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Review

Multidrug-resistant tuberculosis in Lithuania – Still a long way ahead

Greta Musteikienė^{a,*}, Skaidrius Miliauskas^a, Raimundas Sakalauskas^a,
Astra Vitkauskienė^b, Marius Žemaitis^a

^aDepartment of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

^bDepartment of Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

ARTICLE INFO

Article history:

Received 30 July 2015

Received in revised form

1 February 2016

Accepted 13 February 2016

Available online 3 March 2016

Keywords:

Tuberculosis

Multidrug-resistant tuberculosis

Extensively drug-resistant
tuberculosis

Anti-tuberculosis drugs

ABSTRACT

Despite the recent advances in the diagnosis of tuberculosis, treatment of the disease, for the most part, remains the same as it was half a century ago. In recent years only two new anti-tuberculosis drugs have been approved by the European Medicines Agency and Food and Drug Administration. Though the prevalence of this disease is slowly decreasing all over Europe, new challenges appear. One of them is multidrug-resistant tuberculosis (MDR-TB). This problem is especially prominent in Lithuania, which is one of the 27 high MDR-TB burden countries in the world and falls behind neighboring countries in terms of the prevalence of the disease. The objective of this paper was to review the situation of tuberculosis and MDR-TB in Lithuania, and current available methods of treatment, control and diagnosis of this disease.

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1. Introduction

Regardless of the presence of a global strategy for tuberculosis (TB) this disease remains one of the leading causes of mortality among treatable infections [1]. Stop TB Partnership aims to

eliminate TB by 2050, but it can be seen that this will be challenging to achieve with this pace of TB prevalence decline [2,3].

Since 2001 TB incidence in the European Region has been dropping at about 4.5% per year [4]. Nevertheless, to reach the milestones, indicated in the TB Regional Action Plan

* Corresponding author at: Department of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Eivenių 2, 50161 Kaunas, Lithuania. Tel.: +370 631 73730.

E-mail addresses: greta.musteikiene@gmail.com (G. Musteikienė), skaidrius.miliauskas@kaunoklinikos.lt (S. Miliauskas), raimundas.sakalauskas@kaunoklinikos.lt (R. Sakalauskas).

Peer review under the responsibility of the Lithuanian University of Health Sciences.



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<http://dx.doi.org/10.1016/j.medici.2016.02.002>

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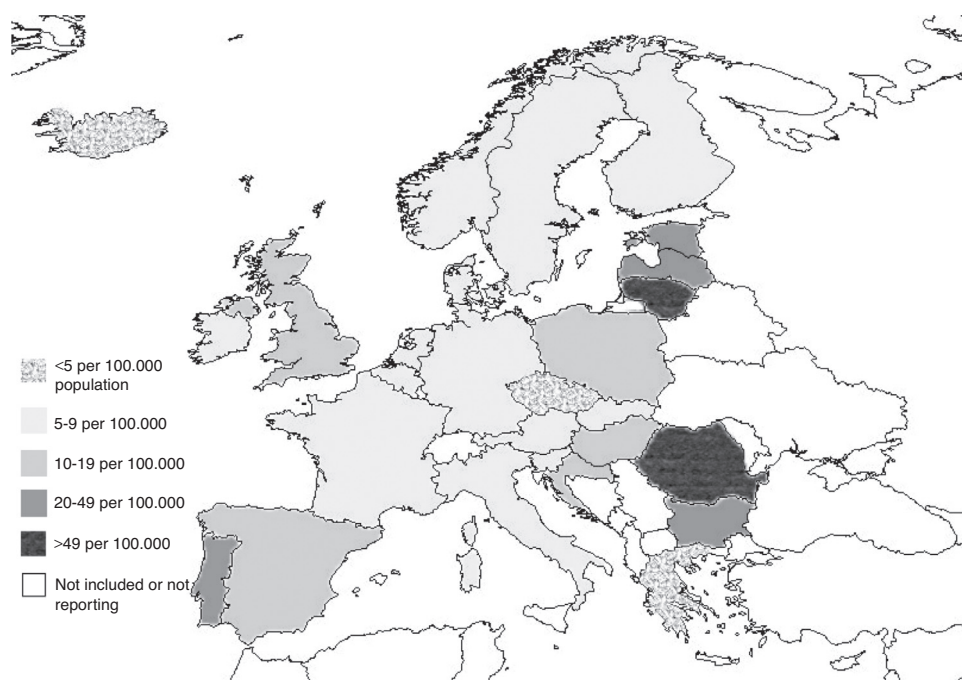


Fig. 1 – Tuberculosis notifications per 100,000 population, by country in European Union, 2013 [13].

2016–2020, we need to ensure the acceleration of this reduction [5].

A total of 18 high-priority countries in European Region (Azerbaijan, Armenia, Bulgaria, Belarus, Estonia, Georgia, Kyrgyzstan, Kazakhstan, Latvia, Lithuania, Moldova, Russia, Romania, Tajikistan, Turkmenistan, Turkey, Uzbekistan, and Ukraine) account for the most of the burden of TB (85% of incidence, 86% of prevalence, 90% of the mortality caused by TB, 90% of TB/human immunodeficiency virus (HIV) co-infections). This means that the key efforts to combat TB need to be focused here [5].

Multidrug-resistant TB (MDR-TB) is also an emerging issue in the European region. The mentioned 18 countries also account for the majority (99.5%) of MDR-TB in the region [5]. MDR-TB is caused by *Mycobacterium tuberculosis* strains resistant to, at minimum, rifampicin (R) and isoniazid (H) [6,7]. The cause of resistance can be multifactorial: improper treatment, transmission of bacteria in public, poor management of drug quality and supply and others [8].

Two paths leading to TB drug resistance are the following: (1) acquired drug resistance is an outcome of inadequate treatment, which allows selection of resistant mutant strains, and (2) primary drug resistance is a consequence of infection with a drug-resistant TB strain that developed resistance, when mutations occurred in genes, encoding drug targets or drug metabolism mechanisms [9].

In 2006, TB with further resistance to second-line drugs was defined as extensively drug resistant TB (XDR-TB). XDR-TB is caused by *M. tuberculosis* resistant to H, R, any fluoroquinolone (FQ), and at least one of three injectable drug: capreomycin (Cm), amikacin (Am) or kanamycin (Km) [7,10]. This makes even fewer options available for the treatment of this disease [8]. By the end of 2013, XDR-TB had been reported in 100 countries (including Lithuania) [5]. Appearance of XDR-TB is a

direct result of mismanagement of MDR-TB cases, and treatment of XDR-TB depends on drugs that are even more toxic and less effective than the ones used for MDR-TB [11].

2. Rates of MDR-TB in Lithuania

The highest rates of TB in the European Union (EU) in 2012 were reported by Romania (85.2 per 100,000 population), Lithuania (59.2), Latvia (48.6), Bulgaria (31.1), Portugal (25.2) and Estonia (21.6) [12]. All three Baltic countries belong to the high-incidence countries for TB (Fig. 1), though the rates of TB vary considerably among them (Fig. 2).

While MDR-TB prevalence among new cases in all EU countries was 4% or lower, in the Baltic States it varied from 8.8% in Latvia, to 17.3% in Estonia, Lithuania being in the middle [5]. The World Health Organization (WHO) placed Lithuania among the high TB burden countries in 2007 and since 2008 among high MDR-TB burden countries [14].

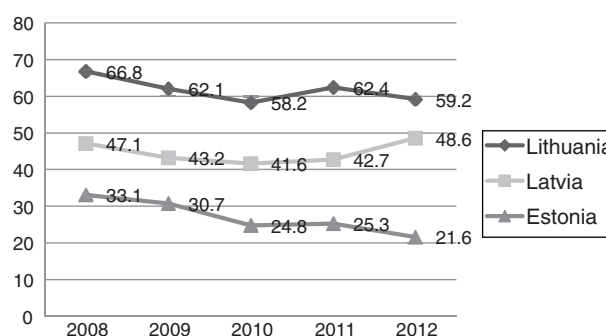


Fig. 2 – Prevalence of tuberculosis in Baltic countries, 2008–2012 (per 100,000 population) [12].

The higher prevalence of TB in Lithuania, compared to western European countries, is frequently associated with Lithuania's history of being in the former Soviet Union [15]. Countries in the Soviet Union were affected not by HIV, but by other amplifiers of TB, like poverty, social dislocation, malnutrition, war, and after the union's collapse – the political and economic difficulties and changes in the health care system. However, the Soviet Union collapsed more than 20 years ago, and the prevalence of TB in Lithuania is still high. The highest reported incidence of TB (85.7 per 100,000) in Lithuania was in 1998–1999 [16]. Currently, it is believed to be influenced by social reasons (poverty, alcohol abuse, etc.), misunderstanding of the disease and reluctance to finish the full treatment course and nationwide organizational problems (anti-TB drugs are not free of charge; patients do not have the funds to reach medical institutions, etc.). One of the biggest issues is that though the directly observed treatment strategy (DOTS) is fully implemented in hospitals, it is not ensured in outpatient settings. This occurs due to lack of funds and organization. For example, of 60 municipalities in Lithuania, 12 do not provide services of pulmonology, and TB patients have to be referred to a neighboring region [17]. This causes further inconvenience and decreases the possibility that the patient even visits a pulmonologist. Furthermore, about 75% of all TB patients in Lithuania are unemployed [18] or do not have a permanent job, many are alcohol abused and regularly violate treatment regimens or do not complete the full course of treatment due to absence of good control of outpatient treatment.

Neighboring Baltic countries seem to be managing TB better (Fig. 2). For example, in 2013 Estonia reached a TB incidence of 22 per 100,000 population [19]. This achievement was supported by a strong political and financial commitment to TB control by the country's government.

Comparing all three Baltic countries, Estonia has the lowest TB rate. This country has devised an ambulatory system of TB control that surpasses the one in Lithuania by providing a better functioning DOTS in ambulatory settings, social support for the TB patients, incorporated methadone therapy for intravenous drug users, antiretroviral therapy for HIV infected, the possibility to treat alcoholism free of charge and a better organized involuntary treatment for patients who deliberately avoid treatment.

In the EU, in 2013 resistance to at least one TB drug was reported in 3891 (10.7%) of the overall TB cases tested, and in

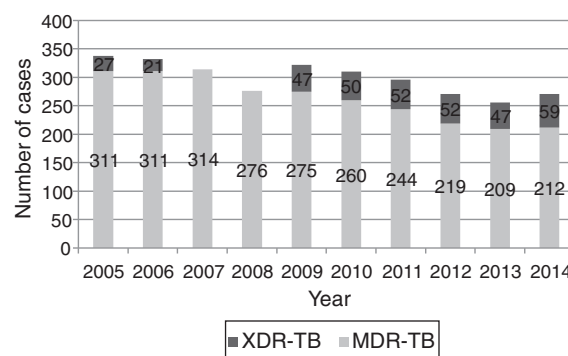


Fig. 3 – Prevalence of MDR/XDR-TB in Lithuania [22]. MDR-TB, multi drug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis (no data for XDR-TB cases in 2007–2008).

Lithuania it was 34.8% [12,20]. In Lithuania these numbers did not change much during recent years: in 2006 resistance to at least one anti-TB drug among the new TB cases was 29.2% [21].

As mentioned, though the prevalence of drugs susceptible TB is decreasing, the prevalence of MDR-TB in Lithuania is quite stable (Figs. 3 and 4). This shows an on-going transmission of primary MDR-TB, and increasing numbers of TB with acquired drug resistance. MDR-TB is registered in all districts of Lithuania (Fig. 5) [22], but the differences among the regions do not appear to be influenced by any known factors. If we would compare the situation among the neighboring countries and would only take the percentage into account, Estonia has a higher percentage of MDR-TB than Lithuania (19% from all new TB cases compared to 14% in Lithuania in 2014) [23,24]; however, HIV-positive patients account for 10% of Estonia's TB cases (compared to 3% in Lithuania) [23,24]. A higher percentage of MDR-TB in Estonia also could be explained by the increase in HIV prevalence, although in Western Europe, a substantial proportion of TB cases occur among immigrants [25]. That is still not the case in the Baltic countries – in Lithuania most of the infected with MDR-TB are Lithuanian born [26].

In 2014 it was estimated that 11% of the new TB cases in Lithuania were MDR-TB [14]. In 2014, Lithuania also reported the highest XDR-TB prevalence of 24.7% among MDR-TB cases

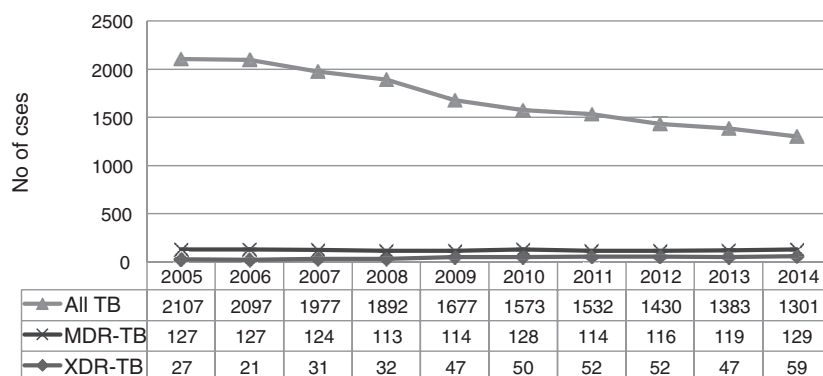


Fig. 4 – New cases of tuberculosis in Lithuania [22]. TB, tuberculosis; MDR-TB, multi drug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

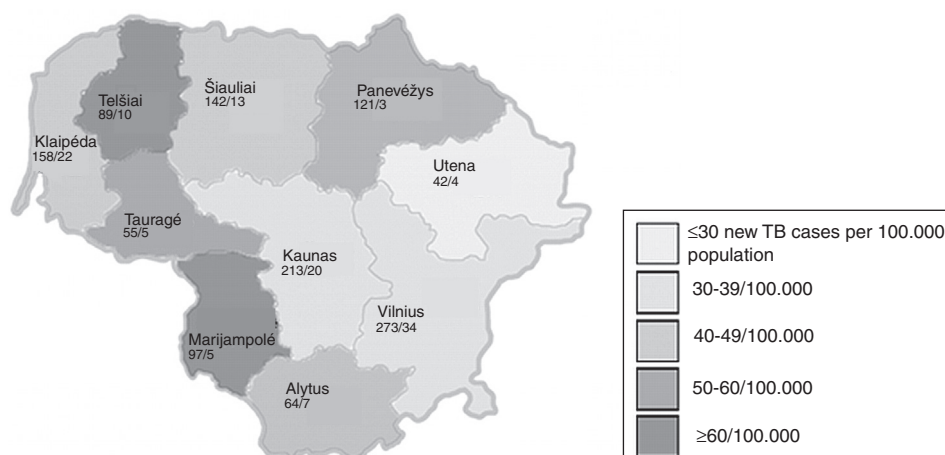


Fig. 5 – New tuberculosis (TB) and multidrug-resistant tuberculosis (MDR-TB) cases registered in the districts of Lithuania in 2014 [22]. Numbers in the map show the incidence of TB/MDR-TB cases per 100,000 population.

in the European region (second highest being Latvia [21.7%] and third, Georgia [19.2%]) [5]. There were 271 patients in Lithuania registered as having MDR-TB, of which 59 had XDR-TB [22]. These trends can be seen in Figs. 3 and 4. The majority of Lithuanian MDR-TB patients are described as unemployed males, with primary or secondary education, aged between 30 and 49 years, living in urban settings and frequently consuming alcohol [26].

In Lithuania, MDR-TB is accompanied by high rates of default (around 30%) and low treatment success (35.1% in 2013) [5], despite a TB control program that has relatively good treatment success among patients with drug-sensitive TB (80.4% among new and 70.8% previously treated, 2013) [22] and low default rates (5.8% among new and 8.3% previously treated, 2013) [22,26] (Table 1). Treatment of MDR-TB seems to be more successful in other Baltic countries (53.7% treatment success in Latvia, 50.0% in Estonia) [5]. Reasons for these differences were already discussed in this article.

Increasing prevalence of MDR-TB overshadows the improvement of situation concerning drug susceptible TB. In 2014, the Ministry of Health of the Republic of Lithuania approved the Action Plan for the Reduction of Health Inequalities in Lithuania for 2014–2023 [17] that sets the following goals for TB in Lithuania:

- To decrease the incidence of TB to 15/100,000;
- To decrease the TB death rate to 2/100,000;
- To reach treatment success in at least 85% of new bacteriologically confirmed TB cases (yearly, from 2018);
- To reduce MDR-TB proportion among all TB cases up to 10%.

However, if we hope to reach these goals, a substantial progress has to be made.

3. Diagnosis of MDR/XDR TB

Drug resistance can occur in pulmonary or extra-pulmonary, new or retreatment cases, smear-negative or positive TB, but previously treated patients are at the highest risk for MDR-TB.

It is considered that in good DOTS programs, failures after first-line retreatment are MDR-TB in 85%–90% of cases [27].

Quick detection of drug resistance permits early effective treatment and has significant impact on TB control. Definitive diagnosis of drug-resistant TB can be confirmed if *M. tuberculosis* is identified and anti-TB drug resistance is determined. It can be achieved by isolating a culture belonging to *M. tuberculosis* complex and by conducting drug susceptibility testing (DST) or performing a WHO-endorsed tests to detect TB DNA or mutations associated with drug resistance [28].

DST methods are divided into conventional (phenotypic) and genotypic. The conventional methods of DST can show that TB bacteria grow on culture media with anti-TB drugs. Genotypic methods are used to detect *M. tuberculosis* mutations associated with resistance.

3.1. Phenotypic methods

Phenotypic (conventional) methods require extended lengths of time (for example solid culture methods may require up to 8 weeks to produce an answer and liquid culture up to 6 weeks for smear-negative TB). During this long testing time patients could be ineffectively treated, drug resistance extension can take place and resistant strains continue to spread. However, culture in liquid media is still the reference method for bacteriological validation of TB [8]. Automated liquid culture methods offer a reduced time to diagnosis (8 days for smear positive; 2–6 weeks for smear negative) and approximately 10% higher yields in comparison with solid culture methods [29], but are prone to contamination, expensive, and require considerable laboratory infrastructure [1]. Liquid culture methods are used more rarely than solid culture methods in Lithuanian laboratories, mostly due to their higher cost. As mentioned, solid culture methods are time consuming; however, they have lower contamination rates [30].

Phenotypic methods permit detection of resistance unrelatedly to the mechanism. That is why currently, phenotypic DST is considered the reference for identifying XDR-TB [8].

As reported by the European Centre for Disease Prevention and Control, in 2013, Lithuania had 6 laboratories performing

Table 1 – Treatment results MDR/XDR-TB [22].

| | 2006 | | 2007 | | 2008 | | 2009 | | 2010 | | 2011 | |
|---|-------------|------------|-------------|------------|------------|------------|------------|------------|-------------|------------|-------------|------------|
| | MDR-TB | XDR-TB | MDR-TB | XDR-TB | MDR-TB | XDR-TB | MDR-TB | XDR-TB | MDR-TB | XDR-TB | MDR-TB | XDR-TB |
| Treatment success | 92 (27.7%) | 2 (9.5%) | 94 (29.9%) | 3 (9.7%) | 81 (29.4%) | 9 (28.1%) | 99 (30.7%) | 6 (12.8%) | 98 (31.6%) | 4 (8.0%) | 116 (39.2%) | 12 (23.1%) |
| Treatment failure | 15 (4.5%) | 3 (14.3%) | 20 (6.4%) | 4 (12.9%) | 31 (11.2%) | 2 (6.3%) | 31 (9.6%) | 3 (6.4%) | 30 (9.7%) | 13 (26.0%) | 24 (8.1%) | 4 (7.7%) |
| Treatment default | 111 (33.4%) | 5 (23.8%) | 88 (28%) | 8 (25.8%) | 83 (30.1%) | 12 (35.7%) | 99 (30.7%) | 20 (42.5%) | 102 (32.9%) | 12 (24.0%) | 87 (29.4%) | 18 (34.6%) |
| Death | 111 (33.4%) | 11 (52.4%) | 111 (35.4%) | 16 (51.6%) | 77 (27.9%) | 9 (28.1%) | 92 (28.6%) | 18 (38.3%) | 75 (24.2%) | 20 (40.0%) | 67 (22.6%) | 16 (30.8%) |
| Lost to follow up (left the country) | 3 (1%) | 0 | 1 (0.3%) | 0 | 4 (1.4%) | 0 | 1 (0.3%) | 0 | 5 (1.6%) | 1 (2.0%) | 2 (0.7%) | 2 (3.8%) |
| Total number of cases | 332 | 21 | 314 | 31 | 276 | 32 | 322 | 47 | 310 | 50 | 296 | 52 |

MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

phenotypic DST for TB with acceptable performance [5]. These laboratories perform the same tests at present.

3.2. Genotypic methods

Genotypic DST detects mutations linked with specific drug resistance. In order to accurately identify resistant phenotypes, mutation locations associated with resistance must be known and included in testing.

Molecular line probe assays (LPAs) and the Xpert MTB/RIF are the only genotypic technologies endorsed by the WHO for detection of R resistance [31,32]. These methods identify mutations in *rpoB* region of *M. tuberculosis* DNA, which account for more than 95% of R resistant strains [8,31,33]. Negative result usually excludes R resistance and no other testing to confirm is required, though rarely, culture-based DST may be used to check for R resistance, resulting from less than 5% of mutations outside the *rpoB* region [8]. In about 90% of TB cases R resistance is related to H resistance, and the percentage is higher among patients who were previously treated [27]. Due to this, detection of R resistance serves as a proxy for MDR-TB [8].

Xpert MTB/RIF [34] is a fully automated genotypic test that uses polymerase chain reaction in sputum specimens to identify DNA of *M. tuberculosis* complex and R resistance associated mutations in less than 2 h. It can be performed with minimal training in locations outside of reference laboratories [1]. Use of this test decreased the mean time to treatment introduction among smear-negative culture-positive TB from 56 to 5 days [35] and it has a strong recommendation from WHO to be used in all patients with suspected MDR-TB and/or co-infected with HIV [34].

Other rapid alternative to phenotypic DST is specialized nucleic acid amplification techniques known as line probe assays (LPAs). They identify *M. tuberculosis* and common mutations causing resistance to R and H, with results available in 1–2 days [8]. LPAs are appropriate to use with smear-positive sputum samples or culture isolates grown by conventional methods. Sensitivity for R resistance is 98%; for H resistance, only 84% [36,37] (due to the presence of resistance mutations outside *inhA* and *katG* genes detected by the assays) [36,38]. One of the newer additions to genotypic testing is GenoType MTBDRsl assay, used to detect resistance to FQ, Km, Cm and E in *M. tuberculosis* strains.

There are 2 laboratories performing LPAs and 8 performing Xpert MTB/RIF assay in Lithuania [5]. Though, genotypic tests are more rapid and could identify MDR-TB cases earlier, due to their higher cost, they are used more rarely than conventional methods.

One of the newest technologies used is whole genome sequencing (WGS) of *M. tuberculosis*. WGS can be used in molecular epidemiology, contact tracing, detection of known drug resistance mutations [39]. This technology is not used routinely, due to high cost, lack of accessible genomic databases and software to process the sequences produced, but it may become available in the future.

3.3. Noncommercial technologies

The WHO also endorsed alternative, simpler and cheaper non-commercial culture and DST technologies, intended to be used

in the settings with limited access to sophisticated laboratory infrastructure [40]. Most advanced technologies are colorimetric redox indicator, microscopic observation of drug susceptibility, thin-layer agar, mycobacteriophage-based and nitrate reductase assays [40]. To our knowledge, these technologies are not used in Lithuania.

4. Treatment strategies for MDR-TB

4.1. Designing and administrating an MDR-TB regimen

Treatment of TB is based on two essential rules: first, treatment has to be protracted to ensure that all bacteria in different phases of growth are eliminated and, secondly, combination of drugs is needed to avoid resistance selection. Effective regimens must have a core of at least 2 very active drugs responsible for eliminating TB bacteria, and 2 or more other drugs which may kill little, but protect the core drugs from acquiring resistance.

The best drugs to treat TB so far are H and R and if it is not possible to use them (like in the cases of MDR/XDR-TB), treatment becomes very complicated. The MDR-TB regimen is made of 2 phases: (1) when an injectable drug is used, and (2) after injectable drugs are stopped. TB programs use standardized and individualized tactics. Preferably, a TB treatment regimen ought to combine the highest available number of bactericidal drugs accompanied with sterilizing drugs [27]. Drugs with bactericidal properties kill many bacteria in a short time; sterilizing drugs kill *M. tuberculosis* in latent phases.

At least 4 drugs should be chosen to design a MDR-TB regimen. It should be constructed using the remaining effective drugs, while following these steps [8]:

- 1) Choose one injectable agent from group 2: Km, Am, or Cm;
- 2) Choose one fluoroquinolone from group 3: ofloxacin (Ofx); moxifloxacin (Mfx); levofloxacin (Lfx); gatifloxacin (Gfx);
- 3) Add up to 2 drugs from group 4: ethionamide (Eto), prothionamide (Pto), cycloserine (Cs), para-aminosalicylic acid (PAS), para-aminosalicylate sodium (PAS-Na), and terizidone (Trd) until there are at minimum 4 second-line drugs that are likely to be effective.
- 4) Add pyrazinamide (Z) or/and ethambutol (E) if effective.
- 5) Add group 5 drugs: delamanid (Dlm), bedaquiline (Bdq), linezolid (Lzd), amoxicillin/clavulanate (Amx/Clv), clofazimine (Cfz), meropenem (Mpm), imipenem/cilastatin (Ipm/Cln), high-dose isoniazid (high-dose H), clarythromycin (Clr), thioacetazone (T) if up to this step, there are less than 4 second-line anti-TB drugs that are likely to be effective.

Only one drug should be chosen from group 2 and one from group 3 because of cross-resistance within the groups.

Duration of the first phase in the MDR-TB treatment of 8 months is suggested [8]. When bacillary load is significantly reduced, fewer drugs are needed and injectable drugs can be stopped. The best indicator for finishing intensive treatment phase is sputum smear conversion [27]. In the case of patients with MDR-TB that has not been previously treated, the total treatment period of 20 months is suggested [8] and in some

programs, at least 12 months after conversion. In Lithuania treatment regimens of MDR-TB also follow these recommendations.

4.2. Classes of anti-tuberculosis drugs and data of resistance

4.2.1. Group 1: First-line anti-tuberculosis drugs

The most powerful first-line drugs must be used if there are clinical history and laboratory evidence suggesting that these drugs are effective [8]. However, in MDR-TB the most effective anti-TB drugs (R and H) cannot be used due to resistance, so treatment duration is prolonged and treatment success is lower.

M. tuberculosis resistance to R in Lithuania in 2012 was 20.8% [12]. Mono-resistance to R was documented in 10 cases (0.7%) [43]. This leads to the conclusion that if resistance to R is detected by genotypic tests, MDR-TB should be strongly suspected.

H develops resistance due to mutations in *katG* and *InhA* genes. *InhA* is a genetic target of Eto and Pto as well, so mutations here results in cross-resistance to Eto/Pto also [45]. In Lithuania, reported resistance of *M. tuberculosis* to H in 2012 was 30.3% [12]. Mono-resistance to H was reported in 56 cases (4.1%) [43]. In other countries, prevalence of H resistance varies from 0% in Malta and Iceland, and 40.8% in Baku city, Azerbaijan [41].

In MDR-TB cases, drugs from group 1, which are sometimes left to use are Z and E. If there are contraindications, Z is usually added to MDR-TB treatment, because in many cases, chronically inflamed lungs in TB patients, produce acidic environment where Z is effective, but the activity of other anti-TB drugs is diminished. DST to Z is also considered not to be reliable, so it is acceptable to use Z, even when DST shows resistance [8].

E has good tolerability, ability to prevent resistance to other drugs and very low initial resistance rate in most countries [41,42]. WHO reported resistance to E of 2.5% amongst new and 10.3% amongst previously treated cases of TB, globally [43]. In Lithuania in 2012 resistance to E was 13% [22]. However, because of difficulties susceptibility testing, E is not considered a key drug in an MDR-TB treatment regimen [8].

One of the two main laboratories in Lithuania performing *M. tuberculosis* DST is located in the Lithuanian University of Health Sciences (LUHS). A total of 52 new and previously treated MDR/XDR-TB cases were diagnosed here in 2012. Of these, 58.82% were found to be resistant to E also and 50% to Z. The percentage of both E and Z resistance in 49 cases of chronic MDR/XDR-TB and patients tested during treatment was even higher – 85.71% (Table 2).

4.2.2. Group 2: Injectable anti-tuberculosis drugs

Group 2 drugs are bactericidal and have strong extracellular activity. All patients should receive an injectable drug in the intensive treatment phase, unless resistance to these drugs is proven or highly suspected [8]. Streptomycin (S) is infrequently used in MDR-TB treatment, even if susceptibility is shown by DST, due to the wide use as a first-line drug and high resistance rates in MDR-TB patients [8]. In Lithuania resistance to S was reported to be 28.5% in all TB cases (2012) [12,20]; it is even

Table 2 – Drug susceptibility testing results from the laboratory of Lithuanian University of Health Sciences in patients with MDR/XDR-TB (2012).

| Resistance to: | Z | E | S | Km | Cm | O | Eto | Cs | PAS |
|--|-------------|-------------|-------------|-------------|----|-------------|-------------|-----------|-----------|
| MDR/XDR-TB (new and relapse cases) | 26 (50%) | 30 (58.82%) | 51 (98.07%) | 16 (31.37%) | 0 | 12 (23.07%) | 12 (23.07%) | 0 | 0 |
| Chronic and tested during treatment MDR/XDR-TB | 42 (85.71%) | 42 (85.71%) | 45 (91.84%) | 34 (69.39%) | 0 | 33 (67.35%) | 27 (55.1%) | 3 (0.61%) | 5 (1.02%) |

Z, pyrazinamide; E, ethambutol; S, streptomycin; Km, kanamycin; Cm, capreomycin; O, ofloxacin; Eto, ethionamide; Cs, cycloserine; PAS, para-aminosalicylic acid; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

higher in MDR-TB cases. For example, in our laboratory (Lithuanian University of Health Sciences Kaunas Clinics Laboratory), all but one patient diagnosed MDR-TB in 2012, was resistant to S. In the EU in 2012, new MDR-TB was resistant to Am in 19.1% of cases; Cm, 18.3%; and Km, 23.5% [44]. In Lithuania the resistance to group 2 drugs in MDR-TB patients was reported to be 42.9% (2012) [45]. The results from the LUHS laboratory are presented in Table 2.

4.2.3. Group 3: Fluoroquinolones

Fluoroquinolones (FQs) are frequently the most effective drugs in a treatment regimen of MDR-TB and were significantly associated with cure of MDR-TB [46–48]. This effect was more pronounced in later-generation FQs (moxifloxacin and levofloxacin) [49]. FQs are also of affordable cost and well tolerated. Use of only one per regimen is reasonable. New MDR-TB cases in the EU were resistant to ofloxacin and moxifloxacin in 11.7% and 8% of cases, respectively (2012) [44]. In the LUHS laboratory, 23.07% of diagnosed MDR-TB cases were resistant to ofloxacin.

4.2.4. Group 4: Oral bacteriostatic second-line anti-tuberculosis drugs

It includes the following drugs: thioamides (Eto and Pto), Cs or its derivative terizidone (Trd), and PAS. These drugs belong to different classes with different genetic targets, so it is rational to choose more than one drug from this group if there is a possibility.

Cs compared with other drugs in group 4 has higher gastric tolerance and lacks cross-resistance to other agents [50], but has psychiatric side effects and a short shelf life. In the LUHS laboratory, only chronic cases of MDR-TB were found to be resistant to Cs (0.61% of all tested MDR-TB cases) in 2012.

PAS is a quite weak drug, is very poorly tolerated and very expensive [27], though no cross-resistance with remaining anti-TB drugs is known [51]. In the LUHS laboratory, only chronic MDR-TB cases were rarely found to be resistant to PAS in 2012 (1.02%) (Table 2). For these reasons PAS is used quite often in Lithuania.

4.2.5. Group 5: Anti-tuberculosis drugs with limited data on efficacy and/or long term safety and new anti-tuberculosis drugs

Though all drugs in group 5 have shown activity against drug-resistant *M. tuberculosis* at least in vitro, the evidence of their safety and efficacy in humans varies. With the exception of Dlm and Bdq, most of these drugs are not registered for treatment of MDR-TB. They can also be expensive and require intravenous administration; however, they remain an option

when acceptable treatment regimens are not possible to design with group 1–4 drugs.

Lzd has been shown to improve the consequences of XDR-TB [52]. It is believed to be one of the most effective drugs of the group 5, however, is expensive and displays a high toxicity profile. Also, it is used in the treatment of other infections, so resistance development is an issue. There is no data on resistance of *M. tuberculosis* to Lzd in Lithuania.

The efficacy of Cfz against TB remains unclear and experience is limited, but it has potential intracellular and extracellular activity [53]. Current availability of Cfz in the market is not assured as it has been restricted for the treatment of leprosy. In some countries where Cfz is available it is included in standard treatment regimens; however it is not registered for use in Lithuania.

β -Lactam antibiotics are not considered very valuable for TB treatment because of the fact that *M. tuberculosis* is resistant to most of these drugs in vitro. Still this resistance may be overcome by inhibition of the β -lactamase or by the use of an antibiotic that is not a substrate for it. One of the examples of this strategy is Amx/Clv that is a combination of a β -lactam and a β -lactamase inhibitor. Though, the use of this drug for the treatment of MDR-TB has been met with skepticism, due to a low cost and high availability this drug is sometimes added to treatment regimens in Lithuania.

Carbapenems can overcome the natural β -lactam resistance of *M. tuberculosis*, but due to difficulty in dosing and a high cost, they are not commonly used. Experience with these drugs is very limited. Mostly it involves isolated XDR-TB patients, but outcomes appear to be rather successful [54,55].

T is one of the oldest drugs in TB treatment, but has always been considered a very weak bacteriostatic drug. It has cross-resistance with Eto [56] and H [57] and is contraindicated for use in HIV-infected patients [58] due to a high risk of Stevens-Johnson syndrome and death. It is believed that this drug should be restricted only to cases with a very extensive drug resistance. To our knowledge, it is not used in Lithuania.

Though Clr is included in anti-TB drug list, its activity against *M. tuberculosis* is unclear. Clr is used only when no other drug is left and the only advantage is a relatively good tolerance and low toxicity profile.

Several new composites for TB treatment are now at different stages of development (Table 3). One of these is Bdq, for which the US Food and Drug Administration gave conditional approval in 2012. Other new anti-TB drug is Dlm, which was approved by the European Medicines Agency in 2013 [8].

Table 3 – Global TB drug pipeline [64].

| Discovery | Preclinical development | Clinical development | | |
|---------------------------------|-------------------------|----------------------|---|--|
| | | Phase I | Phase II | Phase III |
| Cyclopeptides | TBI-166 | TBA-354 | Sutezolid (PNU-100480) | Bedaquiline with optimized background regimen for MDR-TB |
| Diarylquinolines | CPZEN-45 | | SQ109 | Delamanid with optimized background regimen for MDR-TB |
| DprE inhibitors | SQ609 | | Rifapentine for drug-susceptible TB | Rifapentine for latent TB |
| InhA inhibitor | SQ641 | | AZD5847 | Pretomanid–Moxifloxacin–Pyrazinamide regimen |
| Indazoles | DC-159a | | Bedaquiline–Pretomanid–Pyrazinamide regimen | |
| LeuRS inhibitors | PBTZ169 | | | |
| Ureas | Q203 | | | |
| Macrolides | | | | |
| Azaindoles | | | | |
| Mycobacterial gyrase inhibitors | | | | |
| Pyrazinamide analogs | | | | |
| Ruthenium (II) complexes | | | | |
| Spectinamides SPR-113 | | | | |
| Translocase-1 inhibitors | | | | |

Bdq is a diarylquinoline, a bactericidal drug that inhibits adenosine triphosphate synthesis [59]. It is the first anti-TB drug with a totally new mechanism of action in the last 40 years. It can be added to a treatment regimen in adult MDR-TB patients under the following conditions: (1) when an effective treatment regimen containing 4 second-line drugs in addition to Z according to WHO recommendations cannot be designed; (2) when there is documented evidence of resistance to any FQ in addition to MDR-TB [60]. Bdq can cause elongation of the QT interval, which in turn, can lead to a fatal heart rhythm; therefore it cannot be used with Mfx that can also cause prolongation of QT. Though it is a new drug, resistance to it was already documented [61,62].

Dlm is a new anti-TB drug that inhibits the synthesis of mycolic acid. In one study 45.4% of patients receiving Dlm in their treatment regimen had sputum-culture conversion after 2 months, (compared with 29.6% of the ones who received placebo plus the background regimen) [63]. These new drugs are still not widely available in Lithuania. Bdq and Dlm are sometimes used in cases of XDR-TB, but experience with these drugs in Lithuania is limited.

5. Concluding remarks

Lithuania still holds a place among the high MDR-TB and high TB countries. Though prevalence of TB in Lithuania is decreasing, the prevalence of MDR-TB is only rising or, at best, is remaining stable.

Lithuania is facing a number of operational problems in a fight against TB. Current interventions for TB have to be used more efficiently, and could benefit from being supplemented by more new and effective ones. Treatment success rate of drug susceptible TB is satisfactory, but treatment results of MDR-TB are not. MDR-TB is becoming even more challenging

to treat and treatment success remains low. This, for the most part, is influenced by high treatment default rates, and inability to adequately observe and control TB treatment in the ambulatory setting. The mechanisms for effective follow-up of patients to prevent them from defaulting are underdeveloped. Action must be taken to ensure DOTS in the ambulatory setting, if we hope to ensure that the treatment is continued after discharge from hospital.

There is a need for expansion of outpatient TB case management, assistance for primary health care facilities, and social support for TB patients during treatment. While preventing treatment default, treatment success rates may be improved. More intensive efforts should be made to manage drug-susceptible TB in order to prevent appearance of MDR-TB and XDR-TB and to minimize its spread.

So, as mentioned, there are still a lot of problems; however, we hope that while following international recommendations as closely as possible, and handling TB with good operational and clinical case management, all forms of drug-resistant TB may have the possibility to be cured.

Conflict of interest

The authors state no conflicts of interest.

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