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.

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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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proposed an alternative explanation: POA is primarily active against PanD but PanD is only essential if the bacteria are expressing a stringent response, the other genes associated with resistance in some way disrupt the stringent response and eliminate the sensitive phenotype. This suggests a critical role for the stringent response in the life cycle of *M. tuberculosis* as compounds are being developed that target this pathway we suggest these compounds are particularly promising compounds for the treatment of tuberculosis.

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IDENTIFICATION OF MUTATIONS OF RESISTANCE TO FLUOROQUINOLONES, AMINOGLYCOSIDES AND ETHAMBUTOL IN RIFAMPICIN-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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The aim of the study was to identify resistance mutations to second-line anti-tuberculosis drugs in patients with *Mycobacterium tuberculosis* clinical samples resistant to rifampicin. Samples of biomaterial from 35 adult residents of the Tyumen region in West Siberia with established by GeneXpert system presence of rifampicin-resistant mycobacteria tuberculosis (MBT) were examined in our study using the MTBDRsl kit (Hain Lifescience, Nehren, Germany) according to the manufacturer's instructions. The mutations were identified in genes encoding DNA gyrase *(gryA)*, 16S RNA *(rrs)*, and arabinosyltrasferase *(embB)* associated with resistance to fluoroquinolones (FLQ), injectable aminoglycosides/cyclic peptides (AG/CH) and ethambutol (EMB) respectively.

In 9 of the examined samples (25.7%) the MBT resistance to all three groups of drugs was revealed. In the remaining 26 samples, the MBT sensitivity to one or two groups of drugs can be assumed. Samples from 24 patients (92%) were genetically susceptible to AG/CH (in 2/3 cases solo, in 1/3 — in combination with sensitivity to ethambutol (5 samples) and fluoroquinolones (1)). Most samples demonstrated genetic resistance to fluoroquinolones (97%) and ethambutol (80%), and 30% of samples are resistant to aminoglycosides/cyclic peptides.

Among the *gyrA* mutations, 11 were in codon 90 (A90V), 43 — in codon 94 (of which 4 — D94A, 6 — D94N, D94Y, 30 — D94G and 3 — D94H). No mutation in codon 91 (S91P) was detected. In 22 samples 1 mutation was detected, in 4 — 2 mutations, in 6 — 3 mutations, and in 1 — 5 mutations. Among the mutations found in the *rrs* gene, 8 are in codons 1401–1402 (A1401G, C1402T) and 10 — in codon 1484 (G1484T), all mutations in codons 1401–1402 are combined with the presence of a mutation in codon 1484. Among mutations in *embB* (codon 306) in three cases replacement of M306I = 306 ATG/ATA, M306V, in 26 — replacement of M306I = 306 ATG/ATC/ATT was revealed. In 24 cases only one variant of the mutation is found, in 2 — both, and in 18 samples in the presence of a mutation there is no marker of wild type.

In conclusion, preliminary data on the genetic structure of MBT strains resistant to rifampicin and second-line anti-tuberculosis drugs were obtained in tuberculosis patients from the Tyumen Region in Siberia.

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MOLECULAR TYPING OF *MYCOBACTERIUM KANSASII* — A GLOBAL PERSPECTIVE

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To date, over 180 nontuberculous mycobacteria (NTM) species have been identified and almost 30 of these species have been reported as the causative agents of pulmonary and extrapulmonary diseases. *Mycobacterium kansasii* is the sixth most frequently isolated NTM species across the world. The isolation rate of this pathogen, among other NTM, has been calculated at 5% in Europe and 4% globally. In Poland and Slovakia, the recovery of *M. kansasii* from respiratory samples is particularly high, being 36% and 35%, respectively.

The genetic heterogeneity of *M. kansasii* is defined by the presence of seven molecular subtypes. Most of the disease-related strains belong to subtype I and II, while the others (III-VII) have usually been linked to environmental sources. Therefore, subtyping of *M. kansasii* isolates from human samples may be helpful for clinical diagnosis.

The aim of this study was to determine the distribution of *M. kansasii* subtypes among clinical isolates from 19 countries on 4 continents.

A total of 475 isolates recovered between 2000 and 2017 from as many patients with suspected *M. kansasii* disease were analyzed. The isolates were collected from 19 coun-