6.8 doi: 10.15789/2220-7619-2018-4-6.8

GENOTYPING OF MULTIDRUG AND PRE-EXTENSIVELY DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM A HIGH INCIDENCE TB AREA IN MOROCCO

I. Chaoui[1], A. Oudghiri[1,2], F. Chetioui[3], A. Laglaoui[2], M.D. El Messaoudi[3], M. El Mzibri[1]

[1]Unité de Biologie et Recherches Médicales, Centre National de l'Energie, des Sciences et Techniques Nucléaires, Rabat, Morocco; [2]Equipe de Recherche en Biotechnologies et Génie des Biomolécules, Faculté des Sciences et Techniques de Tanger, Tanger, Morocco; [3]Laboratoire de la Tuberculose, Institut Pasteur du Maroc, Casablanca, Morocco

The emergence of extensively drug-resistant tuberculosis (XDR-TB) has raised public Health concern for global control of TB. Although drug resistance-associated mutations in multidrug-resistant *Mycobacterium tuberculosis* complex (MTBC) isolates in Morocco were characterized, mutations in Pre-XDR/XDR isolates and their genotypes have not been reported previously. Resistance to second line antituberculosis drugs (SLDs) is mainly due to mutations in specific genes: *gyrA* and *gyrB* for resistance to fluoroquinolones (FQs), *rrs*, *eis* and *tlyA* for resistance to injectable drugs (kanamycin (KAN), amikacin (AMK), and capreomycin (CAP).

A collection of 70 MTB isolates already characterized as MDR and 100 pan-susceptible isolates randomly selected from a high incidence TB area in Morocco were enrolled in this retrospective study. The mutation profiles associated with resistance to SLDs: FQs and injectable drugs were assessed by DNA sequencing. Target sequences for four genes were examined: *gyrA* and *gyrB* (FQs), and *rrs* (KAN, AMK, and CAP) and *tlyA* (CAP). The fingerprint of each isolates was established by spoligotyping and 15-loci MIRU-VNTR typing methods.

Molecular analysis showed that 26.7% of MDR isolates are pre-XDR strains harboring mutations in *gyrA* gene. The most prevalent mutation involved in FQ resistance was Asp94Gly (50%). None of the isolates harbored mutations neither in *gyrB* nor in *rrs and tlyA* genes. Likewise, none of the pan-susceptible isolates displayed mutations in targeted genes. Spoligotyping of MDR MTB isolates resulted in 4 and 5 orphan and unique patterns respectively, and 61 strains in 9 clusters (2–26 strains per cluster) with a resulting clustering rate and recent transmission rate of 87.1% and 74.3% respectively. The most prevalent spoligotype was SIT42 (LAM; 37% of isolates). The repartition of strains according to major MTBC clades was as follows LAM (46.1%) > Haarlem (59%) > ill-defined T superfamily (17%) Haarlem (7%) > clade S (6%) > Beijing (3%) > T2-T3 and Beijing-like (1%). Of note, CAS (Central Asian) and EAI (East-African Indian) strains were absent in this setting. Subsequent 15-Loci MIRU typing failed to find any cluster of SIT/MIT, all clusters established by spoligotyping were splited, 70 unique MLVA-MtbC15 profiles were generated with a resulting clustering and recent transmission rate equal to zero meaning that all MDR strains are not a part of an established transmission chain and that the developpement of drug resistance in this setting is likely a result of inadequate treatment rather than primary resistance. HGDI analysis of the 15 MIRU loci showed that loci 10, 40 and Mtub04 were highly discriminative in our setting. All pre-XDR isolates harboring mutations in *gyrA* gene had not specific/paticular pattern generated by any of the two methods.

This study provides a first global snapshot of MDR MTBC population structure in Morocco. The results obtained (i) highlight the need for rapid detection of mutations associated with resistance to SLDs in order to adjust timely the treatment and to interrupt the propagation of virtually untreatable form of the disease, (ii) confirm that TB in Morocco is almost exclusively transmitted through evolutionary-modern MTBC lineages belonging to principal genetic groups 2 and 3 (Haarlem, LAM, T), with extremely high level of biodiversity seen by 15-MIRU typing, (iii) validate the use of spoligotyping in conjunction with 15-MIRU-VNTR scheme for future investigations in Morocco that should ideally use modified 15-loci MIRU-VNTRs (to include MIRU 23 instead of MIRU16), (iv) confirm the use of both the two typing methods to understand the transmission dynamic of tuberculosis in this setting.

6.9 doi: 10.15789/2220-7619-2018-4-6.9

THE CORRELATION BETWEEN LEVELS OF PHENOTYPIC RESISTANCE AND GENOTYPIC MUTATIONS OF *M. TUBERCULOSIS*

N. Ciobanu[1], S. Alexandru[1], D. Chesov[1], A. Codreanu[1], C. Lange[2], V. Crudu[1]

[1]Phthisiopneumology Institute, Chisinau, Moldova; [2]Research Center Borstel, Borstel, Germany

Rapid identification of drug resistance of *Mycobacterium tuberculosis* allows an earlier initiation of an adequate treatment regimen that potentially can reduce TB morbidity, mortality and transmission. New diagnostic methods have provided a promising solution for rapid and reliable detection of drug resistant TB. Despite the fact that rapid molecular assays are less accurate than the culture-based methods and raise the possibility of false negative results, the molecular characterization of the resistance spectrum of *M. tuberculosis* isolates offers the opportunity for overcoming the phenotypically detected resistance. So far, mutations within the *rpoB* gene confers a different level of phenotypic resistance for rifampicin (RIF) as well as mutation in *inhA* is link with low resistance to isoniazid (INH).

Objective was to study the correlation between phenotypic and genotypic resistance of *M. tuberculosis* on different levels of drug concentrations.

The genotypic resistance profiles for isoniazid and rifampicin of *M. tuberculosis* sputum isolates were assess by MTBDR*plus* and where correlated with culture based (MGIT-960) drug sensitivity test results. The different level of inhibitory concentrations of rifampicin and isoniazid of individual strains, assessed by MGIT-960 equipped with EpiCenter TB eXiST, were correlate correspondingly with the mutation types in the *rpoB* gene, and the presence of *inhA* mutation in the same *M. tuberculosis* isolates.

The *M. tuberculosis* isolates from 4568 patients with pulmonary tuberculosis were assess. 64.2% of them were INH resistant, and in 1,9% (n = 86) of these isolates, resistance was conferred by *inhA* mutation only. RIF resistance was detected in 61.9% of subjects, and in 27.2% (n = 762) of these the mutation *rpoB531* was missing. 30.6% of INH resistant *M. tuberculosis* strains, conferred by inhA mutation only and 28.6% of RIF resistant *M. tuberculosis* strains without S531 mutation, were sensitive to high concentrations of drugs by phenotypic DST.

The correlation of genotypic tests results with phenotypic resistance levels can be crucial forward a personalized approach in TB patient treatment, stopping the spread of drug resistance and promotion of the optimum use of the few drugs available for resistant TB treatment.