

Dynamics and Development of Extensively Drug-resistant Tuberculosis, Portugal

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The development of multidrug-resistant (MDR) and extensive drug-resistant (XDR) tuberculosis (TB) combined with subsequent transmission constitutes a serious threat to the effective control of tuberculosis in several countries. Lisbon Health Region, despite great progresses in TB management still presents a high number of MDR/XDR-TB cases. The development of this type of resistance is the result of adaptative selection of *Mycobacterium tuberculosis* strains that acquire and accumulate specific mutations at specific genes. The presently known mechanisms of drug resistance include the modification or overexpression of drug targets, inactivation of drug-activator enzymes and overexpression of drug-modifying enzymes. Although the molecular basis of resistance of MDR/XDR-TB strains circulating in Lisbon has already been addressed in different studies, the dynamics or mode of resistance acquisition that have led to the different circulating strains is still partially unclear. In the present study we have genotyped and screened a set of 44 MDR/XDR-TB isolates for mutations in *tlyA*, *gyrA*, *rrs* and *eis* genes. We have determined the most prevalent mutations found in each gene to be Ins755GT in *tlyA*, A1401G in *rrs*, G-10A in *eis* and S91P in *gyrA*. Two genetic clusters previously known to be associated with XDR-TB were detected, Lisboa3 and Q1, containing 27 and 17 isolates, respectively. Lisboa3 strains isolated in the 90's with the same mutational profile of recent XDR-TB Lisboa3 strains were found, emphasizing the ancient XDR-TB problem in the region. Also investigated was the resistance level conferred by *eis* G-10A mutations, revealing that *eis* G-10A mutations may result in an undetectable AMK resistance. We concluded by analyzing the mutational distribution found by genetic cluster that in Q1 cluster two mutations, *gyrA* D94A and *rrs* A1401G, were enough to ensure development of XDR-TB from a multidrug resistant strain. Moreover, in Lisboa3 cluster it was possible to determine that the development of kanamycin low-level resistance mediated by *eis* promoter mutations was at the origin of independent emergence of several XDR-TB strains that can be discriminated within Lisboa3 genetic cluster by *tlyA* mutations.