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GENOTYPIC DIVERSITY AND DRUG RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS

313.02 – MICROBIOLOGY, MEDICAL VIROLOGY

Summary of the PhD thesis in medical sciences

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CONTENT

CONCEPTUAL LANDMARKS OF THE THESIS

Actuality

Tuberculosis (TB) is one of the largest public health challenges globally. In 2022, the World Health Organization estimated 10.6 million new cases and 1.3 million deaths due to TB [1]. Currently, one of the most significant obstacles in reducing TB's impact on public health is the rise of multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR strains) that are resistant to both isoniazid and rifampicin [2]. The incidence of MDR-TB cases is particularly alarming in Eastern European countries, where MDR rates among newly diagnosed TB patients exceed 30%, and among retreatment cases, exceed 50% [3]. Disruption of drug supply, inadequate implementation of treatment regimens in previous decades, alongside poor infectious control and delayed diagnosis, have facilitated the selection and sustained transmission of drug-resistant strains, perpetuating the MDR-TB endemic in this region [4–6]. Moreover, the genetic adaptability and diversity of *Mycobacterium tuberculosis complex* strains may directly influence the perpetuation and spread of drug resistance. Genetic features such as compensatory mutations and homoplastic mutations could provide advantages to drug-resistant strains of the *M. tuberculosis complex* [7].

Studies utilizing whole genome sequencing data of *Mycobacterium tuberculosis* complex strains from the Republic of Moldova have investigated the circulating genotypic lineages and presumed transmission history within this geographic area [8, 9]. Similarly, sequencing data have been employed to assess nosocomial transmission of *Mycobacterium tuberculosis complex* strains in specialized medical facilities treating TB patients [10]. However, the genetic determinants influencing the successful spread of MDR strains in this region and their associations with clinical characteristics of TB have remained largely understudied.

This study evaluates the genetic structure of MDR strains of the *Mycobacterium tuberculosis complex* isolated in the Republic of Moldova over six consecutive years, using whole genome sequencing data. Analysis of sequencing data allowed characterization of genetic diversity, evolutionary patterns, and phylogenetic relationships among MDR strains of

the *M. tuberculosis complex*. Specifically, the potential role of genomic features such as compensatory mutations and homoplastic mutations in the evolution of MDR-TB and their association with clinical characteristics of tuberculosis was determined. Special attention was given to the association between mycobacterial genetic characteristics and potential clinical management deficiencies leading to resistance to new or re-introduced anti-tuberculosis drugs like bedaquiline and linezolid.

Keywords: TB, *M. tuberculosis complex*, MDR, laboratory diagnosis, whole genome sequencing, risk factors, genotypic lineages.

Study Domain: Microbiology

Study Aim:

To study the genotypic diversity and molecular-genetic characteristics of *Mycobacterium tuberculosis* strains from the Republic of Moldova impacting drug resistance.

Research Objectives

1. To assess the genotypic diversity and temporal evolution of multidrug-resistant *Mycobacterium tuberculosis* strains in the Republic of Moldova.

2. To identify genetic determinants of *Mycobacterium tuberculosis* potentially involved in the spread of multidrug-resistant strains in the Republic of Moldova.

3. To establish potential associations between genotypic determinants impacting antimicrobial resistance in *Mycobacterium tuberculosis* and the clinical evolution of tuberculosis.

4. To identify genotypic correspondences of mycobacterial resistance to key drugs used in the treatment of multidrug-resistant tuberculosis.

General Methodology of Research

To achieve the study objectives, three studies were conducted involving several research cohorts and a variety of statistical analysis solutions.

Methodologically, the first study comprised a descriptive component of molecular epidemiology data derived from whole genome sequencing of *M. tuberculosis complex* genomes from 2013 to 2018, utilizing comparative statistics it highlights differences among genotypic lineages of *M. tuberculosis complex* in the Republic of Moldova. Specifically, genetic determinants associated with resistance to anti-tuberculosis drugs, frequency and types of compensatory mutations, and homoplastic mutations were compared. This study also included an analytical component where a multiple logistic regression model was applied to identify associations between genotypic determinants of different *M. tuberculosis complex* lineages and clinical characteristics of tuberculosis, such as severity of imaging lesions and negative treatment outcomes.

The second study documented the emergence of bedaquiline resistance shortly after its introduction in treatment regimens for MDR-TB patients in the Republic of Moldova, along with geno-phenotypic correlations of resistance to this drug. Additionally, an analytical component involved a multiple logistic regression model to evaluate factors associated with negative outcomes of anti-tuberculosis therapy in these patients, including potential associations with the genotypic lineage of *M. tuberculosis* in MDR-TB patients receiving bedaquiline-containing treatment regimens.

The third study is a cohort study identifying risk factors for *Mycobacterium tuberculosis complex* acquisition of linezolid resistance during anti-tuberculosis treatment, concurrently describing the frequency of linezolid resistance and genetic mutations associated with resistance to this drug in MDR strains of the *M. tuberculosis complex* from the Republic of Moldova.

Novelty and Originality

The results of this study describe the genotypic diversity of the *M. tuberculosis complex* in the Republic of Moldova based on whole genome sequencing analysis of mycobacterial isolates selected over a significantly longer period (6 years) than previous studies. This allowed for the description of temporal evolution of mycobacterial genotypes and genetic determinants of resistance to anti-tuberculosis drugs. Using the same sequencing dataset, the study analyzed for the first time in a sample from Eastern Europe the

potential role of compensatory mutations and homoplastic mutations in the evolution of the MDR-TB epidemic in this region. Previously, associations between these mutation types and characteristics of MDR-TB strains had only been described in isolates from Central Asia and South Africa. Another innovative aspect of the study is the identification of associations between clinical management deficiencies and the emergence of resistance to new or re-introduced anti-tuberculosis drugs in TB patients treated within the National Tuberculosis Response Program in the Republic of Moldova.

Key Results Addressing a Major Scientific and Applied Problem

The vast majority of circulating MDR strains of the *M. tuberculosis complex* in the Republic of Moldova belong to relatively stable proportions (over the study period) of genotypic lineages L2 (2.2.1 Central Asia 56%, 2.2.1 Europe/Russian W148 outbreak 22%, 2.2.1 Central Asia outbreak 17%) and L4 (4.2.1 Ural 91%), which exhibit significant differences in resistance to anti-tuberculosis drugs.

- MDR strains of the *M. tuberculosis complex* belonging to L2 genotypic lineages in the Republic of Moldova are characterized by more severe imaging lesions of pulmonary tuberculosis, associated with the presence of the Rv2828c T141R mutation in the mycobacterial genome, manifesting positive selection for this mutation.

- MDR strains of the *M. tuberculosis complex* belonging to L4 genotypic lineages in the Republic of Moldova are characterized by a particularly high rate of population transmission, potentialy associated with the presence of compensatory mutations for rifampicin in the rpoC gene of the mycobacterium.

- Under programmatic management of tuberculosis patients in the Republic of Moldova, resistance of MDR strains of the *M. tuberculosis complex* to new and reintroduced anti-tuberculosis drugs like bedaquiline and linezolid is acquired secondary to MDR-TB treatment using deficient treatment regimens.

Theoretical significance

The results of this work provide theoretical arguments for the successful spread of phylogenetic lineages of *M. tuberculosis complex* L2 and L4 in the Republic of Moldova, specifically identifying genetic

determinants associated with positive selection of L2 genotypes and those advantageous for the spread of L4 genotypes. For L2 strains, the identified genetic characteristics would predispose to more severe forms of pulmonary tuberculosis (cavitary forms with increased risk of treatment failure) and potentially prolonged infectivity of these patients. In the case of L4 strains, the acquisition of compensatory mutations (rpoC) would enhance the biological adaptability of these strains, with each mentioned genetic change favoring transmission within the population. Similarly, the thesis provides a theoretical illustration of the acquisition of resistance to new anti-tuberculosis drugs, secondary to their inclusion in deficient therapeutic regimens used in MDR-TB patients.

Applicative value

Data on the specifics of geno-phenotypic correlation and their differences between the two identified genotypes (L2 and L4) in this study can find application in improving methods for testing drug susceptibility of *M. tuberculosis complex* strains. Similarly, the genetic determinants found in this study, associated with the highly efficient spread of L2 and L4 genotypes of MDR *M. tuberculosis complex* in the Republic of Moldova, can be applied in molecular epidemiological studies of tuberculosis outbreaks during epidemiological investigations. Additionally, data on the acquisition of resistance to bedaquiline and linezolid can be used by specialized physicians in the treatment of MDR-TB and by public health specialists to guide therapeutic decisions and reduce the risk of secondary resistance development to essential drugs currently used in MDR-TB treatment, as well as to new drugs to be implemented in the National Tuberculosis Control Program.

Implementation of results

The thesis results have been implemented in the National Reference Laboratory for Tuberculosis Microbiology at the Chiril Draganiuc Institute of Phthisiopneumology, resulting in two acts of implementation in scientific practice and two innovator certificates. The thesis results have been reflected in 15 scientific publications, including 3 articles in Web of Science indexed journals, 2 articles in national registry journals, and 10 theses. Additionally, research results have been presented at 12 scientific forums and events.

THESIS CONTENT

1. *MYCOBACTERIUM TUBERCULOSIS* - GENETIC DETERMINANTS OF EPIDEMIOLOGICAL AND CLINICAL ASSOCIATIONS

The chapter presents and synthesizes relevant data from the specialized literature regarding the genotypic lineages of *M. tuberculosis complex*. Molecular analysis techniques and phenotypic and genotypic drug sensitivity testing are described in detail. Known mutations in the mycobacterial genome associated with resistance to anti-tuberculosis drugs are described, as well as potential genotypic characteristics that could favor the evolutionary selection of *M. tuberculosis complex* strains.

2. GENOTYPIC DIVERSITY OF *MYCOBACTERIUM TUBERCULOSIS COMPLEX* IN THE REPUBLIC OF MOLDOVA IN THE CONTEXT OF ANTIMICROBIAL RESISTANCE DEVELOPMENT AND CLINICAL CHARACTERISTICS OF THE DISEASE

The chapter describes the methodology and research results dedicated to the phylogenetic reconstruction of MDR strains of *M. tuberculosis* complex in the Republic of Moldova and their temporal evolution, identifying associations between genotypic lineages of *M. tuberculosis* complex (MTBC) and cavitary lesions as well as negative treatment outcomes in MDR-TB patients. For this purpose, a retrospective cohort study was conducted on MTBC isolates retrieved from the biobank of the National Reference Laboratory for Tuberculosis Microbiology (LNR) in Chisinau, Republic of Moldova. Sixty isolates were randomly selected for six consecutive years (2013-2018). The samples resulting from the selection (n=360) were re-inoculated on Lowenstein-Jensen media and subjected to DNA extraction and whole-genome sequencing. MTBC strains containing mixed genotypes and/or major discrepancies between phenotypic and genotypic drug resistance profiles were excluded from the final analysis. For the remaining isolates (n=288), available epidemiological and clinical data (age, sex, TB history, relevant comorbidities, number of drugs in the therapeutic regimen, cavitary lesions on chest radiography, treatment

outcome of each patient) were extracted from the National Electronic Monitoring and Evaluation System for Tuberculosis (SIME-TB).

2.1 Phylogenetic reconstruction and transmission of drug-resistant *M. tuberculosis complex* strains in the Republic of Moldova, 2013-2018

Among the 288 analyzed strains of *M. tuberculosis complex*, 124 (43%) were classified as belonging to Lineage 2 (L2), and 164 (57%) strains belonged to Lineage 4 (L4). L2 strains included the following sublineages: Central Asia (56%), W148 outbreak from Europe/Russia (22%), Central Asia outbreak (17%), and by one strain from Ancestral 1 and Ancestral 2. The vast majority (91%) of L4 isolates belonged to a single sublineage, namely 4.2.1 (Ural). The rates of L2 and L4 strains were similar and did not change over time ($p>0.09$). The clustering rate among sequenced strains was 51.7%, higher for L4 strains (63% for L4 vs. 36.3% for L2, $p < 0.001$).

L4 strains of *M. tuberculosis complex* were part of 11 molecular clusters (phylogenetic reconstruction with a genetic distance of 5 SNPs). The largest L4 cluster comprised 75 4.2.1/Ural strains, alongside 10 smaller clusters containing 2 to 8 strains each. Among L2 genotype strains, 45 strains (36.3%) were part of 16 clusters, including 3 clusters each containing 6 strains. The clustering rate of L4 strains was higher than that of L2 strains ($p < 0.001$), (63% for L4 vs. 36.3% for L2, $p < 0.001$).

To quantify recent transmission of mycobacterial strains, the terminal branch lengths in the phylogenetic tree for L2 and L4 strains were evaluated. L4 strains had a shorter median terminal branch length compared to L2 strains (5.479×10^{-4} [95% CI (confidence interval) 3.656×10^{-4} - 2.019 x 10-3] vs. 8.233×10^{-4} [95% CI 1.826×10^{-4} - 1.097 x 10-3], $p = 0.0002$), suggesting higher transmissibility of L4 strains (Figure 2).

More L2 than L4 strains were observed to be resistant to fluoroquinolones, PAS, pyrazinamide, ethambutol, amikacin, and capreomycin (Figure 2D). Conversely, resistance to ethionamide and kanamycin was more frequent among L4 strains. No differences were found between L2 and L4 regarding resistance to cycloserine and streptomycin. Additionally, statistically significant differences were observed in the frequency of specific mutations in L2 and L4 strains conferring resistance to isoniazid, rifampicin, ethambutol, ethionamide, PAS, and kanamycin.

2.2 Genetic determinants of MTBC with potential role in the spread of multidrug-resistant strains in the Republic of Moldova

The proportion of putative compensatory mutations in rpoC was higher in L4 strains compared to L2 strains (81.1% vs. 63.7%, p=0.001), primarily due to clonal expansion of 4.2.1/Ural strains with the rpoC V483G mutation. Compensatory mutations in rpoB were more prevalent among L2 isolates compared to L4 isolates (18.5% vs. 9.1%, p=0.02). Mutations in rpoA were only present in L2 strains (6.45% vs. 0%, p=0.001). Additionally, potential compensatory mutations in thyX were more commonly found in L2 strains (29.03% vs. 1.2%, p<0.00001). Mutations in the ahpC gene promoter region were identified in only 3 isolates (two L4 and one L2).

Specifically, isolates with a compensatory mutation in rpoC had a higher clustering rate than isolates without such a mutation (57.2% vs. 33.3%, p=0.0007) (Figure 3 B). By genetic lineage, this observation was statistically significant only in L4 isolates (67.7% vs. 45.2%, p=0.02) and not in L2 isolates (41.8% vs. 26.7%, p=0.1). Strains with rpoA mutations had a lower clustering rate and longer terminal branch lengths (0% vs. 53.2%, p=0.003) and ($2.2*10^{-3}$ vs. $6.09*10^{-4}$, p=0.0002), respectively (Figure 3 C, G). It is noteworthy that the number of strains with rpoA mutations was limited (only 8 strains, all belonging to L2). Thus, comparing the clustering rate and terminal branch lengths between L2 isolates with rpoA compensatory mutations and those without mutations showed a significant difference only for terminal branch lengths ($2.2*10^{-3}$ [95% CI $2.0*10^{-3}$ - $2.3*10^{-3}$] vs. $7.3*10^{-4}$ [95% CI $3.6*10^{-4}$ - $1.6*10^{-3}$], p=0.002, respectively) and not for the clustering rate (Figure 3 G, K).

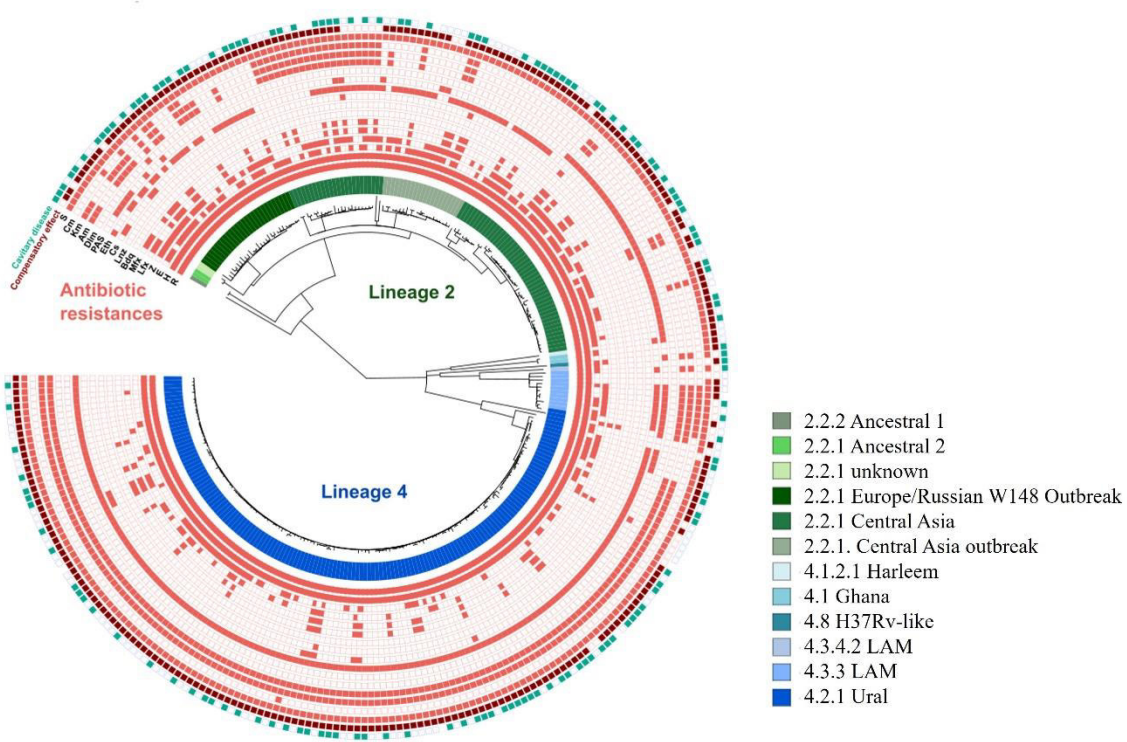

Figure 1. **Phylogenetic tree of MTBC M/XDR strains in the Republic of Moldova, 2013-2018**

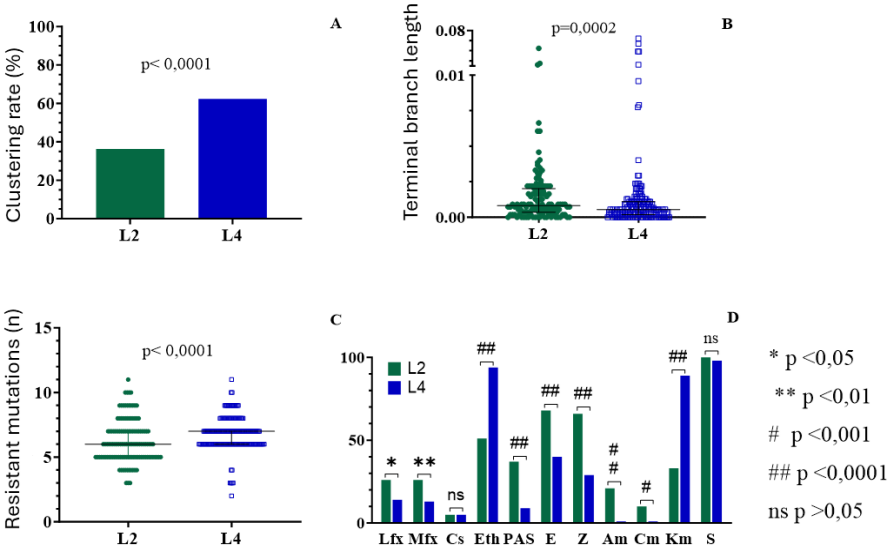

Figure 2. **MTBC transmission and drug resistance in the Republic of Moldova 2013-2018**

There was no difference in the presence/absence of rpoA compensatory mutations segregated by the number of drug-resistant mutations in the entire cohort and in each of the two main lineages (Figure 3 L). Strains with a mutation in the thyX gene promoter region had fewer drug resistance mutations than strains without thyX mutations (6 [95% CI 5-7] vs. 7 [95% CI 6-8], p=0.005); no differences were observed in clustering rate or terminal branch lengths compared to strains without thyX mutations (Figure 3 H, M). Similarly, L2 strains with mutations in the thyX gene promoter had a higher clustering rate (61.1% vs. 26.1%, p=0.0004) and shorter terminal branch lengths ($5.4*10^{-4}$ [$1.8*10^{-4}$ - $8.6*10^{-4}$] vs. $1.0*10^{-3}$ [$5.4*10^{-4}$ - $2.2*10^{-3}$], p<0.0001) compared to L2 strains without such mutations, while L4 strains with mutations in the thyX promoter region had longer terminal branch lengths than those without such mutations ($2.0*10^{-2}$ [$2.0*10^{-2}$ - $3.8*10^{-2}$] vs. $5.4*10^{-4}$ [$1.8*10^{-4}$ - $1.0*10^{-4}$], p=0.02) (Figure 3 H, M).

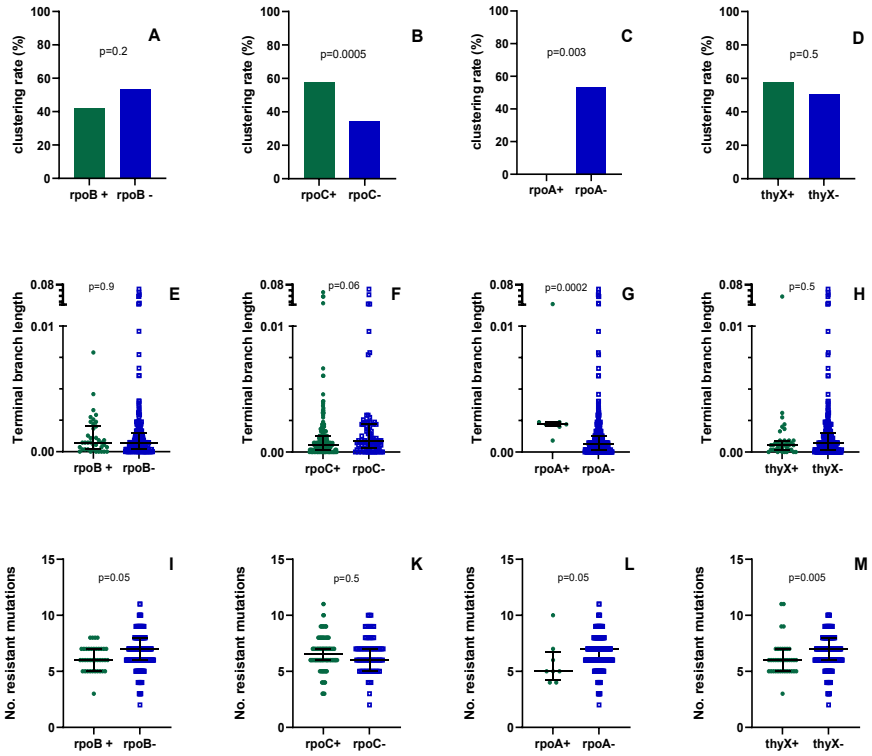

Figure 3. **Compensatory mutations and transmissibility in the entire cohort of *M. tuberculosis complex* strains**

Homoplasy and Possible Positive Selection

In the sequenced strains, 145 mutations showing signs of homoplasy (identical mutations in subgroups without phylogenetic linkage) were detected, which could indicate positive selection at these genomic loci. Of these, 75 out of 145 mutations were not considered, as they were synonymous mutations or mutations located in repetitive regions such as the PE/PPE gene family, pseudogenes, insertion sequences, and phages, as well as undifferentiated SNPs for each genetic lineage.

Each of the 70 homoplastic mutations considered for the final analysis was found in a variable number of the analyzed genomes, ranging from 2 to

285 strains out of the total 288, resulting in a total of 2624 homoplastic alleles across the cohort. Fifty-three (75.7%) of these SNPs (1822 alleles; 69.4%) were present in coding regions of the 25 annotated genes, while 17 SNPs were found in intergenic regions. Forty-three SNPs showing positive selection were identified in 15 genes associated with resistance, and 7 SNPs in 4 genes known to compensate for fitness deficits induced by mutations associated with rifampicin (rpoC, rpoB, rpoA) and PAS resistance (thyX) (Figure 3).

Homoplastic mutations in prpR (Rv1129c I433T and H355R), a gene associated with drug tolerance [11], were observed, with both mutations evolving independently in two L2 isolates. Overall, both L2 and L4 isolates had a similar median number of homoplastic SNPs (9 [95% CI 8-10] vs. 9 [95% CI 9-10], $p < 0.9$, respectively).

The ratio of homoplastic SNPs to total differentiating SNPs was analyzed according to genetic lineages (phylogenetic SNPs L2 and L4 were not considered). In this context, L2 strains had a higher ratio ($p<0.0001$) of homoplastic SNPs to all differentiating SNPs (0.039 [95% CI 0.038 - 0.043]) compared to L4 isolates (0.047 [95% CI 0.039 - 0.052]) (Figure 4A).

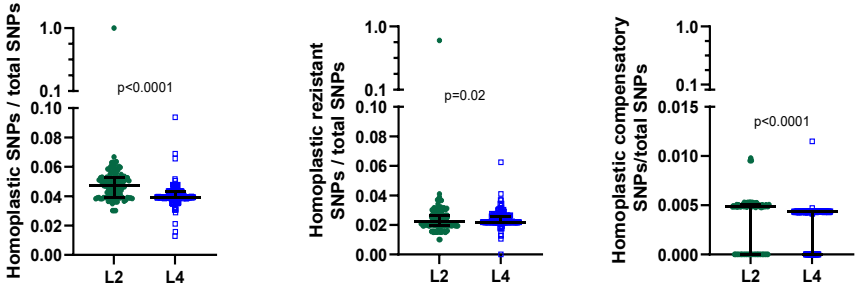

Figure 4. **Homoplasy and positive selection by MTBC lineage**

This difference was also observed when comparing homoplastic SNPs in resistance genes ($p = 0.03$) and compensatory genes ($p < 0.0001$), suggesting increased positive selection for L2 isolates (Figure 4 B, C).

It is worth noting that the Rv2828c T141R mutation, identified in the homoplasy analysis of this study and previously associated with more

widespread radiological pathology [11], was found almost exclusively among L2 isolates (98% in L2 vs. 1.2% in L4, $p < 0.0001$).

2.3 Associations between genotypic determinants impacting MTBC antimicrobial resistance and clinical course of tuberculosis

Within the study, potential associations between the genotypic lineage of *M. tuberculosis complex* and clinical characteristics of tuberculosis disease were evaluated in patients from whom the analyzed strains were isolated. The basic clinical-demographic characteristics of the cohort of MDR-TB patients included in the study were characterized by a young age (median 33 years [95% CI, 42-53]), male gender (79.2%), and presence of cavitary lesions on chest radiography (51.6%). Among these patients, 7.4% were HIV-positive. Fifty-nine percent of patients were newly diagnosed tuberculosis cases. The cure rate in the studied cohort was 51.8%, while treatment completion was 4.4%, treatment failure cases constituted 7.7%, and those lost to follow-up and deaths were 19.5% and 16.5%, respectively.

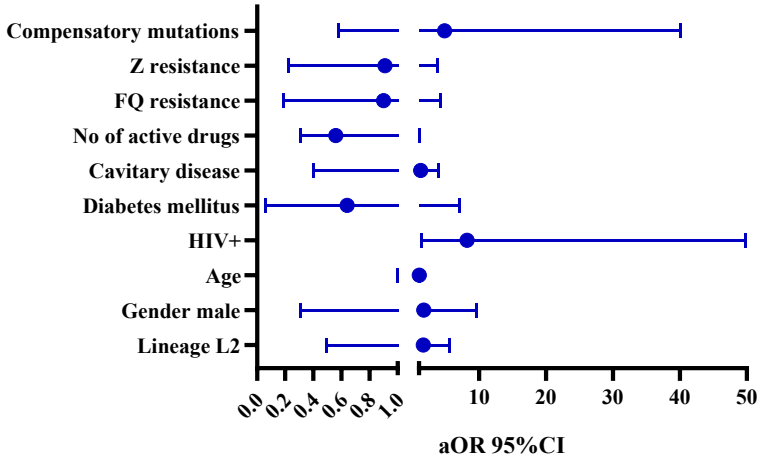

Figure 5. **Associations with negative treatment outcomes, new cases**

To confirm the representativeness of the randomly selected cohort for this study, all available clinical parameters of the study patients (n=288) were compared with those of MDR-TB patients diagnosed during 2013-2018 but not included in the study (n=5608). Thus, there were no significant differences regarding patient gender, frequency of cavitary lesions and diabetes mellitus, as well as the number of patients with disease recurrence and those treated after being lost to follow-up. However, patients in the study cohort were on average 3 years older, had a lower rate of TB/HIV coinfection, but a higher rate of new cases and a lower rate of patients after previous treatment failure.

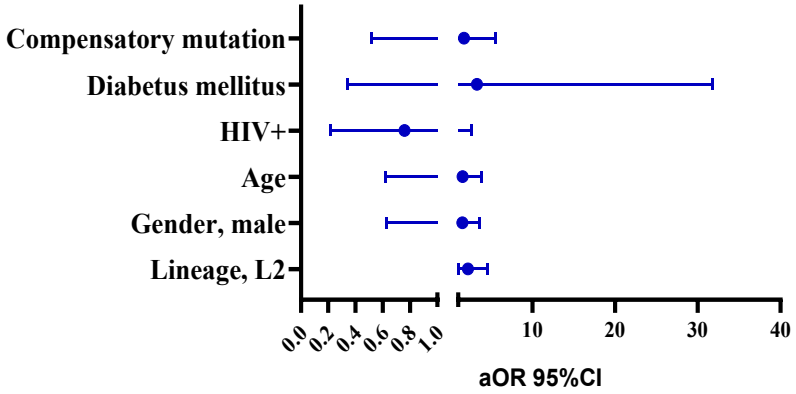

Figure 6. **Associations with cavitary lesions, new cases**

In the univariate regression, no significant differences were observed in variables such as age, sex, HIV status, diabetes mellitus, or previous episodes of TB between patients with different genotypic lines of the *M. tuberculosis complex*. However, the analysis showed that patients infected with the L2 genotypic line of *M. tuberculosis complex* had a higher probability of presenting with cavitary lesions and a higher treatment failure rate compared to those infected with L4. This association remained significant in the multivariate model, adjusting for covariates including age, sex, HIV status, diabetes mellitus, and the presence of compensatory mutations.

In the first predictive model for unfavorable outcomes of anti-tuberculosis treatment, an association was demonstrated between treatment outcome and age (OR 1.06 per year, 95% CI 1.00-1.11, p=0.04), as well as HIV status of the patient (OR 8.19, 95% CI 1.34-49.80, p=0.02) (Figure 5).

In the second logistic regression model (associating with cavitary lesions), a significant association was observed with the L2 genotypic line (OR 2.20, 95% CI 1.07-4.55, p=0.03) (Figure 6).

3. DEVELOPMENT OF BEDAQUILINE RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS* STRAINS IN THE REPUBLIC OF MOLDOVA

The chapter presents the results of a cross-sectional cohort study investigating the acquisition of resistance to bedaquiline among patients with MDR-TB who received bedaquiline as part of their treatment regimen, along with an analysis of risk factors associated with treatment failure and death in patients with bedaquiline-resistant TB. Whole genome sequencing (WGS) and phenotypic testing of *M. tuberculosis complex* strains isolated from sputum samples of MDR-TB patients treated with bedaquiline in the Republic of Moldova between 2016 and 2018 were conducted for this purpose.

The study included isolates stored in the Biobank of the National Reference Laboratory for Tuberculosis Microbiology (LNR) in Chisinau, Republic of Moldova, from all MDR-TB patients who started a bedaquiline-containing treatment regimen during the specified period and had at least one *M. tuberculosis complex* isolate stored from sputum collected before treatment initiation for that episode of disease.

Out of the total MDR-TB patients treated from 2016 to 2018, 203 (6.8%) received bedaquiline as part of their MDR-TB treatment regimen. Consequently, 82/203 (40.4%) *M. tuberculosis complex* strains were identified for study purposes. Isolates from the remaining 121 patients were not available in the LNR Biobank. For 9 of these patients, only post-treatment isolates were available, hence they were excluded from the final analysis. Additionally, *M. tuberculosis complex* isolates from another eleven patients were excluded due to failure to grow or contamination.

Thus, the final analyzed cohort included 18 new cases of TB and 44 retreatments (total 62 isolates). The median age of patients from whom the analyzed strains were isolated was 39 years (interquartile range (IQR) 34-45 years). The majority of patients were male 50/62 (80.6%), diagnosed with cavitary disease 45/62 (72.6%), and HIV-seronegative 54/62 (87.1%).

To assess the representativeness of the study cohort, available clinical and epidemiological characteristics of patients with included isolates were compared with those of patients diagnosed during the reference period but not included in the study. Analyzed characteristics included residence, sex, age, microscopy results, case definition, HIV status, and treatment outcome. No significant differences were observed between the included and excluded study cohorts ($p > 0.09$).

Based on WGS results, 56.5% of patients were infected with an *M. tuberculosis complex* Lineage 2 (L2) strain (35/62), while the remaining 43.5% (27/62) were infected with a Lineage 4 (L4) strain.

Phenotypic and genotypic resistance to bedaquiline:

The study identified nine *M. tuberculosis complex* strains with mutations in the atpE and/or Rv0678 genes. One isolate had mutations only in atpE, six strains had mutations only in Rv0678, and two strains had mutations in both genes. Eight *M. tuberculosis complex* strains had a Minimum Inhibitory Concentration (MIC) for BDQ of 2.0 mg/L or higher, as determined by MGIT960 testing. Concurrently, one isolate (CAR-84) with two mutations in Rv0678 was found to be sensitive to bedaquiline (MIC 1.0 mg/L) (Figure 7).

Notably, seven isolates had multiple mutations in atpE and/or Rv0678 with varying frequencies, suggesting the presence of distinct subpopulations in these patients. All bedaquiline-resistant *M. tuberculosis complex* strains were obtained after initiation of MDR-TB treatment regimens containing bedaquiline, within a timeframe of 77 to 451 days post-treatment initiation. Among the 26 patients with MTBC isolates available both before and after bedaquiline treatment initiation, the baseline and follow-up strains differed by a maximum of 4 SNPs. Four patients (15.4%) were likely reinfected with a second strain, exhibiting a difference

of 26-1126 SNPs compared to the baseline strain.

In total, 4/26 (15.4%) of MTBC isolates acquired resistance to bedaquiline after 90, 159, 348, and 451 days of bedaquiline administration. One post-treatment isolate (patient 29) with the atpE p.I66M mutation (97% frequency) was phenotypically resistant to bedaquiline but sensitive to clofazimine.

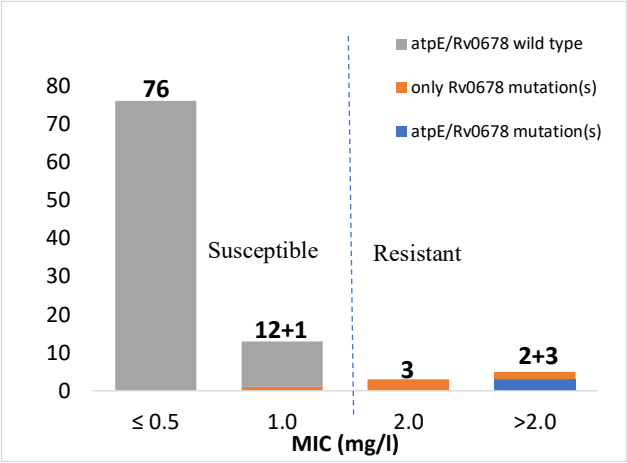

Figure 7. **Distribution of bedaquiline minimum inhibitory concentration (MIC) for MTBC strains and sequencing results (n=97)**

Another follow-up isolate (patient 12) harbored three mutations in the Rv0678 gene with varying frequencies (p.D5fs - 57%, p.G24D - 19.8%, and p.S64fs - 13%). Two other post-exposure isolates acquired mutations atpE p.A63P (25%) combined with Rv0678 p.S64fs (2%) (patient 2), and atpE p.E61D (28%), atpE p.I66M (3%), Rv0678 p.S63fs (5%), and p.S64fs (44%) (patient 57).

Among the four patients reinfected with a different MTBC strain, patient 37 was reinfected with a bedaquiline-resistant strain carrying the Rv0678 p.T58P mutation at a frequency of 100%. Although the second isolate had the same genotype (L 2.2.1/Europe/Russia W148 Outbreak), a genetic distance of 26 SNPs from the baseline isolate clearly indicated reinfection (Table 1).

Table 1. **Results of genotypic and phenotypic testing for bedaquiline and clofazimine of MTBC strains isolated before and after inclusion of bedaquiline in the therapeutic regimen in MDR TB patients, 2016 - 2018**

Patient no., (isolate ID), sampling time	**Rv0678 (mutation frequency %)**	**atpE (mutation frequency %)**	**BDQ MIC MGIT960 (mg/mL)**	**CFZ MIC MGIT960 (mg/mL)**
Patient29, (CAR-13), prior bedaquiline	wild type	wild type	≤0,5 (S)	≤0,5 (S)
Patient29, (CAR-38), after bedaquiline, acquired resistance	wild type	I66M# (97%)	>2,0	≤0,5 (S)
Patient12, (CAR-52), prior bedaquiline	wild type	wild type	≤0,5 (S)	≤0,5 (S)
Patient12, (CAR-61) after bedaquiline, acquired resistance	16_del_g (57.4%); 193_del_g# (12.7%); G24D (19.8%)	wild type	2.0	1.0 (S)
Patient2, (CAR-78), prior bedaquiline	wild type	wild type	≤0,5 (S)	≤0,5 (S)
Patient2, (CAR-87), after bedaquiline, acquired resistance	192insG# (2%)	A63P# (25%)	>2,0 (R)	2.0 (R)
Patient37, (CAR-10), prior bedaquiline	wild type	wild type	≤0,5 (S)	≤0,5 (S)
Patient37, (CAR-18), after bedaquiline, re-infection	T58P (100%)	wild type	2.0 (R)	1.0 (S)
Patient57, (CAR-45), prior bedaquiline	wild type	wild type	≤0,5 (S)	≤0,5 (S)
Patient57, (CAR-55), after bedaquiline, acquired resistance	193_del_g# (44.4%); S63G# (5.5%)	E61D# (27,5); I66M# (2,6%)	>2,0 (R)	1.0 (S)
Patient32, (CAR-84), after bedaquiline	192_ins_g# (74.2%); 193_del_g# (5.7%)	wild type	1.0 (S)	1.0 (S)
Patient71, (CAR-40), after bedaquiline	192ins_g# (23.8%); L142P (64%)	wild type	>2,0 (R)	2.0 (R)
Patient61, (CAR-1), after bedaquiline	136_ins_g (7,1%); 141_ins_c# (69,0%); 195_ins_t# (5,8%); G66W (6.0%)	wild type	>2,0 (R)	2.0 (R)
Patient33, (CAR-43), after bedaquiline	436_ins_t (90.2%); R72W (28.5%)	wild type	>2,0 (R)	>2,0 (R)

4. LINEZOLID RESISTANCE OF *M. TUBERCULOSIS* RISK FACTORS AND GENETIC DETERMINANTS

In this analysis, all MTBC strains isolated and stored between 2017-2018 in the LNR biobank were identified, obtained from sputum of adult MDR-TB patients who received linezolid (LNZ) in their treatment regimen for any duration over the past two years and had a cumulative exposure to linezolid exceeding 30 days. Only one MTBC strain isolated from sputum was included per eligible patient.

The MTBC strains included in the study were isolated from 52 patients with a mean age of 38.8 ± 8.5 years, of whom 67.3% were male. The majority of patients (84.6%) had cavitary pulmonary lesions detected on chest radiography, and 17.3% were HIV-positive.

The median number of prior linezolid doses administered to patients included in the study was 347 (IQR 165-470.5). Simultaneously, the median number of active drugs included in the treatment regimen with linezolid for the analyzed patients was 3.0 (IQR 2.0-4.0).

The rate of MTBC strains isolated from patients during treatment failure was higher among LNZ-resistant strains - 91.7% compared to LNZ-sensitive strains - 60%. Patients with linezolid-resistant strains had a higher number of prior linezolid doses administered and a lower number of active drugs in their treatment regimen compared to those with linezolid-sensitive strains (576.5 (IQR 404.5-705.5) vs 257.5 (IQR 120.5-376.5), $p = 0.0001$ and 1.5 (IQR 1.0-3.0) vs 4.0 (IQR 2.5-4.0), $p = 0.0001$, respectively). No other significant differences were observed between patients with linezolid-resistant and linezolid-sensitive strains, except for their ages (33.5 ± 5.3 vs 39.9 ± 8.8, $p = 0.01$).

The majority of analyzed strains belonged to Lineage 2 genotypic lineage - 63.5%, with the remaining strains belonging to Lineage 4. WGS confirmed the MDR profile of all 52 MTBC strains included in the study. Of these, 72% exhibited compensatory mutations in the rpoA and rpoC genes.

According to sequencing data, 12 (23.1%) MTBC strains were resistant to linezolid. The number of strains with genotypic resistance to linezolid (12/52) differed from those detected by phenotypic DST based on culture, which identified 15 linezolid-resistant strains. This difference was attributed to specific genetic mutations, such as the T460C substitution in the rplC gene and other mutations in the rrl gene. For 8 linezolid-resistant strains, the T460C substitution mutation was found in the rplC gene, while in 4 other strains, multiple substitution mutations were found at different positions in the rrl (Rvnr02) gene encoding ribosomal protein L4.

In one strain with phenotypic resistance to linezolid, no mutations were found in the rplC and rrl genes, while in another strain with phenotypic

resistance, two nucleotide substitution mutations were detected at different positions in the rrl gene: 2814G>T (98%); 2810A>C (98%), with no mutations found in the rplC gene for both strains.

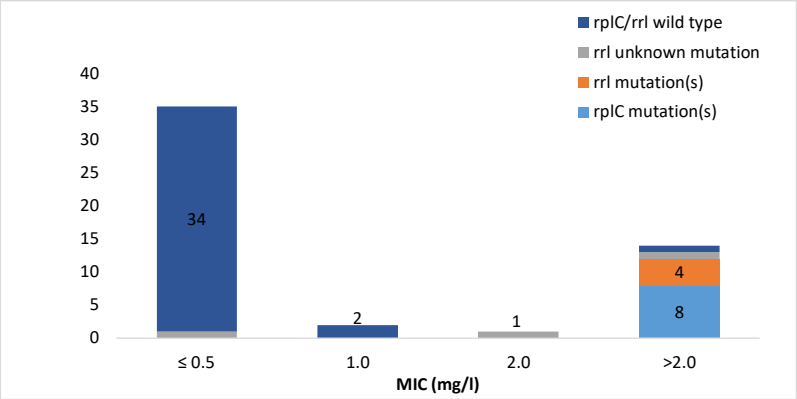

Figure 8. **Distribution of minimum inhibitory concentrations (MICs) of linezolid for MTBC strains and sequencing results**

For two other phenotypically sensitive strains, mutations 2691A>T (100%) and 219G>C (1.3%) were identified, these have not been previously described in the literature, thus classified as linezolid-sensitive strains.

All strains with resistant mutations in the rplC gene had a linezolid MIC greater than 2 mg/l, as did strains with resistant mutations in the rrl gene. The majority of strains without mutations in rplC and rrl had a linezolid MIC ≤ 0.5 mg/l. The strain with phenotypic resistance but no mutations found in the rplC or rrl genes had a linezolid MIC of 2 mg/l (Figure 8).

It is noteworthy that a significant proportion (76.9%) of linezolid-resistant strains also showed additional resistance to fluoroquinolones.

To identify risk factors associated with linezolid resistance in initial MDR MTBC strains, the following clinical and microbiological variables were evaluated through univariate logistic regression: gender, age, place of residence, presence of cavities on radiography, MTBC genotypic lineage, presence of compensatory mutations, number of doses of linezolid administered, history of interruption of linezolid treatment for more than 2

months, number of active drugs in the tuberculosis treatment regimen (evaluated based on sequencing data), and HIV status (Figure 9). Among the variables analyzed, four showed a significant association in the univariate analysis - patient age, number of doses of linezolid administered, number of drugs in the treatment regimen, and HIV status ($p<0.25$).

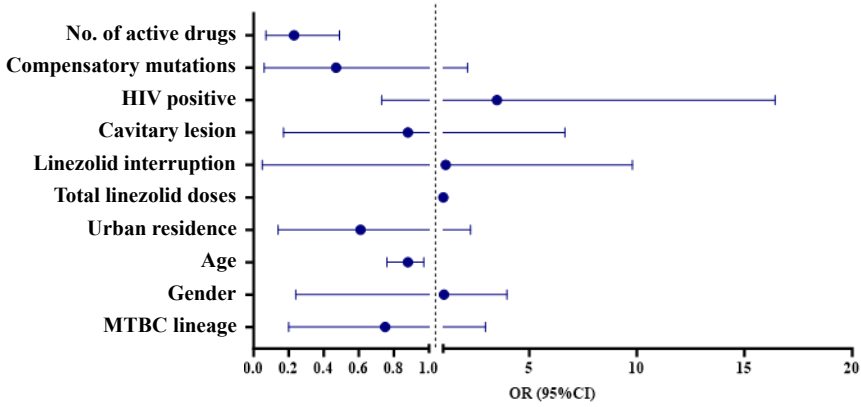

Figure 9. **Risk factors for linezolid resistance**

Subsequently, these variables were evaluated in a multivariate logistic regression model which highlighted a significant association between linezolid resistance and the number of active drugs in the MDR-TB treatment regimen (OR 0.23; 95% CI 0.03 - 0.70; $p = 0.04$). A weaker association was found with the number of previously administered linezolid doses (OR 1.01; 95% CI 1.004 - 1.03; $p = 0.03$). At the same time, no significant associations were found for the other two tested factors (age and HIV status).

Based on WGS data, it was determined that the median number of active drugs in treatment regimens is significantly lower than the number calculated based on phenotypic sensitivity test results and presumed sensitivity to untested drugs. This means that in practice, patients receive fewer active drugs than initially estimated based on laboratory tests.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

1. MDR strains of *Mycobacterium tuberculosis complex* in the Republic of Moldova are predominantly represented by genotypes L2 and L4 (Ural), with their proportion remaining stable over the six-year observation period of this study.
2. There are significant differences in phenotypic resistance as well as genotypic determinants to anti-tuberculosis drugs between genotypic lineages L2 and L4 of *Mycobacterium tuberculosis complex*, indicating differences in adaptability to applied treatments.
3. Pulmonary tuberculosis caused by L2 strains is associated with more severe clinical characteristics, such as a higher frequency of cavity lesions and a higher treatment failure rate compared to L4 strains, with potential genetic determinant for this fact.
4. Lineage L4 exhibits a higher rate of resistance and transmission, possibly due to the presence of compensatory mutations that promote bacterial adaptation to anti-tuberculosis treatments.
5. Resistance acquisition to newer anti-tuberculosis drugs such as bedaquiline occurs shortly after introduction into clinical practice. This can reach rates of up to 15% in MDR tuberculosis patients, with therapy failure in these patients being associated with the presence of cavitary disease and a reduced number of confirmed sensitive drugs in the treatment regimen.
6. Linezolid resistance in *Mycobacterium tuberculosis complex* strains is common in patients previously treated with this drug, and is associated with a suboptimal number of active drugs in the previous anti-tuberculosis treatment regimen containing linezolid.

Recommendations:

1. Implement systematic surveillance and monitoring of MDR strains of *Mycobacterium tuberculosis complex*, with a focus on L2 and L4

genotypes, to identify the evolution of drug resistance and guide therapeutic strategies.

2. Develop a personalized approach to anti-tuberculosis treatment, considering specific mutations of *Mycobacterium tuberculosis complex* strains, to maximize therapeutic effectiveness and minimize the risk of resistance acquisition.

3. Implement rapid molecular techniques, potentially based on mycobacterial genome sequencing, to detect *Mycobacterium tuberculosis complex* sensitivity to the full spectrum of anti-tuberculosis drugs used in MDR tuberculosis treatment.

4. Adhere to current guidelines for MDR tuberculosis treatment, including ensuring an adequate number of drugs to prevent secondary resistance to key anti-tuberculosis medications.

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- **Articles in scientific journals abroad:**

✓ **articles in ISI journals, SCOPUS and other international databases**

1. **Chesov, E**., Chesov, D., Reiman, M., Dreyer V., Utpatel, C., Groschel M., Ciobanu N., Crudu, V., Lange, C., Heyckendorf, J., Merker, M. Impact of *Mycobacterium tuberculosis* strain type on multidrug-resistant tuberculosis severity, Republic of Moldova. In Journal of Infection, 2023, no 87(6), pp 588-591. ISSN 0163-4453. **(IF: 28,2)**
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Articles in accredited national scientific journals:

1. **Chesov, E.,** Ciobanu, N., Chesov, D., Lange C., Heyckendorf, J., Merker, M., Crudu, V. Rezistenţa *Mycobacterium tuberculosis* la linezolid - mutaţii asociate și factori de risc: studiu transversal, retrospectiv, analitic. În: Revista de Ştiinţe ale Sănătăţii din Moldova. 2021, nr. 26, pp. 43-56. ISSN 2345-1467.
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ADNOTARE

Elena Chesov

„Diversitatea genotipică și rezistența la medicamente a tulpinilor de *Mycobacterium tuberculosis*”

Teză de doctor în științe medicale, Chișinău, 2024

Structura tezei: introducere, patru capitole, concluzii generale și recomandări, bibliografie din 210 surse, două anexe, 15 tabele și 22 figuri. Rezultatele sunt publicate în 15 lucrări științifice.

Cuvinte cheie: TB, *M. tuberculosis complex,* MDR, diagnostic de laborator, tratament antituberculos, secvențierea întregului genom, factori de risc, linii genotipice.

Domeniul de studiu: 313.02 – Microbiologie, virusologie medicală.

Scopul lucrării: De a studia diversitatea genotipică și caracteristicile molecular-genetice ale tulpinilor de *Mycobacterium tuberculosis* din Republica Moldova cu impact asupra fenomenului de rezistență.

Obiectivele lucrării: 1. Aprecierea diversității genotipice și a evoluției temporale a tulpinilor multidrog-rezistente de *Mycobacterium tuberculosis* în Republica Moldova. 2. Identificarea determinantelor genetice ale *Mycobacterium tuberculosis* cu rol putativ în răspândirea tulpinilor multidrog-rezistente în Republica Moldova. 3. Stabilirea potențialelor asocieri dintre determinantele genotipice cu impact asupra rezistenței la antimicrobiene a *Mycobacterium tuberculosis* și evoluția clinică a tuberculozei. 4. Identificarea corespondentelor genotipice ale rezistenței micobacteriene la medicamentele cheie utilizate în tratamentul tuberculozei multidrog-rezistente.

Noutatea și originalitatea științifică: Rezultatele prezentului studiu descriu diversitatea genotipică a *M. tuberculosis complex* din Republica Moldova în baza analizei datelor de secvențiere a întregului genom micobacterian (Whole Genome Sequencing) obținute în baza unui lot de izolate micobacteriene selectate dintr-o perioadă de timp semnificativ mai lungă (6 ani) decât în studiile anterioare. Utilizând același set de date de secvențiere a fost analizat pentru prima dată un lot de izolate *M. tuberculosis complex* provenit din Europa de Est, a fost determinat rolul putativ al mutațiilor compensatorii și a unor mutații homoplazice în evoluția endemiei de TB MDR în această regiune. La fel, elementul novatoriu al lucrării este asigurat de evidențierea asocierilor dintre deficiențele de management clinic și apariția rezistenței la preparatele antituberculoase, la bolnavii TB, tratați în cadrul Programului național de răspuns la tuberculoză din Republica Moldova.

Rezultatele noi pentru știință și practică: Majoritatea covârșitoare a tulpinilor MDR de *M. tuberculosis complex* circulante în Republica Moldova aparțin într-o proporție relativ stabilă (pe durata observată în studiu) liniilor genotipice L2 și L4, care manifestă diferențe semnificative în rezistența la medicamentele antituberculoase. Tulpinile MDR ale *M. tuberculosis complex* ce aparțin liniei genotipice L2, circulante în Republica Moldova, se caracterizează prin forme cu leziuni imagistice mai severe de tuberculoză pulmonară, fapt asociat cu prezența în genomul micobacterian a mutației Rv2828c T141R, fiind observată o selecție pozitivă pentru această mutație. Tulpinile MDR ale *M. tuberculosis complex* ce aparțin liniilor genotipice L4, circulante în Republica Moldova, se caracterizează printr-o rată deosebit de mare de transmitere în populație, unul dintre factorii potențiali asociați cu succesul epidemiologic al acestor tulpini fiind prezența mutațiilor compensatorii pentru rifampicină în gena rpoC a micobacteriei.

În condițiile managementului programatic al bolnavilor de tuberculoză în Republica Moldova, rezistența tulpinilor de *M. tuberculosis complex* la medicamentele noi și repropuse precum bedaquilina și linezolidul este achiziționată secundar tratamentului TB MDR prin aplicarea unor scheme de tratament deficitare.

Semnificația teoretică: Rezultatele acestei lucrări oferă argumentarea teoretică pentru succesul răspândirii liniilor filogenetice de *M. tuberculosis complex* L2 și L4 în Republica Moldova, în special identificând unele determinante genetice asociate cu selecția pozitivă a genotipurilor L2 precum și a altora care avantajează răspândirea genotipurilor L4. La fel, teza aduce ilustrarea teoretică a achiziționării rezistenței la medicamentele antituberculoase noi, secundar includerii acestora în scheme terapeutice deficitare utilizate la bolnavii de TB MDR.

Valoarea aplicativă: Datele privitor la particularitățile corelației rezistenței geno-fenotipice și diferențele acestora dintre cele două genotipuri identificate (L2 și L4) în prezentul studiu își pot găsi aplicația în perfecționarea metodelor de testare a sensibilității la medicamente a tulpinilor *M. tuberculosis complex,* îmbunătățirii anchetării epidemiologice a focarelor de tuberculoză și identificarea măsurilor de reducere a riscurilor de achiziționare a rezistenței la medicamentele antituberculoase.

Implementarea rezultatelor: Rezultatele tezei au fost implementate în cadrul Laboratorului Național de referință în Microbiologia Tuberculozei din cadrul Institutului de ftiziopneumologie ”Chiril Draganiuc”, fiind obținute două acte de implementare în procesul științifico-practic și două certificate de inovator.

АННОТАЦИЯ

Елена Кесов

"Генотипическое разнообразие и лекарственная устойчивость штаммов *Mycobacterium tuberculosis*."

Диссертации на соискание ученой степени кандидата медицинских наук, Кишинев, 2024 год.

Структура диссертации: введение, четыре главы, общие выводы и рекомендации, библиография из 210 источников, два приложения, 15 таблиц и 22 рисунков. Результаты диссертации опубликованы в 15 работах.

Ключевые слова: ТБ, *M. tuberculosis*, МЛУ, лабораторная диагностика, противотуберкулезное лечение, полногеномное секвенирование, факторы риска, генотипические линии.

Область исследования: 313.02 – Микробиология, медицинская вирусология.

Цель работы: Изучение генотипического разнообразия и молекулярно-генетических характеристик штаммов *Mycobacterium tuberculosis* в Республике Молдова, влияющих на лекарственную устойчивость.

Задачи работы: 1. Оценка генетического разнообразия и временной эволюции штаммов *Mycobacterium tuberculosis* с множественной лекарственной устойчивостью в Республике Молдова. 2. Идентификация генетических детерминант *Mycobacterium tuberculosis*, играющих роль в распространении штаммов с множественной лекарственной устойчивостью в Республике Молдова. 3. Установление потенциальных ассоциаций между генетическими детерминантами, влияющих на лекарственную устойчивость *Mycobacterium tuberculosis*, и клиническим течением туберкулеза. 4. Идентификация генетических изменении обусловливающих устойчивость *Mycobacterium tuberculosis* к ключевым препаратам, используемым в лечении туберкулеза с множественной лекарственной устойчивостью.

Научная новизна и оригинальность: Результаты данного исследования описывают генотипическое разнообразие *M. tuberculosis complex* в Республике Молдова на основе анализа данных полногеномного секвенирования *M. tuberculosis complex* (Whole Genome Sequencing), полученных на основе выборки микобактериальных штамов, за значительно более длительного периода (6 лет) по сравнению с предыдущими исследованиями. Кроме того, впервые была определена потенциальная роль компенсаторных мутаций и гомоплазматических мутаций в эволюции эндемии МЛУ ТБ в этом регионе. Также инновационный элемент работы состоит в выявлении ассоциаций между отрицательными клиническими проявлениями туберкулеза и появлением устойчивости к противотуберкулезным препаратам у пациентов с ТБ, леченных в рамках Национальной Программы по Борьбе с Туберкулезом в Республике Молдова.

Новые результаты для науки и практики: Подавляющее большинство МЛУ штаммов *M. tuberculosis*, циркулирующих в Республике Молдова, принадлежат к генотипических линиям L2 и L4, в относительно стабильном соотношении (на протяжении наблюдаемого периода в данном исследовании), которые характеризуются значительными различиями в резистентности к противотуберкулезным препаратам. МЛУ штаммы *M. tuberculosis*, принадлежащие генотипической линии L2, циркулирующие в Республике Молдова, характеризуются более тяжелыми радиологическими поражениями легких, что связано с наличием мутации Rv2828c T141R в микобактериальном геноме, с наблюдением положительной селекции для этой мутации. МЛУ штаммы *M. tuberculosis*, принадлежащие генотипической линии L4, циркулирующие в Республике Молдова, характеризуются особенно высокой степенью передачи в населении, одним из потенциальных факторов, ассоциированных с эпидемиологическим успехом этих штаммов, является наличие компенсаторных мутаций для рифампицина в гене rpoC микобактерии.

В условиях программного лечения больных туберкулезом в Республике Молдова резистентность штаммов *M. tuberculosis complex* к новым и препаратам, таким как бедаквилин и линезолид, приобретается вторично во время лечения МЛУ ТБ вследствие применения неполноценных схем лечения.

Теоретическое значение: Результаты данной работы предоставляют аргументы, объясняющие успех распространения филогенетических линий L2 и L4 *M. tuberculosis complex* в Республике Молдова, в частности, были идентифицированы генетические особенности, ассоциированные с положительной селекцией генотипов L2, а также те, которые способствуют распространению генотипов L4. Также диссертация дает теоретическое объяснение приобретению резистентности к новым противотуберкулезным препаратам, вторично использованию неполноценных терапевтических схем лечения, у больных с МЛУ ТБ.

Прикладная ценность: Данные касающиеся корреляции гено-фенотипической устойчивости и их различиях между двумя выявленными генотипами (L2 и L4) полученные в данном исследовании могут быть применены для улучшения методов тестирования чувствительности штаммов *M. tuberculosis complex* к противотуберкулезным препаратам. Также представление данные могут быть применены для улучшения эпидемиологического исследования очагов туберкулеза и выявление мер по снижению рисков приобретения устойчивости к противотуберкулезным препаратам.

Внедрение результатов: Результаты диссертации были внедрены в Национальной Лаборатории по Микробиологии Туберкулеза при Институте Фтизиопульмонологии им. Кирила Драганюка, в Кишиневе Республика Молдова. Также были получены два свидетельства о внедрении результатов диссертации в научно-практический процесс и два сертификата о инновации.

SUMMARY

Chesov Elena

"Genotypic Diversity and Drug Resistance of *Mycobacterium tuberculosis* Strains"

PhD Thesis in Medical Sciences, Chisinau, 2024

Thesis structure: introduction, four chapters, general conclusions and recommendations, bibliography 210 sources, two annexes, 15 tables, and 22 figures. The results are published in 15 papers.

Keywords: TB, *M. tuberculosis*, MDR, laboratory diagnostics, anti-tuberculosis treatment, whole genome sequencing, risk factors, genotypic lineages.

Field of study: 313.02 – Microbiology, medical virology.

Aim: To study the genotypic diversity and molecular-genetic characteristics of *Mycobacterium tuberculosis* strains from the Republic of Moldova with impact on mycobacterial drug resistance.

Objectives: 1. To assess the genotypic diversity and temporal evolution of multidrug-resistant *Mycobacterium tuberculosis* strains in the Republic of Moldova. 2. To identifying the genetic determinants of *Mycobacterium tuberculosis* potentially involved in the spread of multidrug-resistant strains in the Republic of Moldova. 3. To establish potential associations between genotypic determinants impacting the antimicrobial resistance of *Mycobacterium tuberculosis* and the clinical evolution of tuberculosis. 4. To identify genotypic correlates of mycobacterial resistance to key drugs used in the treatment of multidrug-resistant tuberculosis.

Scientific novelty and originality: The results of the present study describe the genotypic diversity of *M. tuberculosis complex* in the Republic of Moldova based on the analysis of Whole Genome Sequencing data obtained from a set of mycobacterial isolates selected over a significantly longer period (6 years) than in previous studies. Additionally, for the first time on a set of *M. tuberculosis complex* isolates from Eastern Europe, the putative role of compensatory and homoplastic mutations in the evolution of the MDR-TB endemic in this region, was determined. Likewise, the work is ensured highlights the associations between clinical management deficiencies and the emergence of resistance to anti-tuberculosis drugs in TB patients treated under the National Tuberculosis Response Program in the Republic of Moldova.

New results for science and practice: The overwhelming majority of MDR *M. tuberculosis complex* strains circulating in the Republic of Moldova belong in a relatively stable proportion (over the study period) to genotypic lineages L2 and L4, with significant differences in resistance to anti-tuberculosis drugs. MDR strains of *M. tuberculosis complex* belonging to genotypic lineage L2, circulating in the Republic of Moldova, are characterized by more severe radiographic lesions in pulmonary tuberculosis, a fact associated with Rv2828c T141R mutation in the mycobacterial genome, with positive selection observed for this mutation. MDR strains of *M. tuberculosis complex* belonging to genotypic lineage L4, circulating in the Republic of Moldova, are characterized by a particularly high rate of transmission in the population, one of the potential factors associated with the epidemiological success of these strains being the presence of compensatory mutations for rifampicin in the rpoC gene of the mycobacterium. Under the conditions of programmatic management of tuberculosis patients in the Republic of Moldova, resistance of *M. tuberculosis complex* strains to new and repurposed drugs such as bedaquiline and linezolid is acquired secondary to TB MDR treatment through the application of deficient treatment regimens.

Theoretical significance: The results of this work provide putative theoretical explanation for the successful spread of phylogenetic lines of *M. tuberculosis complex* L2 and L4 in the Republic of Moldova, especially by identifying some genetic determinants associated with positive selection of L2 genotypes as well as others that potentially favor the spread of L4 genotypes. Likewise, the thesis provides theoretical illustration of the acquisition of resistance to new anti-tuberculosis drugs, secondary to their inclusion in deficient therapeutic MDR-TB treatment regimens.

Applicative value: The data regarding the peculiarities of the correlation between geno-phenotypic resistance and their differences between the two identified genotypes (L2 and L4) in this study can be applied to improve methods for testing the susceptibility of *M. tuberculosis complex* strains to anti-tuberculosis drugs. Improving the epidemiological investigation of tuberculosis outbreaks and identifying measures to reduce the risks of acquiring resistance to anti-tuberculosis drugs.

Implementation of results: The results of the thesis have been implemented at the National Reference Laboratory for Tuberculosis Microbiology at the Chiril Draganiuc Institute of Phthisiopneumology, resulting in two implementation and innovation certificates.

ELENA CHESOV

GENOTYPIC DIVERSITY AND DRUG RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS STRAINS

313.02 – MICROBIOLOGY, MEDICAL VIROLOGY

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