Grave impact of undetected rpoB I572F mutation on clinical course of multidrug-resistant tuberculosis: a case report

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Hong Kong Med J 2023;29:70–2
https://doi.org/10.12809/hkmj219735

Case report

Accelerating diagnosis and treatment of rifampicin-resistant/multidrug-resistant tuberculosis (MDR-TB) are key components of the World Health Organization's End TB Strategy.[1] Rifampicin resistance that arises from mutations outside the 81-base pair (bp) rifampicin-resistance determining region (RRDR) of the rpoB gene such as the I572F mutation nonetheless cannot be detected by existing World Health Organization–endorsed Xpert MTB/RIF assay or line probe assays (LPAs).[2] Mutations located outside the rpoB hotspot may also be missed by the liquid medium–based BACTEC Mycobacteria Growth Indicator Tube (MGIT) culture system. We report a rare case of MDR-TB with rpoB I572F mutation (*Escherichia coli* numbering system) that was missed by LPA and liquid culture but confirmed by full rpoB gene sequencing to illustrate the negative impact of the mutation being undetected.

A 54-year-old Chinese man presented with symptoms of TB in July 2019. He had no history of anti-TB treatment. Chest radiograph on presentation showed bilateral cavitary lesions (Fig a). Sputum acid-fast bacilli smear examination was positive. Xpert MTB/RIF assay confirmed *Mycobacterium tuberculosis* (MTB)–positive/rifampicin-negative pulmonary TB. He was prescribed standard anti-TB treatment with isoniazid, rifampicin, ethambutol, and pyrazinamide. Initial sputum culture (performed with an MGIT culture system) later confirmed MTB organisms susceptible to all first-line drugs. Nonetheless lung shadows did not improve on serial chest radiographs (Fig b and c) despite good compliance with directly observed therapy. Sputum culture was transiently negative between November 2019 and January 2020. Culture result of a sputum specimen saved in October 2019 became available in January 2020 and showed MTB organisms resistant to isoniazid. The LPA (GenoType MTBDRplus Version 2.0; Hain Lifescience GmbH, Nehren, Germany) performed at that juncture showed an inhA C-15T mutation but no mutation in the rpoB gene. The regimen was switched to rifampicin, levofloxacin, ethambutol and pyrazinamide. Sputum culture reverted to positive in subsequent months and acid-fast bacilli smear also reverted to positive at 12 months. The LPA repeated at 12 months showed inhA C-15T and gyrA D94A mutations. Mutation of rpoB, rrs and enhanced intracellular survival genes was not detected although whole genome sequencing (WGS) performed on isolates obtained at 12 months revealed mutations of rpoB I572F, inhA C-15T, embB D354A, pncA D63G, and gyrA D94A. Whole genome sequencing performed retrospectively on isolates obtained earlier confirmed that rpoB I572F and inhA C-15T mutations had been present since the beginning of treatment. Culture result (using liquid culture system) of a sputum specimen saved at 12 months later became available and showed bacillary resistance to streptomycin, isoniazid, ethambutol and levofloxacin, but rifampicin resistance was again missed. In view of the gene sequencing result, the regimen was switched to a bedaquiline-containing regimen at 12 months. The patient responded well to treatment thereafter (Fig d).

Discussion

Although most cases of rifampicin resistance are linked to mutations in the 81-bp hotspot region of the rpoB gene, notably mutations at codons 526 to 531, our case illustrates the occurrence of rare mutations outside the RRDR, namely I572F, and its negative impact on treatment outcome due to amplification of further drug resistance if left undetected. In our case, initial rifampicin resistance conferred by rpoB

I572F mutation was missed by Xpert MTB/RIF assay, LPA and liquid medium–based culture system. Due to the exceptionally slow growth of this strain, prolonged incubation using MGIT was required and there was also difficulty in selecting log phase growth for subsequent drug susceptibility testing (DST). Not only was turnaround time increased, but initial isoniazid resistance was also missed although it was detected in the subsequent strains phenotypically. The patient therefore received an inadequate number of drugs during the early course of anti-TB treatment resulting in further acquired drug resistance to ethambutol, pyrazinamide, and levofloxacin. Amplification of drug resistance in our case likely emerged from the segregation of a single strain into two lineages of drug-susceptible and drug-resistant organisms under the selective pressure of insufficient TB therapy. This was suggested by the presence of both wild type and resistant subpopulations during the transition from susceptibility to resistance with regard to levofloxacin from the WGS data on D94A mutation.

Review of the database at the TB Supranational Reference Laboratory, Centre for Health Protection, Department of Health of Hong Kong revealed a total of five cases of MDR-TB with rpoB I572F mutation (including the present case) out of 340 rifampicin-resistant isolates between 2011 and 2020, corresponding to a prevalence of 1.5%. The prevalence of rpoB I572F in Hong Kong in this study is similar to that (2%) reported from a previous local study.[3] On the contrary, a much higher prevalence of rpoB I572F mutation (corresponding to Ile491Phe mutation in MTB numbering system) has been reported recently in some countries with high TB prevalence such as Eswatini (formerly Swaziland) and South Africa (30% and 15%, respectively).[4] The highly variable prevalence of rpoB I572F mutation in different geographical regions highlights the importance of expanding the geographical database of this mutation to better understand its global prevalence.

To improve the accuracy of phenotypic DST, the World Health Organization has recently lowered the critical concentration for rifampicin susceptibility testing in MGIT from 1 mg/L to 0.5 mg/L.[5] The revised recommendation helps reduce but does not eliminate the discordance observed between phenotypic and molecular methods to detect rifampicin resistance and the potential false-susceptible results from phenotypic tests due to the presence of mutations outside the RRDR. Given the potential impact of rifampicin resistance conferred by an rpoB I572F mutation on treatment outcomes, and that an increasingly higher prevalence of such mutations has been reported recently in some countries, new molecular tests that expand the drug target coverage to help guide the formulation of treatment regimens are warranted. Expanding the target 81-bp hotspot RRDR of the rpoB gene to include codon 572 has been suggested.[3] More recently, a multiplex allele-specific polymerase chain reaction (PCR) assay to detect I572F mutation in rpoB has been designed using a one-step real-time PCR.[6] Although novel PCR assays may enable efficient and rapid detection of rpoB I572F mutation

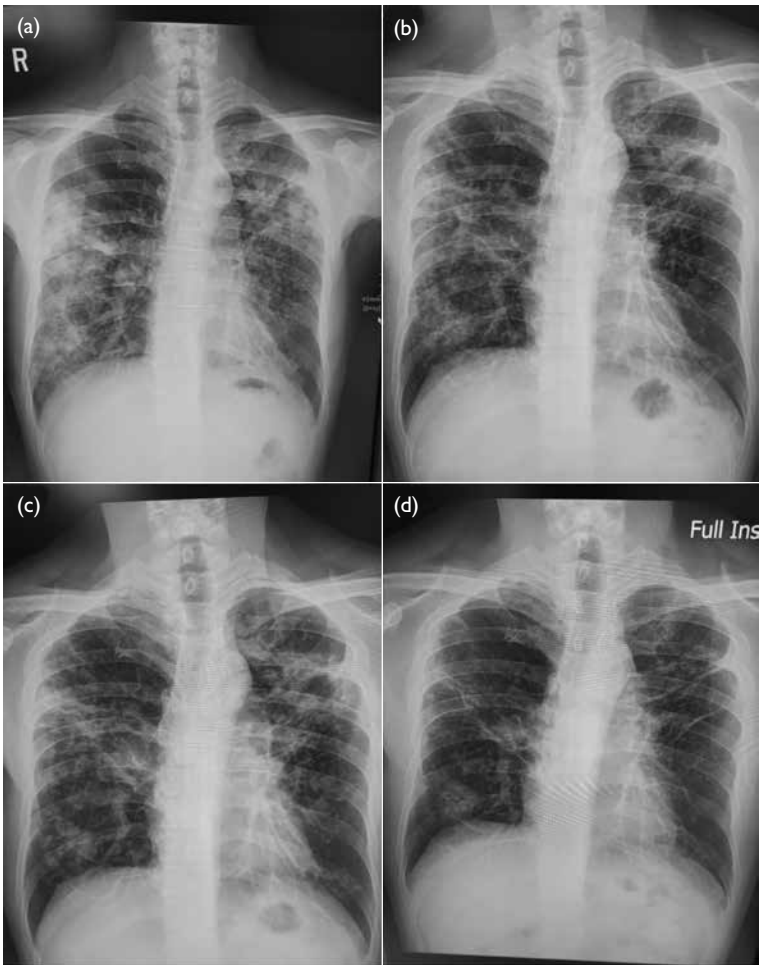

FIG. (a) A 54-year-old Chinese man with pulmonary tuberculosis. Chest radiograph on presentation showed extensive bilateral shadows with cavitation, especially in both upper zones and right lower zone. Xpert MTB/RIF assay showed MTB positive/RIF negative. Initial sputum culture (liquid medium–based) showed *Mycobacterium tuberculosis* (MTB) organisms susceptible to all first-line drugs. (b) Chest radiograph showing persistent shadows after about 6 months of treatment with first-line drugs when culture result of sputum specimen collected at 3 months revealed isoniazid-resistant MTB organisms. Line probe assay showed inhA C-15T mutation. Mutation associated with rifampicin resistance was not detected. The regimen was switched to rifampicin, levofloxacin, ethambutol and pyrazinamide. (c) Chest radiograph at 12 months showing persistent cavitary shadows in the left upper zone. Some improvement in shadows in right lung field was seen. The rpoB I572F mutation that conferred rifampicin resistance was detected by gene sequencing but not by line probe assay. Rifampicin resistance was again missed by phenotypic drug susceptibility testing repeated at this juncture. The regimen was changed to a bedaquiline-containing regimen. (d) Chest radiograph taken at 24 months showing improvement in bilateral lung shadows after being put on treatment with bedaquiline-containing regimen

and are simpler than sequencing methods, their implementation requires further validation and strengthening of laboratory capacities.

Until novel PCR assays and WGS gain widespread use, clinicians should remain alert for the more rare rpoB mutations such as the I572F mutation, and communicate promptly with laboratories for further tests if a patient does not respond well to standard first-line treatment. This is vital even if Xpert MTB/RIF assay, LPAs or phenotypic DST do not suggest the presence of rifampicin resistance to ensure the patient receives an adequate number of effective drugs for treatment success. Our case also calls for continued surveillance of the prevalence of rpoB I572F mutation and other rifampicin-resistance conferring mutations outside the RRDR to inform region-specific TB diagnostic and treatment strategies.

Author contributions

All authors contributed to the concept or design of the study, acquisition of the data, analysis or interpretation of the data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

The authors have disclosed no conflicts of interest.

Declaration

Part of the findings of this study has been presented at the Hong Kong Thoracic Society clinical meeting on 26 May 2022, which was an internal meeting attended by members of the Society held in virtual format.

Funding/support

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics approval

The patient was treated in accordance with the Declaration of Helsinki and provided written informed consent for publication.

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