Title: A rare D94F change in gyrA gene of multi-drug resistant Mycobacterium tuberculosis possibly contributing to an unfavorable treatment outcome.

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We read the study by Seifert et al on *Mycobacterium tuberculosis* single nucleotide polymorphisms to predict treatment outcome published in May 2019 with interest. Various gyrA mutations in QRDR region were grouped into different level of drug resistance to fluoroquinolones. In India, the fluoroquinolone resistance is detected in 21.8% of the multidrug resistant tuberculosis patients. Various studies have defined the role of mutations in QRDR region of gyrA of *M. tuberculosis* genome. The most common mutations are reported at the position of codon 88, 90, 91 and 94, and changes on these positions correspond to different level of drug resistance in clinical isolates. Although other rare mutations have also been reported on these positions but the clinical outcome of these mutations are not documented. We report a rare D94F mutation found in a multidrug resistant tuberculosis patient with an unfavorable clinical outcome. The sputum sample of a 30-year old female was received in July 2015. The smear was positive and line probe assay (Genotype MTBDRplus ver 2.0, Hain life sciences) testing showed rifampicin resistance with isoniazid resistance. The patient was non-reactive for HIV. She had a body weight of 35 kg and 170 cm height, and was started on conventional MDR regimen of weight band of 26-45 in August 2015. The follow-up cultures were received as per RNTCP (Revised National Tuberculosis Control Program) protocol from months 3 to 6 in intensive phase and at every 3rd month of continuation phase. The first three follow-ups were culture positive for *M. tuberculosis* and the intensive phase was extended. In May 2016, the continuation phase was started and three
consecutive culture were found positive showing not-responsive to therapy. The drug
sensitivity was performed by MGIT 960 (Becton Dickinson, USA) for levofloxacin and
kanamycin on the cultures which were positive during the continuation phase, and showed
resistance to levofloxacin and sensitivity to kanamycin. However, the patient was succumbed
to the illness in December 2016.

Retrospectively, we tested the minimal inhibitory concentration (MIC) of different
fluoroquinolones like ofloxacin (2µg-64µg), levofloxacin (1.5µg-64µg) and moxifloxacin
(0.25µg-8µg) by REMA (resazurin microtiter assay) method. The MIC’s for ofloxacin,
levofloxacin and moxifloxacin were 32µg/ml, 16µg/ml and 2µg/ml, respectively. All the
culture positive samples were sequenced for gyrA and gyrB gene by sanger sequencing. The
first three month culture had shown a mixed sequence of wild type GAC (94 codon) and TTC
(Figure 1). The last 15th month follow-up culture of *M. tuberculosis* was sequenced for gyrA
gene and found only TTC from GAC at the 94th position (Figure 2). We searched in the
literature for this rare mutation and found that there are only two reports from China and
Philippines showing this change in single strains but the clinical outcome was not
mentioned. The WHO has published the MICs of different drug with their corresponding
drug resistance related mutations, but this rare mutation is not mentioned in the WHO’s
technical guideline. A wider reporting of drug resistance related SNPs for tuberculosis with
their treatment outcomes from different geographical areas should be encouraged.
References


Figure 1. The sequencing results showing mixed chromatogram of wild type GAC and mutant TTC at codon 94 in gyrA gene of *M. tuberculosis*. 
Figure 2. The sequencing results showing the complete change of wild type GAC codon 94 to mutant TTC codon in gyrA gene of *M tuberculosis*.
Figure 1. The sequencing results showing mixed chromatogram of wild type GAC and mutant TTC at codon 94 in gyrA gene of *M tuberculosis*. 
Figure 2. The sequencing results showing the complete change of wild type GAC codon 94 to mutant TTC in gyrA gene of *M. tuberculosis*. 

![Sequence readouts showing the complete change of wild type GAC codon 94 to mutant TTC in gyrA gene of *M. tuberculosis*.](Image)