Methods For Diagnosing Drug-Resistant Tuberculosis

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ABSTRACT

The widespread prevalence of drug-resistant tuberculosis is a global problem and can negatively affect the tuberculosis situation on a global scale. Rapid diagnosis of the disease and early initiation of effective treatment based on the selection of personalized chemotherapy regimens are the basis for preventing the spread of tuberculosis. Microbiological methods are of particular importance for the diagnosis of tuberculosis, which make it possible to substantiate the etiology of the process and determine the drug sensitivity of the pathogen. The review examines current and developing methods for the microbiological diagnosis of tuberculosis, including classical microbiological and modern molecular genetic tests.

Keywords: Mycobacterium tuberculosis, drug resistance, molecular genetic methods, culture diagnostics, PCR, sequencing.

Introduction

The widespread prevalence of drug-resistant tuberculosis is a global problem. In Uzbekistan, over the past 10 years, against the background of a decrease in the main indicators of tuberculosis (incidence - by 43.2%, prevalence - by 42.4%, mortality - by 64.2%), an increase in the prevalence of multidrug-resistant tuberculosis has been recorded (MRT) from 23.4 (in 2013) to 55.3% (in 2023) and an increase in primary drug resistance from 13.6 (in 2013) to 29.3% (in 2023) [1].

Materials And Methods

Rapid diagnosis of the disease and early initiation of effective treatment based on the selection of personalized chemotherapy regimens are the basis for preventing the spread of tuberculosis. The World Health Organization (WHO) Global Report on Tuberculosis notes: "If all people with tuberculosis had access to timely diagnosis and high-quality treatment, patient mortality rates would be low in all countries" [2, 3].

Results And Discussion

Traditional microbiological methods for diagnosing tuberculosis - microscopy of diagnostic material and cultural studies - remain relevant.

Microscopy is not directly related to the diagnosis of drug resistance, despite the low sensitivity and specificity of the method, it allows you to quickly and with minimal financial costs assess the massiveness of bacterial excretion and monitor the effectiveness of chemotherapy, including drug-resistant forms of tuberculosis [4].

Since microscopy methods have low throughput and place a high load on the microscopist's vision, modern developments in this area are aimed at digitizing the image and transmitting it to a monitor screen. Great importance is also paid to the portability of the equipment. For example, the battery-powered

digital fluorescence microscope CellScope (Fletcher Lab, UC Berkeley, USA) has small dimensions (20 × 20 × 10 cm) and weight (about 3 kg). The system was created on the basis of a microscope with a magnification of 200, but digital images generated on the screen can be enlarged directly on the monitor screen up to 3500 without loss of sharpness [2].

The automated TBDx system (Signature Mapping Medical Sciences, USA), which automatically loads stained smears and analyzes the digital image, will significantly reduce the labor intensity of the microscopy process and increase the throughput of the method. This platform consists of a high-quality microscope and an imaging system. In the minimum configuration, the system can automatically process 1-4 strokes, and when equipped with a robotic carousel for loading glass, from 50 to 200 strokes. Patented software reads the images to detect stained cells. Reading each smear takes about 5 minutes, therefore, the analysis of 200 smears takes about 16 hours without operator participation [3]. Automated high-throughput staining platforms, such as RAL Stainer (RAL Diagnostics, France) or Aerospray TB Series 2 (ELITechGroup, France), can be used to stain smears before entering the system. A study of the feasibility of using TBDx for the diagnosis of tuberculosis in South Africa demonstrated that the results obtained using a stand-alone system were comparable to those obtained by experienced microscopists, making TBDx an ideal diagnostic solution in situations where qualified specialists are not available [1].

In general, systems with automatic recording of culture growth in a liquid medium are an ideal tool for conducting cultural diagnostics of tuberculosis and performing drug susceptibility tests, but the high cost of the equipment makes them inaccessible for widespread use. Therefore, for countries with low economic levels, WHO recommends the use of inexpensive non-commercial methods such as MODS (from microscopic-observation drug-susceptibility - microscopic detection of drug sensitivity). The MODS method consists of microscopic detection of the beginning of culture growth in a liquid medium, and detection of the growth of Mycobacterium tuberculosis and determination of drug sensitivity are carried out in parallel by simultaneous inoculation of diagnostic material on a medium for detecting Mycobacterium tuberculosis and on media with anti-tuberculosis drugs. This approach, according to a multicenter study, made it possible to obtain the result of resistance to antituberculosis drugs in an average of 14.3 days, while with BACTEC MGIT a similar result was obtained in an average of 24.7 days [2]. An improvement to the MODS method was proposed by a group of researchers from the Laboratory of Infectious Diseases at the University of Peruana Cayetano Heredia (Lima, Peru) with support from the Wellcome Trust. The use of the indicator 3-diphenyl-5-(2-thienyl) tetrazolium, which changes color as the culture grows, made it possible to monitor the growth of microcolonies by changing the color of the medium and only after that carry out confirmatory microscopic studies [3].

Molecular genetic test systems in the diagnosis of drug-resistant tuberculosis

The use of molecular genetic methods for diagnosing tuberculosis makes it possible to reduce the time required to obtain an analysis result to 1 day, which makes this direction the most promising and in demand [5]. The polymerase chain reaction (PCR) method, which is the basis of molecular genetic diagnostics, in its various variants allows the use of diagnostic material isolated from patients for analysis: identification of species-specific DNA fragments of the pathogen indicates the presence of mycobacterium tuberculosis in the sample, and identification of mutations in genes associated with resistance.

The market for molecular genetic technologies today is maximally saturated with various developments, and it is important to understand which of the existing methods is advisable to use in clinical diagnostic laboratories at different levels. Currently, several approaches are used to determine drug sensitivity by molecular genetic methods. All of them are based on knowledge about mutations associated with drug resistance to a particular anti-tuberculosis drug [2].

Hybridization technologies for determining genotypic drug resistance, based on the binding of PCR products to specific oligonucleotide probes immobilized on a matrix, which is a biological microchip or DNA strip, are widely used in our country and abroad.

DNA strips are a relatively inexpensive method, but have a fairly low sensitivity, and therefore can be used for samples with a positive microscopic examination or for determining genotypic resistance in mycobacterial cultures. There are several companies on the world market that produce DNA strips for determining genotypic resistance, two of which, kits produced by Hain Lifescience and INNO-LiPA, are approved by WHO [4].

Conclusion

However, despite the advances in therapy, it is necessary to further improve the diagnosis of drug resistance of tuberculosis. Considering that, according to our data, there is an unfavorable dynamics of expanding the spectrum of drug resistance of the tuberculosis pathogen, the possibility of determining drug resistance by molecular genetic methods to only 3 drugs seems clearly insufficient [3]. It is urgent to develop new molecular domestic test systems that make it possible to determine drug resistance to a wide range of 1st and 2nd line anti-tuberculosis drugs with high reliability for the earliest possible prescription of adequate chemotherapy.

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