TUBERCULOSIS RAPID METHODS OF DIAGNOSIS

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Abstract: *Tuberculosis is infection with a high risk fatality when untreated or unfairly treated. Nowdays, when have taken changes in clinical appearance of mycobacterial infections (mainly due to association of comorbidities), and define new multidrug resistant forms with increased frequency, required major changes in laboratory diagnosis. It proves the necessity of implementing modern methods of rapid detection mycobacterial division and their susceptibility to antibiotics, increasing therapy efficiency and decreasing the risk of suplementary developing of multidrug resistance. Methods currently available in the world are BACTEC system 460, BACTEC 9000, MB /BacT method, nitrate reductase method (NRA). The genetic methods, involving DNA hybridization probes, Polymerase Chain Reaction (PCR), less accessible, are options for the future.*

Cuvinte cheie: *tuberculoza, multidrogrezisten□ ,ă metode moleculare*

Rezumat: *Tuberculoza reprezintă o infec□ie transmisibilă care netratată sau tratată incorect are o mortalitate crescută. În contextul actual, odată cu apari□ia numeroaselor schimbări în tabloul clinic al infec□iilor micobacteriene (în principal datorită asocierii comorbidită□ilor), □i definirii noilor forme multidrogrezistente cu frecven□ ărescută, se impun modificari majore în diagnosticul de laborator, cu necesitatea implementării metodelor moderne de detectare rapidă a diviziunii micobacteriene □i a susceptibilita□ii acestora la antibiotice, crescând eficien□a terapiei □i scăzând riscul dezvoltării suplimentare a multidrogrezisten□ei. Metode disponibile la ora actuală în lume sunt sistemul BACTEC 460, BACTEC 9000, metoda MB/BacT, metoda nitrat-reductazei (NRA). Metodele genetice, mai pu□in accesibile implicând sondele de hibridizare ADN, reac□ia lan□urilor de polimerază PCR (Polimerase Chain Reaction) reprezintă op□iuni de viitor.*

SCIENTIFICAL ARTICLE OF THEORETICAL PREDOMINANCE

In recent decades, multidrug resistant tuberculosis (MDR TB), and in recent years through extensive form (extensive drug resistant tuberculosis, XDR TB) has become a significant threat in the therapeutic control of disease (20). It is estimated that only 2% of MDR TB cases worldwide are treated properly, this partly due to the still inefficient laboratory services. (1). The antibiogramm for Mycobacterium tuberculosis is required especially in case of relapse or multidrug resistance (MDR) as reference method in detecting resistant strains. The testing on conventional solid media requires two months or more to confirm the diagnosis. Bacteriological examinations will be performed before starting any treatment, thus prolonged time until the beginning of targeted therapy according to antibiogramm. During this period, the patients infected with resistant germs can spread disease in the community. Sometimes death occurs before the results, especially in HIV co-infected patients.

Progress in molecular biology has lead to new methods that significantly improved the performance of the classic methods. (3)

Nowdays it is necessary to use rapid culture methods as BACTEC or newer method and analyze such as DNA hybridization probes or the RFLP (DNA restriction fragment length polymorphisms). In modern current laboratories, methods using liquid medium with radiometric growth detection (BACTEC-460), have replaced traditional methods of isolation on solid media and biochemical identification tests. These new methods have reduced the time needed to isolate the stem to 2-3 weeks.

BACTEC 460 system is a radiometric detection system, while the **MB / BacT** is a colorimetric method, monitoring bacterial multiplication on liquid medium. Positive cultures can appear starting from the 4th day. Respirometry BACTEC detection requires 7-25 days. (11)

BACTEC is a radiometric detection system that uses medium containing palmitic acid marked with 14C. This is catabolised of mycobacteria, releasing 14CO2, measured by automatic system (BACTEC 460) on a scale calibrated from 0 to 999, specifing the growth index (GI), which is directly proportional to the growth rate of mycobacteria. The system uses a type environment Middlebrook, supplemented with an antibiotic solution containing polymyxin B, azlocillin, nalidixic acid, trimethoprim and amphotericin B. (2) BACTEC bottles should be read twice weekly for the first 3 weeks and once each subsequent week for a total of 6 weeks before being designated negative. The time required to obtain positive cultures in this system is much lower than that required using a solid medium. For some non-tuberculous mycobacterial positivity can occur in less than seven days, and for M. tuberculosis in 4-25 days. With the BACTEC system is tested also the sensitivity to chemotherapy, and time decreases to less than 10 days, compared to 3 weeks for conventional antibiogramms. The BACTEC 9000 system eliminates the risk of using radioactive substances. (5)

The MB /BacT is a colorimetric method, monitoring the bacterial multiplication, eliminating the disadvantages of radiometric methods. The tubes containing a liquid medium

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have a colorimetric detector. When the bacterial multiplication reaches a density of 106-l07 bacteria/ml., the device signalized optically and acustically the positive culture. Culture medium is added in advance a solution with factors that stimulate the multiplication of germs ("recovery solution"), and a mixture of antibiotics to avoid the suprainfection of culture. In the case of pulmonary tuberculosis, a positive result is obtained on average 10 days faster than when using conventional solid media. In 20-25% of cases, positive signals may occur in 5-7 days. (5) (9) (4)

Another simple, fast and accesible method proposed to be introduced is the **nitrate reductase (NRA) (direct and indirect),** compared with the standard method to determine the proportions of M.tuberculosis antibiogram in order to evaluate its usefulness and efficiency as a screening method. The method is rapid and inexpensive and can be applied directly to biological samples of sputum, bringing greater benefit to the National Tuberculosis Control Programmes, giving rapid monitoring of MDR-TB patients liable (1). The method evaluates the sensitivity of M. tuberculosis to antibiotics using Löwenstein-Jensen medium. M. tuberculosis has the ability to reduce nitrate to nitrite due nitrate-reductase enzyme action, reported by changing color after adding Griess reagent. NRA is able to detect mycobacteria rapid growth, with rapid results in 10 to 14 days versus 42 days by the method of proportions. (7) The sensitivity and specificity of the direct method are recorded in 75% and 100% for rifampicin and 75% and 98.4% for isoniazid. For the indirect method are 77.7% and 92% for rifampicin, respectively 85.7% and 85.2% for isoniazid. (8)

One of the most promising diagnostic techniques involve the amplification and detection of specific segments of DNA – **the Polymerase Chain Reaction (PCR).** PCR is the maximum utility in the diagnosis of pulmonary or extrapulmonary paucibacilare forms of tuberculosis. Due to high costs, this method is not routinely available

CONCLUSIONS

Molecular methods for identification and determination of antibiotic susceptibility of germs are extremely valuable for slow-growing germs, such as M. tuberculosis. TB diagnosis depends on isolation and identification of M. tuberculosis and the efficiency of MDR TB control depends on speed and sensitivity of laboratory results.

BACTEC method (BACTEC 460TB system, BACTEC 9000, MB /BacT) or the NRA have the advantage of faster result compared to conventional methods and reduce working time and expense claimed by these methods.

Early recognition of MDR TB /XDR cases, using rapid methods for resistance detection, would considerably reduce the duration of conventional ineffective therapy, promoting the administration of adequate therapy according to individual resistance, reducing the costs of diagnosis and treatment

Widespread use of these sensitive and rapid methods is necessary and would bring great benefits beneficial in the future for the identification, treatment and eradication of TB and especially resistant forms of disease.

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