6. TUBERCULOSIS AND MYCOBACTERIA: MOLECULAR APPROACH*

6.1

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PERFORMANCE OF GENEXPERT MTB/RIF IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS IN MOROCCO

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Tuberculosis is commonly associated with lung diseases, but can also affect other parts of the body (extrapulmonary tuberculosis [EPT]). A rapid diagnosis is essential to initiate a specific and effective treatment. The diagnosis of EPT is a real challenge because of the paucibacillary nature of samples. GeneXpert MTB/RIF is a rapid automated diagnostic test that allows the detection of the presence of *M. tuberculosis* as well as mutations in the hot-spot region of the *rpoB* gene associated with rifampicine resistance. The objective of this study was to evaluate the performance of the GeneXpert MTB/RIF test for the diagnosis of EPT.

We analyzed 304 clinical samples collected in the Laboratory of Mycobacteria and Tuberculosis of Pasteur Institute of Morocco, between 2016 and 2017. Of these samples, 113 were pleural fluids decontaminated using the Petroff method and 191 biopsies (78 lymph nodes and 113 pleural biopsy), decontaminated using the Loeweinstein method. All samples underwent smear microscopy, culture on Loeweinstein–Jensen medium and tested with Xpert MTB/RIF.

The study population included 192 patients, 54.2% were men and 45.8% women. The age of the patients ranged from 2–78 years with the majority of the patients in the age group 25–45 years. The sensitivity of GeneXpert was 51.47% for all samples and 83.3% for lymph nodes.

Our study clearly shows that GeneXpert MTB/RIF test presents limitations in the diagnosis of EPT. In view of these results, it would not be appropriate to use only this technique for the diagnosing of EPT.

6.2

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ANALYSIS OF GENE MUTATIONS ASSOCIATED WITH *MDR AMONG MYCOBACTERIUM T*UBERCULOSIS STRAINS ISOLATED IN MOSCOW REGION

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The aim of this study was to determine the prevalence and variants of mutations in *M. tuberculosis* genes associated with the development of multidrug-resistance (MDR) as well as their correlation with genotypes in the study of clinical isolates obtained from patients with tuberculosis (TB) in hospitals from the Moscow region.

179 randomly selected *M. tuberculosis* clinical isolates from TB patients collected from 2008 to 2016 years were included in this study. One isolate from each patient was used. The molecular characteristics of *rpoB*, *katG* genes and *inhA* promoter, resulting in rifampin and isoniazid resistance (MDR), were obtained by Sanger sequencing. All specimens were subjected to spoligotyping; spoligotypes were compared to SITVIT_WEB database. Pearson χ^2 test was used to check pairwise differences.

All clinical isolates were divided into 2 groups of genotypes according to the results of spoligotyping: Beijing (72.6%, n = 130) and other genotypes collectively named "non-Beijing" genotypes (27.4%, n = 49). Beijing genotype had *rpoB* Ser 531> Leu mutation in 62.9% of cases whereas non-Beijing genotypes in only 15.8% of cases. Other variants of *rpoB* mutations were detected in only 6.3% of Beijing strains versus 28.1% of non-Beijing strains. The wild-type *rpoB* gene was observed in Beijing genotype in 30.8% of cases whereas in non-Beijing genotype in 56.1%. Statistically significant differences were obtained for all comparisons between two groups ($\chi^2 = 9.21$, $p < 0.01$).

We also obtained statistically significant differences in the analysis of combinations of *katG* gene and *inhA* promoter for Beijing and non-Beijing genotypes, respectively: in the case of simultaneous presence of mutations in them, in 13.9% and in 34.9% of cases ($\chi^2 = 8.59$, $P = 0.0036$) or wild-type in 20.4 and 39.5% of cases ($\chi^2 = 20.64$, $p < 0.0001$), as well as in the presence of genetic changes in only *katG* gene, 62.0 and 20.9% ($\chi^2 = 20.64$, $p < 0.0001$). However, no statistically significant differences were noted when comparing *inhA* promoter mutations occurred alone without *katG* mutations which was observed in a small proportions in both genotypes — 3.7 and 4.7%.

We established some specific features in clinical isolates of *M. tuberculosis* in Moscow region.

6.3

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COULD THE NEW INSIGHTS INTO PZA RESISTANCE PROVIDE ROUTE TO SHORTER MORE EFFECTIVE TB THERAPY?

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Pyrazinamide (PZA) is a pro-drug that is transformed into pyrazinoic acid (POA) by mycobacterial PncA enzyme. A very wide range of mutations in *pncA* result in clinical and *in vitro* resistance. In the last two years multiple groups have demonstrated that a low pH is not required for the activity of POA against tuberculosis as was previously widely assumed. Furthermore, laboratory mutants against POA have been generated in multiple laboratories under different conditions. Mutations in a range of genes have been observed but always including *clpC1* and/or *panD*. A direct activity of POA against mycobacterial PanD has been demonstrated but evidence of activity against other genes associated with *in vitro* resistance is disputed or lacking. It has been suggested that PZA is a dirty drug with multiple targets but we recently

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