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MDR, pre-XDR and XDR drug-resistant tuberculosis in Poland in 2000–2009

Gruźlica lekooporna typu MDR, pre-XDR i XDR w Polsce w latach 2000–2009

Work co-financed from a grant No. R1302103

Abstract

Introduction: Tuberculosis (TB) is a curable disease and its spread can be prevented by using appropriate diagnostics methods and effective treatment. The obstacle to the rapid eradication of the disease from a population may be strainsresistant to essential and most effective antibiotics. In many places in the world MDR, pre-XDR and XDR-TB was reported. These forms of TB do not respond to the standard six-month treatment with first-line anti-TB drugs and the therapy should be conducted two years or more with drugs that are less potent, more toxic and much more expensive.

Material and methods: This study included MDR-TB strains isolated from 297 patients in 2000–2009. To determine the XDR-TB population structure, the 19 isolates were genotyped by spoligotyping and MIRU-VNTR (mycobacterial interspersed repetitive units-variable number of tandem repeats) method.

Results: Among 297 MDR-TB cases, 36 (12.1%) were pre-extensively drug-resistant (pre-XDR), 19 (6.4%) were XDR and 1 (0.3%) was pre-totally drug-resistant (pre-TDR). Four of the 19 XDR isolates exhibit a unique spoligopattern, while the rest 15 belonged to one of 5 clusters. The MIRU-VNTR analysis reduced the number of clustered isolates to 11.

Conclusions: The study documented the emergence of pre-extensively and extensively drug-resistant tuberculosis in Poland among patients with multidrug-resistant TB. Genotyping methods showed clonal similarity among XDR strains and may suggest the possible transmission among patients with newly diagnosed and with recurrent TB.

Key words: MDR-TB, pre-XDR-TB, XDR-TB, spoligotyping, MIRU-VNTR

Pneumonol. Alergol. Pol. 2011; 79, 4: 278–287

Introduction

Results of epidemiological studies showed that the risk factors for tuberculosis infection or TB development afterwards include socioeconomic factors, particularly poverty and poverty-associated housing conditions and malnutrition, homelessness, addictions (alcohol, tobacco, illicit drugs), as well as biological factors, such as elderly age, diseases resulting in a weaker immune response, or immunosuppressive treatment. The risk of tuberculosis is particularly high in HIV-positive patients [1, 2].

Today, despite the availability of the state-of-the-art prevention methods that include both diagnostics and treatment, the drug resistance of the bacilli poses the greatest threat to programs aimed at fighting tuberculosis. It is increasingly common

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The article was submitted to edition on 16 November 2010

ISSN 0867–7077

Table 1. Definitions of drug-resistant tuberculosis

Mycobacterium tuberculosis *drug resistance*	*Type of resistance*
INH + RMP	**MDR**
INH + RMP + SM	
INH + RMP + EMB	
INH + RMP + SM + EMB	
MDR + fluoroquinolone + one of the injectable drugs (amikacin or kanamycin or kapreomycin)	**XDR**
MDR + fluoroquinolone or MDR + one of the injectable drugs (amikacin or kanamycin or kapreomycin)	**pre-XDR**
INH + RMP + SM + EMB + fluoroquinolone + aminoglycoside + polypeptide + tioamide + cycloserine + paraaminosalicylic acid)	**TDR**

Table 2. Number of strains isolated from patients covered by the three programs of the WHO Drug Resistance Surveillence

WHO program	Number of strains	Number of MDR strains (%)
2000	3705	88 (2.4)
2004	3238	51 (1.6)
2008	4380	97 (2.2)
Total	11 323	236 (2.1)

for multidrug-resistant (MDR) strains to acquire additional resistance and reach the pre-extensively drug-resistant (pre-XDR) or extensively drug-resistant (XDR) status [3, 4]. The definition of multidrug-resistant tuberculosis is presented in Table 1.

Tuberculosis caused by multidrug-resistant bacilli should be suspected in every case of a standard treatment failure [5, 6]. Multidrug resistance is caused by monotherapy, e.g. as a result of administration of a single drug upon failure of previous treatment and by irregular periodic administration of antituberculosis drugs, leading to excessively low levels of individual drugs in patient's tissues. Suboptimal concentrations of antituberculosis drugs prevent their bactericidal effect against populations of bacilli in pathogenic lesions, which favors proliferation of mutated bacilli resistant to these drugs. Currently, studies are under way to verify the hypothesis whether particular molecular patterns of tuberculosis bacilli predispose these bacteria to a faster acquisition of drug resistance-associated mutations.

Factors contributing to worldwide spread of multidrug-resistant tuberculosis include migrations, mostly from the developing countries, and transmission within the so-called closed populations, including prisoners, the homeless, social welfare inmates or HIV-positive patients [7–12].

A World Health Organization (WHO) report estimates that there were nearly 400 000 new cases of multidrug-resistant tuberculosis (MDR-TB), which accounted for approximately 3.6% of all tuberculosis patients. Among the MDR-TB cases, there were 5.4% cases of extensively drug-resistant tuberculosis (XDR-TB), and the presence of strains with XDR-type resistance was observed in 58 countries worldwide by 2009.

The highest percentage of MDR-TB cases was observed in non-industrialized countries (85% of MDR-TB); nearly 50% of these cases were observed in China and India. Among MDR-type strains, 7.2% of XDR strains were identified in China in 2007, 12.5% and 14.8% of XDR strains were identified in 2008 in Estonia and Japan, respectively, and 21% of XDR strains were identified in 2009 in Tajikistan [13]. In Poland, the first case of XDR-TB was observed in 2000 [3, 14].

The aim of this work was to analyze the incidence of pre-XDR and XDR tuberculosis in Poland in years 2000–2009 and to find an answer to the question whether XDR strains are transmitted between patients in Poland.

Material and methods

The studies were of both retrospective and prospective character. The programs were carried out in line with the WHO protocol and included more than 10 000 tuberculosis patients examined in years 2000–2008 (Table 2). Besides the strains listed in Table 2, the analysis included bacilli collected from 51 MDR-TB patients and received from

regional laboratories. The overall number of MDR strains subject to analysis was 297.

In years 2000–2009, among 297 patients with microbiologically-confirmed MDR-TB, 19 patients (6.4%) were identified as excreting bacilli of XDR-type resistance and 36 (12.1%) were identified as excreting bacilli of pre-XDR-type resistance.

Of the 55 examined patients, 9 were the residents of the Mazowieckie region, 8 were the residents of the Lubelskie, 8 of the Kujawsko-Pomorskie and 8 of the Łódzkie regions, 6 were the residents of the Śląskie region, 3 of the Dolnośląskie region, 4 were the residents of the Podlaskie region, 2 were the residents of: the Podkarpackie, the Wielkopolskie, the Lubuskie, the Warmińsko-Mazurskie and the Małopolskie regions, and 1 was the resident of the Świętokrzyskie region.

The study population included 10 women at the age of 23–61 and 45 men at the age of 33–75 years, 52 Poles and 3 foreigners. There were 19 newly diagnosed and 36 previously treated patients. All strains belonged to the *Mycobacterium tuberculosis* species and were cultured according to standard methods. Species identification was performed by high performance liquid chromatography (HPLC) and molecular methods (spoligotyping). Drug resistance of strains was determined [15].

There were 19 XDR-TB strains subjected to spoligotyping and MIRU-VNTR (mycobacterial interspersed repetitive units variable number tandem repeat). The spoligotyping method was described in earlier articles, while MIRU-VNTR was used for the first time in Poland as a part of this study [16, 17].

MIRU-VNTR

MIRU sequences were described for the first time in 1997, and in 2001 they were proposed to be used for tuberculosis typing [18, 19].

MIRU-VNTR typing is based on identification of 41 highly polymorphic microsatellite sequences in the genome of *M. tuberculosis*. Each of the sequences may be present in several or a dozen or so repetitions in the genomes of various *M. tuberculosis* strains, giving a total number of over 16 000 000 potential combinations. This study used the analysis of 15 most polymorphic *loci* of the length of 52–77 nucleotides. Fifteen pairs of primers complementary to regions neighboring the MIRU sequences were used in 15 amplification reactions. In order to identify the number of MIRU-sequence repetitions in individual *loci,* the polymerase chain reaction (PCR) products were separated on 2% agarose gels against the reference compounds. The obtained results are presented as numerical patterns (codes) representing the numbers of repetitions of successive MIRU sequences [20].

The reference *M. tuberculosis* $H_{37}Rv$ and *M. bovis* BCG strains originated from own collection of the Department of Microbiology of the Institute of Tuberculosis and Lung Diseases in Warsaw.

Results

Among 297 MDR strains, 36 were identified as pre-XDR (12.1%) and 19 were identified as XDR (6.4%). Table 3 presents the drug-resistance patterns within the analyzed strain pool.

The resistance pattern dominant among the pre-XDR and XDR strains included the resistance to 4 primary drugs: streptomycin (SM), isoniazid (INH), rifampicin (RMP) and ethambutol (EMB) — observed in 14 (38.9%) and 15 (78.9%) strains, respectively.

The analysis of the resistance to additional drugs showed that the largest group within the studied pool of XDR strains consisted of strains resistant to all 3 additional drugs, i.e. ofloxacin (OFL),

Table 3. Analysis of patterns of drug resistance XDR and pre-XDR strains isolated from patients in Poland in 2000–2009

Additional drug resistance		Primary drug resistance/Number of strains (%)				Total	
		S + I + R + E	S + I + R	I + R + E	I + R		
pre-XDR	O	11 (30.5)	5 (13.8)	2 (5.6)	5 (13.8)	23 (63.7)	36
	A	1 (2.8)	4 (11.1)	0	3 (8.4)	8 (22.3)	
	K	1 (2.8)	1 (2.8)	0	0	2 (5.6)	
	A + K	1 (2.8)	2 (5.6)	0	0	3 (8.4)	
XDR	O + A	4 (21)	0	1 (5.3)	0	5 (26.3)	19
	O + K	0	0	0	1 (5.3)	1 (5.3)	
	O + A + K	11 (57.8)	2 (10.6)	0	0	13 (68.4)	
Total		**29**	**14**	**3**	**9**	**55**	

R — drug-resistant strain
S — drug-sensitive strain

Table 4. Analysis of patterns of additional drug resistance among 11 XDR strains resistant to SM, INH, RMP and EMB

No	Date of culture	N*/W**	Additional drugs resistance				
			OFL	AMC	CAP	ETA	CS
1	2000	N	R	R	R	R	S
2	2006	N	R	R	R	S	S
3	2007	W	R	R	R	S	S
4	2008	W	R	R	R	S	S
5	2008	W	R	R	R	S	S
6	2008	W	R	R	R	S	S
7	2008	W	R	R	R	S	S
8	2008	W	R	R	R	S	S
9	2008	N	R	R	R	S	S
10	2009	W	R	R	R	S	S
11	2009	W	R	R	R	S	S

*N — patient newly detected
**W — patient previously treated
S — streptomycine; I — isoniazyde; R — rifampicine; E — etambutol; O — ofloksacyde; A — amikacyde; K — kapreomycyde
Other abbreviations in the text

amicacine (AMC) and capreomycin (CAP) — 13 strains (68.4%). The largest subgroup of pre-XDR strains was comprised of OFL-resistant strains — 23 strains (63.7%).

Among the 19 XDR strains, 11 (58%) were identified as resistant to SM, INH, RMP, EMB, OFL, AMC and CAP; the resistance patterns for cycloserine (CS) and ethionamide (ETA), i.e. drugs to which TDR (totally drug-resistant) strains are also resistant, were analyzed for these strains. The results are presented in Table 4.

One patient with bacilli resistant to ETA was identified in the analyzed pool (Table 4). This was a newly identified patient, registered in the Central Registry for Tuberculosis in 2000. No CS-resistant strains were identified.

In the further part of the study, all XDR strains (19) were submitted to a two-stage molecular analysis. The first stage consisted of spoligotyping. The results are presented in Table 5.

Nine spoligotypes were identified. Two of these spoligotypes were new patterns, hitherto not registered in the *Spoligotyping Database* (SpolDB4); registry entries were found for 7 molecular patterns. The most popular was the T1 1558 family, consisting of 5 strains (26.3%). Four strains belonged to the T4_CEU1 39 family, while 2 strains (10.5%) belonged to each of the Beijing 1, T1 53 and H3 180 families. One strain was identified in each of the molecular families U(likelyH) 46 and T3 442, as well as in two hitherto SPOLDB4-unregistered families. Next, all XDR strains were submitted to MIRU-VNTR molecular analysis. The results are presented in Table 6.

Table 5. Spoligotypes of 19 XDR strains

No	Spoligotype	Number of strains (%)
1	T1 1558	5 (26.3)
2	T4_CEU1 39	4 (21.0)
3	T1 53	2 (10.5)
4	H3 180	2 (10.5)
5	Beijing 1	2 (10.5)
6	U(likelyH) 46	1 (5.3)
7	T3 442	1 (5.3)
8	767777775660771	1 (5.3)
9	777773037760771	1 (5.3)
		19 (100)

Eleven different DNA patterns were identified. The analysis ruled out molecular similarity for 8 XDR strains (42.4%). This group included two Beijing 1 strains — one isolated from a foreigner in the Mazowieckie region, and the other one from a Polish resident of the Lubelskie region, two T1 53 strains isolated from Polish residents of the Podkarpackie region, a U(likelyH) strain, A T3 442 strain, a strain characterized by spoligotype 777773037760771 and one of 4 strains of the T4_CEU1 39 family.

Three MIRU-VNTR patterns were identified for the remaining 11 strains. The largest molecular family was comprised of 6 strains (31.6%) with the numerical code 342635444243125. Of these, 5 (26.3%) belonged to spoligotype T1 1558, and one (5.3%) to a spoligotype hitherto not registered in the SpolDB4 database. Three strains of the

Table 6. MIRU-VNTR patterns of 19 XDR strains

No	MIRU-VNTR pattern	Spoligotypes of strains clustered in MIRU-VNTR analysis	Number of strains (%)
1	342635444243125	T1 1558	5 (26.3)
	767777775660771		1 (5.26)
2	353554423333325	T4_CEU1 39	3 (15.80)
3	364624434744227	H3 180	2 (10.53)
4	333534544441456	Beijing 1	1 (5.3)
5	333753544441457	Beijing 1	1 (5.3)
6	331534342231125	T1 53	1 (5.3)
7	342635442233125	T1 53	1 (5.3)
8	364631434444135	U(likelyH) 46	1 (5.3)
9	334434343241325	T3 442	1 (5.3)
10	334532342242425	777773037760771	1 (5.3)
11	343444333441345	T4_CEU1 39	1 (5.3)
			19 (100)

Table 7. Epidemiological data, molecular patterns and resistance phenotypes of *M. tuberculosis* strains isolated from patients belonging to epidemiological groups

Group of patients	A		B			C					
Patient	1	2	1	2	3	1	2	3	4	5	6
N*/W**	W	N	W	W	N	N	N	W	W	W	W
Province	Dolnośląskie	Mazowieckie	Łódzkie			Kujawsko-pomorskie					
Date of culture	2004	2007	2008			2000	2007	2007	2007	2008	2008
Spoligotype	H3 180		T4_CEU1 39			T1 1558					767777775660771
MIRU-VNTR pattern	364624434744227		353554423333325			342635444243125					
Primary drug resistance	SM^R INH^R RMP^R EMB^R		SM^R INH^R RMP^R EMB^R			SM^R INH^R RMP^R EMB^{R***}					
Additional drug resistance	OFL^R AMC^R CAP^S		OFL^R AMC^R CAP^R			OFL^R AMC^R CAP^{R****}					

*N — patient newly detected
**W — patient previously treated
***C3 strain sensitive to EMB
****C2 strain sensitive to CAP

T4_CEU1 39 family had a pattern of 353554423333325, while two strains of the H3 180 family had a pattern of 364624434744227.

Table 7 lists the molecular patterns and strain-resistance phenotypes in three identified patient groups.

Strains belonging to the H3 180 family were isolated from 2 patients from group A. The patients were the residents of the Mazowieckie and Dolnośląskie regions. One of these patients was previously treated for tuberculosis in 2004, while the other one, newly identified, fell ill for the first time in 2007. Strains isolated from these patients had identical molecular profiles and resistance patterns.

Group B included 3 residents of the Łódzkie region; of these, 2 patients were previously treated for tuberculosis and 1 patient was newly identified. In 2008, bacilli with the same molecular pattern and drug-resistance phenotype were isolated from each of these patients. Group C consisted of 6 patients from the Kujawsko-Pomorskie region. The earliest registration dated back to 2000; 3 cases were registered in 2007 and 2 cases were registered in 2008. Three patients were registered as newly identified cases, and three patients had been treated previously. Bacilli isolated from these patients had identical DNA profiles and resistance

patterns, except for the EMB-resistant strain C3 and the CAP-resistant strain C2.

Discussion

The problem of drug-resistant tuberculosis affects the countries, where the elimination of the disease from the society is impossible due to e.g. difficult socioeconomic conditions, inappropriate diagnostics of patients, improper treatment regimens and coincidence with HIV infections.

Early detection and diagnosis in patients with this form of tuberculosis facilitates introduction of an appropriate drug regimen and prevents transmission of drug-resistant strains into the environment [21–24].

The analysis of drug resistance of MDR strains isolated in Poland in years 2000–2009 allowed to assess the epidemiological situation of patients with XDR and pre-XDR tuberculosis. Nineteen XDR cases and 36 pre-XDR cases were identified in the group of 297 MDR-TB patients. The first case of XDR-TB was registered in 2000; as shown by this analysis, the number of newly identified and previously treated patients with this type of TB in Poland has increased each year [25]. Two XDR strains were identified in 2004; one strain was identified in each of the years 2005 and 2006; 4 strains were identified in 2007, 8 strains were identified in 2008 and another 2 strains were identified in 2009.

Resistance to 4 primary drugs was predominant among 19 XDR-TB patients registered in Poland in the years 2000–2009 — 15 patients (78.9%) were identified. Bacilli that were resistant to both OFL and CAP were isolated form 13 patients (68.4%).

The analysis of the resistance of the XDR strains to additional drugs showed that no TDR-TB patient has been registered in Poland to date; however, one XDR strain additionally resistant to ETA was identified among 11 XDR strains resistant to 4 primary drugs that determine total drug resistance, i.e. SM, INH, RMP and EMB. Therefore, the strain may be described as pre-TDR. The identified strain was sensitive to cycloserine which, along with ETA and para-aminosalicylic acid (PAS), determines the total drug resistance. To date, no case of resistance to CS was registered in Poland, and PAS is not used in resistance tests.

No cross-resistance between RMP and rifabutine was detected among the 19 strains tested. Ten strains (53%) were resistant to both RMP and rifabutine, while 9 strains (47%) were sensitive to rifabutine but showed no resistance to RMP. These data are in line with the reports from the studies conducted by O'Brien, as well as with Polish studies conducted by Augustynowicz-Kopeć [25–27].

Analysis of the resistance patterns showed in case of 2 patients, 2 strains of bacilli with different resistance patterns were cultured from isolates collected at different time points. The first case was a case of a 40-year old woman, from whom a pre-XDR strain was isolated in 2004, followed by an XDR strain in 2008, while the second case was that of a 75-year-old man, from whom a pre-XDR strain was isolated in 2008, followed by an XDR strain in 2009. Molecular analysis of the strains showed that bacilli isolated from patients at different time points and having different resistance patters had identical DNA patterns. Therefore, one may conclude that in both cases, the recurrence of the disease was due to the same strain of *M. tuberculosis*, which has changed its drug resistance phenotype during the treatment. The strain isolated from a female patient in 2004 was resistant to INH, RMP, OFL and was a pre-XDR type, while the strain isolated from the same patient in 2008 was characterized by an XDR resistance pattern. The strain isolated from another patient in 2008 was a pre-XDR strain resistant to SM, INH, RMP, AMC and CAP; one year later, the strain was already an XDR strain.

A patient from the Kujawsko-Pomorskie region is an example of exogenous reinfection with tuberculosis. The patient was registered for the first time as a newly identified patient in 2001, presenting with a strain that was sensitive to all drugs. In 2008, treatment was initiated again in this patient. The results of microbial studies showed the same sensitivity as in the case of the 2001 strain. Since the clinical status of the patient did not improve following initiation of the treatment, and microbial analyses, bacterioscopy and inoculates gave positive results, another drug resistance test was performed in April 2008 and identified the XDR-TB strain. The analysis of DNA patterns of strains cultured in January and April 2008 ruled out their possible molecular relationship and showed that an exogenous infection with XDR bacilli had taken place and a new form of tuberculosis had developed. Epidemiological survey showed that at the same time, a patient secreting XDR bacilli with the same resistance phenotype was hospitalized next room. Genomic studies showed that both strains were identical.

Considering the close contact and the genetic similarity of the cultured strains one may suspect that XDR-TB transmission had occurred between both patients.

These two examples illustrate practical application of molecular epidemiological studies. Ge-

notyping of bacillar strains showed that reinfection with tuberculosis is not always due to endogenous reactivation of the disease. A patient who had suffered from tuberculosis in the past may become ill again as a result of infection with another strain of bacilli, i.e. due to exogenous causes [28].

How to define the case for registration purposes in the situation of reinfection with a strain having a new molecular pattern? Is this a newly diagnosed patient or a patient who was previously treated? Taking into account the frequency of registration as a tuberculosis patient, the patient will be considered a previously treated patient. However, taking into account that the disease is due to infection with another strain — in this case, an XDR strain — this is a case of primary drug resistance, and therefore the patient is a newly identified one.

This analysis was used to determine the scale of the spread of drug-resistant strains and pointed out possible transmission of these trains among patients in Poland.

Molecular typing of bacilli is one of the basic tools to identify transmission of tuberculosis within a population. At highest risk of infection are individuals in the vicinity of the patient, primarily individuals living together with the patient or frequently contacting the patient. There are many reports regarding proven transmission of tuberculosis within small societies, such as prisons, social welfare institutions, kindergartens and schools [29–32]. The literature contains reports of transmission between the hospital personnel and the patients, as well as between individuals commuting by public transportation or among homeless individuals [33, 34]. If a bacilli-secreting individual is found within such population, infection spread may occur. Therefore, it is more important to take appropriate measures to identify all patients and to break the transmission chain as soon as possible.

Strain similarity is observed not only among individuals in close contact with one another, but also in patients without proven epidemiological connections [35, 36]. In this case, tracing of the route of transmission from the infection source is very difficult. The only reliable proof of transmission is the result of molecular epidemiological analysis, suggesting identity of DNA patterns of both cultured strains. In order to identify genetic relationships between bacilli, standardized typing methods with high differentiation potential are used [37–40].

This study is an example of such analysis. The molecular epidemiological investigation conducted as part of the study allows to suppose, that there might have been a recent transmission of XDR-TB in the 3 test groups, or that the infection source had been common in individual groups.

In the group of two patients secreting bacilli of H3 180 family, one previously treated patient was identified in 2004, while the newly identified patient was identified in 2007. It was impossible to find out if the patients were in contact with each other and whether any epidemiological connection existed between them. There is also no information regarding a potential common source of the infection. Despite the above, transmission of tuberculosis between these patients may not be ruled out.

The second group of patients from whom bacilli of the same DNA profile were identified consisted of 3 residents of the Łódzkie region, including one XDR-TB patient newly diagnosed in 2008 and two previously treated patients — one diagnosed in 2005, secreting bacilli sensitive to all drugs at that time and of the XDR type in 2008, and the other diagnosed with MDR-TB in 2006 and with XDR-TB in 2008. In 2008, bacilli with the same molecular pattern and drug resistance phenotype were isolated from each of these patients. Medical history revealed that in 2008, patients were hospitalized at the same time in one of hospitals in Łódź, which was probably the place where the XDR bacilli were transmitted. Analysis of drug resistance patterns shows that a possible source of infection was the patient secreting MDR bacilli in 2006, who secreted XDR bacilli in 2008 due to treatment discontinuation.

The third epidemiological group consisted of 6 patients from the Kujawsko-Pomorskie region. The earliest patient was registered in 2000; another 3 were infected for the first time in 2007, and another 2 in 2008. Three patients had been previously treated, while 3 were newly identified. Bacillar strains isolated from these patients had identical DNA profiles.

It was impossible to find out whether the patients had been in contact with one another and whether there were any circumstances that favored transmission of bacilli between these patients. However, just as in the case of the first group, transmission may not be ruled out.

Molecular analysis conducted as part of the study was used to identify potential epidemiological groups among MDR-TB and allowed to assess the usefulness of the methods.

The analysis made use of two genotyping methods — spoligotyping was used in the first stage, followed by the MIRU-VNTR analysis.

Worldwide literature reports suggest that spoligotyping is a screening method that needs to be supplemented by additional typing with a method

allowing to differentiate the DNA profiles of strains having the same spoligotype. Such methods include, among others, restriction fragment length polymorphism (RFLP) analysis and MIRU-VNTR analysis [37, 38].

Numerous studies conducted in the recent years showed that epidemiological analysis of *M. tuberculosis* strains by the MIRU-VNTR method is as efficient as RFLP, and is characterized by a higher differentiation potential in case of strains containing a small number of copies of insertion sequence 6110 (IS6110). This method is much more cost-effective and less work-consuming than RFLP, and can be performed using small amounts of the DNA [39].

Spoligotyping analysis allowed to identify 5 molecular families (spoligotypes) encompassing 15 of 19 XDR strains. On the other hand, MIRU-VNTR analysis reduced the number of strains with similar DNA patterns to 11 and indicated that they belonged to 3 families with characteristic MIRU codes. This result is a proof of the screening character of spoligotyping and points out a need to supplement the molecular investigations with a method characterized by a higher differentiation potential to be used in the analysis of strains having the same spoligotype [37, 38].

Conclusions

1. The analysis of resistance of strains isolated from 297 MDR-TB patients indicated 19 patients (6.4%) with XDR bacilli and 36 patients (12.1%) with pre-XDR bacilli.
2. No patients with TDR resistance pattern bacilli were identified in the study population.
3. The molecular analysis allowed for identification of three potential epidemiological groups among XDR-TB patients. The molecular similarity of cultured strains isolated from patients in individual groups may suggest probable transmission from common source of infection.

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