Letter to the Editor

Preliminary remarks on assembly whole genome sequencing of MDR M. tuberculosis isolated in Vietnam

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Tuberculosis remains a major cause of morbidity and mortality in many countries and a significant public health problem worldwide. The emergence of drug resistant strains and particularly multidrug-resistant strains of *Mycobacterium tuberculosis* has become a significant public health problem in a number of countries and an obstacle for an effective control of tuberculosis.

Vietnam is a high-burden country for tuberculosis [1]. In Vietnam, almost 30,000 people die every year from TB (one death every 18 minutes). Vietnam, with its estimated 175,000 new cases per year, ranks 12th among the 22 countries that bear 80% of the global TB burden [1,2]. If TB is detected early and treated properly by using a combination treatment for six to nine months, the patients quickly become non-infectious and are eventually cured. Multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), HIV-associated TB, and weak health systems are major challenges in Vietnam. There are an estimated 7,000 new MDR-TB cases and 6,400 new TB/HIV cases every year. Both of these forms of complicated TB carry a high risk of early mortality [1]. Although tuberculosis is still a public health problem in Vietnam, there is little information about the genetic characteristics of the isolates. A better knowledge of the molecular characteristics of *M. tuberculosis* strains will contribute to our understanding of the transmission dynamics of the disease within the country.

One strain of *M. tuberculosis* (MTB_HUE_20 strain), which was isolated from a smear-positive sputum specimen of a 47-year-old male patient with typical clinical features of new tuberculosis in Hue, Vietnam, was resistant to isoniazid and rifampicin (multidrug resistant), positive with IS6110 PCR. Furthermore, its spoligotyping pattern did not match any described genotype in the SpolDB4 database (unknown genotype). This strain was chosen for re-sequencing of the whole genome based on paired-end sequencing on the Illumina GAIIx platform (Illumina Inc, San Diego, CA, USA). Thirty micrograms of the genomic DNA was purified by the CTAB method and sent to BaseClear Co DNA Sequencing Service in the Netherlands to perform the sequencing. De Novo genomic assembly was performed with the Velvet program (EMBL-EBI, Hinxton, Cambridge, UK) [3]. Gene prediction and translate predicted genes into proteins with GeneMarkS [4]. All ORFs obtained were annotated with the Blast2Go program [blast2go] (BioBam Bioinformatics S.L., Valencia, Spain), and GFF files were created to annotate the circular genome MTB_HUE_20 strain with Geneious software (Biomatters Ltd, Auckland, New Zealand) [5].

Some preliminary observations on the assembled whole genome sequencing of MTB_HUE_20 strain were as follows: the full length is 4,397,928 bp, less than that of H37Rv strain of about 14Kb; percentages of A, T, C, G and high percentages of G and C are similar to those of H37Rv strain; this strain harbors only one copy of IS6110 and has 2329 SNP (single-nucleotide polymorphisms), of which 1257 lead to change of amino acid and 159 DIP (Deletion Insertion Polymorphisms), of which 105 lead to change of amino acid when compared to whole genome of

H37Rv strain; the lack of 15 copies of IS6110 combining 159 DIPs cause the length of MTB_HUE_20 strain shorter than H37Rv strain; interestingly this multidrug resistant strain has a mutation in the katG gene but no mutation in the rpoB gene.. Mutations in DNA-dependent RNA polymerase (rpoB) gene is among the most frequent in RIF-resistant strain [6] and catalase-peroxidase (katG) gene in INH-resistant strain [6,7]. Previous studies indicated that less than 5% of resistant strains do not show a mutation in the *rpoB* resistance region [7,8] and rare inconsistencies of mutated *M. tuberculosis* strains, one of them may be clinically resistant, which could suggest other mechanism for resistance (9]. Maybe the MTB_HUE_20 strain is one of these strains.

This report confirms a multidrug resistant *M. tuberculosis* strain with KatG mutation. Multidrug resistant tuberculosis could become an emerging problem in Vietnam; therefore early detection of drug resistance and proper treatment are needed for the effective control of drug resistant tuberculosis.

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