CASE REPORT

Missed rifampicin and isoniazid resistance by commercial molecular assays

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Drug-resistant tuberculosis (TB) has poor outcomes unless resistance is detected early, ideally by commercially available molecular tests. We present a case of occult multidrug-resistant TB where both rifampicin and isoniazid resistance were missed by molecular testing and were only identified by phenotypic testing.

Keywords: Tuberculosis, drug resistance, diagnosis, mutations, molecular markers, challenges

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South Africa (SA) has a high burden of tuberculosis (TB), drug-resistant TB and TB-HIV co-infection, and is among the top 30 high-burden countries for these three populations. In 2021, 304 000 people were diagnosed with TB, and there were 6 381 laboratory-confirmed cases of rifampicin (RIF)-resistant TB cases in SA. Treatment success for drug-sensitive TB is 78% (2020 cohort), and 66% for multidrug-resistant TB (MDR-TB) (2019 cohort), which has increased from previous years.[1]

Delays in the initiation of drug-resistant TB (DR-TB) treatment are associated with poorer outcomes, and phenotypic drug susceptibility testing (DST), the reference standard for resistance detection, has a long turnaround time of 3 - 8 weeks.[2,3] Therefore, molecular tests are employed to rapidly identify resistance to key antituberculous drugs. RIF resistance is particularly amenable to genotypic resistance testing, since 95% of resistance is caused by mutations in the RIF resistance-determining region (RRDR), an 81 base-pair segment of the *rpoB* gene of *Mycobacterium tuberculosis* (MTB).[4,5] Similarly, 85 - 90% of isoniazid (INH) resistance is attributable to mutations in the *katG* and/or *inhA* genes. The World Health Organization (WHO) and SA National TB guidelines recommend screening all patients under investigation for TB for RIF drug resistance by means of a rapid molecular assay such as the Xpert MTB/RIF or Xpert MTB/RIF Ultra assays. In low- to middle-income countries (LMICs), INH resistance is not routinely screened for unless RIF resistance is detected.[6-8]

In SA, the Xpert MTB/RIF assay was implemented in 2011 and Xpert MTB/RIF Ultra in 2017. Once a patient is diagnosed with RIF-resistant TB by the Xpert MTB/RIF Ultra assay, the GenoType MTBDR*plus* (Hain Lifescience, Germany) line-probe assay (LPA) is then performed to predict high- and low-level INH resistance by detecting mutations in the *katG* gene and the promoter region of the *inhA* gene, respectively.[9] There is, however, potential for DR-TB to be missed by these molecular assays, since resistance mutations may occur in genetic loci other than those that are targeted, such as the flanking regions of the RRDR within the *rpoB* gene. In addition, in LMIC settings, current diagnostic algorithms do not generally recommend that phenotypic resistance testing be routinely performed for patients diagnosed with RIF-sensitive TB by frontline molecular assays. Phenotypic resistance testing is only performed if there is clinical treatment failure and undetected resistance is suspected.[7,10] Discordance between genotypic and phenotypic DST for both RIF and INH, the two core TB drugs, in the same individual is extremely rare, occurring with a frequency of 0.01 - 0.5%.[11-13] In this case report we describe one such case, which to our knowledge is the first to be reported in SA.

Ethical approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (ref. no M2008136).

Case report

A 47-year-old HIV-uninfected male was referred from a provincial clinic to the specialised TB clinic at Helen Joseph Hospital (HJH) in Johannesburg in September 2019 with pulmonary TB (PTB) unresponsive to conventional treatment. The patient had been treated for drug-susceptible TB four times prior to his current presentation (Table 1). He was first diagnosed with rifampicin-sensitive PTB (RS-PTB) in October 2014 by GeneXpert MTB/RIF assay on a sputum sample. He completed standard anti-TB treatment, consisting of 2 months of RIF, INH, pyrazinamide and ethambutol (RHZE), and 4 months continuation phase of RIF and INH (RH), after which he felt relatively well for ~2 months. Thereafter he started to feel unwell again, with symptoms of cough, weight loss, night sweats and fatigue. His second diagnosis of RS-PTB occurred in July 2015 by GeneXpert MTB/RIF assay on sputum, at which time he again completed 6 months of conventional TB treatment. Thereafter, he felt well until mid-2017, when the symptoms recurred and he was re-initiated on a course of conventional therapy for a third time. He was switched to the continuation phase after 2 months despite ongoing symptoms and sputum acid-fast bacilli (AFB) positivity. The GenoType MTBDR*plus* (LPA) assay tested on a sputum sample in August of the same year showed sensitivity to both RIF and INH. In February 2018, a decision

Table 1. Basis of tuberculosis diagnoses, monitoring and treatment of the patient over the period of 2014 - 2019

Diagnosis date	Diagnositic test	Monitoring	Treatment	Rx duration
1st diagnosis: October 2014	GXP + Rif S		RHZE	2 months
			RH	4 months
2nd diagnosis: July 2015	GXP + Rif S	August 2015: AFB –	RHZE RH	2 months 4 months
3rd diagnosis: mid 2017	Missing data	Aug 2017: AFB + LPA INH/Rif S Culture: MTB Nov 2017: LPA INH/RIF S Culture: MTB Dec 2017: AFB + Jan 2018: AFB+	RHZE RH	2 months 6 months
February 2018		March 2018: AFB + July 2018: AFB + Aug 2018: LPA INH/Rif S Oct 2018: AFB + Culture: MTB Dec 2018: AFB + Culture: MTB Feb 2019: LPA INH/Rif S Aug 2019: AFB +	RHZE	19 months
September 2019	Phenotypic DST: INH/Rif R	Nov 2019: AFB – Culture: MTB Dec 2019: AFB – Jan 2020: AFB – Culture: Neg	MDR regimen MDR regimen MDR regimen MDR regimen	

GXP + Rif S = sputum GeneXpert MTB/RIF assay positive with rifampicin sensitivity; RHZE = rifampicin, isoniazid, pyrazinamide and ethambutol;
AFB +/– = sputum acid-fast bacilli positive/negative; LPA = GenoType MTBDRplus (Hain Lifescience) molecular line-probe assay; Culture:
MTB = sputum-cultured *Mycobacterium tuberculosis*; DST = drug sensitivity test; INH/Rif S/R = isoniazid and rifampicin sensitive/resistant;
MDR regimen = multidrug-resistant regimen consisting of bedaquiline, linezolid, levofloxacin, clofazimine, pyrazinamide, high-dose INH and ethambutol.

was made to restart RHZE, as he was clinically deteriorating and remained sputum AFB-positive. He continued on this regimen until referral to our hospital in September 2019. During the period from February 2018 to September 2019, he remained sputum AFB-positive despite enrolment in a directly observed therapy programme – a strategy to improve adherence to TB treatment by requiring health workers, community volunteers or family members to observe and record patients taking each dose.[14] The GenoType MTBDR*plus* assay was repeated in August 2018, February 2019, June 2019 and August 2019, all showing sensitivity to RIF and INH.

Once referred to HJH, the patient was empirically started on an MDR regimen consisting of bedaquiline, linezolid, levofloxacin, clofazimine, pyrazinamide, high-dose INH and ethambutol, owing to suspected occult drug resistance to first-line therapy. A sputum sample was submitted for TB culture with a specific request for phenotypic DST.

The results of the phenotypic DST revealed resistance to both RIF and INH. Due to the unexpected genotypic-phenotypic discordance for both drugs, the cultured isolate was processed for whole genome sequencing (WGS) by the Centre for TB at the National Institute for Communicable Diseases, which serves as the National TB reference laboratory. For resistance detection by WGS, the 2021 WHO catalogue of mutations associated with drug resistance was used – essentially the catalogue is derived from all available data sources and contributions of unpublished data to grade mutations based on statistical support or expert rules.[15] WGS revealed the following mutations associated with resistance: Val170Phe in the *rpoB* gene, and Ile563 frame shift in the *katG* gene. Of concern, these mutations fall outside of the areas detected by both routine commercial diagnostics, the Xpert MTB/RIF Ultra and GenoType MTBDR*plus*, as well as the areas targeted by other commercially available assays conventionally used for the detection of RIF and INH resistance. Thus this patient's MDR-TB was undetectable by all commonly available genotypic diagnostic methods.

The patient responded well to the MDR-TB regimen, as evidenced by a weight gain of 2.1 kg and conversion to AFB negativity on sputum for the first time in 2 years after 1 month. His sputum TB culture became negative after 4 months of treatment.

Discussion

Discordance simultaneously between the genotypic and phenotypic DST for both RIF and INH, core drugs for treating susceptible TB,

is not a common occurrence. Solari *et al.*[11] screened TB isolates for discordance nationally in Peru between the years 2013 and 2015, and found genotype-phenotype discordance to both INH and RIF in just 1/7 194 samples (0.01%). Kang *et al.*[12] found that 5/1 069 (0.5%) of patients infected with TB in South Korea had discordance for both RIF and INH. Both studies used the GenoType MTBDR*plus* as the molecular diagnostic test. Yakrus *et al.*[13] performed a study in the USA comparing molecular and phenotypic DST in TB isolates submitted to the Centers for Disease Control and Prevention (CDC). Despite this being a 'high risk' cohort for resistance (it is not routine practice to submit all TB isolates to the CDC, and this is generally only done when resistance is a concern), none of the 227 isolates were reported to have discordance for both RIF and INH.[13] However, the USA is a low-burden TB setting, and Peru and South Korea are medium-burden TB settings; it is not clear what the prevalence is in high-burden settings such as SA.

The patient described in this case report had apparent RIF and INH sensitivity by rapid genotypic assays tested at multiple time points. Resistance detection and confirmation of MDR-TB was only determined upon phenotypic DST supported by WGS. The mutations detected by WGS in our patient are predictors of RIF and INH resistance. The Val170Phe mutation has been graded by the WHO catalogue as associated with (RIF) resistance.[15] The INH frameshift mutation Ile563fs has been graded by the catalogue as associated with resistance interim (the WHO catalogue term for probable resistance but not confirmed according to available data) according to the expert rule based on the loss of function due to a frameshift event. [4,5,11,15-18] The phenotypic DST for INH in our patient, however, confirms resistance.

Our patient could have initially been infected with an INH monoresistant strain from the community in 2014. This is more likely than RIF monoresistance on epidemiological grounds (INH monoresistance is far more common than RIF monoresistance in SA) and the patient's initial favourable clinical response followed quickly by relapse is also more typical of INH monoresistance than RIF monoresistance.[19] Once INH monoresistance was established, he would effectively have been treated with RIF monotherapy in his continuation phase, exerting significant pressure to select for subsequent RIF resistance. However, we cannot rule out the possibility that he was infected with this MDR strain or a RIF monoresistant strain to begin with.

Any patient who remains sputum AFB- or sputum TB-culture positive at the end of the intensive phase requires careful evaluation and consideration of additional laboratory testing. Current SA guidelines recommend additional resistance testing by commercial molecular assays in this scenario, which does not make provision for the detection of resistance outside the targeted regions of these assays.[7] While simultaneous occult phenotypic resistance to both INH and RIF is very rare, resistance to either drug alone is not uncommon, occurring with a frequency of 0.5 - 5.4% in microbiologically confirmed TB cases.[11-13] Thus in a high-burden TB setting such as SA, there may be additional such cases annually. We therefore propose that in cases of treatment failure in which a suspicion for resistance remains despite apparent drug susceptibility according to current routine diagnostics, a sample should be submitted for phenotypic DST or potentially next-generation sequencing, as both technologies will detect the mutations. Furthermore, ongoing routine surveillance should be undertaken to identify the prevalence of resistance markers outside the targeted regions of rapid molecular assays.

Data availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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