IDENTIFICATION OF THE MYCOBACTERIUM TUBERCULOSIS’ STEM (STRAIN) AT RIFAMPICINA AND IZONIAZIDA RESISTANCE THROUGH PCR TECHNIQUE

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Summary

To prevent the multi-drug-resistant tuberculosis extension (TB - MDR), major modifications in laboratory diagnosis are imposed and, in the same time new modern methods for a fast detection of mycobacterial splitting as well as their sensitiveness to antibiotic are needed, increasing this way the therapy and decrease the risk of multi-drug-resistance over development. In this experiment it was analyzed the resistance to RMP and INH of Mycobacterium tuberculosis culture; in this way the physician will be able to prescribe the proper treatment, because these methods get more benefit for people to win the battle against TB making easier its identification, treatment and eradication.

Key words: Mycobacterium tuberculosis, multi-drug-resistant tuberculosis (TB - MDR), PCR technique

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Introduction

Pulmonary tuberculosis continues to represent a major problem of the public health in Romania. Together with numerous changes of mycobacterial infections, clinical description and defending the new multi-drug resistant forms with high incidence, major modifications in laboratory diagnosis are imposed and, in the same time new modern methods for a fast detection of mycobacterial splitting as well as their sensitiveness to antibiotic are needed, increasing this way the therapy and decrease the risk of multi-drug-resistance over development (Mitrea and Rădulescu, 2011).

Thus, at present, the available methods are BACTEC 460 system, BACTEC 9000, BACTEC MGIT 960 and MB/ BacT method, NRA (nitrate reductosis); among genetic methods, DNA hybridization probe, PCR (Polimerase Chain Reaction).

Although they are expensive, these methods have a big advantage: they give much faster results, establishing an efficient therapy and diminishes the risks of a therapeutic failure (Homorodean et al., 2005; Martin et al., 2006; Negi et al., 2006).

To prevent the multi-drug-resistant tuberculosis extension (TB - MDR), the National Program of Tuberculosis Control (PNCT) assisted by OMS, has drawn up the National Preventing and Management Programme of TBMDR for middle term, starting from 2010 to 2015, with a purpose of diagnosing and treating at least 80% of the TB cases, up to 2015 and achieving a rate of therapeutic succes of 70% from the new cases and 50% of old cases retreated (Colebunders et al. 2002; www.tbnews.ro/download.php.article; World Health Organization, 2006).

Material and methods

This study is meant to size up the resistance to RMP and INH of Mycobacterium Tuberculosis culture; in this way the physician will be able to prescribe the proper treatment. The GENOTYPE MTBDR PLUS 96, ver. 1.0, analysis kit was used, a quick and reliable test. There are more stages in the procedure: DNA extraction, PCR amplification, samples hybridization, the results, evaluation and interpretation.
Using the PCR method (simultaneous with the classic methods of the absolute concentration) antibiograms were done (determination of the germs’ sensitiveness to antibiotics) on 115 *Mycobacterium tuberculosis* isolated stems, the compatibility between the 2 methods being 100%.

The DNA extraction was done from *Mycobacterium Tuberculosis* cultures, an inoculation dowser is put into 300 µl PCR water, then 15 min of centrifugation at 10,000 rotations; add again 300 µl PCR water and it is incubated 25 min at 90°C, following an ultrasonic bath.

![Figure 1. Ultrasonic bath](image1)

To amplify PCR for each reaction is transferred 5 µl supertanant inside the tubes and 45 µl Master Mix are added; the next step – all the typical cycles.

![Figure 2. Labcycler](image2)

The samples’ hybridization is worked out in a water bath with TwinCubator agitation at 45°C. 20 µl DEN (denaturing solution) in every corner of the tray’s bucket. 20 µl of the amplified sample, is well homogenized and for 5 min, an incubation at the room’s temperature. At the same time the strips are numbered.

1ml of preheated hybridization buffer is added in each tray’s bucket, then it is slightly agitated to homogenize and the strips are very carefully introduced so they are perfectly covered with water. The tray is fixed up into the Twin Cubator and follow the next stages – washing and rinsing.

![Figure 3. TwinCubator](image3)

![Figure 4. The results interpretation](image4)

**Results and discussion**

The 115 *Mycobacterium tuberculosis* isolated stems have been processed for a year and the following were obtained:

- Stems both antibiotics (RMP and INH) resisting: 27
- Stems INH resisting: 6
- Stems RMP resisting: 2
- Stems with total sensitiveness: 78

The same results were obtained through the classic method of antibiograms (of the absolute concentration).

The micro-bacteria population from a stem is heterogeneous regarding the mutation/ alteration proportion of resistance.
confronted by various anti-phthisical drugs (remedy).

The heterogeneity is due to the fact that spontaneous mutations take place and the rate of frequency varies according to drugs and so, the result is 1 bacillus of 10^8 RMP resisting; 1 bacillus of 10^6 INH resisting. The “wilde” stems which never been treated with anti-phthisical (tubercular) drugs, are also heterogens, but the bacteria with resistance mutation do not exceed 1% from all, over this limit is considered that it could be a correlation with the therapeutic result. Resistant mutants selection is made only in the presence of the drug (Homorodean et al., 2005).

To evaluate and explain the results, the presence of control strip (Conjugate Control - CC) and amplification strip (Amplification Control - AC) certifies the efficiency of the link between the conjugate and the amplification process’ precision. For the *Mycobacterium tuberculosis* (TUB) complex the presence of the strip certifies that the zone interbreed with the amplicon generated by all the members of the complex of *Mycobacterium tuberculosis*. Also, the presence of the strip results in locus control (rpoB, katG, inhA) is compulsory and the TUB zone certifies the presence of *Mycobacterium tuberculosis* stem (strain).

The “wilde” probes contain the most important and resistant zones of those gene; if all the wilde probes for a certain gene stay positive, these are no detected mutations in that region and the stem/strain is responsive to that specific antibiotic. In case that a mutation appears that amplicon is uncapable of linking a suitable wilde probe. The lack of any signal for at least one wild probe, indicates a resistance of that tested stem/strain to the specific antibiotic.

The strips obtaines with rpoB specific probe give us conclusions about the stem resistance vs. rifampicina. The strips obtained with specific katG, specific stems indicate a high resistance at izoniazida and the strips you get with the inhA specific stems, give us information about a low resistance at izoniazida of the tested stems. The other types – the mutant probes detect the most important resistant mutations.

The GENOTYPE MTBDR PLUS 96 is quite easy and represent another modern and useful instrument for tuberculosis management, because it allows the detection of the resistance at INH and RPM in stems or directly from the products having a positive smear (Hillemann et al., 2007; Lacoma et al., 2008).

**Conclusions**

Precocious acknowledgement of TB MDR/XDR (extensive drug resistance), using rapid methods of resistances detection, would considerably reduce the therapy duration and allow to fit the therapy to individual resistance. In this way, to diagnose and treat could be much cheaper.

It’s really a necessity to utilize these rapid methods, to get more benefit for people to win the battle against TB making easier its identification, treatment and eradication.

**References**


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