CURRENT APPROACHES FOR CONTROL OF ISONIAZID-RESISTANT TUBERCULOSIS

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ABSTRACT

Isoniazid (H; INH) is an important first-line drug for the treatment of active tuberculosis (TB) and latent TB infection because of its potent early bactericidal activity against *Mycobacterium tuberculosis*. Currently, TB resistant to INH, alone or in combination with other drugs, is the most common type of drug-resistant TB. Epidemiology of INH-resistant TB, the molecular mechanisms of drug resistance, current methods for diagnosis and therapeutic regimens of this TB form are presented.

Studies in the last years have shown that resistance to INH reduces the probability of treatment success and increases the risk of acquiring resistance to other important first-line drugs.

Based on the most recent meta-analyses, the last WHO recommendations for treatment of INH-resistant TB are to include rifampicin (RIF), ethambutol, pyrazinamide and levofloxacin for 6 months, and not to add streptomycin or other injectable agents to the drug regimen. The guideline emphasizes the importance of excluding resistance to RIF before starting the regimen for INH-resistant TB because of the risk for development of multidrug-resistant TB during the treatment course.

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The WHO recommendations are based on observational studies, not randomized controlled trials, and are thus conditional and based on low certainty in the estimates of effect. Therefore, further work is needed to optimize the treatment and control of INH-resistant TB.

Keywords: *Tuberculosis; Isoniazid; Drug resistance; Diagnosis; Treatment*

INTRODUCTION

Tuberculosis (TB), an ancient communicable disease caused by *Mycobacterium tuberculosis* (MTB), is one of the top 10 causes of death worldwide. Until the Coronavirus disease 2019 (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent. Based on World Health Organization (WHO) data, in 2020, an estimated 9.9 million people fell ill with TB worldwide, equivalent to 127 cases per 100,000.. Among all incident cases of TB, 8% were people living with Human immunodeficiency virus (HIV). Globally in 2020, there were an estimated 1.3 million deaths among HIV-negative individuals and an additional 214,000 deaths among HIV-positive individuals (1).

Drug-resistant TB (DR-TB) continues to be a public health threat. In MTB, drug resistance develops through spontaneous genetic mutations. The development of acquired drug resistance usually occurs when there is a large bacterial population, such as in pulmonary cavities (2) or when an inadequate drug combination or dosages of the first-line drugs (FLDs) has been prescribed (3,4). Rarely, malabsorption of anti-TB drugs may account for acquired resistance (5).

WHO currently uses five categories to classify cases with DR-TB:

- Multidrug-resistant TB (MDR-TB) – with resistance to both isoniazid (H; INH) and rifampicin (R; RIF) – the two most effective FLDs;
- Rifampicin-resistant TB (RR-TB);
- Resistant to INH and sensitive to RIF TB (Hr-TB);
- Pre-extensively drug-resistant TB (pre-XDR-TB) – MDR/RR-TB plus resistance to any fluoroquinolone (FQ) – a class of second-line anti-TB drugs (SLDs);
- Extensively drug-resistant TB (XDR-TB) – MDR/RR-TB plus resistance to any FQ, plus to at least one of the drugs bedaquiline (Bdq) and linezolid (Lzd) (1).

EPIDEMIOLOGY OF ISONIAZID-RESISTANT TUBERCULOSIS

INH (isonicotinylhydrazide) is one of the most powerful FLDs for treatment of active and latent TB infection because of its potent early bactericidal activity against MTB. INH has been in clinical use since the 1950s, and drug resistance was expected because its use became widespread, but drug-susceptibility testing (DST) for Hr-TB or special drug regimens for Hr-TB were not widely used. Indeed, for decades, no DST for any drug has been done unless patients failed treatment with FLDs or had risk factors for DR-TB.

Currently, Hr-TB alone or in combination with other drugs, is the most common type of resistance to FLDs worldwide. Based on WHO estimates, globally in 2019 an estimated 1.4 million cases were with INH-resistant TB (any type of DR-TB, including resistance to INH) – 13.1% (95% CI: 9.9–16.9%) of new TB cases and 17.4% (95% CI: 0.5–54%) of previously treated cases. The cases with Hr-TB (only resistant to INH and sensitive to RIF) were 1.1 million or 11% of all incident TB cases (6). The global burden of INH-resistant TB is keeping higher in different regions worldwide. In a representative study published in 2008, Hoopes et al. reported that the prevalence of INH-resistant TB in the United States for the period 1993-2003 had not declined, despite the downward trend in the prevalence of overall TB (7). Data from the WHO showed that resistance to INH was detected in 30% of TB cases in Eastern Europe and 14% of TB cases in West/Central Europe and Africa for the period 1994-2009 (8). Hr-TB is much more common than RR-TB and could seriously jeopardize progress in the fight against TB. This is confirmed by an analysis of aggregated DR-TB data for the period 2002-2018 across 156 countries presented in the research study by Dean et al., showing that – on average – 7.4% (95% CI 6.5–8.4) of new TB cases and 11.4% (9.4–13.4) of previously treated patients have Hr-TB. The overall prevalence of INH resistance (with or without concomitant RR) ranged between 10.7% (9.6–11.9) and 27.2% (24.6–29.9) depending on the treatment history and reached even more alarming levels in certain countries, particularly in the European and Western Pacific WHO regions.

According to the data presented in the study of Dean et al., the prevalence of Hr-TB among new TB cases in Bulgaria for the above mentioned period was between 3 and 5.9%, lower than the global prevalence (7.4%) (9).

Bulgaria is one of the 18 TB high priority countries in the WHO European Region. In 2020, 930 TB cases were notified in the country, i.e. 13.4 per 100,000 – almost double than the average for the European Union/European Economic Area (EU/EEA) TB notification rate (7.3 per 100,000). Since 2006, the number of TB cases and the TB notification rate have been declining. A national drug resistance survey conducted in 2010 showed that MDR-TB was detected in 2.1% of new TB cases and 11.1% of previously treated TB cases. In 2020, 12 pulmonary RR/MDR-TB cases (5% of all tested with DSTs) were identified through the national TB register, which is less than expected. Out of them, 2 pre-XDR-TB cases (22.2%) were notified (10). Data from the joint review of the Bulgarian National TB Control Programme conducted in 2014 by the European Centre for Disease Prevention and Control (ECDC) and the WHO Regional Office for Europe shows that out of 1,932 TB cases reported in 2013, 951 were culture positive, of which 734 (77%) had a DST result for FLDs. The proportion of INH-resistant TB was 6.1% among new cases and 23% among previously treated cases (calculated among cases with known DST results) (11), i.e. the prevalence of INH-resistant TB in the country seems to be higher than MDR-TB rates.

MOLECULAR-GENETIC MECHANISMS OF DEVELOPMENT OF ISONIAZID-RESISTANT TUBERCULOSIS

MTB has the ability for spontaneous, slow but steady mutations, leading to the development of drug resistance. This natural phenomenon is genetically determined and varies for the different drugs. Drug intake increases the likelihood for its appearance.

Isoniazid exerts its effects only in metabolically active mycobacterial cells. The probability for development of spontaneous resistance to INH is 1 in every 10^6 cell divisions (12). Drug resistance is the result of the selection of mycobacteria with mutations among the bacterial population due to destruction of the susceptible bacteria by anti-TB drugs. The problem is exacerbated if the patient is treated with monotherapy or with combination of FLDs in suboptimal concentrations – most bacteria die, but those with mutations survive, multiply and dominate the bacterial population (13).

Table 1 presents the main MTB genes, affected by specific molecular mechanisms (mutations) leading to development of resistance to the anti-TB drugs (14).

Table 1. Anti-tuberculosis drugs and *Mycobacterium tuberculosis* genes associated with development of drug resistance.

Anti-tuberculosis drug	Gene	Responsible for encoding of:
Isoniazid (INH; H)	*inhA*	NADH-dependent enoyl-ACP reductase (mutation is related with low level of resistance to H)
	katG	Catalase-peroxidase (mutation is related with high level of resistance to H)
	ahpC32	Alkyl hydroperoxide reductase
	oxyR	Oxidative stress regulator
	kasA	β-ketoacyl-ACP M synthase
Rifampicin (RIF; R)	*rpoB*	β-subunit of RNA polymerase
Pyrazinamide (PZA; Z)	*pncA*	Pyrazinamidase
Streptomycin (STR; S)	*rpsL*	30S ribosomal protein S12
	rrs	16S ribosomal RNA
	strA	Aminoglycoside phosphoryltransferase
Capreomycin (Cm)	*tlyA*	2'-O-methyltransferase
Ethambutol (ETH; E)	*emb A, emb B* and *emb C*	Arabinosyl transferases
Fluoroquinolone (FQ)	*gyr A* and *gyr B*	DNA gyrase

NADH: Nicotinamide adenine dinucleotide hydrogen; ACP: Acyl carrier protein; RNA: Ribonucleic acid; DNA: Deoxyribonucleic acid

Resistance to INH is usually due to a mutation in *katG* or *inhA*, and more seldom - in other genes, such as *ahpC32* (15,16).

In order to be effective against TB, INH must be activated by catalase-peroxidase, an enzyme regulated by *katG* gene,. Mutations in *katG*, most commonly at Ser315Thr (Ser → Thr), result in insufficient activation of the drug and are associated with a high level of resistance – Minimum inhibitory concentration (MIC) > 1 μg/ml (17,18).

The *inhA* gene encodes an enoyl ACP reductase involved in fatty acid synthesis in MTB. As these fatty acids are targeted by the active derivative of INH, mutations in *inhA* or its promoter region block INH binding and result in low-level INH resistance (MIC <1 μg/ml). The most frequent mutation in the promoter region is at position 15C/T (Cys → Thr) (19,20). Isolates with an *inhA* mutation are also typically resistant to ethionamide (Eto) and prothionamide (Pto) – SLDs for treatment of DR-TB (21).

According to most of the studies, risk factors for developing INH resistance include a history of TB, and origin from regions with a higher TB prevalence (Asia, Pacific Islans, etc.) (22).

DIAGNOSIS OF ISONIAZID-RESISTANT TUBERCULOSIS

Drug susceptibility tests (DSTs), which determine the sensitivity of MTB to FLDs and SLDs, and can detect the presence of DR-TB, including Hr-TB, are phenotypic and genotypic (PCR; Polymerase Chain Reaction based). DSTs are very important for developing the drug regimen, treatment outcome monitoring, disease prognosis and Drug Resistance Surveillance (DRS).

1. Phenotypic DSTs are performed with MTB strain isolated from cultures and determine if a strain is resistant to an anti-TB drug by evaluating the growth (or metabolic activity) in the presence of the drug. They are used for TB diagnosis and treatment follow-up. The reliability of DSTs varies from one drug to another. For the FLDs, DSTs are very reliable for RIF and INH, but less so for PZA and much less for ETH. DSTs for aminoglycosides, polypeptides and FQs have relatively good reliability and reproducibility. DSTs to other SLDs – para-aminosalicylic acid (PAS), Eto and cycloserine (Cs) are much less reliable and reproducible.

1.1. Automated methods – **BACTEC MGIT 960°**, using liquid culture media MGIT (Mycobacterium

Growth Indicator Tube), are currently the most widely-used option, as they are quick and reliable. It is recommended that they be performed on pure MTB cultures. The following concentrations of the FLDs are used: STR – 1.0 μg/ml; INH – 0.1 μg/ml; RIF – 1.0 μg/ml; ETH – 5 μg/ml; and of the SLDs: amikacin (Am) – 1.0 μg/ml; kanamycin (Km) – 5.0 μg/ml, capreomcin (Cm) – 2.5 μg/ml, and ofloxacin (Ofx) – 2.0 μg/ml (23).

1.2. Nitrate reductase assay (NRA) – a rapid method for detection of resistance to RIF and INH. It is a simple technique based on the capacity of MTB to reduce nitrate to nitrite, which is detected by adding the Griess reagent to solid Löwenstein–Jensen media, used for cultures of *Mycobacterium* species, and in the presence of the corresponding concentration of FLDs (RIF, INH, ETH and STR). The technique is in use since the 1980s and was developed by assoc. prof. Emil Kalfin from the Scientific Institute of Pneumology and Phthisiatry at Medical Academy, Sofia, Bulgaria. The NRA was shown to be highly sensitive and specific for detection of RIF and INH resistance when used on clinical isolates (24).

2. Genotypic DSTs – modern automated molecular technologies for rapid diagnosis of MTB using genetic material from biological samples, including determination of drug resistance. These molecular tests can only be performed by specialized laboratories with strict quality assurance in place. It is possible to test DNA of an isolated MTB strain, and also extracted DNA from the investigated clinical sample. The genotypic DSTs are used only for initial TB diagnosis but are not recommended for treatment follow-up.

2.1. Line Probe Assays (LPAs) based on DNA-STRIP® technology allow DNA detection and testing from isolated MTB strain or directly from the clinical sample, but only from the pulmonary system. This method has the advantage of giving fast results, within few hours, for smear-positive patients (referred to as direct testing, because the sputum can be directly tested). For smear-negative patients, a primary culture is needed prior to testing (referred to as indirect testing, because a culture has to be grown first from the patient's sputum).

- **GenoType® MTBDR*plus*** (Hain Lifescience GmbH, Nehren, Germany) – LPA for DSTs to **FLDs;** rapid MTB detection for 2 days; can identify mutations on *katG* and *inhA* genes associated with resistance to INH, and mutation on *rpoB* gene, encoding **β-subunit of RNA polymerase**, associated with resistance to RIF. Using this method, it is possible to determine the presence of **Hr-TB, RR-TB and MDR-TB**;

- **GenoType® MTBDR*sl*** (Hain Lifescience GmbH, Nehren, Germany) – LPA for DSTs to **SLDs;** can identify mutation on *gyrA* gene, encoding the enzyme DNA-gyrase, corresponding with resistance to FQs; mutation on *rrs* gene, encoding **16S rRNA**, corresponding with resistance to aminoglycosides and polypeptides. The second version of the test allows identification of mutation on *gyrB* gene, encoding the enzyme DNA-gyrase, associated with resistance to FQs; and mutation in the promoter of *eis* gene, encoding the enzyme **N-acetyltransferase**, associated with resistance to Km. Using this method, it is possible to determine the presence of **pre-XDR-TB and XDR-TB**. LPA to SLDs can be used as a triage test on smear-positive patients to guide the initial treatment of XDR-TB suspects while awaiting confirmatory results from conventional phenotypic testing. However, LPAs cannot be used as replacement tests for conventional phenotypic SLD DSTs.

2.2. Xpert MTB/RIF® (GeneXpert; Real-Time PCR, Cepheid®, Sunnyvale, USA) – automated nucleic acid amplification technology for rapid detection of MTB within 2 hours, as well as for detection of possible mutations in *rpoB* gene, causing RR-TB of the isolated MTB strain. The method was approved by the WHO in 2010 (25).

2.3. Whole Genome Sequencing (WGS) – where the entire genetic code of MTB strains isolated from patients is described and compared to a reference set of genomes. The WGS has been introduced routinely in England, Germany and many EU/EEA countries to guide clinical decision-making, earlier detection of resistance, and support of outbreak and epidemiological investigation. However, WGS requires sophisticated laboratory and bioinformatics infrastructure, and currently requires MTB isolation and DNA extraction before sequencing. Therefore the routine applicability of

the method beyond research especially in low resource settings is uncertain. Nevertheless, the advances in the sequencing field may rapidly turn WGS to a TB diagnostic tool (26). Between January 2017 and December 2019 Bulgaria participated in the pilot study of the European Center for Disease Prevention and Control (ECDC), evaluating the systematic use of a WGS-based approach for MTB surveillance involving all European Union/European Economic Area (EU/EEA) countries and highlighting the challenges to be considered for the future development of a WGS-based surveillance system (27).

Yordanova et al. investigated MTB isolates from 36 TB cases from all over Bulgaria in a retrospective study for the period 2015-2016. All the cases were confirmed by BACTEC MGIT 960 with mono-resistance to INH and additionally tested with GenoType MTBDR*plus* in the National Reference Laboratory of Tuberculosis (NRL TB) at the NCIPD. The authors found that only 25% of the tested MTB isolates with phenotypic INH mono-resistance had the S315T1 mutation in *katG*; all isolates were with MIC over 0.4 µg/ml. Resistance type C15T in the promoter region of *inhA* was detected in 22.22% of cases and only 1 of them showed MIC below 0.4 µg/ml. No mutations were detected in nearly half of the cases (n=19, 52.78%) and most of these isolates were with lower MIC values (n=12). The authors supposed that the resistance among the cases without mutations in *katG* or *inhA* can be explained with mutations in many other loci or genes (*furA-katG*, *fabG1-inhA*, *ahpCoxyR* intergenic region, *efpA*, *fadE24*, *iniA*, *iniB*, *iniC*, *kasA*, *nat*, *ndh*), which cannot be found using only GenoType MTBDR*plus* (28).

At the end of 2021, WGS of all isolated DR-TB strains in Bulgaria started in the NRL TB at the NCIPD, which will expand our knowledge about the mutations of MTB in the country, including those corresponding with resistance to INH.

TREATMENT OF ISONIAZID-RESISTANT TUBERCULOSIS

The management of INH-resistant TB is important because the last systematic meta-analyses have shown that resistance to INH reduces the probability of treatment success and increases the risk of acquiring resistance to other important FLDs such as RIF, thereby increasing the risk of MDR-TB. Moreover, INH-resistant TB generally requires longer treatment than drug-susceptible TB, increasing the burden of the disease (29,30).

The recommended regimens for INH-resistant TB differ among countries and have differed over time. Table 2 summarizes the previous and current international guidelines for the treatment of INH-resistant TB (22).

There is a standard code for writing out anti-TB regimens. Each drug has an abbreviation (shown in the table). A DR-TB regimen consists of two phases, separated by a slash: the first is intensive phase and the second is prolonged phase. The number shown before each phase stands for the minimal required phase duration in months . The number in subscript (e.g., 3) after a letter is the number of drug doses per week. If there is no number in subscript, treatment is daily. Alternative drug(s) appears as a letter(s) in parentheses. The drugs in the higher groups are written first followed by the others in descending order of potency.

Based on the most recent meta-analyses on the management of INH-resistant TB, in 2019 the WHO published the last key recommendations for Hr-TB treatment – with RIF-EMB-PZA-Lfx for 6 months and no addition of SM or other injectable agents to the drug regimen.

The WHO guidance emphasizes the importance of excluding resistance to RIF before starting the regimen for INH-resistant TB because of the risk for development of MDR-TB during the treatment course.

The guidelines are based primarily on individual patient data or observational studies conducted in various settings. They indicate that addition of an FQ to RIF-EMB-PZA regimens compared to ≥6 months of RIF-EMB-PZA is leading to higher treatment success rate (aOR, 2.8; 95% CI, 1.1–7.3). The addition of an FQ to a 6-month RIF-EMB-PZA regimen tended to reduce the number of deaths (aOR, 0.4; 95% CI, 0.2–1.1) and the acquisition of RIF resistance (aOR, 0.10; 95% CI, 0.01–1.2).

The main recommendations are to include Lfx rather than Mfx as a first choice because Lfx has a better safety profile than other FQs, and fewer drug interactions than Mfx; the plasma peak concentration of Lfx is not affected by the addition of RIF. Additionally, there are no contraindications for the use of Lfx with antiretroviral agents for the treatment of patients co-infected with HIV (31,32).

Table 2. International guidelines for treatment of isoniazid-resistant tuberculosis

Guideline	Recommended regimen	Duration (months)
ATS/CDC (1994)	REZ	6
	RE	12
ATS/CDC (2003)	REZ (FQ for extensive disease)	6
BTS (1998)	2 SREZ / 7 RE	9
	2 REZ / 10 RE	12
NICE (2011)	2 SREZ / 7 RE	9
	2 REZ / 10 RE	12
NICE (2016)	2 (H)REZ / 7 RE (10 months for extensive disease)	9-12
Canadian Tuberculosis Standards (2014)	2 (H)RZE / 4-7 REZ	6-9
	2 (H)RZE / 10 RE	12
	2 (H)RZE FQ / 4-7 RE FQ	6-9
WHO (2006)	REZ (FQ)*	6-9
WHO (2008)	REZ (FQ)*	6-9
WHO (2014)	REZ (FQ)* †	6-9
WHO (2018)	REZ FQ (Lfx > Mfx) ‡	6

*A fluoroquinolone may strengthen the regimen for patients with extensive disease.

†Use Xpert MTB/RIF at month 0, 2, and 3, and if RR-TB is switched to full MDR-TB treatment.

‡The new 2018 WHO guidelines recommend Lfx as the first choice, rather than Mfx.

ATS: American Thoracic Society; CDC: Centers for Disease Control and Prevention; BTS: British Thoracic Society; NICE: National Institute for Health and Care Excellence; WHO: World Health Organization; H: isoniazid; R: rifampicin; E: ethambutol; Z: pyrazinamide; FQ: fluoroquinolone; Lfx: levofloxacin; Mfx: moxifloxacin.

CONCLUSION

INH-resistant TB poses a significant challenge before public health systems. Many patients with Hr-TB would be missed by current diagnostic algorithms driven by RIF testing, thus receiving incomplete drug regimen. The development of new rapid molecular technologies is needed in order to ensure access to appropriate treatment and care.

The WHO recommendations for Hr-TB treatment are based on observational studies, but not on randomized controlled trials, and are thus conditional and based on low certainty in the estimates of the effect. Therefore, further work is needed to optimize the treatment and control of INH-resistant TB.

ACKNOWLEDGMENTS

REFERENCES

1. Global tuberculosis report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. Xi Y, Zhang W, Qiao R-J, Tang J (2022) Risk factors for multidrug-resistant tuberculosis: A worldwide systematic review and meta-analysis. PLoS ONE 17(6): e0270003. https://doi.org/10.1371/journal.pone.0270003..
3. Mahmoudi A, Iseman MD. Pitfalls in the care of patients with tuberculosis: common errors and their association with the acquisition of drug resistance. *JAMA* 1993;270:65-8.
4. Mitchison DA. How drug resistance emerges as a result of poor compliance during short course chemotherapy for tuberculosis. *Int J Tuberc Lung Dis* 1998;2:10-5.
5. Patel KB, Belmonte R, Crowe HM. Drug malabsorption and resistant tuberculosis in HIV-infected patients. *N Engl J Med* 1995;332:336-7.
6. Global tuberculosis report 2020. Geneva: World Health Organization; 2020. License: CC BY-NC-SA 3.0 IGO.
7. Hoopes AJ, Kammerer JS, Harrington TA, Ijaz K, Armstrong LR. Isoniazid-monoresistant tuberculosis in the United States, 1993 to 2003. *Arch Intern Med* 2008;168:1984-92.
8. Jenkins HE, Zignol M, Cohen T. Quantifying the burden and trends of isoniazid resistant tuberculosis, 1994-2009. *PLoS One* 2011;6:e22927.
9. Dean AS, Zignol M, Cabibbe AM, Falzon D, Glaziou P, Cirillo DM, et al. Prevalence and genetic profiles of isoniazid resistance in tuberculosis patients: A multicountry analysis of cross-sectional data. *PLoS Med.* 2020; 17(1): e1003008. https://doi.org/10.1371/journal.pmed.1003008.
10. European Centre for Disease Prevention and Control, WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2022 – 2020 data. Copenhagen: WHO Regional Office for Europe and Stockholm: European Centre for Disease Prevention and Control; 2022. Licence: CC BY 3.0 IGO.
11. European Centre for Disease Prevention and Control. Report of the joint ECDC and WHO review of the national tuberculosis programme in Bulgaria. Stockholm: ECDC; 2014. ISBN 978-92-9193-598-7. doi 10.2900/37158.
12. Goering R., R.I.M., Mims C. Medical Microbiology, Chapter 5.

Diagnosis and control; Resistance to Antibacterial Agents. 4th ed 2007: Mosby.

13. Pfyffer, G.E. Drug-resistant tuberculosis: resistance mechanisms and rapid susceptibility testing. *Schweizerische medizinische Wochenschrift*, 2000. 130(49):1909-13.

14. Miotto P, Tessema B, Tagliani E, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis. *Eur Respir J* 2017; 50: 1701354 [https://doi.org/10.1183/13993003.01354-2017].

15. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. *Nature* 1992;358:591-3.

16. Piatek AS, Telenti A, Murray MR, El-Hajj H, Jacobs WR Jr, Kramer FR, et al. Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing. *Antimicrob Agents Chemother* 2000;44:103-10.

17. Ramaswamy SV, Reich R, Dou SJ, Jasperse L, Pan X, Wanger A, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 2003;47:1241-50.

18. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. *Tuber Lung Dis* 1998;79:3-29.

19. Dalla Costa ER, Ribeiro MO, Silva MS, Arnold LS, Rostirolla DC, Cafrune PI, et al. Correlations of mutations in katG, oxyR-ahpC and inhA genes and in vitro susceptibility in Mycobacterium tuberculosis clinical strains segregated by spoligotype families from tuberculosis prevalent countries in South America. *BMC Microbiol* 2009;9:39.

20. Palomino JC, Martin A. Drug Resistance Mechanisms in Mycobacterium tuberculosis. *Antibiotics.* 2014; 3(3):317-340.

21. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. *Science* 1994;263:227-30.

22. Jhun BW, Koh WJ. Treatment of isoniazid-resistant pulmonary tuberculosis. *Tuberc Respir Dis (Seoul)*. 2020;83:20–30. doi:10.4046/trd.2019.0065.

23. Ministry of Health of the Republic of Bulgaria. Methodological guideline on microbiological diagnosis of tuberculoosis, 2016. Approved by Order No. RD-01-72 / 10.03.2016. Available on: http://www.mh.government.bg/. In Bulgarian.

24. Martin A, Panaiotov S, Portaels F, Hoffner S, Palomino JC, Angeby K. The nitrate reductase assay for the rapid detection of isoniazid and rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis. *J Antimicrob Chemother.* 2008 Jul;62(1):56-64. doi: 10.1093/jac/dkn139. Epub 2008 Apr 10. PMID: 18407918.

25. Ministry of Health of the Republic of Bulgaria. Methodological guideline on organization of the performance of fast methods for diagnosis of tuberculosis on the territory of the Republic of Bulgaria, 2015. Approved by Order No. RD -01-237/16.10.2015. Available on: http://www.mh.government.bg/. In Bulgarian.

26. MacPherson, P. (2018). Emerging Issues in tuberculosis. K4D Reading Pack. Brighton, UK: Institute of Development Studies; p. 56.

27. Tagliani E, Anthony R, Kohl TA, et al. Use of a whole genome sequencing-based approach for Mycobacterium tuberculosis surveillance in Europe in 2017–2019: an ECDC pilot study. *Eur Respir J* 2021; 57: 2002272 [https://doi.org/10.1183/13993003.02272-2020].

28. Yordanova, S., Baykova, A., Atanasova, Y., Todorova, Y., Bachiyska, E. Isoniazid-monoresistant tuberculosis in Bulgaria. *Probl. Inf. Paras. Dis*., Vol. 48, 2020, 1, 21-24.

29. Menzies D, Benedetti A, Paydar A, Martin I, Royce S, Pai M, et al. Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. *PLoS Med* 2009;6:e1000146.

30. Menzies D, Benedetti A, Paydar A, Royce S, Madhukar P, Burman W, et al. Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS Med* 2009;6:e1000150.

31. WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

32. WHO treatment guidelines for isoniazid-resistant tuberculosis: Supplement to the WHO treatment guidelines for drug-resistant tuberculosis. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.