strains – two of them originated from foreigners from Asian countries. The Beijing strains are considered to be among the most dangerous and the most contagious strains, ones that heal badly, are a large part of the pool of drug-resistant strains, often mutate, and are associated with a dangerous course of the disease and a poor prognosis, resulting in the patient's death. They are typical for the region of China, but are quite quickly spreading all over the world, especially in the former Soviet Union countries. Globally, they constitute approximately 13% of all the strains (approx. 50% in Asia).[20,21] They were discovered for the first time in Poland in 2000 among patients with the multidrug-resistant tuberculosis; the strains were isolated predominantly among foreigners – immigrants from Asian countries and from former Soviet Union countries. Currently, the majority – 61% of the isolated strains – come from Poles and 39% from foreigners.[22]

Table 6: The profile of the patients belonging to the *Mycobacterium tuberculosis* 1-*Mycobacterium tuberculosis* 2 families

Characteristics of patients	Number
Quantity of patients	96
Sex	
Male	78
Female	18
Age	
0-40	33
41-60	46
>60	17
Pulmonary/extrapulmonary form	
P	92
EP	4
AFB	
AFB+	54
AFB−	42
Sensitivity to drug	
Streptomycin	
Sensitive	77
Resistant	19
Isoniazid	
Sensitive	84
Resistant	12
Rifampicin	
Sensitive	92
Resistant	4
Ethambutol	
Sensitive	94
Resistant	2
Belonging to risk groups (the homeless, prisoners, and nursing home residents)	21

AFB: Acid-fast bacilli, AFB+: Acid-fast bacilli positive, AFB−: Acid-fast bacilli negative, EP: Extrapulmonary form, Mtb: *Mycobacterium tuberculosis,* P: Pulmonary form

In order to clarify the genetic relation of the strains undergoing spoligotyping, the strains belonging to the spoligo families were examined using the second method – IS6110Mtb1–Mtb2 CRP. The genetically identical ones as per the second method were the families – clusters Mtb1–Mtb2. The above method has a high differentiating potential – it is thought that the genetically identical strains as detected by the second method may transmit between one another.[12,23,24] In the Mtb1–Mtb2 families, there were 96 individuals, 21 of whom were in the so-called tuberculosis risk groups (prisoners, the homeless, and nursing home residents). Ninety-six patients have created 18 families with an identical genetic pattern – with a high probability, there has been transmission of tuberculosis between them. Among these 18 Mtb1–Mtb2 families, we have found as many as 11 in which there were at least two patients who belonged to the so-called risk groups, which seems to significantly indicate these groups as highly responsible for the transmission of the disease. The homeless are a serious risk group with a possibility of a latent tuberculosis infection and an active form of the disease. In the group of the homeless, Kruczak *et al*. indicated a

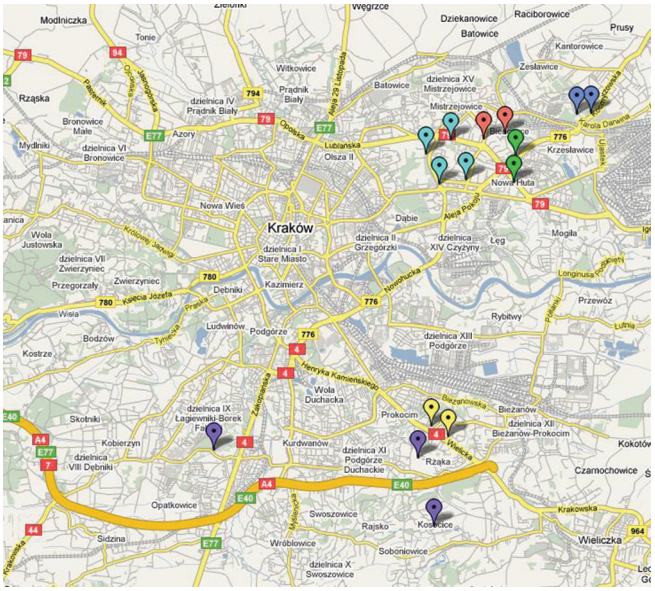

Figure 1: The map of Krakow with the spoligo families marked. H3 50, 177777677760771, H1 382, T1 53, 777737777700771, T 544

very high degree of latent tuberculosis infection (being as high as 36.7%) assessed by the QuantiFERON-TB Gold In-Tube test. In the same group, the tuberculin skin test (TST) results were positive in 55.8% of the participants.[25,26] Within the group of the homeless, alcoholism, malnutrition, nicotinism, and the presence of chronic diseases all affect the decrease of general immunity. Tuberculosis incidence among the homeless is tremendous. In their work published in 2007, Story *et al*.[27] researched the problem of tuberculosis in groups such as the homeless, drug addicts, and prisoners in London and assessed the tuberculosis incidence rate among the homeless being at the level of 788/100,000 as compared to the general incidence rate among the residents of London being at the level of 27/100,000. The investigation indicated that homelessness was more frequently associated with the infectious form of tuberculosis (odds ratio [OR]: 2.2; 95% confidence interval [CI]: 1.6–2.9), multidrug-resistant tuberculosis occurred more frequently (OR: 2.1; 95% CI: 1.1–4.1), and the homeless showed poorer cooperation with the medical personnel (OR: 2.5; 95% CI: 1.6–3.7), as well as were more commonly lost from further follow-up (OR: 3.8; 95% CI: 2.0–7.4). The transmission of tuberculosis among the homeless occurs very often. Curtis *et al*.[28] concluded that eight out of ten cases of tuberculosis among the homeless were genetically identical (fingerprinting and spoligotyping), and that it was highly advisable to apply routine screening in a search of latent mycobacterial infections and to apply tuberculosis chemoprophylactics – which is very difficult. Yun *et al*.[29] assessed the degree of Mtb infection with the assistance of TST, and it was 17.6% among the homeless in Denver in 1996; only 44% of the infected individuals did complete the suggested chemoprophylactic treatment. The homeless often do not return for the second visit for the TST assessment;

Table 7: The profile of the patients investigated in the individual *Mycobacterium tuberculosis* 1-*Mycobacterium tuberculosis* 2 spoligo families

Spoligo families (number of strains)	Sex Male/ female	Age (0-40/ 41-60/>60)	Pulmonary/ extrapulmonary form	AFB+/ AFB−	Sensitivity to drugs				Belonging to risk groups (the homeless, prisoners, nursing home residents)
					Streptomycin Sensitive/ resistant	Isoniazid Sensitive/ resistant	Rifampicin Sensitive/ resistant	Ethambutol Sensitive/ resistant	
T1 53 (20)	15/5	7/8/5	19/1	10/10	20/0	19/1	19/1	20/0	2
H3 50 (14)	8/6	7/5/2	14/0	4/10	14/0	14/0	14/0	14/0	2
777737777700771 (10)	9/1	6/3/1	10/0	7/3	10/0	10/0	10/0	10/0	2
177777677760771 (8)	7/1	4/4/0	8/0	5/3	2/6	8/0	8/0	8/0	2
H1 47 (7)	5/2	3/2/2	6/1	4/3	7/0	7/0	7/0	7/0	2
H2 2 (6)	6/0	0/4/2	6/0	6/0	0/6	2/4	6/0	6/0	3
T2 52 (6)	6/0	2/4/0	6/0	3/3	6/0	6/0	6/0	6/0	0
LAM3 and S/ convergent 4 (3)	2/1	1/1/1	3/0	3/0	3/0	3/0	3/0	3/0	1
T5 44 (3)	3/0	1/2/0	3/0	2/1	3/0	3/0	3/0	3/0	2
H3 746 (3)	3/0	1/2/0	3/0	3/0	0/3	0/3	2/1	3/0	0
000002004020771 (2)	1/1	0/2/0	2/0	2/0	0/2	0/2	2/0	2/0	0
577777777720711 (2)	2/0	0/2/0	2/0	1/1	2/0	2/0	2/0	2/0	0
777777770000000 (2)	2/0	0/0/2	1/1	1/1	1/1	2/0	2/0	2/0	0
Beijing 1 (2)	2/0	1/1/0	1/1	0/2	2/0	2/0	2/0	2/0	0
H1 382 (2)	2/0	0/2/0	2/0	1/1	2/0	1/1	1/1	1/1	0
H3 37 (2)	2/0	0/1/1	2/0	1/1	2/0	2/0	2/0	2/0	2
H4 35 (2)	1/1	0/1/1	2/0	0/2	2/0	2/0	2/0	2/0	1
U 1928 (2)	2/0	0/2/0	2/0	1/1	1/1	1/1	1/1	1/1	2

AFB+: Acid-fast bacilli positive, AFB−: Acid-fast bacilli negative, Mtb: *Mycobacterium tuberculosis*

therefore, it is justified to use the interferon-gamma release assay (IGRA) as the screening method because it requires only one visit from a patient; unfortunately, the procedure is quite expensive. It is worthwhile to emphasize that a positive predictive value of IGRA and TST in the assessment of the incidence of the active form of the disease among individuals with a latent tuberculosis infection is low.[30] In Poland, homeless people are one of the risk groups of tuberculosis.[31]

Another risk group endangered with developing tuberculosis constitutes nursing home residents and staff. In this group, more often than in the case of healthy people, there are numerous factors leading to a mycobacterial infection, such as the presence of chronic diseases affecting the immunological status of the organism, the concentration of numerous individuals in a small area, as well as the possibility of more frequent contacts between healthy residents and those who are undiagnosed which may infect others. According to the European Consensus from 2010 regarding the screening research of contacts in countries with a low tuberculosis incidence, one should conduct studies addressing the contacts in nursing homes for the elderly, but the subsequent chemoprophylactics is not routinely advisable due to the risk of a higher number of undesired effects of the drugs, which is increasing with age.[32] In this group, it is recommended to pay special attention to the occurrence of symptoms of active tuberculosis with the antiretrograde performance of prophylactic chest X-ray and the propagation of extensive education among the staff that would allow them for early recognition of the symptoms of tuberculosis.[32] In our investigation, we found one Mtb1–Mtb2 (H22) family in which there were two nursing home residents.

Conventionally, prisoners constitute a risk group for tuberculosis. Confinement in a small area and being plagued by numerous factors promoting the conversion of the latent to the active form of the disease (malnutrition, alcoholism, drug addiction, and HIV infection) are the reasons behind such a high prevalence of the disease in this group of patients.[3,10] In our research, two prisoners were found within the same H350 family.

In the spoligo and Mtb1–Mtb2 families, we did not find either related patients being the so-called close contacts or individuals being the so-called periodic contacts as per the World Health Organization definition.[32] The standard epidemiological interview conducted with the patients belonging to the Mtb1–Mtb2 families did not indicate the existence of any close or periodic contacts between them – apart from the patients belonging to the specified risk groups (the homeless, prisoners, and nursing home residents). In the literature, a known phenomenon of the transmission of tuberculosis among closely related individuals and those who are frequently or even periodically in contact has been reported. Kozińska *et al*. indicated that patients who were closely related and suffered from tuberculosis and who belonged to the same

molecular groups constituted 89% of the entire investigated material. Such individuals are at risk of getting infected with mycobacteria and develop active disease – they are among the group of patients with a high infection priority.[33,34] However, in our investigation, the vast majority of the patients belonging to the Mtb1–Mtb2 molecular families were individuals who – according to the WHO definition – constituted the so-called third circle of contact, i.e., maintaining occasional contacts. Among these patients were those who could have used the same public utility institutions such as shops, trams, churches, and health centers. An individual being the so-called infection source in such a group is difficult to capture.[32] Analyzing the places of residence of our patients, we were able to identify patients from a few Mtb1–Mtb2 molecular families living within the close vicinity of each other and thus characterized with a high probability of having used the same institutions. Daley[35] formulated similar conclusions in his investigations on the contacts of patients suffering from tuberculosis. He discovered that the standard epidemiological interview covered the results of genetic typing to a small degree only, applying solely to approximately 30% of the individuals among whom it was possible to prove tuberculosis transmission through the epidemiologic interview (initial and deepened after molecular research). The remaining 62% involved the transmission of tuberculosis among the patients who were most probably in occasional contact with each other. Nevertheless, molecular research may be a valuable tool in the research on the circumstances, risk places, and groups in which the disease transmission may occur. Epidemiological research based on epidemiological interviews and molecular research may assist in the determination of suspected and unsuspected transmission of tuberculosis; specify the proportion of cases resulting from fresh transmission, as well as from reactivation of a latent tuberculosis infection; and determine the disease transmission source or groups. 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. The absence of epidemiological data to confirm a clonal relationship among the isolates was an important limitation of this study.[36] The second – in fact, the verification of the risk groups by using MIRU-VNTR method and/or IS6110-RFLP – would make the study stronger, but the calculated discriminatory power of Mtb1/Mtb2-PCR method seems to be high.[37]

Hence, we may conclude that standard epidemiological investigation and molecular methods maybe very helpful tool in tuberculosis transmission taken together. In addition to the immediate treatment of active tuberculosis, they may be an indispensable element of combating the disease.

Financial support and sponsorship

This preventive program was funded by the Health Department, Krakow City Hall.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. Global Tuberculosis Report 2017. Geneva, Switzerland: World Health Organization; 2017. p. 1-147.
2. Korzeniewska-Koseła M, Grzelewska-Rzymowska I, Kruczak K, Augustynowicz-Kopec E, Zwolska Z. Gruźlica i mykobakteriozy. In: Gajewski P, editor. Interna Szczeklika 2018. Kraków: Medycyna Praktyczna; 2018. p. 783-99.
3. Korzeniewska-Koseła M. Tuberculosis in Poland 2017. Report of IGiChP. Warszawa; 2017. p. 1-112.
4. Moström P, Gordon M, Sola C, Ridell M, Rastogi N. Methods used in the molecular epidemiology of tuberculosis. Clin Microbiol Infect 2002;8:694-704.
5. Augustynowicz-Kopeć E, Jagielski T, Kozińska M, Zabost A, Zwolska Z. The significance of spoligotyping method in epidemiological investigations of tuberculosis. Pneumonol Alergol Pol 2007;75:22-31.
6. Krawczyk M, Brzostek A, Gorna A, Knapska K, Ziolkiewicz M, Wojtasik A, *et al.* Epidemiological analysis of *Mycobacterium tuberculosis* strains isolated in Lodz, Poland. Int J Tuberc Lung Dis 2011;15:1252-8, i.
7. Jagielski T, Klatt M, Zwolska Z, Augustynowicz-Kopeć E. Spoligotype-defined population structure of drug-resistant *Mycobacterium tuberculosis* isolates in Eastern Poland. Postępy Nauk Medycznych 2015;4:235-41.
8. Guernier V, Sola C, Brudey K, Guégan JF, Rastogi N. Use of cluster-graphs from spoligotyping data to study genotype similarities and comparison of three indices to quantify recent tuberculosis transmission among culture positive cases in French Guiana during an eight year period. BMC Infect Dis 2008;8:46.
9. Jagielski T, Augustynowicz-Kopeć E, Zwolska Z. Genotyping of *Mycobacterium tuberculosis*. An overview of the major research techniques (Part I). Pol Merkur Lekarski 2010;29:206-11.
10. Brzezińska S, Zabost A, Kozińska M, Janicka-Sobierajska G, Zwolska Z, Augustynowicz-Kopeć E, *et al.* Molecular analysis of strains from tuberculosis patients in Polish prisons in 2004-2008. Initial analysis of the project. Pneumonol Alergol Pol 2012;80:209-13.
11. Brzostek A, Dziadek J. Molecular genotyping methods in epidemiological investigations of TB infections. Pneumonol Alergol Pol 2012;80:193-7.
12. Kotłowski R, Shamputa IC, El Aila NA, Sajduda A, Rigouts L, van Deun A, *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;42:372-7.
13. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, *et al.* Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997;35:907-14.
14. Barnes PF, Cave MD. Molecular epidemiology of tuberculosis. N Engl J Med 2003;349:1149-56.
15. Gori A, Esposti AD, Bandera A, Mezzetti M, Sola C, Marchetti G, *et al.* Comparison between spoligotyping and IS6110 restriction fragment length polymorphisms in molecular genotyping analysis of *Mycobacterium tuberculosis* strains. Mol Cell Probes 2005;19:236-44.
16. Van Soolingen D. Molecular epidemiology of tuberculosis and other mycobacterial infections: Main methodologies and achievements. J Intern Med 2001;249:1-26.
17. Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, Al-Hajoj SA, *et al. Mycobacterium tuberculosis* complex genetic diversity: Mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol 2006;6:23.
18. Augustynowicz-Kopeć E. Drug resistant tuberculosis in Poland. Epidemiological, Microbiological and Genetic Analysis. Warszawa: Warszawski Uniwersytet Medyczny; 2007. p. 1-230.
19. Kozińska M, Zientek J, Augustynowicz-Kopeć E, Zwolska Z, Kozielski J. Transmission of tuberculosis among people living in the border areas of Poland, the Czech republic, and Slovakia. Pol Arch Med Wewn 2016;126:32-40.
20. Park YK, Shin S, Ryu S, Cho SN, Koh WJ, Kwon OJ, *et al.* Comparison of drug resistance genotypes between Beijing and non-Beijing family strains of *Mycobacterium tuberculosis* in Korea. J Microbiol Methods 2005;63:165-72.

21. Lasunskaia E, Ribeiro SC, Manicheva O, Gomes LL, Suffys PN, Mokrousov I, *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;12:467-75.
22. 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.
23. Sajduda A, Dziadek J, Kotłowski R, Portaels F. Evaluation of multiple genetic markers for typing drug-resistant *Mycobacterium tuberculosis* strains from Poland. Diagn Microbiol Infect Dis 2006;55:59-64.
24. Jagielski T, Augustynowicz-Kopeć E, Pawlik K, Dziadek J, Zwolska Z, Bielecki J, *et al.* A two-step strategy for molecular typing of multidrug-resistant *Mycobacterium tuberculosis* clinical isolates from Poland. Pol J Microbiol 2011;60:233-41.
25. Kruczak K. The Assessment of the Degree of Mycobacteria Infection with the Assistance of the Quantiferon-TbGold In Tube test in Risk Groups of Developing Tuberculosis and in Healthy Persons. Doctoral thesis. Kraków: Jagiellonian University Medical College; 2011.
26. Kruczak K, Mastalerz L, Sładek K. Interferon-gamma release assays and tuberculin skin testing for diagnosing latent *Mycobacterium tuberculosis* infection in at-risk groups in Poland. Int J Mycobacteriol 2016;5:27-33.
27. Story A, Murad S, Roberts W, Verheyen M, Hayward AC; London Tuberculosis Nurses Network. Tuberculosis in London: The importance of homelessness, problem drug use and prison. Thorax 2007;62:667-71.
28. Curtis AB, Ridzon R, Novick LF, Driscoll J, Blair D, Oxtoby M, *et al.* Analysis of *Mycobacterium tuberculosis* transmission patters in a homeless shelter outbreak. Int J Tuberc Lung Dis 2000;4:308-13.
29. Yun LW, Reves RR, Reichler MR, Bur S, Thompson V, Mangura B, *et al.* Outcomes of contact investigation among homeless persons with infectious tuberculosis. Int J Tuberc Lung Dis 2003;7:S405-11.
30. Kruczak K, Duplaga M, Sanak M, Cmiel A, Mastalerz L, Sladek K, *et al.* Comparison of IGRA tests and TST in the diagnosis of latent tuberculosis infection and predicting tuberculosis in risk groups in Krakow, Poland. Scand J Infect Dis 2014;46:649-55.
31. Korzeniewska-Koseła M, Kuś J, Lewandowska K, Siemion-Szcześniak I. Tuberculosis in homeless persons in Poland. Przegl Epidemiol 2015;69:445-51, 575-80.
32. Erkens CG, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, Haas W, *et al.* Tuberculosis contact investigation in low prevalence countries: A European consensus. Eur Respir J 2010;36:925-49.
33. Kozińska M, Brzostek A, Krawiecka D, Rybczyńska M, Zwolska Z, Augustynowicz-Kopeć E, *et al.* MDR, pre-XDR and XDR drug-resistant tuberculosis in Poland in 2000-2009. Pneumonol Alergol Pol 2011;79:278-87.
34. Kozińska M. Molecular Epidemiological Investigation among People Closely Related and Evaluation of Usefulness of Typing Methods. Doctoral Thesis. Instytut Gruźlicy i Chorób Płuc, Warszawa, Poland; 2011.
35. Daley CL. Tuberculosis contact investigations: Please don't fail me now. Am J Respir Crit Care Med 2004;169:779-81.
36. Braden CR, Templeton GL, Cave MD, Valway S, Onorato IM, Castro KG, *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;175:1446-52.
37. Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Żaczek A, *et al.* Methodological and clinical aspects of the molecular epidemiology of *Mycobacterium tuberculosis* and other mycobacteria. Clin Microbiol Rev 2016;29:239-90.