

Effectiveness of Molecular-Genetic and Bacteriological Research Methods in Drug-Resistant Forms of Pulmonary Tuberculosis

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Abstract Numerous scientific studies on the effectiveness of treatment are being conducted worldwide. The issue of drug resistance of *Mycobacterium tuberculosis* is the subject of numerous studies by domestic and foreign authors. The meaning of the term "drug-resistant strain" has changed as knowledge about the mechanisms of drug resistance formation has accumulated. At the stage of introducing anti-tuberculosis drugs, it was proposed to designate a strain as resistant if it was isolated from a patient for whom treatment did not improve. This approach lost its significance after the complete rejection of monotherapy. Currently, in our country, special attention is paid to the problems of improving the activities of the healthcare system, including a healthy lifestyle of the population, early diagnosis of diseases, treatment and prevention of tuberculosis.

Keywords Molecular genetic diagnostics, Treatment effectiveness, Drug resistance, Tuberculosis

1. Introduction

Tuberculosis (TB) remains a major global health challenge, particularly with the emergence of drug-resistant strains, such as multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis*. The rise of these resistant forms complicates treatment strategies, leading to higher morbidity and mortality rates. Accurate and timely diagnosis is crucial for effective patient management and preventing further transmission.

Traditional bacteriological methods, such as sputum smear microscopy and culture-based techniques, have been the cornerstone of TB diagnosis for decades. However, these methods have limitations, including long turnaround times and variable sensitivity, particularly in cases of drug resistance. In contrast, molecular-genetic techniques, such as polymerase chain reaction (PCR), whole-genome sequencing (WGS), and line probe assays (LPAs), offer rapid and precise detection of resistance-conferring mutations.

This study evaluates the effectiveness of molecular-genetic and bacteriological research methods in diagnosing drug-resistant pulmonary tuberculosis. By comparing their sensitivity, specificity, speed, and clinical applicability, we aim to determine the optimal diagnostic approach for improving patient outcomes and TB control programs. The findings will

contribute to the ongoing efforts to combat antimicrobial resistance in tuberculosis and enhance diagnostic protocols in high-burden settings.

2. Materials and Methods of the Study

The work is based on the data of examination of 244 patients from the southern regions of the Republic of Uzbekistan, who received treatment in the centers of phthisiology and pulmonology of the Bukhara region - 148 (60.7%), Navai region - 30 (12.3%), Kashkadarya region - 32 (13.1%) and Surkhandarya region - 34 (13.9%) cases in the period from 2016 to 2020.

The age of patients ranged from 18 to 86 years, the average age was 52.1 ± 2.9 years. Men were ill 2.2 times more often than women, and the incidence of patients was 65.2% at the age of 19-59 years (working age), 18.4% at 60-69 years and 16.4% over 70 years.

It should be noted that the most frequent incidence of the disease occurred after forty years - 75.4% of patients and the peak age period was noted from 50 to 59 years in 23.4% of cases. Patients of the rural population were observed - in 185 (75.8%), the urban population - in 56 (23.0%) and homeless people - in 3 (1.2%) cases. When analyzing, it is noted that the disease was 3.3 times more common in rural populations. Of the 244 patients - 73 (29.9) patients had an employee profession, 13 (5.3%) - agricultural workers, 4 (1.6%) - students, a single (0.4%) case - medical personnel, and most often people without a certain job, who often do not undergo preventive medical examination - in 153 (62.8%) cases.

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Table 1. Localization of the tuberculosis process

Localization of the process in the lungs	Number of patients	Lobe lesions		
		top	lower	total
Right	86(35.2%)	69(28.3%)	7(2.9%)	10(4.1%)
Left	69(28.3%)	47(19.3%)	10(4.1%)	12(4.9%)
Both	89(36.5%)	53(21.7%)	7(2.9%)	29(11.9%)
Total:	244 (100%)	169(69.3%)	24(9.8%)	51(20.9%)

Localization of the tuberculosis process in most patients was observed in both lungs - in 89 (36.5%), in 86 (35.2%) - damage to the right lung and less cases of the left lung - in 69 (28.3%) patients. From Table 1 it is evident that frequent localization of tuberculosis lung damage was noted in the upper lobe of the lungs - in 169 (69.3%), less often in the lower lobe of the lung - in 24 (9.8%) patients. It should be noted that total damage of one lung was observed - in 51 (20.9%) cases, and total damage of both lungs - in 29 (11.9%) patients, for whom the clinical manifestation of the disease was severe. Of the 244 patients, 151 (61.9%) patients were diagnosed with the disease for the first time, and 93 (38.1%) - repeatedly, i.e. they had previously received treatment. It should be noted that 21 (8.6%) patients were previously prisoners.

Of the 244 patients, generalized forms of tuberculosis were found in 11 ($4.5 \pm 2.5\%$) cases, of which pulmonary tuberculosis with tuberculous pleurisy was found in 7 ($2.9 \pm 1.1\%$) cases, genitourinary organs were determined in 2 ($0.8 \pm 0.2\%$) and with tuberculous spondylitis in 2 ($0.8 \pm 0.2\%$) patients.

It is evident that the generalization of the tuberculosis process is often observed in more advanced stages of the disease - cavernous or fibro-cavernous tuberculosis of the lungs - in 7 (63.6%) cases.

All patients underwent bacteriological examination of sputum and in 100% of cases the clinical diagnosis was verified bacteriologically.

In 74 ($30.3 \pm 2.7\%$) patients, the disease developed slowly over more than 1 year, with a characteristic progression of general malaise, rare rises in temperature to subfebrile, sometimes accompanied by a dry cough. Subacute course of the disease with progression during 1 year, weight loss, subfebrile temperature, dry cough and sweating in the evenings was noted in 24 ($9.4 \pm 1.8\%$) patients, in 26 ($10.6 \pm 1.9\%$) cases the duration of the above symptoms was up to 6 months, it should be noted that in 120 ($47.6 \pm 2.4\%$) patients it was up to 3 months - the clinical course of the disease was acute, with a rise in temperature over 38.0°C , with intoxication, weight loss of more than 10% of the total, with a strong increasing dry or wet cough with sputum production and deterioration of the general condition of patients.

The duration of patients' complaints before the final diagnosis was established ranged from 1 month to 10 years, with an average of 6.3 ± 1.7 months.

The general condition of patients upon admission to the clinic was as follows: relatively satisfactory - 11 (4.5%),

moderately severe - 217 (88.9%), and with a severe condition - 16 (6.6%) cases. The consciousness of patients was normal - in 240 (98.4%), and a soporous state - in 4 (1.6%) cases.

Most often, general weakness of patients was observed - 99.6%, sweating - 90.2%, cough with sputum production - 89.3%, weight loss - 77.9% of cases, and in rare cases, dry cough - 3.7% and an increase in body temperature above 38° - 6.2% of patients (who are infected with mixed flora MBT + normal microflora or complicated by caseous pneumonia).

The patients were examined using all general clinical methods: anthropometric, palpation, percussion, auscultation of organs and instrumental methods.

During examination, chest pain was detected in 115 ($47.1 \pm 3.2\%$) patients, changes in the configuration of the chest in 57 ($23.4 \pm 2.7\%$), atrophy of the pectoral muscles in 30 ($12.3 \pm 2.1\%$) and expansion of the intercostal space in 42 ($17.2 \pm 2.4\%$) cases.

Palpation revealed increased vocal fremitus in 70 cases ($28.7 \pm 2.9\%$), decreased vocal fremitus in 129 cases ($52.7 \pm 3.2\%$), and no change in the remaining 45 cases ($18.4 \pm 2.5\%$). Percussion of the lungs revealed dullness of the pulmonary sound in 208 patients ($85.2 \pm 2.3\%$), box sound in 12 cases ($4.9 \pm 1.4\%$), and pulmonary sound in 27 cases ($11.1 \pm 2.0\%$). Auscultation of the lungs plays an important role in identifying pulmonary diseases. During auscultation of the lungs, weakened vesicular breathing was heard in 211 ($86.5 \pm 2.2\%$) patients, bronchial breathing was heard in 12 ($4.9 \pm 1.4\%$), breathing was not heard in 4 ($1.6 \pm 0.8\%$), and vesicular breathing was heard in 21 ($8.6 \pm 1.8\%$) cases.

The pathological symptoms, anatomical features of the lungs and chest organs, routes and location of pleurisy spread were carefully examined. A 6-minute walk test was performed - this is the most common exercise test in the world used to determine the functional status of patients with respiratory pathology, assess the prognosis of the disease and the effectiveness of therapy. This test is highly sensitive to changes in the methodology of its implementation, so it is recommended to strictly adhere to the technical requirements developed jointly by the European Respiratory Society and the American Thoracic Society: patients who have covered more than 551 m - FC 0, 426-550 m - FC I, 301-425 m - FC II, 151-300 m - FC III and those who have covered a distance of less than 150 m - FC IV, as recommended by the New York Heart Association (NYHA - New York York Heart Association).

In 223 cases (91.4%) there were concomitant diseases: chronic obstructive pulmonary disease (COPD) and chronic bronchitis - in 18 (8.1%), anemia - in 51 (22.9%), cardiovascular diseases - in 51 (22.9%), hepatobiliary system diseases - in 32 (14.3%), genitourinary system diseases - in 14 (6.3%), diabetes mellitus - in 41 (18.4%), mental illness - in 6 (2.7%), AIDS - in 5 (2.2%) and MDR pulmonary TB + diabetes mellitus + hepatitis - in 5 (2.2%) patients. In many cases, anemia was observed with other diseases. In 85 (34.8%) patients, more than 2 types of concomitant diseases with pulmonary tuberculosis were observed.

3. Results and Discussion

When analyzing statistical data, it is noted that drug-resistant pulmonary tuberculosis, often develops in people living with chronic diseases, weakened immunity or with background diseases. The disease is most often detected in patients with concomitant diseases of the cardiovascular, hematopoietic, hepatobiliary system and diabetes. The clinical course was severe and the treatment of patients in this category was quite complex.

In diagnostics and treatment of LU forms of pulmonary tuberculosis, bacteriological verification of mycobacteria with determination of sensitivity to anti-tuberculosis drugs plays a particularly important role. There is treatment without determination of sensitivity or bacteriologically without verification of diagnosis, based on indirect instrumental methods or clinical. In many cases, these irrational activities lead to the emergence of more resistant forms of mycobacterium tuberculosis, while the general condition of patients worsens. Targeted timely adequate treatment serves to cure this severe pathology and prevents its spread among the population.

In modern times, molecular genetic research methods are a particularly important method for studying infectious diseases, as well as tuberculosis caused by Mycobacterium tuberculosis - Koch's shelf. PCR (polymerase chain reaction) diagnostics is based on the detection of mycobacterium DNA in pathological material, such as sputum, pus, pleural fluid, urine. The purpose of detection of the MTB complex + resistance to rifampicin and the determination of MDR-TB and there is no need for a separate detection stage, allows multiplexing, internal control can confirm negative results, high resolution, high specificity, very good reproducibility. Molecular genetic methods are used to multiply and detect genes of interest.

Table 2. Results of bacteriological diagnostic methods

Diagnostic methods	Total	MBT +	MBT-
Bacterioscopy	244	154(63.1%)	90(36.9%)
GenExpert (PCR)	244	237(97.1%)	7(2.9%)
HAIN- test (PCR)	164	164(100%)	0
Bacterial culture in solid medium (Lowenshtein-Jensen)	229	206(90%)	23(10%)
Bacterial culture in liquid medium (MGIT BACTEC-960)	15	11(73.3%)	4(26.7)

In 244 (100%) cases, sputum bacterioscopic examination was performed and MBT was detected in 154 (63.1%) cases.

Sensitivity – Se; Specificity – Sp;

Number of true positive results (NTPR) – A;

Number of true negative results (NTNR) – B;

Total number of cases under study (TNCS) – A+B = C

$$Se = \frac{A}{C} \cdot 100\% = \frac{154}{244} \cdot 100\% = 63,1\%;$$

$$Sp = \frac{B}{C} \cdot 100\% = \frac{90}{244} \cdot 100\% = 36,9\%$$

The sensitivity of bacterioscopic examination of sputum is

63.1%, and the specificity is 36.9%.

PCR diagnostics were performed using the Gene / Xpert device – Mycobacterium tuberculosis DNA was detected in 244 (100%) patients and 237 (97.1%) cases.

$$Se = \frac{A}{C} \cdot 100\% = \frac{237}{244} \cdot 100\% = 97,1\%;$$

$$Sp = \frac{B}{C} \cdot 100\% = \frac{7}{244} \cdot 100\% = 2,9\%$$

The sensitivity of molecular genetic testing of sputum on the GenExpert device is 97.1%, and the specificity is 2.9%.

PCR diagnostics in the HAINtest device were performed in 164 (67.2%) cases and 100% of cases were positive. The most effective methods are those based on hybridization with DNA probes - DNA strip technology and the most widely used in the world is the HAIN test. This method allows: to differentiate mycobacteria of the tuberculosis complex - M.tuberculosis complex: M.tuberculosis, M.bovis, M.africanum, M.microti allows to determine drug sensitivity to isoniazid, rifampicin, fluoroquinolones, aminoglycosides, cyclic peptides and ethambutol.

$$Se = \frac{A}{C} \cdot 100\% = \frac{164}{164} \cdot 100\% = 100\%;$$

$$Sp = \frac{B}{C} \cdot 100\% = \frac{0}{164} \cdot 100\% = 0\%$$

The sensitivity of the molecular genetic study of sputum on the HAINtest device is 100%, and the specificity is 0%. It should be noted that in these cases the study was carried out on patients who excrete MBT with sputum in 100% of cases.

Sowing of pathological material was carried out on a solid Lowenstein - Jensen medium - in 229 (93.9%) patients and in 206 (90%) cases an increase in colonies of Mycobacterium tuberculosis was noted.

$$Se = \frac{A}{C} \cdot 100\% = \frac{206}{229} \cdot 100\% = 90\%;$$

$$Sp = \frac{B}{C} \cdot 100\% = \frac{23}{229} \cdot 100\% = 10\%$$

-Jensen solid medium culture method was 90%, and the specificity was 10%.

Sowing pathological material on liquid BACTEC medium MGite 960 was performed – 15(6.1%) cases and 11(73.3%) cases had a positive result. Purpose of BACTEC MGite 960: Mycobacterium detection and recovery, principle: bacterial growth in appropriate liquid media, semi-automated liquid medium system, reduction of time to result - reduced from 8 weeks to 14 days.

$$Se = \frac{A}{C} \cdot 100\% = \frac{11}{15} \cdot 100\% = 73,3\%;$$

$$Sp = \frac{B}{C} \cdot 100\% = \frac{4}{15} \cdot 100\% = 26,7\%$$

The sensitivity of sputum testing using the MGIT- BACTEC-960 liquid medium culture method was 73.3%, and the specificity was 26.7%.

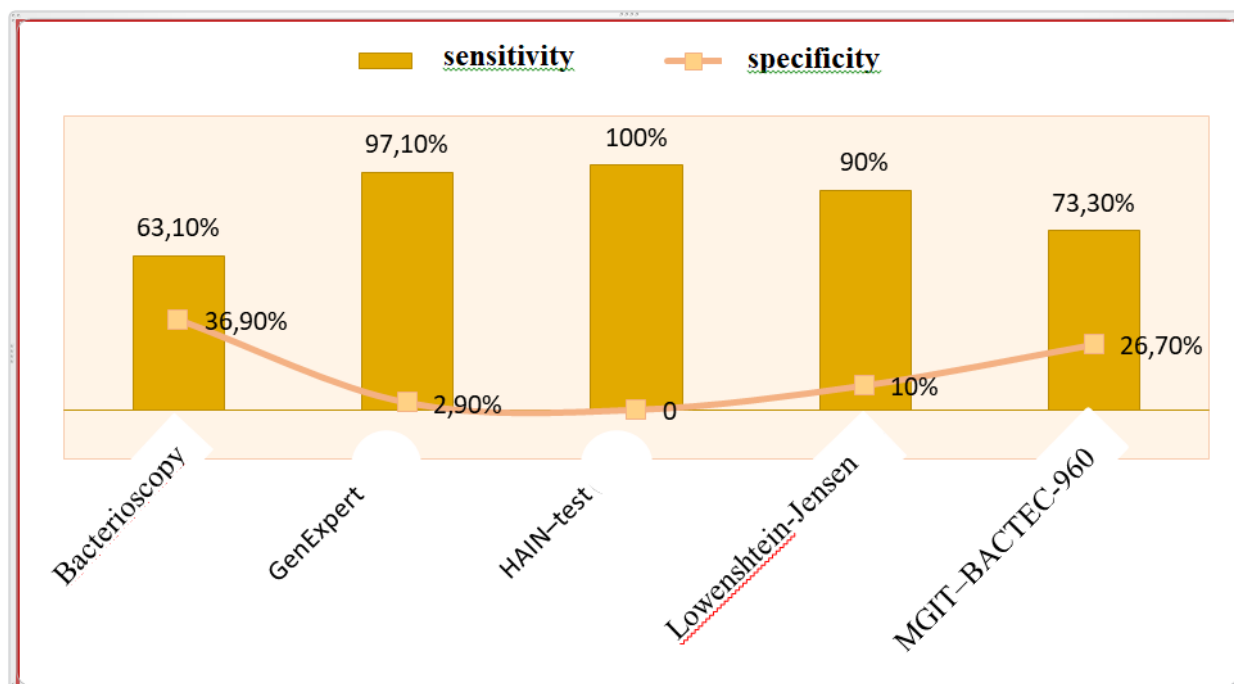


Diagram 1. Comparison of sensitivity and specificity of bacteriological research methods

From diagram 1 it is evident that a comparative assessment of the methods used for bacteriological examination of sputum showed that the most sensitive are molecular genetic methods, and the specificity of the research methods is higher with the bacterioscopic method - in 36.9%, with the methods of sowing on a liquid medium - in 26.7% and on a solid medium in 10% of cases.

Molecular genetic methods of sputum examination are highly effective diagnostic methods, this is due to the fact that PCR diagnostics detect mycobacteria DNA regardless of whether they are alive or dead. Mycobacterium tuberculosis, and with the methods of bacteriological culture from dead mycobacteria, colonies are not formed.

4. Conclusions

Timely diagnostics and adequate treatment of LU forms of pulmonary tuberculosis remains a very urgent problem in phthisiology and society. This section examines the clinical type of the disease, the mistakes made in both somatic and phthisiological medical institutions, the advantages and disadvantages of diagnostic research methods.

The sensitivity of bacterioscopic examination of sputum is 63.1%, and the specificity is 36.9%.

The sensitivity of molecular genetic testing of sputum on the GenExpert device is 97.1%, and the specificity is 2.9%.

The sensitivity of the molecular genetic study of sputum on the HAINtest device is 100%, and the specificity is 0%. It should be noted that in these cases the study was carried out on patients who excrete MBT with sputum in 100% of cases.

-Jensen solid medium culture method was 90%, and the specificity was 10%.

The sensitivity of sputum testing using the MGIT-BACTEC-960 liquid medium culture method was 73.3%, and the specificity was 26.7%.

Molecular genetic methods are highly effective diagnostic methods, this is due to the fact that PCR diagnostics detect mycobacterial DNA regardless of whether the mycobacteria are alive or dead, while bacteriological culture methods do not form colonies from dead mycobacteria.

REFERENCES

- [1] Caminero J.A. Multidrug-resistant tuberculosis: epidemiology, risk factors and case detection // International journal "Tuberculosis and lung diseases". Vol. 2, No. 1, 2011. -pp. 33-44.
- [2] Campbell J. R., Menzies D. What's next for the standard short-course regimen or treatment of multidrug-resistant tuberculosis // Am. J. Trop. Med. Hygiene. American Society of Tropical Medicine and Hygiene. – 2019. – Vol. 100, № 2. – P. 229-230.
- [3] Dalcolmo M. et al. Resistance profile of drugs composing the "shorter" regimen for multidrug-resistant tuberculosis in Brazil, 2000-2015 // Eur. Respir. J. European Respiratory Society. – 2017. – Vol. 49, № 4.
- [4] Hong H., Budhathoki C., Farley J. E. Increased risk of aminoglycoside-induced hearing loss in MDRTB patients with HIV coinfection // Int. J. Tuberc. Lung Dis. International Union against Tubercul. and Lung Dis. – 2018. – Vol. 22, № 6. – P. 667-674.
- [5] Liu Q. et al. Practical considerations to implement the shorter regimen to MDR-TB patients in China // Clini. Microbiol.

Infect. Elsevier B.V. – 2018. –Vol. 24, № 10. – P. 1035-1036.

- [6] Khomova N. A., Kolomiets V. M., Tashpulatova F. K. Adherence to treatment in patients with tuberculosis as a risk factor for reducing its effectiveness // University Science: A Look into the Future. - 2020. - pp. 314-319.

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