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TRANSMISSION OF *MYCOBACTERIUM TUBERCULOSIS* AND THE CAUSES OF GENERATION OF RECURRENT TUBERCULOSIS CASES IN LATVIA

Doctoral Thesis Summary
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Clinic of Tuberculosis and Lung Diseases of Infectology Center of Latvia

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Doctoral Thesis is available in the library of Riga Stradins University and on webpage: www.rsu.lv.

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ANNOTATION

Latvia is among these countries in the world where there still are many cases of multi-drug resistant (MDR) tuberculosis (TB). To follow emergency and spread of these extremely dangerous TB forms, for the first time in Latvia we used methods for molecular epidemiology of TB, like genotyping of *M.tuberculosis (MT).* By providing the risk assessment of recent TB transmission from 1995 to 2002, we founded that the epidemic of MDR TB was associated with nosocomial MDR *MT* reinfection among patients, previously continuously treated in TB hospitals. We identified two MDR *MT* genotypes, i.e., Beijing and LAM9, spread in the community during that period of time.

The gradual implementation of the infection control (IC) measures in TB hospitals was started in 1997 and completed by 2003 with the development of the first TB infection control plan and implementation of it in TB hospitals. To decrease the risk of nosocomial TB transmission, a strict adherence to IC measures in hospitals and ambulatory treatment is required.

TB transmission risk analysis was repeated and reviewed after the implementation of IC measures when we found the decrease of range of recent MDR TB transmission and we did not find definite links among hospitalized MDR TB patients. As a result of implemented IC strategy the epidemiology of TB, especially MDR-TB, has changed. Until 2002 the most important risk factor for MDR-TB epidemic was nosocomial MDR *MT* transmission. After implementation of IC program, a reduction of recent transmission of MDR *MT* was observed. The effectiveness of TB infection control measures, including the necessity and promotion of TB diagnostics and treatment on ambulatory basis were demonstrated in this doctoral thesis an in future also it should be an important component in Latvian National TB program.

CONTENT

1. ABBREVIATIONS

AIDS	-	acquired immune deficiency syndrome
AFB	-	acid - fast bacillae
ATLD	-	Agency of Tuberculosis and Lung Diseases
BCG	-	*Bacillus Calmette-Guerin* vaccine
B genotype	-	*Beijing* genotype
CDC	-	Centers for Disease Control and Prevention
CI	-	confidence interval
CTLD	-	Clinic of Tuberculosis and Lung Diseases
DNS	-	dezoxyribonucleic acid
DOTS	-	tuberculosis control strategy
DOTS-Plus	-	multi-drug resistant tuberculosis control strategy
DR	-	drug resistant *M.tuberculosis*
DS	-	drug sensitive *M.tuberculosis*
DST	-	drug sensitivity test
HIV	-	human immune deficiency virus
H37Rv	-	virulent drug-susceptable *M.tuberculosis* standard strain
IS6110	-	insertion segment 6110
LIC	-	Latvian Infectology centre
LJ	-	*M.tuberculosis* cultivation on Lovenstein-Jensen media
MT, MTc	-	*Mycobacterium tuberculosis, Mycobacterium tuberculosis* complex
MDR-TB	-	multi-drug resistant tuberculosis
OR	-	odds ratio
RFLP	-	restriction fragment length polymorphism
rt-PCR	-	real time polymerase chain reaction
SATLD		States Agency of Tuberculosis and Lung Diseases
ST or SIT	-	spoligotype according to SIT VIT international database
TB	-	tuberculosis
XDR-TB	-	extensively-drug resistant tuberculosis
WHO	-	World Health Organisation

2. GENERAL DEFINITIONS

1. Definite case of TB: a patient with *MTc* identified from a clinical specimen, either by culture or by a molecular line probe assay [1].
2. TB case definitions and registration according to previous treatment [1].
   - 2.1. New TB case: patient has never had treatment for TB, or has taken anti-TB drugs for less than 1 month.
   - 2.2. Retreatment TB cases: TB relapse, retreatment after failure and retreatment after default.
     - 2.2.1. TB relapse: patient has received TB treatment and cured or treatment completed.
     - 2.2.2. Retreatment after failure: patient has received 1 month or more of anti-TB drugs and failed.
     - 2.2.3. Retreatment after default: patient has received 1 month or more anti-TB drugs and defaulted it for 2 or more months.
3. *MT* drug resistance definitions [2].
   - 3.1. Drug sensitive TB case: TB in patients whose infecting isolates of *MT* are confirmed to be sensitive in vitro to all first-line antituberculosis drugs.
   - 3.2. Mono-resistant TB case: TB in patients whose infecting isolates of *MT* are confirmed to be resistant in vitro to one first-line antituberculosis drug.
   - 3.3. Poly-resistant TB case: TB in patients whose infecting isolates of *MT* are confirmed to be resistant in vitro to more than one first-line antituberculosis drug, other than both isoniasid and rifampicin simultaneously.
   - 3.4. MDR TB case: TB in patients whose infecting isolates of *MT* are confirmed to be resistant in vitro to at least isoniasid and rifampicin.

3.5. XDR TB case: TB in patients whose infecting isolates of *MT* are confirmed to be multi drug-resistant and resistant to fluoroquinolones and one of second line injectable drugs (amikacinum, kanamycinum, capreomycinum).

3.6. Primary drug resistance: TB in patients whose infecting isolates of *MT* are confirmed to be resistant without previous use of anti-tuberculosis drugs or its use for less than one month.

3.7. Acquired drug resistance: TB in previously treated patients whose infecting isolates of *MT* are confirmed to be resistant

4. Treatment outcome definitions [1].

4.1. Cured: a patient whose sputum smear or culture was positive at the beginning of treatment but who was smear- or culture- negative in the last month of treatment and on at least one previous occasion.

4.2. Treatment completed: a patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion.

4.3. Treatment failure: a patient whose sputum smear or culture is positive after 5 months of the treatment or later during the treatment.

4.4. Default: a patient whose treatment was interrupted for 2 consecutive months or more.

5. Clustering definitions.

5.1. Similarity family or group: two and more *MT* isolates with at least 60% similarity by *IS6110* RFLP of DNA.

5.2. Cluster or clustered cases: two and more *MT* isolates with identical *IS6110* RFLP and spoligotype patterns.

5.3. Non-clustered cases: TB cases, which *MT* DNA according to *IS6110* RFLP and/or spoligotyping pattern is distinct, i.e., unique.

5.4. Recent TB transmission: getting infected or reinfected with *MT* in recent 2 to 5 years.

3. INTRODUCTION

Tuberculosis (TB) is an ancient disease and it is still widespread in the world, despite recent decline in TB incidence globally: 9.4 million new cases were registrated in 2009, i.e., 137 per 100 000 world's population [3].

TB is the disease with an ancient history also in Latvia, but the first registrated detailed TB epidemiological analysis in Latvia was dated in 1950 when the incidence of TB was 607/100 000. Over the following years until 1989 the incidence declined and it was 26.9/100 000 then, but the epidemiological situation has worsened dramatically in 1990s [4]. In 1998 the incidence was 74/100 000. Over the following years the TB incidence declined by 3-10% per year until 2010 when there were 36.6/100 000 newly detected TB patients in our country. However, the TB incidence in Latvia is still higher than the average for European Union countries.

The resolution of the World Health Assambley in 1991 was to detect 70% and to cure 85% of new sputum smear positive TB cases [5] and in Latvia it has been successfully carried out in the field of diagnostics, but the implementation of the whole decision in general is still urgent around the world and actualized in the last Global Plan „TO STOP TB" 2011-2015 [6]. Since the implementation of DOTS strategy in Latvia, a gradual improvement of treatment outcomes has been observed: in cohort from 2007 a positive treatment outcome was observed in 82% of cases that almost complies with the WHO target.

Drug resistant, especially MDR TB in Latvia is extremely important problem discovered in the middle of ninetees when TB data from Latvia was included in the first WHO Global Drug resistance surveillance project. According to the output of the project, Latvia with 14.4% of primary MDR and 54.4% of acquired MDR had the most unfavourable epidemiologic situation among 35 contries [7].

In 1995 the DOTS strategy [6] and in 1997 the DOTS-Plus strategies were implemented in Latvia. In addition an implementation of TB infection control measures were started. As the result of all previously mentioned activities, the decline of MDR TB, especially acquired MDR TB, has been achieved year by year (figure 1.1). There were 11% of primary MDR TB cases in 2010 [8]. The treatment success rate for MDR-TB cases in the period 2000-2003 was 67% [9].

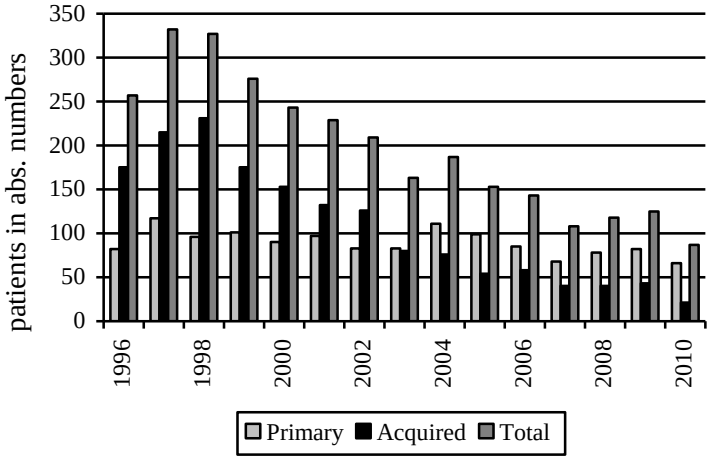

Figure 1.1 Patients with primary and acquired MDR TB in Latvia, 1996-2010

There was a continuous decrease of TB relapses in Latvia from 271 cases in 2001 to 102 cases in 2010. There was also a decrease of the drug resistance among retreatment cases from 245 cases (42.5%) in 2003 to 173 cases (35.8%) in 2009 [8]. It takes a long time to treat TB. The treatment of drug sensitive TB takes 6 to 8 months, but MDR treatment - at least 2 years. For many years drug sensitive and drug resistant, including MDR TB cases

were treated in hospitals, where appropriate infection control measures were not fully implemented. Under these conditions a nosocomial TB transmission was possible. The gradual implementation of the infection control measures in TB hospitals was started in 1997 and completed by 2003 with the development of the first TB infection control plan and implementation of it in the SATLD.

Recurrent TB cases was a serious problem in the beginning of this century in Latvia being a real threat to public health. There were 375 previously mentioned retreatment cases registered in 2000. The treatment success rate for these cases was only 41%. Improvement was observed after some years and only 176 retreatment cases were registrated in 2007 with improved treatment success rate up to 58%. Still high (22%) proportion of MDR TB cases was observed in Latvia in 2009, and patients, who defaulted TB treatment, e.i 11% [3]. Delayed MDR TB case finding and treatment defaults, especially without sputum smear conversion, prolongs infectiousness of these patients and further MR TB transmission at the same time. In accordance to the above-mentioned, there is a necessity for epidemiological studies, development and implementation of additional prophylactic infection control measures within the framework of National Tuberculosis programme.

The longer TB patients stay in hospital, the higher likelihood for nosocomial TB transmission. In the 1990s we started to implement the infection control measures with a isolation of drug sensitive and MDR TB patients in separate wards, in 2001 we also isolated patients who were at risk of MDR TB. Strict isolation of infectious TB cases within the TB ward was finished in 2003. There was a decrease of amount and length of TB hospitalizations due to the improvement of ambulatory care.

4. IMPORTANCE OF THE PROBLEM

Latvia is one of these countries in the world where there are still many cases of multi-drug resistant (MDR) tuberculosis (TB), including extensively-drug resistant TB.

To follow conditions of emergency and spread of these extremely dangerous TB forms, for the first time in Latvia we used methods of TB molecular epidemiology, like spoligotyping and *IS 6110* RFLP typing of *M.tuberculosis (MT).*

We ascertained major risk factor in TB spread, including MDR TB spread and its association with reinfection with another *MT* in high risk areas. By implementing of infection control measures and reassessment of risk the effectiveness of Infection control plan was proved resulting in reduction of nosocomial MDR transmission. The above mentioned confirms that the Infection control plan needs to be a constituent part of National Tuberculosis programme aiming for improvement of public health currently and in future.

5. OBJECTIVE AND TASKS OF THE STUDY

5.1. Objective

To evaluate importance of nosocomial TB transmission in conditions of development of MDR-TB epidemic and discover solutions for its derogation.

5.2. Tasks

1. To detect risk factors for development of acquired multi-drug resistant tuberculosis.
2. To determine risk factors of recent TB, including MDR and assess the role of nosocomial TB transmission in MDR-TB epidemiology.
3. To find out the conditions and risk factors for tuberculosis reinfection and reactivation and to evaluate impact of infection control measures on them.
4. To work out recommendations for providing of tuberculosis infection control measures in TB facilities. Improvement of fast-track diagnosis of TB by using molecular detection methods of *M.tuberculosis* and drug resistance.
5. To evaluate the effectiveness of current TB infection control measures and their impact on nosocomial TB transmission.

6. HYPOTHESES

1. There is a high TB, especially MDR TB, transmission risk in TB hospitals.
2. The risk of TB recurrence is associated with MDR and particular genotype of *MT*.
3. Development and implementation of comprehensive and evidence based TB Infection control plan according to existing risks, reduces nosocomial TB transmission.

7. SCIENTIFIC NOVELTY AND PRACTICAL IMPLICATION OF THE STUDY

The study of molecular epidemiology of TB was carried out for the first time in Latvia, involving assessment of TB transmission risks.

The outcomes of the study, i.e., information about high TB transmission risk areas and factors, were used for definition and implementation of infection control measures, aimed to decrease TB transmission risk. The effectiveness of implemented infection control measures were assessed by repeated evaluation of TB transmission risks. In our study we confirmed substantial restriction of nosocomial transmission of MDR TB after implementation of Infection control plan. Methods of *MT* molecular epidemiology were used for assessment of the circumstances of TB transmission, especially facts and conditions of nosocomial transmission.

In general the study provides recent discoveries on TB, especially MDR TB transmission and progression of both epidemics in medical institutions, in Latvia and in former USSR countries overall.

Recurrent risk analysis of TB recent transmission will allow National Tuberculosis programme supervisors and health care authorities to develop high standard TB control directives, including TB infection control strategy, selection of treatment place and system for diagnosing, isolating and treating TB patients at lower costs and with lower risk of TB nosocomial transmission.

8. STRUCTURE AND ETHICAL CONSIDERATIONS OF THE STUDY

8.1. Structure and wordage

The Doctoral Thesis consists of introduction, objective of the study, tasks and hypotheses, review of literature, methodology, results, conclusions, discussion, publications and approbation. Practical recommendations are presented in task № 3 as well as in a separate chapter. Reading list comprises 168 references. The total volume of the Doctoral Thesis is 113 A4 format pages; symbols size 12 and 1.5 rows spaces, including 24 pictures and 6 tables.

8.2. Ethical considerations

Ethics approval for this study was obtained from the Medical Ethics Committee of State Agency of Tuberculosis and Lung Diseases, Riga district (No. 23 dated March 15, 2006 and No. 102 dated April 22, 2009) and from the Ethics Committee of Riga Stradins University and dated June 10, 2010.

9. STUDY POPULATION AND METHODS

9.1. Study population

It is described separately for each task, depending on study framework and selection criteria.

9.2. Cultivation of *MT*

All clinical specimens were collected and processed by the N-acetyl-L-cysteine-sodium hydroxide procedure, inoculated and cultured on solid Lowenstein-Jensen medium. Drug susceptibility was determined by the absolute concentration method on Lowenstein-Jensen medium and BACTEC culture. The H37Rv strain was used as a "gold standard" for susceptible strains.

9.3. *MT* molecular typing

IS6110-based RFLP *MT* typing was performed according to a standardized protocol [10]. The isolates were grouped by the UPGMA (unweighted pair group method with arithmetic mean). Isolates with identical *IS6110* patterns were defined as clusters. Isolates with at least 60% similarity were defined as similarity groups or families.

Spoligotyping performed according to a standardized protocol [11] was used to detect the *Beijing* genotype [12] and in cases where genomic DNA was insufficient for RFLP analysis.

Cases were clustered, if genotyping patterns had been similar by both genotyping methods. Then clustered cases represented recently, mostly in the last 2 to 5 years, occured transmissions [13].

9.4. Statistical analysis

Odds ratio (OR), Cornfield 95% confidence interval (CI) and p-value were detected. For detection of recent TB transmission risk factors, binary logistic regression of SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) was used. For risk assessment, the OR was calculated using the Woolf method. Statistical significance was determined by the Fisher test. $P<0.05$ was considered statistically significant and associated with clustering.

10. RISKS FOR ACQUIRED MDR TB

Task № 1: To detect the risk factors for acquired MDR TB.

10.1. Material and methods

Retrospective case control study.

There were 122 patients with recurrent MDR TB and 19 patients with DS TB cases diagnosed in Riga city and district from January, 1998 to December, 1999. 67 out of 122 MDR TB cases with registered drug susceptibility test in previous episode and 19 drug sensitive retreatment cases were included in the study.

Inclusion criteria: patient, diagnosed as retreatment case, *MT* culture positive, MDR or drug sensitive, with known drug sensitivity test data's in both episodes, diagnosed in Riga city and district from 1998 to 1999.

Dependent variables: recurrent MDR and drug sensitive TB cases. Independent variables: place of residence, gender, age, year of previous TB episode, place of treatment, received treatment and its outcome, TB contact, anamnesis of incarceration, social status, comorbidities.

Cases: recurrent MDR TB cases. Controls: recurrent drug sensitive TB cases.

10.2. Results

MDR TB cases were younger than controls and in the age group from 20 to 30 they had higher risk of acquiring MDR TB (OR 1.31; $p<0.05$). By

analysing a previous TB episode we found the essential difference between groups in the context of time period, when previous occasion was registered (Figure 10.1). MDR TB risk was two times higher for patients, whose first TB episode was registered between 1994 and 1999 (OR 1.98; $p<0.05$), because widespread MDR TB cases were characteristic for epidemiological situation in Latvia during that period of time. The possibility that recent transmission might be a causative agent in development of MDR recurrent TB cases was confirmed also by the following: in 45 out of 67 MDR TB cases the patients had drug resistance to previously never used TB drugs.

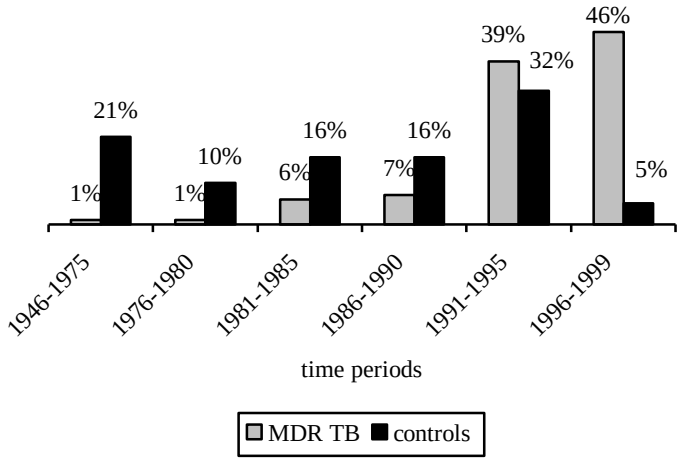

Figure 10.1 Distribution of cases (n=67) and controls (n=19) in previous TB episodes during different periods of time.

11. NOSOCOMIAL TRANSMISSION OF MDR TB

Task № 2: To determine the proportion of tuberculosis, including MDR-TB cases, attributable to recent transmission and risk factors associated with clustering and to assess the role of nosocomial transmission in the epidemiology of MDR-TB.

11.1. Material and methods

Retrospective nested case-control study.

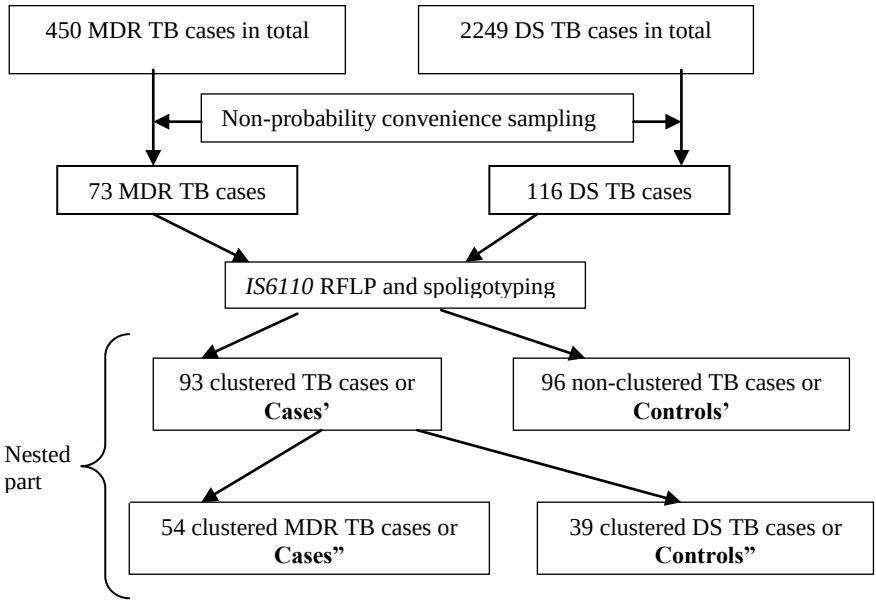

Figure 11.1 Design of the second task

2249 patients with culture-confirmed drug sensitive TB and 450 patients with MDR-TB were diagnosed in Latvia from January, 1999 to December

2001. Using non-probability convenience sampling, all MDR cases and up to 20 drug sensitive samples, cultured at the same time at the Latvian State Agency of Tuberculosis and Lung Diseases laboratory were selected every 4 months. Altogether 73 MDR and 116 drug sensitive isolates were obtained from 189 patients (one *MT* isolate per patient), representing 16.2% of MDR and 5.2% of drug sensitive TB patients diagnosed during that period of time.

Case definition: all patients registered in Latvia from January 1, 1999 to December 31, 2001 as new, relapse, treatment after failure or default were defined as *MT* culture-positive, drug sensitive or MDR.

Dependent variables were clustered and non-clustered cases. For the nested study - clustered MDR and clustered drug sensitive TB cases.

Independent variables for previous anti-TB treatment episode were age, sex, place of residence, drug resistance profile, HIV status, drug addiction, alcohol abuse, smoking, concomitant diseases, characteristics of TB contacts, history of incarceration, homelessness, disability, hospitalization and received treatment. For the current episode we included all the above variables and performed *MT* genotyping.

Cases: clustered TB cases. Cases in nested part: clustered MDR TB cases.

Controls: non-clustered TB cases. Controls in nested part: clustered drug sensitive TB cases.

11.2. Results

Demographic characteristics of the study population. A total of 189 patients with molecular characterization of isolates were included in the study. The predominant gender of the sampled group was male (n=141; 75%). The mean age of the patients was 42 years (range 15-84). The majority of the study

population lived in cities (n=120; 64%), and the remainder - in rural areas (n=63; 33%); six patients (3%) were homeless. There were 35 (19%) previously incarcerated patients.

Characteristics of the *IS6110* RFLP-profile typed isolates and their clustering. *MT* isolates from the 189 cases studied by clustering method were divided into several distinct groups or unique isolates. Almost half (49.2%) of cases were clustered. MDR TB cases were clustered more frequently (74.0%) than drug sensitives (33.6%). The largest similarity group was the B family, consisting of 71 isolates in 11 clusters, with 2-11 members in each cluster. *IS6110* patterns and the spoligotype SIT1 identified the B family as the Beijing or W genotype [12]. Of the 189 isolates tested, 71 belonged to the Beijing and 118 to the non-Beijing genotype. MDR TB isolates predominantly had the Beijing genotype 58 (79%); only 15 (21%) showed the non-Beijing genotype (OR = 30.65, CI 12.75-75.51). The MDR TB Beijing genotypes were clustered in 86%, which indicated more recent transmission than the MDR non-Beijing genotype (13%). In contrast with the B group, in family C (n=40), with 3 clusters, the only closely related subgroup, Cb (LAM9 or SIT42 strain according to spoligotyping), had a MDR phenotype. The remaining non-Beijing MDR isolates (6/120) had unique genotypes. In resume the majority of the MDR isolates belonged to the SIT1 and SIT42 families according the spoligotyping.

Clustered isolates and risk factors. If clustered TB cases represented recently transmitted TB cases [13], then risk factors were associated with clustering, as well as with recent transmission. Risk factors for recent transmission were identified by comparing patients with clustered and non-clustered *MT* isolates (Table 11.1).

Table 11.1

Evaluation of risk factors for recent transmission by cluster analysis

Risks	Total n=189 (%)	Clustered n=93 (%)	Non-clustered n=96 (%)	OR	p-value
B genotype	71 (37)	59 (62)	12 (13)	12.15	0.000
MDR *MT*	73 (39)	54 (58)	19 (20)	5.61	0.000
Alcohol abuse	85 (45)	56 (60)	29 (30)	3.92	0.000
Incarceration	33 (19)	23 (27)	10 (10)	2.95	0.008
Tobacco smoking	87 (46)	53 (57)	34 (35)	2.58	0.003
Prev. treatment	73 (39)	44 (47)	29 (30)	2.,07	0.017
Prev. hospit.	78 (41)	46 (50)	32 (33)	2.04	0.018
Age 26-55 years	137 (73)	79 (85)	58 (60)	3.70	0.000

OR - odds ratio, MDR - multidrug-resistant; *MT* - *M.tuberculosis*; TB - tuberculosis; HIV - human immunodeficiency virus; NA - not applicable; B - *Beijing*; hosp.- hospitalization; prev.- previous

Patients, aged 26-55 years were more likely to have clustered *MT* isolates. Clustered TB cases were 6 times more likely to have MDR and 12 times more likely to have Beijing genotype than non-clustered cases. Alcohol abuse, incarceration, smoking and previous TB treatment in hospitals were also significantly associated with clustering.

The *MT* Beijing genotype and previous TB treatment in hospitals were strongly associated with clustering of MDR cases in comparison with drug sensitive cases in nested part of the study (Table 11.2).

Table 11.2

Evaluation of risk factors for recent transmission of MDR *MT*

Risks	Clustered MDR n=54 (%)	Clustered DS n=39 (%)	OR	95%CI
B genotype	50 (93)	9 (21)	41.67	18.14-134.65
Prev. hospitalization.	40 (74)	6 (15)	18.33	4.91-53.06
Prev. TB treatment.	39 (72)	5 (13)	17.68	5.25-63.68

OR - odds ratio, MDR - multidrug-resistant; *MT* - *M.tuberculosis*; TB - tuberculosis; prev.- previous; B - *Beijing*

12. THE CAUSES OF GENERATION OF RECURRENT TB CASES

Task № 3: To identify the risk factors for tuberculosis reinfection and reactivation and to evaluate impact of infection control on them.

12.1. Material and methods

Retrospective case control study.

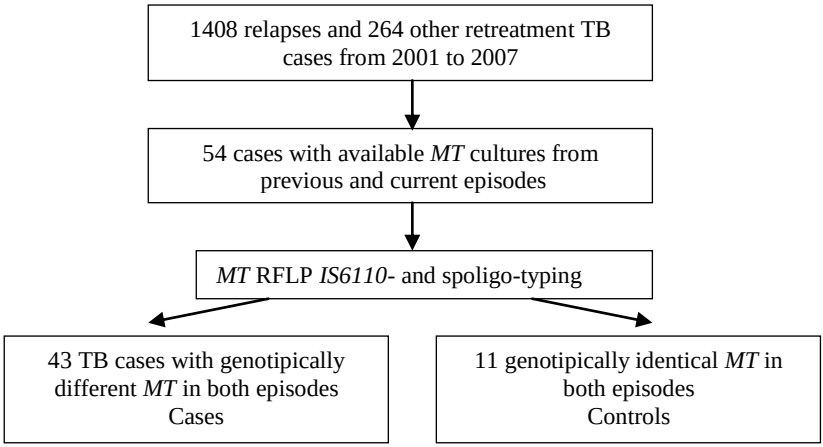

Figure 12.1 Design of the third task

54 retreatment TB cases (relapses, failures and defaults according to WHO definitions) from 2001 to 2007 with available *MT* cultures from previous and current episodes were analyzed, representing 3.2% of all retreatment cases in Latvia during that period of time. *MT* identification, drug sensitivity test by absolute concentration and/or BACTEC cultivation methods, spoligotyping and *IS6110* RFLP genotyping were performed for both episodes.

First TB episode: case registration in previous TB episode. Second TB episode: case registration in current TB episode. Other case definitions (see chapter “General definitions”).

Dependent variables: Retreatment TB cases with genotipically similar or different *MT* in both episodes.

Independent variables: gender, age, place of residence, social status, alcohol and drug addiction, incarceration, TB contact, HIV status, diabetes mellitus, prolonged usage of steroids, low body mass index, irradiation, previous case registration - definition, year, sputum smear AFB status, drug sensitivity test of *MT*, genotype, inpatient and outpatient treatment received, length of treatment and sputum smear conversion, current case registration - definition, year, sputum smear AFB status, drug sensitivity test of *MT*, genotype.

Cases (reinfection): retreatment TB cases with genotipically different *MT* in both episodes, suggesting reinfection of different *MT* strain. Controls (reactivation): retreatment TB cases with genotipically identical *MT* in both episodes, suggesting endogenous reactivation of existing *MT* strain.

12.2. Results

According to genotyping results, all retreatment cases were divided into two groups, consisting of 43 genotipically different reinfection cases and 11 genotipically similar reactivation cases. Reinfection with other *MT* strain (80%) is more common than reactivation of the same strain (20%) in the pathogenesis of TB recurrence in this study.

Demographic and social characteristics of both groups were similar: predominance of male gender (reinfection cases 100%, reactivation controls 82%) and urban citizens (both 70%). Average age of patients in recurrent

episode ~ 43 years, but it differed by age groups (Figure 12.2), where we observed typical curves for recent transmission and endogenous reactivation for each group.

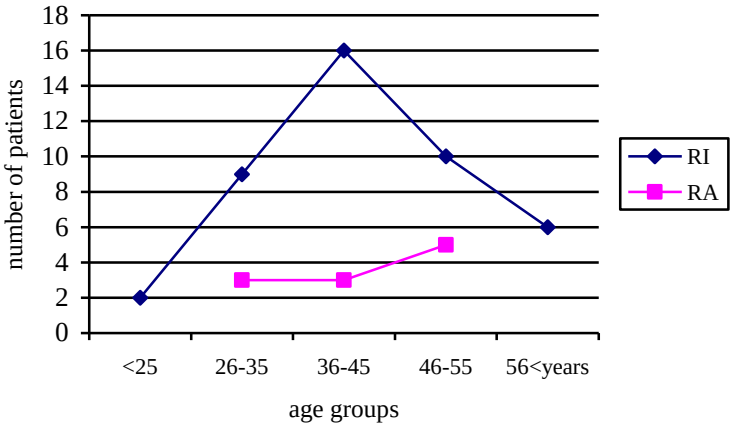

Figure 12.2 Patient's distribution by age in recurrent TB episode

RI - exogenous reinfection cases; RA - endogenous reactivation controls

Evaluation of risk factors by comparative analysis of previous TB episode in both groups was performed (Table 12.1). Previous treatment time, place and drug sensitivity test were statistically significant risk factors for TB recurrence. Majority of reinfection cases (72%) in previous TB episode were diagnosed and treated in hospitals from 1999 to 2002 so long as reactivation controls (62%) from 2003 to 2007. Most of reinfection cases (70%) in previous episode were drug sensitive, with comparatively faster (80 days on average) sputum smear AFB conversion to negative, versus reactivation controls converted in approximately 141 days. Reinfection cases stayed in hospital longer time (229 days) than reactivation controls (154 days). As a result of that 30 drug sensitive, 7 drug resistant and 6 MDR TB cases became reinfected with

MDR in 72% of cases and with XDR *MT* strains in 5%. 70% of reinfected MDR and XDR cases were *Beijing* (SIT1) and LAM9 (SIT 42) genotypes (Figure 12.3).

Table 12.1

Characteristics of patients in previous TB episode

Risks	RI, # 43	RA, # 11	OR, p-value
DST - sensitive	30 (70%)	3 (27%)	6.15, p<0.05
DST - multi-drug resistant	6 (14%)	5 (45%)	0.19, p<0.05
In - patient from 1999 to 2002	31 (72%)	4 (38%)	4.52, p<0.05

RI - reinfection cases; RA - reactivation controls; DST - drug sensitivity test

Figure 12.3 Characteristic spoligotypes of *MT*

SIT1 – *Beijing* genotype; SIT42 – LAM9 (Latin-America's) genotype;
SIT53, SIT766 – other genotypes

5 out of 11 reactivation cases (45%) were MDR, 3 – drug resistant and 3 – drug sensitive in previous TB episode. After received treatment *MT* became more resistant: 5 MDR and 2 XDR cases. In 8 cases (73%) from all Beijing (SIT1) and LAM9 (SIT42) genotypes were observed in reactivation group.

TB reactivation risk were almost 7 times higher, if *MT Beijing* and LAM9 genotypes (OR 6.89; $p<0.05$) and 5 times higher, if MDR *MT* were detected in previous TB episode (OR 5.14; $p<0.05$). Other analyzed risk factors had no impact on TB recurrence.

13. INFECTION CONTROL

Task № 4: To work out recommendations for providing of TB infection control measures in TB treatment facilities. Improvement of fast-track diagnosis of TB by using molecular detection methods of *MT* and drug resistance.

Based on results of tasks № 2 and 3, which confirmed the role of prolonged hospitalization in nosocomial transmission of MDR *MT,* task № 4 contains practical issues of developing and implementation of infection control strategy. According to WHO and CDC recommendations [14], the infection control team, including me, under the guidance of Andra Cirule, developed the Infection control plan and implemented it in the central unit of Latvian National Tuberculosis programme.

Content of infection control measures [14]**.** Administrative infection control measures were worked out for reduction of formation of infectious droplet nuclei, consisting of *M.tuberculosis.* These control measures include risk assessment of institution, fast-track diagnosis, isolation and adequate treatment of infectious TB cases, as well as shortening of time of hospitalization, education of personnel and patients, reduction and control of procedures contributing to formation of infectious aerosols. Engineering control measures are next to administrative controls and works on reduction of concentration of infectious droplet nuclei, containing *M.tuberculosis.* These control measures include ventilation, HEPA filtration and ultra-violet irradiation.

Protection of respiratory airways of medical personnel and in case of necessity of visitors as well against inhalation of *M.tuberculosis* containing droplet nuclei in areas with high TB transmission risk.

14. INFECTION CONTROL REDUCES NOSOCOMIAL TB TRANSMISSION

Task № 5: To evaluate the effectiveness of established TB infection control measures and the impact of these measures on nosocomial TB transmission.

14.1. Material and methods

Prospective cohort study.

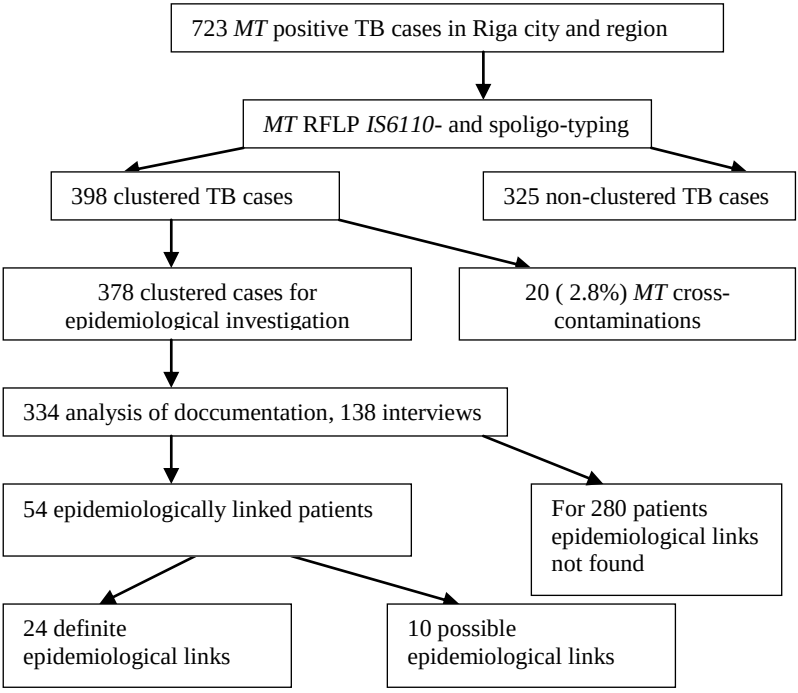

Figure 14.1 Design of the fifth task

MT cultures from TB cases (723 altogether, one culture per case) diagnosed in Riga city and district from November, 2007 to June, 2010 were selected. Drug sensitivity testing and genotyping (IS*6110*-RFLP and spoligotyping) of isolates were performed. Interviews, data abstractions from medical documentation and cluster analysis were performed for clustered TB cases.

Definite epidemiological link: common place and time linkages between 2 cases were documented.

Possible epidemiological link: at least 2 patients mentioned the same location, but without precise time or they did not recognize each other.

14.2. Results

From 723 TB cases tested, 398 were clustered (55%) in 76 clusters from 2 to 40 cases in each. 138 patients (35%) in 69 clusters were interviewed.

M.tuberculosis laboratory and/or bronchoscopy related cross-contaminations were detected for 20 cases (2.8%) in 10 clusters. Up to 4 other samples were cross-contaminated from one sputum smear AFB positive sample. In 1 case bronchoscopic cross-contamination were suspected. In 5 clusters we observed only cross-contamination related isolates.

After the exclusion of 20 cases with cross-contaminations, we observed proportion of clustering, suggesting recent transmission among 378 (54%) from 703 cases. More recent clustering was observed among 122 (57%) from 214 phenotipically MDR TB cases. For further epidemiological analysis we also excluded 44 cases from clusters without any interview. Further analysis were done for 334 patients. Predominantly they were males 237 (71%). After interviews and analysis of medical documentation, we detected that 54 patients

(16%) were epidemiologically linked with 34 links: 24 definite (Table 14.1) and 10 possible links (Table 14.2).

Table 14.1

Characteristics of definite epidemiological links

Type of contact	Description of link	DST	Spoligo-type (ST)	Pairs in clusters
Household	Family	DS	1, 40, 42, 262	7
Household	Family	DR	1, 254, 283, 1292	4
Social	Acquaintances, shelter, relatives	DS	1, 40, 53, 766, 254	10
Social	Church, friends	DR	53, 283	2
Social	Neighbors	MDR	42	1

DST - drug sensitivity test; DS - drug sensitive; DR - drug resistant; MDR - multi-drug resistant

Table 14.2

Characteristics of possible epidemiological links

Type of contact	Description of link	DST	Spoligo-type (ST)	Pairs in clusters
Nosocomial	One wide-profile hospital in 5 years	DR	283	1
Nosocomial		MDR	1	1
Social	Acquaintances, school-mates, neighbors	DS	1, 119, 1597	3
Social	Bars, prisons, born abroad	MDR	1, 42	5

DST - drug sensitivity test; DS - drug sensitive; DR - drug resistant; MDR - multi-drug resistant

Possible recent nosocomial MDR TB transmissions were observed in 2 cases, but patients did not accurately define the ward and time of admission in this wide-profile hospital in Riga city.

15. DISCUSSION

The dynamics of TB transmission and its risk factors in Latvia from 1995 to 2010 were investigated. In the first study of TB molecular epidemiology, which covers a period from 1995 to 2001, we observed clustering in 49% of cases, indicating a moderate rate of recent transmission in Latvia. Globally the highest rate of recent transmission has been observed in the high TB incidence area of French Guinea (67-79%) [15]. A little lower clustering rate (28 to 51%) was observed in countries with low TB incidence: Maryland, USA and Japan [16, 17]. TB epidemiological situation in country and risk of *MT* exposure can be estimated in accordance by *MT* clustering.

The higher rate of recent transmission in Latvia was observed among MDR *MT* isolates (74%) in comparison with clustering among drug sensitive strains (34%). Similar differences have been observed in studies in Norway and New-York, USA where MDR *MT* clustered in 53 to 73% of cases, but drug sensitive only in 16 to 33% of cases [18, 19]. Previously described results indicate more extent recent MDR TB transmission due to the more prolonged period of infectiousness of these patients: delay of MDR TB diagnosis and adequate treatment, worse treatment outcomes. There were less cured and treatment completed (66%) cases among MDR TB cases [8] in comparison with non-MDR TB cases (72%) [3], MDR TB cases defaulted (13%) and failed (14%) treatment more often [8] compared to non-MDR TB cases (7% and 3% respectively) [3].

All or at least most of clustered cases should be epidemiologically linked, but the highest epidemiological linkage (86%) till nowadays has been described by *van Deutecom* in Netherlands [13]. In some studies in Spain, USA (Arkansas) and France (Gironde) epidemiological links have been found among 27 to 35% of clustered cases [20, 21, 22]. In our studies, we found

epidemiological links in 32% of clustered MDR cases nosocomially in time period from 1999 to 2001 and only in 16% of all clustered cases without definite links in hospitals in time period from 2007 to 2010.

Young age, black race, male gender, urban residency, homelessness, HIV infection, pulmonary diseases, including destructive forms as well, previous TB treatment and incarceration have been described as risk factors associated with recent TB transmission in studies from different countries [16, 18, 20]. In our study there were no patients of black race. All other risk factors, except HIV, pulmonary diseases, male gender and urban residency, were also found in Latvia.

The association of *MT* Beijing genotype with drug resistance, including MDR, has been described in the former Soviet Union countries: Estonia (34%), Uzbekistan and Turkmenistan (27%), Azerbaijan (61%) [23, 24, 25], as well as in Taipei (Taiwan) [26] and Singapore, where higher transmission level of these strains has been observed [27]. The majority (80%) of MDR *MT* isolates in Latvia belong to the Beijing molecular genotype, as well as the ones from the Aral Sea region of Central Asia (75%) and Singapore (58-76%) [28, 24, 27]. For the first time, a strong association among recent TB transmission, multi-drug resistance and the Beijing genotype, has been observed by molecular genotyping and epidemiological investigation in Eastern Europe. The clustering rate of 74% among MDR TB cases indicates high level of recent transmission. In our study we confirmed the role of previous hospitalization and treatment in recent MDR TB transmission.

Exogenous drug sensitive, drug resistant and MDR TB re-infection in medical facilities has been described since 1990s [29, 30], while Kenyon et al. have described a nosocomial outbreak of MDR TB due to the absence of infection control [31].

Several studies have been done in different countries for pathogenetical analysis of recurrent TB cases. Exogenous reinfection among

recurrent TB cases in countries with high TB incidence, like South-Africa 225 / 100 000, also is high 75-77% [32]. It was observed in our study that from 1991 to 1997 *MT* reinfection among recurrent cases had been very high: 80%. It was characteristic for epidemiological situation in our country for that time period. In early 1990s TB incidence in Latvia was 27.4 / 100 000, which increased untill 1998 (74/100 000). Since DOTS strategy has been implemented in 1996, TB treatment was mostly hospital-based. Due to the recent, mostly nosocomial MDR TB transmission, in 1997 there were a peak (332 cases) of detected MDR TB cases [8]. After implementation of directly observed treatment in 1996, the out-patient treatment has become more widespread.

Predominance of different *MT* genotypes in two consecutive TB episodes could be overestimated due to selection constraints, namely, only 3.2% from recurrent cases in time period from 2001 to 2007, possible multi-strain *MT* infection or *MT* heterogenity and laboratory cross-contamination. Variable drug sensitivity tests in repetitive *MT* cultures from patient has been studied in South-Africa and Georgia, where it has been explained by contamination with several and different *MT* strains [33, 34]. Multiple infections with different strains occur in places with high load of *MT*. Drug sensitivity tests and DNA extraction from isolated and selected *MT* culture does not allow to detect multiple strains. Due to that Rinder et al. have used PCR-RFLP methods for detection of heteroresistance and detected heterogeneity in fresh clinical material in 17% of investigated cases. It was very important for TB case management, because by traditional DST methods 78% of *MT* isolates were drug sensitive [35].

MT cross-contamination in Latvian TB reference laboratory from 2007 to 2010 was 2.8 %, similarly as in Netherlands with 2.4% cases [36], which is within acceptable range.

Patients with prolonged infectious MDR TB are sources for reinfection of other people. According to the results of our study, the *MT*

Beijing genotype is a possible risk factor for TB relapses. It corresponds with other studies in Vietnam (OR 2.8; CI 1.5-5.2) [37] and shows, that the patients, infected with *MT* Beijing genotype form the risk group which needs to be treated and prevented from relapses. Systematic registration and analysis are necessary to evaluate the conditions of spread of different genotypes of *MT* strains in Latvia, especially in cases of MDR and XDR phenotype.

According to the results of our study where we confirmed nosocomial transmission of MDR *MT*, the next step is to develop and implement Infection control plan, to keep strong adherence to it and promote ambulatory TB treatment. Infection control measures of all three levels, i.e., administrative, engineering and personal respiratory protection should be implemented. Administrative control measures are the most important, having more higher effectiveness for reduction of nosocomial TB transmission. They include the fast-track diagnostics of infectious cases and *MT* drug resistance, and timely and adequate isolation and treatment of these cases, taking into account the drug sensitivity test of *MT*.

All traditional *MT* examination methods, like sputum smear AFB microscopy, cultivation of *MT* from sputum, are still important and useful currently, but at the same time some of these methods are inaccurate and some are slower in comparison with molecular examination methods. Thus there are *MT* DNA PCR based methods with possibility to detect *rpoB* mutation, used in medical practice [38].

When a case with *MT* resistance to rifampicin is detected, usually it is MDR case, because isolated rifampicin resistance is rare in Latvia. Accordingly in a short time we can obtain data giving evidence of MDR TB, while using traditional methods it takes no less than two months. Adequate isolation and treatment significantly reduces transmission of MDR TB. Drug sensitivity tests for other TB drugs also require the time from 2 weeks to 3 months, depending on the method used. Therefore much has been done to examine location of

mutations in *MT* DNA, which are responsible for resistance to other TB drugs. This may reduce time required for detection of full drug resistance profile to one day. Generation mechanisms of resistance to antituberculosis drugs, such as mutations in *rpoB* and *katG* genes, which determine 93.6% rifampicine and 98.4% isoniazide resistance have been studied in Latvia [39]. 56 (85%) of the tested 66 streptomycin–resistant isolates had mutations either at *rpsL* or *rrs* genes. 17 (52%) of the tested 33 ethambutol-resistant isolates had mutations in *embB* gene. Point mutations were found in 23 (82%) of the 28 pyrazinamide-resistant isolates and were located in 10 different codons of the *pncA* gene. Based on the aboved mentioned results we can conclude that genetic methods will have the most essential role in detecting *MT* drug resistance patterns in the nearest future. On the other hand there is a necessity to continue scientific evaluation of drug resistance patterns, for example, ethambutol, because in our study we found mutations in *embB* gene in five (15%) of 33 ethambutol-susceptible MDR *MT* isolates. In several studies by Mokrousov and co-authors the fast methods for detection of *MT* drug resistance have been examined: allele-specific *rpoB* PCR assay detects mutations in 86.1% of rifampicin-resistant *MT* [40]. Spanish scientists have identified epidemiological links among patients whose *MT* isolates have identical *IS6110* patterns and *rpoB* genotypes and have concluded that characterization of *rpoB* mutations can provide information about susceptibility to rifampin and be a useful epidemiological tool for discrimination of rifampicin-resistant strains of *MT* with identical *IS6110* fingerprints [41].

Until 2002 a prolonged hospital treatment was provided for the majority (72%, OR 4.52; $p<0.05$) of previously drug-susceptible reinfection cases. This statistically significant risk factor separates two time periods: period till 2002, when infection control measures were implemented in hospitals and period after 2003, when implemented infection control principles significantly reduced nosocomial TB transmission. Reduction of TB, including MDR TB

nosocomial transmission was also confirmed by our prospective molecular epidemiology study in Riga city and Riga district. In our study we did not confirm any definite nosocomial epidemiological link among MDR TB cases. Only two pairs of cases were possibly epidemiologically linked in hospital: one was drug resistant pair, another - MDR, both hospitalized in Riga city hospital within period of 5 years, without specifying ward and time of staying. Definite epidemiological links were found only in 16%, particularly among drug sensitive TB cases in social contacts, incl. shelters. Tuberculosis transmission in homeless shelters has also been described in New York, USA in ninetees. Because of the high rate of infection, routine screening for TB and preventive therapy for eligible persons should be considered in shelters [42].

16. CONCLUSIONS

1. Approximately one half of genotipically analyzed *MT* isolates, were clustered, indicating intermediate range of recent TB transmission.
2. From 1995 to 2001 MDR TB cases were more recently transmitted (74.0%) in comparison with drug sensitive cases (33.6%).
3. The following are the risk factors associated with recent TB transmission: Beijing genotype MDR *MT*, addictions, age from 26 to 55 years, incarceration and previous TB treatment in hospitals.
4. The *MT* Beijing and LAM9 genotypes and prolonged previous TB treatment in hospitals with confirmed epidemiological links in 32% were strongly associated with recent transmission of MDR cases.
5. In the pathogenesis of TB recurrence a reinfection with other *MT* strain (80%) was more common than reactivation of the same strain (20%).
6. Previous episodes of MDR reinfection in 72% of cases before year 2002 were diagnosed.
7. From 2002 Infection control plan was delivered and implemented in SATLD which substantially reduced nosocomial TB transmission.
8. After the implementation of the Infection control plan a decrease of clustering, i.e., reduction of recent MDR transmission till 57% was observed.
9. Previous episodes of MDR *MT* reinfection after year 2002 were diagnosed only in 28% of cases.
10. From 2007 to 2010 in Riga region the spread of sensitive *MT* strains in household and social contacts, including shelters were most common. We did not confirm definite nosocomial MDR TB transmission.
11. Laboratory cross-contamination was observed in 2.8 % of all cases.

17. PRACTICAL RECOMMENDATIONS

1. Rapid detection of infectious and drug-resistant TB cases from clinical specimen already during the phase of outpatient investigation is necessary.
2. If possible, isolation and directly observed outpatient treatment should be provided in patients' place of residence.
3. In case of a necessity to isolate TB patients and provide inpatient care for them, strong adherence to the infection control measures of all 3 levels is essential.
4. Discharge TB patients from hospital as early as possible and motivate for outpatient care, by ensuring patients' compliance with incentives and enablers.
5. Systemic registration and analysis are required for evaluation of *MT* genotypes in Latvia, especially phenotipically MDR and XDR, and assessment of their spread rate and conditions.

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