DOI: https://doi.org/10.2298/SARH240520001G
UDC: 615.33.015.8(517.3)

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

A cross-sectional study on the factors influencing drug resistance in clinical *Mycobacterium tuberculosis* in Hulunbuir, Inner Mongolia

Huijun Guo[1], Yanqin Yu[1], Jinqi Hao[1], Lan Zhang[1], Mingyuan Hao[2]

[1]Baotou Medical College, School of Public Health, Baotou, Inner Mongolia, China;
[2]The Second People's Hospital in Hulunbuir, Zaerdong, Inner Mongolia, China

Introduction/Objective This study aimed to improve the understanding of drug-resistant tuberculosis (TB) by conducting a retrospective analysis of clinical data from TB patients in Hulunbuir, Inner Mongolia, collected between 2015 and 2017.
Methods A retrospective analysis was performed on clinical data from patients with TB. The data were used to determine monodrug resistance rankings for ethambutol, isoniazid, rifampicin (RIF), and streptomycin. Additionally, the study examined drug resistance rates for multidrug resistance (MDR) combinations, specifically isoniazid + RIF + streptomycin and isoniazid + RIF + ethambutol + streptomycin. A multivariate logistic regression analysis was conducted to assess risk factors for drug resistance, including sex, hospitalization status, age, and treatment history.
Results The findings revealed that both MDR combinations had resistance rates of 4.51%, the highest among the combinations analyzed. Ethambutol, isoniazid, and RIF showed the three highest monodrug resistance rates. Patients undergoing retreatment had higher rates of monodrug resistance, MDR, and polydrug resistance compared with those receiving initial treatment. The multivariate logistic regression analysis indicated that women had a lower risk of developing drug resistance than men, and hospitalized patients were found to be at lower risk than outpatients. Being 20–40 years old and undergoing retreatment were identified as significant risk factors for developing drug-resistant TB.
Conclusion In the Hulunbuir region of Inner Mongolia, there was a notable presence of drug resistance among patients with TB, with specific demographic and treatment history factors contributing to this resistance. These findings underscore the importance of considering these factors in developing targeted treatment strategies and public health policies to combat drug-resistant TB.
Keywords: *Mycobacterium tuberculosis*; drug resistance; influence factor

INTRODUCTION

Tuberculosis (TB) is a disease primarily caused by *Mycobacterium tuberculosis* (Mtb) infection and poses a significant threat to public health [1]. Between 2016 and 2020, the World Health Organisation designated China as a high-burden country for TB, TB-HIV co-infection and multidrug-resistant TB (MDR-TB). The high burden of MDR-TB is a key factor hindering TB control [2]. Multidrug-resistant TB is difficult to treat, severely impacting patients' physical and mental health and livelihoods and endangering their lives.

In recent years, with the implementation of the National Tuberculosis Prevention and Control Programme, some progress has been made in the prevention and control of drug-resistant TB in China. However, the epidemic of drug-resistant TB remains inadequately controlled in economically disadvantaged, remote areas [3]. Recent reports indicate a significant upward trend in drug resistance rates, particularly for rifampicin (RIF), multidrug-resistant strains and fluoroquinolones among TB patients in Inner Mongolia [4]. These remote regions face unique challenges due to economic disparities. This study aims to investigate the drug resistance status of TB and its associated influencing factors in the Hulunbuir area to guide individualized treatment plans. It also addresses a gap in the understanding of specific factors affecting drug resistance, which is essential for developing effective control measures.

METHODS

Study design and population

A cross-sectional study was conducted using convenience sampling of 688 patients with TB who were newly diagnosed or previously treated and who sought medical care, were referred to it, or were tracked in the Hulunbuir Second People's Hospital in the Hulunbuir region between January 1, 2015 and December 31, 2017. A questionnaire survey was then undertaken to gather basic information, medical history and treatment history from the patients. The diagnostic criteria used in this study followed the Diagnostic Criteria for Pulmonary Tuberculosis formulated by the Tuberculosis Branch of the Chinese Medical Association [5]. The inclusion criteria were as follows: (1) patients with

Received • Примљено: May 20, 2024
Revised • Ревизија: October 18, 2024
Accepted • Прихваћено: November 15, 2024
Online first: January 9, 2025

Correspondence to:
Jinqi HAO
School of Public Health
Baotou Medical College
31 Jianshe Road
Donghe District
014030 Baotou City
Inner Mongolia
China
btyxyhiq@163.com

positive sputum culture identification for Mtb, (covering pulmonary TB and extrapulmonary TB) and (2) patients who underwent drug susceptibility testing (DST). The exclusion criteria were as follows: (1) patients who were on treatment during the investigation period, (2) patients declining DST, and (3) patients unwilling to participate in the survey.

Research methods

Sputum samples from all active patients with TB included in the study were cultured on a solid medium using Roche culture medium. Positive cultures were subsequently subjected to strain isolation, identification and DST. The DST for TB was divided into two parts. First, sputum samples were collected to culture Mtb; subsequently, the drug susceptibility of the strains was tested using kits. Strain identification was accomplished by qualified researchers using a gene chip method, which employed a DNA microarray chip to qualitatively detect samples from clinically suspected patients for Mtb and non-tuberculous mycobacteria. The detailed procedures followed the Laboratory Testing Guidelines for Tuberculosis [6]. To ensure the accuracy and reliability of the gene chip method in strain identification, a series of quality assurance measures were implemented, including the establishment of standardized operating procedures, the adoption of double-blind testing processes, the introduction of positive and negative controls, inter-laboratory comparisons, the development of quality control metrics, ongoing quality improvement plans, detailed record-keeping and adherence to ethical standards. Through these stringent quality control measures, the consistency and traceability of the experimental results were ensured and enhanced the scientific validity of the research. The acidic Roche culture medium was manufactured by Hangzhou Innovation Biotechnology Co., Ltd (Hangzhou, Zhejiang, China). The DST kit and gene chip were purchased from Beijing Boao Biological Group Co., Ltd. (Beijing, Beijing, China), both compliant with national standards.

Quality control

Patient questionnaires were completed by professional physicians to ensure accuracy in the patients' medical history and treatment records. Smear examination, bacterial culture and DST were conducted in accordance with the Diagnostic Criteria for Pulmonary Tuberculosis [5] and the Laboratory Testing Guidelines for Tuberculosis [6].

Relevant definitions

Newly diagnosed patients were defined as individuals who had never used anti-TB drugs or had used them for less than one month and had discontinued treatment for less than two months. Retreatment patients, by contrast, included those who underwent irregular anti-TB treatment for at least one month, as well as those who experienced treatment failure or relapse. The drug resistance classification was as follows:

1. Any drug resistance: Mtb is resistant to a particular anti-TB drug, including but not limited to that specific drug;
2. Monodrug resistance: Mtb is resistant to a single first-line anti-TB drug;
3. MDR: Mtb is resistant to both isoniazid and RIF concurrently;
4. Polydrug resistance: Mtb is resistant to two or more anti-TB drugs, excluding simultaneous resistance to both isoniazid and RIF.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive and analytic statistics were employed to depict and examine drug resistance profiles and rates. Counts were described using proportions or percentages, and group differences were assessed using the chi-squared (χ^2) test. A logistic regression model was utilized to analyze the factors influencing drug resistance in patients with TB. The outcomes were visualized using GraphPad Prism 9.3 (GraphPad Software, San Diego, CA, USA). Two-tailed tests were performed, with a significance level of $\alpha = 0.05$.

The Ethics Committee of Baotou Medical College approved this study (Ethical number: Baotou Medical College Ethics Committee Approval [2023] No. 16). The Committee approved the waiver of informed consent from parents/guardians of the minors, due to the study being a retrospective analysis of clinical data and all methods were performed in accordance with the ethical guidelines.

RESULTS

Basic information

Among the 688 patients with TB in this study, 393 were newly diagnosed, whereas 295 were previously treated. The male-to-female ratio among these patients was 2.8:1. Most patients were hospitalized due to pulmonary TB.

Drug resistance testing results

By examining the resistance results to four anti-TB drugs – isoniazid, RIF, ethambutol and streptomycin – this study identified a drug resistance rate of 44.04% among the 688 isolates. Fifteen combinations of drug resistance were observed among these four drugs, with each combination exhibiting resistance. The ranking of monodrug resistance was as follows: ethambutol (8.14%), isoniazid (5.67%), RIF (5.52%), and streptomycin (1.45%). Among the multidrug resistance patterns, the highest resistance rates were observed for isoniazid + RIF + streptomycin and isoniazid + RIF + ethambutol + streptomycin, each at 4.51% (see Table 1).

Table 1. Drug resistance frequencies of various drug combinations in treatment-resistant patients with tuberculosis*

Combinations	n	Drug resistance rate (95% CI)	Composition ratio % (n/303)
Any drug resistance	—	—	—
H	166	24.13 (20.93–27.33)	—
R	165	23.98 (20.79–27.17)	—
E	79	11.48 (9.10–13.86)	—
S	164	23.84 (20.65–27.02)	—
Monodrug resistance	143	20.78 (17.75–23.82)	47.19 (143/303)
H	39	5.67 (3.94–7.4)	12.87 (39/303)
R	38	5.52 (3.82–7.23)	12.54 (38/303)
E	56	8.14 (6.1–10.18)	18.48 (56/303)
S	10	1.45 (0.56–2.35)	3.3 (10/303)
Multidrug resistance	99	14.39 (11.77–17.01)	32.67 (99/303)
H+R	26	3.78 (2.35–5.2)	8.58 (26/303)
H+R+E	11	1.6 (0.66–2.54)	3.63 (11/303)
H+R+S	31	4.51 (2.96–6.06)	10.23 (31/303)
H+R+E+S	31	4.51 (2.96–6.06)	10.23 (31/303)
Polydrug resistance	61	8.87 (6.74–10.99)	20.13 (61/303)
H+E	2	0.29 (0.11–0.69)	0.66 (2/303)
H+S	23	3.34 (2–4.69)	7.59 (23/303)
H+E+S	3	0.44 (0.06–0.93)	0.99 (3/303)
R+E	13	1.89 (0.87–2.91)	4.29 (13/303)
R+S	11	1.6 (0.66–2.54)	3.63 (11/303)
R+E+S	4	0.58 (0.01–1.15)	1.32 (4/303)
E+S	5	0.73 (0.09–1.36)	1.65 (5/303)
Total	303	44.04 (40.33–47.75)	100 (303/303)

H – isoniazid; R – rifampicin; E – ethambutol; S – streptomycin;
"—" indicates no corresponding value

Drug resistance distribution among patients with tuberculosis

Among the 688 patients, 385 were drug-sensitive, 143 were monoresistant, 61 were polyresistant and 99 were multidrug-resistant. At a significance level of $\alpha = 0.05$, a significant difference in the distribution of drug resistance was found between newly diagnosed and previously treated patients ($\chi^2 = 49.620$, $p < 0.001$). There were no significant differences in the composition ratios of the four drug resistance categories across sex, age groups, ethnicity, patient types or diagnostic outcome groups (all p-values > 0.05) (see Table 2).

Analysis of factors influencing drug resistance in patients with tuberculosis

The multivariate analysis of the logistic regression model showed that women had a lower risk of developing drug resistance than men (odds ratio [OR] = 0.68, $p < 0.05$). Patients aged 20–40 years had a higher risk of developing drug resistance than those aged 0–20 years (OR = 2.64, $p < 0.05$). Previously treated patients had a higher likelihood of developing drug resistance than newly diagnosed patients (OR = 2.34, $p < 0.05$). Hospitalized patients had a lower risk of developing drug resistance than outpatients (OR = 0.64, $p < 0.05$) (Figure 1).

DISCUSSION

The survey revealed that the prevalence of MDR-TB (14.39%) and overall drug resistance (44.04%) among patients in the Hulunbuir area of Inner Mongolia surpassed the rates reported in a drug resistance survey conducted among four provinces in northwest China between 2005 and 2011 (11.29% and 27.90%, respectively) and the national baseline survey (39.12% drug resistance rate) [7, 8]. These findings indicate a potentially higher drug resistance rate among patients with TB in the Hulunbuir area compared with other regions. The survey ranked the drug resistance rates of various anti-TB drugs as follows: isoniazid (24.13%), RIF (23.98%), streptomycin (23.84%), and ethambutol (11.48%). These rankings were highly similar to the reported drug resistance rates of acid-fast bacilli-positive samples in the Inner Mongolia region, according to Feng and He. [4]. The high resistance rate for streptomycin is notable, as TB treatment guidelines recommend its limited use. However, current guidelines that advocate for reduced use of streptomycin may unintentionally withhold a potentially lifesaving, inexpensive and readily available drug from certain patients with drug-resistant TB. Isoniazid and RIF are the most commonly used first-line oral anti-TB drugs, noted for their strong bactericidal effects, antimicrobial activity and low toxicity [9]. The appearance and progression of the resistant strain of Mtb depends largely on the selection of genetic mutations, which the *Mycobacterium* population exploits under selective pressure to favor antibiotic resistance. This study's OR analysis emphasizes the heightened risk of drug-resistant TB in patients aged 20–40 years and those undergoing retreatment, informing tailored clinical strategies. These findings provide actionable insights for healthcare providers to enhance treatment adherence and outcomes in these high-risk groups.

The virulence of Mtb, genetic factors of the host, HIV infection, and incomplete treatment of patients all contribute to the outbreak of drug-resistant TB [10]. Studies have revealed that risk factors for drug-resistant TB include monotherapy resulting from intermittent treatment, actual monotherapy due to irrational combination therapy, as well as insufficient drug concentrations leading to ineffective treatment [11]. Therefore, in the diagnosis and treatment of patients with TB, it is crucial to prioritize strict adherence to drug use principles and rational use of anti-TB drugs, particularly first-line medications.

In this survey, the proportions of monodrug resistance, MDR and polydrug resistance among newly diagnosed patients were 21.37%, 6.87%, and 7.12%, respectively, indicating possible transmission of drug resistance in the community [12]. Phenotypic DST is considered the gold standard for DST in China. However, TB laboratory tests in primary hospitals mainly consist of time-consuming methods, such as modified acid-fast staining and acid-fast culture [13, 14]. Current clinical practice relies on early anti-TB treatment principles similar to those for MDR-TB. However, these treatment regimens lack phenotypic DST results for

Table 2. Analysis of the distribution of drug resistance among patients with different characteristics

Characteristics	Drug resistance type				χ^2	p
	Sensitivity (%)	Monodrug resistance (%)	Polydrug resistance (%)	Multidrug resistance (%)		
Sex						
Male	269 (53.06)	111 (21.89)	49 (9.66)	78 (15.38)	7.049	0.070
Female	116 (64.09)	33 (18.23)	11 (6.08)	21 (11.6)		
Age (years)						
0–19	22 (75.86)	4 (13.79)	2 (6.9)	1 (3.45)	15.010	0.091
20–39	110 (52.38)	45 (21.43)	20 (9.52)	35 (16.67)		
40–59	165 (52.05)	73 (23.03)	29 (9.15)	50 (15.77)		
60–older	88 (66.67)	22 (16.67)	9 (6.82)	13 (9.85)		
Ethnic group						
Han	326 (56.5)	120 (20.8)	52 (9.01)	79 (13.69)	1.775	0.620
Mongolian	59 (53.15)	24 (21.62)	8 (7.21)	20 (18.02)		
New versus retreatment cases						
new	254 (64.63)	84 (21.37)	27 (6.87)	28 (7.12)	49.620	< 0.001
retreatment	131 (44.41)	60 (20.34)	33 (11.19)	71 (24.07)		
Type						
Outpatients	68 (50.37)	31 (22.96)	17 (12.59)	19 (14.07)	4.168	0.244
Inpatients	317 (57.32)	113 (20.43)	43 (7.78)	80 (14.47)		
Diagnosis						
Pulmonary tuberculosis	380 (55.96)	141 (20.77)	60 (8.84)	98 (14.43)	—	0.788
Extrapulmonary tuberculosis	5 (55.56)	3 (33.33)	0 (0)	1 (11.11)		

"—" represents the use of the Fisher exact probability method to calculate precise probabilities without calculating the χ^2 value

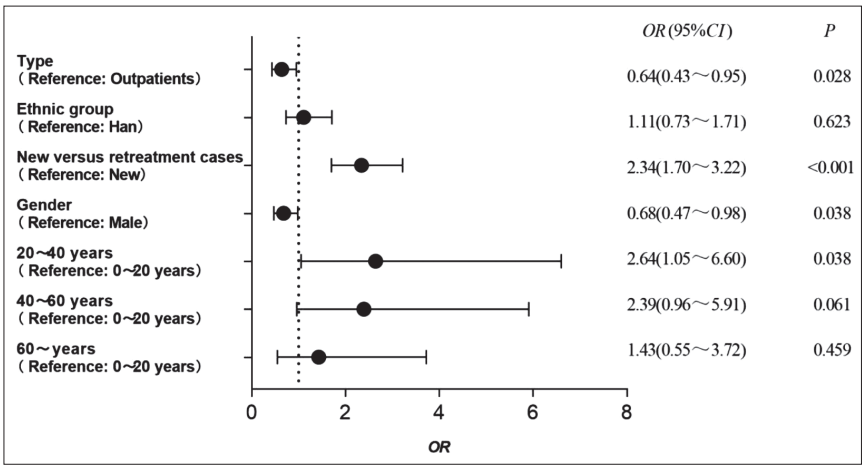

Figure 1. Multivariate analysis of a tuberculosis patient in a logistic regression model

other drugs. Consequently, when resistance to these drugs arises, it significantly impacts treatment efficacy and prognosis. Additionally, the results reveal that drug-resistant TB patients in the Hulunbuir area are distributed differently between newly diagnosed and previously treated patients. Several studies have demonstrated that drug resistance patterns vary between newly diagnosed and previously treated patients, potentially due to differences in drug exposure history and patient compliance [15]. Therefore, it is crucial to consider the characteristics of these patient populations in the clinical diagnosis and treatment process and select appropriate personalized treatment strategies based on specific situations, emphasizing the importance of drug guidance to enhance compliance.

Multivariate logistic regression analysis revealed several independent risk factors for drug-resistant TB, consistent with previous research findings [16]. Patients aged 20–40 had a 2.64 times higher risk of drug resistance compared with those aged 0–20. This may be attributed to higher levels of stress, social activities, and mobility among young and middle-aged patients, resulting in lower treatment compliance. Another reason is the relatively poor physical fitness to resist TB infection, which leads to prolonged disease [17]. This method was used for data collection in this study because of the high feasibility and validity of convenience sampling, but selection bias resulting from the use of convenience sampling is a possible explanation for this result. The risk of drug resistance is also higher in previously treated patients with pulmonary TB compared with newly diagnosed patients, potentially due to factors such as insufficient professional knowledge, improper medication, self-discontinuation of treatment or treatment failure in the initial stages [18]. Additionally, outpatients undergoing follow-up have a higher risk of drug resistance compared with hospitalized patients, which may be due to the non-adherence of patients to their 6-month therapy and/or a lack of physicians in therapy management. Another cause may be that some patients who require hospitalization may exhibit weaker willingness to partake in it, leading to poor compliance among outpatients. This emphasizes the importance of improving patient cooperation during outpatient follow-up.

Among newly diagnosed patients, male sex, age 20–40 years, and outpatient follow-up were identified as risk factors for drug resistance. In the retreatment group, risk factors included male sex, age 20–40 years and a prior history of treatment failure or relapse. These findings highlight the importance of tailoring interventions and management strategies based on each patient's treatment history and specific risk factors.

When compared to international data, our results are particularly striking. A global analysis by the World Health Organization (2023) reported a lower worldwide average for drug-resistant TB, indicating that Inner Mongolia, and particularly the Hulunbuir region, may be a hotspot for drug

resistance. The high resistance to isoniazid and rifampicin, observed in our study, mirrors the findings of Shabani et al. [9], who noted the increasing prevalence of resistance to these cornerstone anti-TB medications. In contrast, a study by Magotra et al. [13] reported lower resistance rates but noted the importance of personalized treatment strategies, which is also a key takeaway from our study. The need for tailored interventions is further emphasized by the high resistance rates to ethambutol, isoniazid, and rifampicin, which are consistent with the patterns reported by Feng et al. [15] in a study on drug sensitivity in Xi'an, China.

This study in Hulunbuir, Inner Mongolia, uniquely revealed high rates of drug resistance to ethambutol, isoniazid, and RIF among patients with TB, emphasizing the need for tailored treatment strategies in the region. Additionally, it identified social support and physical function as critical factors influencing resistance patterns, underscoring the importance of a holistic approach to combat drug-resistant TB. This study's findings prominently indicate that healthcare providers urgently need to adopt individualized treatment strategies that take into account the specific patterns of drug resistance observed in the region. This may include revised guidelines for the initial and ongoing treatment of TB, with a focus on more effective drug regimens and stricter monitoring of patient adherence. From a public health perspective, the findings highlight the importance of strengthening surveillance systems for drug resistance trends and of informing policy decisions. There is a clear need for targeted public health campaigns to raise awareness about the dangers of drug-resistant TB and the importance of completing the full course of medication.

Limitations

1. This analysis lacked detailed information on disease progression, treatment methods and certain laboratory test results, which warrants further research.
2. The molecular correlation could be further studied, and the evaluation index of patients should be improved.
3. Due to the use of pre-existing data, retrospective studies may lack certain specific information that was not fully documented in past records.

CONCLUSION

The drug resistance spectrum exhibited diversity and complexity, with variations in the distribution of drug resistance between newly diagnosed and patients who were treated again. Specifically, for male patients, especially those aged 20–40 years, and patients under outpatient follow-up, it is crucial to strengthen health education, improve healthcare insurance systems and enhance patient management models to improve compliance.

ACKNOWLEDGEMENTS

We would like to thank all the physicians who helped with collecting data from tuberculosis hospitals in Hulunbuir.

Funding: This work was supported by National Natural Science Foundation of China (82260657), Inner Mongolia Natural Science Foundation of China (2023MS08005, 2024MS08086), Inner Mongolia Autonomous Region Health and Technology Program of China (202201375).

Availability of data and materials: The data supporting this study are available from the corresponding author upon reasonable request.

Conflict of interest: None declared.

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Студија пресека о факторима који утичу на резистенцију на лекове клиничких узорака *Mycobacterium tuberculosis* у Хулунбуиру, Унутрашња Монголија

Хуиђун Гуо[1], Јанћин Ју[1], Ђинћи Хао[1], Лан Џанг[1], Мингјуан Хао[2]

[1]Медицински колеџ у Баотоуу, Школа јавног здравља, Баотоу, Унутрашња Монголија, Кина;
[2]Друга народна болница у Хулунбуиру, Заердонг, Унутрашња Монголија, Кина

САЖЕТАК

Увод/Циљ Циљ ове студије био је да побољша разумевање резистенције туберкулозе на лекове спровођењем ретроспективне анализе клиничких података болесника са туберкулозом у Хулунбуиру, Унутрашња Монголија, прикупљених између 2015. и 2017. године.

Методе Студија је укључивала ретроспективну анализу клиничких података болесника са туберкулозом. Подаци су коришћени за одређивање степена резистенције на појединачне лекове (укључујући етамбутол, изониазид, рифампицин и стрептомицин). Студија је такође испитивала стопе вишеструке резистенције на комбинације лекова, посебно изониазид + рифампицин + стрептомицин и изониазид + рифампицин + етамбутол + стрептомицин. Урађена је мултиваријантна логичка регресиона анализа да би се проценили фактори ризика повезани са резистенцијом на лекове, укључујући пол, статус хоспитализације, старост и претходна лечења.

Резултати Резултати су показали да су стопе резистенције на комбинације лекова, тј. вишеструке резистенције за обе комбинације, биле 4,51%, што је највећа стопа међу до сада анализираним комбинацијама. Етамбутол, изониазид и рифампицин имали су најучесталије три стопе резистенције међу свим изолатима. Изолати од болесника који су били подвргнути поновном лечењу показали су веће стопе резистенције на један лек, вишеструке резистенције и резистенцију на више лекова у поређењу са онима који су примали терапију први пут. Мултиваријантна логичка регресиона анализа је показала да су жене у мањем ризику од развоја резистенције на лекове од мушкараца. Поред тога, утврђено је да хоспитализовани болесници имају мањи ризик од појаве резистенције на лекове у поређењу са амбулантним болесницима. Студија је идентификовала старост између 20 и 40 година и поновну терапију као значајне факторе ризика за развој туберкулозе резистентне на лекове.

Закључак Ова студија је показала да је у региону Хулунбуир у Унутрашњој Монголији постојала приметна резистенција на лекове међу болесницима са туберкулозом, а са специфичним демографским факторима и факторима претходног лечења који доприносе развоју резистенције. Налази наглашавају важност разматрања ових фактора у развоју циљаних стратегија лечења и јавноздравствене политике за борбу против туберкулозе резистентне на лекове.

Кључне речи: *Mycobacterium tuberculosis*; резистенција на лекове; фактори од значаја