Comparative Genomics and Evolution

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GENOMIC DIVERSITY OF DRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS ISOLATES IN LISBON PORTUGAL: TOWARDS TUBERCULOSIS GENOMIC EPIDEMIOLOGY

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Multidrug- (MDR) and extensively drug resistant (XDR) tuberculosis (TB) present a challenge to disease control and elimination goals. Lisbon, Portugal, has a high TB incidence rate and, unusual and successful XDR-TB strains that are found in circulation for almost two decades.

In the present study, 56 *Mycobacterium tuberculosis* isolates, mostly recovered in Lisbon, were genotyped by 24-*loci* Mycobacterial Interspersed Repetive Unit - Variable Number of Tandem Repeats (MIRU-VNTR) and the genomes sequenced using a next generation sequencing platform - Illumina HiSeq 2000. The genotyping data revealed three major clusters associated with MDR-TB (Lisboa3-A, Lisboa3-B and Q1), two of which associated with XDR-TB (Lisboa3-B and Q1). Whilst the genomic data contributed to elucidate the phylogenetic positioning of circulating MDR-TB strains, showing a high predominance of a single SNP cluster group 5. Furthermore, a genome-wide phylogeny analysis from these strains, together with 19 publicly available genomes of Mycobacterium tuberculosis clinical isolates, revealed two major clades responsible for M/XDR-TB in the region: Lisboa3 and Q1. On the overall, 9419 different SNPs were identified, ranging between 488 - 1465 per isolate (mean: 928 SNPs/isolate).

The data presented by this study contributes to the expanding knowledge of *Mycobacterium tuberculosis* genomic diversity yielding insights on microevolution and identification of novel compensatory mutations associated with rifampicin resistance in *rpoB* and *rpoC*. The screening for other structural variations revealed putative clade-defining variants. One deletion in PPE41, found among Lisboa3 isolates, is proposed to contribute to immune evasion and as a selective advantage. Insertion sequence (IS) mapping has also demonstrated the role of IS*6110* as a major driver in mycobacterial evolution by affecting gene integrity and regulation. A total of 251 candidate insertion sites were detected, of which 105 were intergenic and 64 were predicted to have a putative upregulatory effect.

Additionally, the analysis of non-synonymous/synonymous ratios revealed heterogeneities across the chromosome, genotype and Clusters of Orthologous Groups, highlighting possible and different evolution strategies. Globally, our data supports the notion of a growing genomic diversity facing both setting and host adaptation.