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CASE REPORT

Overcalling Rifampicin Resistance by Rapid Molecular Xpert MTB/RIF Ultra - A Diagnostic Pitfall and Treatment Dilemma

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ABSTRACT

Tuberculosis (TB) is a worldwide pandemic, with Sarawak among the top three TB-prevalent states in Malaysia. Molecular WHO-recommended rapid diagnostic tests (mWRDs), such as Xpert MTB/RIF Ultra, have been a game-changer in the early detection of *Mycobacterium tuberculosis* (Mtb), providing additional information on rifampicin resistance, enabling prompt and effective treatment of possible multidrug-resistant TB (MDR-TB). However, improved test sensitivity comes at the expense of specificity, resulting in a higher risk of false positives. We report a false-positive rifampicin resistance detected by Xpert Ultra in a newly diagnosed pulmonary TB patient without MDR-TB risk factors. A 39-year-old healthy gentleman presented with a productive cough, increasing breathlessness, and constitutional symptoms for three months. Following acid-fast bacilli (AFB) smear positivity, he was started on first-line anti-TB treatment. Initial culture confirmed susceptibility to first-line antibiotics. During the maintenance phase, a sputum sample was positive for AFB, alerting to the possibility of MDR-TB. Sputum Xpert Ultra detected Mtb with rifampicin resistance; however, a repeat Xpert Ultra test a week later revealed the absence of rifampicin resistance. Additional testing, including line probe assay and cultures, also did not detect the *Mycobacterium tuberculosis* complex (MTBC). The incidental AFB-positive sputum was identified by culture as a non-tuberculous mycobacterium. The

patient responded with the first-line anti-TB re-treatment regimen and was discharged well. False-positive rifampicin resistance in Xpert Ultra is uncommonly reported but may lead to overcalling and complicate Mtb treatment. Patient clinical evaluation and careful assessment of MDR-TB risk should be performed before treatment escalation.

INTRODUCTION

With over 1.25 million deaths last year, tuberculosis (TB) has once again become the world's leading cause of death from a single infectious agent, following the COVID-19 pandemic (World Health Organisation, 2024). Since the country's first case was reported in the early 20th century, its incidence has steadily increased since 2011, despite the National TB Control Programme's establishment spanning more than six decades (Fadzil et al., 2025). In 2023, Sarawak was the third state to record the highest number of TB cases in Malaysia, after Sabah and Selangor (Bernama, 2024). With an annual TB incidence of 104 per 100,000 population, Sarawak is classified as a high TB burden area by the WHO definition. The majority of Sarawak's notified cases originated from the Kuching division (Tie et al., 2018).

The discovery of the Ziehl-Neelsen (ZN) stain in 1882 has provided a basis for TB diagnosis, with improvement using the fluorescent Auramine stain by Paul Ehrlich and Carl Weigert (Prakoeswa et al., 2022). Both methods were pioneering in their time, but the microscopy relies on the visual detection of acid-fast bacilli (AFB) in sputum samples, making it susceptible to operator variability. While inexpensive and easy to use, the test has relatively low sensitivity, especially in paucibacillary TB with low bacterial loads. With a long doubling time, the gold standard culture for *Mycobacterium tuberculosis* is both time-consuming and requires specialised laboratory facilities, further delaying diagnosis and treatment initiation.

Molecular WHO-recommended rapid diagnostic tests for TB (mWRDs), such as the Cepheid Xpert Ultra, have been game-changers in the early diagnosis of TB. Xpert Ultra utilises the nested polymerase chain reaction (PCR) method for detecting *Mycobacterium tuberculosis* DNA, targeting the genes IS6110 and IS1081. It is also capable of detecting rifampicin resistance-associated mutations in the rpoB gene through melting temperature analysis (WHO, 2024). This semi-quantitative, automated, cartridge-based molecular testing platform has improved sensitivity in TB diagnosis, especially among people living with HIV (PLHIV) and those with extrapulmonary TB, compared to traditional smear microscopy. Moreover, as the Xpert Ultra system also provides information on the rifampicin resistance of the isolate, it enables prompt and effective targeted treatment of possible multidrug-resistant TB (MDR-TB). WHO has globally endorsed its utility in its global fight against TB following its introduction in 2010. Cepheid has since upgraded the MTB/RIF assay to MTB/RIF Ultra, an assay with higher sensitivity that allows the detection of lower Mtb bacterial loads (Dorman et al., 2018). A prospective multi-centre diagnostic accuracy demonstrated that Xpert MTB/RIF Ultra has higher sensitivity than Xpert MTB/RIF in detecting paucibacillary TB and in individuals living with HIV (PLHIV) (Dorman et al., 2018).

However, improved test sensitivity comes at the expense of reduced test specificity, increasing the risk of false positivity. False-positive rifampicin resistance in Xpert Ultra can lead to the overdiagnosis of drug-resistant TB and unnecessary second-line anti-TB treatment, exposing patients to higher costs, drug toxicities, and adverse effects. We describe a case of discrepant rifampicin resistance in sputum Xpert Ultra results, leading to diagnostic and therapeutic dilemmas in the management of pulmonary tuberculosis.

CASE PRESENTATION

A 39-year-old gentleman with no comorbidities and a history of smoking cessation presented to the emergency department with a three-month history of productive cough, worsening shortness of breath, and constitutional symptoms. He denied any history of TB contact. On examination, he was cachectic and tachypneic, with generalised crepitations on lung auscultation. Blood investigations revealed leucocytosis (15.24 × 10^3/μL), anaemia (Hb 8.6 g/dL), hypoalbuminemia (albumin 23 g/L), and acute kidney injury (urea 20.6 mmol/L; creatinine 343 μmol/L). A chest X-ray (CXR) demonstrated bilateral lung field opacities, consolidations, and cavitations (Figure 1). His initial sputum AFB smear was 2+ and was promptly reported to the attending clinician. His HIV Ag/Ab Combo serology screening was nonreactive.

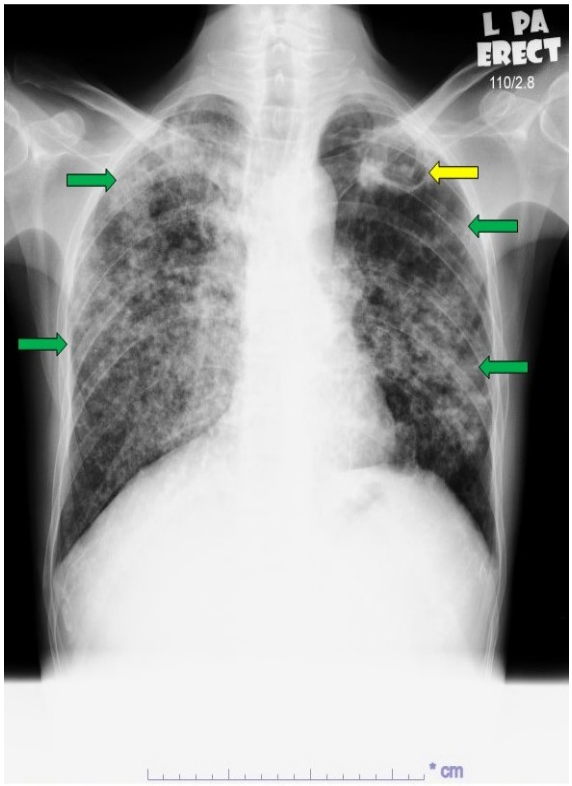

Figure 1: Chest X-ray (CXR) PA view on hospital admission shows consolidations in both lungs, worse on the right, as depicted by the green arrows. The yellow arrow points to the left upper lobe consolidation.

Diagnosis and Treatment Initiation

Following his sputum AFB smear positivity, first-line anti-TB treatment was initiated. He was started on the EHRZ regimen, consisting of isoniazid 250 mg (6 mg/kg) daily, rifampicin 600 mg (14.6 mg/kg) daily, and renal-adjusted doses of ethambutol 800 mg (19.5 mg/kg) and pyrazinamide 1250 mg (30 mg/kg) thrice weekly due to his acute kidney injury. Given the patient's social issues, his attending respiratory physician planned for inpatient directly observed therapy (DOTS). He was subsequently transferred to a district hospital for continuation of DOTS. He completed 84 doses of the intensive-phase anti-TB treatment, achieving sputum AFB seroconversion on Day 68 of the intensive phase. No significant findings were noted during contact tracing.

The initial sputum sample was cultured, and the colony was sent to the National Public Health Laboratory (NPHL) for identification and antibiotic susceptibility testing. The colony was identified as a *Mycobacterium tuberculosis* complex, susceptible to isoniazid, rifampicin, pyrazinamide, and ethambutol, with the final report released four months later.

Incidental Finding During Maintenance Phase

Unexpectedly, on the third day of his maintenance phase with the Akurit-2 tablets, an incidental sputum sample tested for AFB showed scanty AFB positivity (2 in one length). This finding contradicted the patient's improving clinical and radiographic condition (Figure 2). The patient also had no predisposing factors for MDR-TB.

A virtual consultation was held with the respiratory physician at a tertiary care hospital. Persistent smear positivity led to suspicion of treatment failure; hence, the sputum sample was sent to the state hospital for Xpert Ultra to rule out rifampicin resistance.

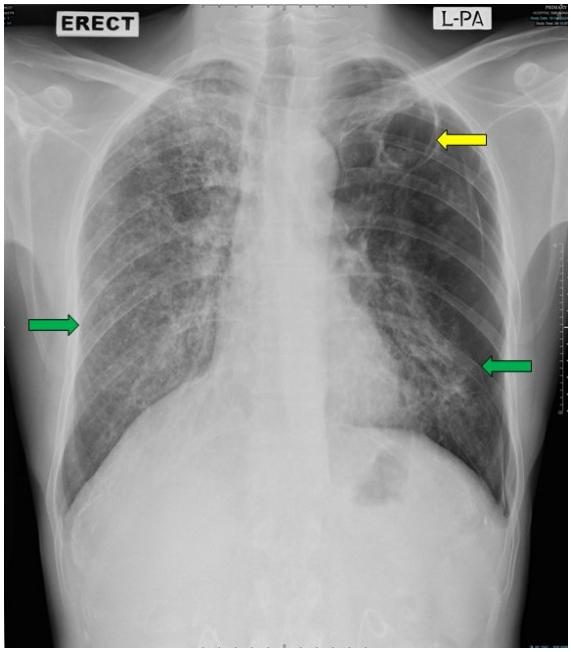

Figure 2: Chest X-ray (CXR) PA view on re-assessment following the GeneXpert finding shows resolving consolidation (green arrows). Note that the previously noted cavitation (yellow arrow) is less conspicuous compared to the previous CXR.

Xpert Ultra (Cepheid, California) was performed, confirming the presence of a very low quantity of M. tuberculosis. In addition, the Ultra rpoB3 mutant's melting temperature of 73.0 °C suggested the presence of rifampicin resistance. The result was promptly informed to the requesting clinician.

In response to the report, the attending physician initiated treatment based on the local MDR-TB regimen. The intensive phase was restarted, replacing the previously used rifampicin with a renal-adjusted dose of ofloxacin of 400 mg (15 mg/kg) daily.

Clinical Reassessment and Further Investigations

A virtual referral was made to the tertiary care hospital's respiratory medicine team for input. Upon the respiratory physician's reassessment, the patient was deemed to be at low risk of developing MDR-TB, and a repeat sputum Xpert Ultra was requested. A very low quantity of *M. tuberculosis* was detected in the second Xpert Ultra testing; however, the rifampicin resistance was not detected in the repeated test. The discrepant result was conveyed to the respiratory physician team, who continued the treatment using first-line anti-TB medication. His ofloxacin was switched back to his previous dosage of rifampicin and continued into the maintenance phase.

Cepheid was consulted, and they had postulated a mixed infection of rifampicin-resistant isolates with susceptible populations, suggesting further testing using alternative methods. Sputum TB culture on Mycobacteria Growth Indicator Tube (MGIT, BD™ Bactec, Becton, Dickinson and Company, New Jersey, USA) was also sent with the repeat sputum Xpert Ultra, noted to have growth following eight days of incubation, but was not proceeded for identification and antimicrobial susceptibility test (AST) due to contamination. Two more sputum samples were later sent to the NPHL reference laboratory for line probe assay detection of MDR-TB, but failed to detect the MTBC. Thus, the rifampicin resistance was not ascertained.

The sputum with incidental positive AFB was cultured and identified to be *Mycobacterium chelonae*, a nontuberculous mycobacterium. Due to its ubiquitous nature and minimal pathogenic association, AST was not performed. Upon completing TB treatment for a total of nine months, the patient was discharged uneventfully.

DISCUSSION

One of the contributing factors to the global endemicity of TB is delay in management, which can be categorised into three types: patient, diagnostic, and treatment delays (Figure 3).

Patient delay, defined as the interval between the onset of TB symptoms and the patient's first visit to a healthcare provider, is significantly associated with education

level and clinic visit frequency. Secondly, a diagnostic delay is the interval between the patient's first visit to the healthcare facility and the confirmation of the TB diagnosis. With the median diagnostic delay for Sarawak pulmonary TB patients of 30 days, female gender and moderate familial stigma for TB significantly predict it. Finally, treatment delay refers to the interval between the confirmation of a TB diagnosis and the initiation of TB treatment. It is predicted considerably by age, minority ethnicity, lower level of education, knowledge of the disease, number of signs and symptoms, and diagnostic tests performed during the first visit (Mohamad et al., 2022).

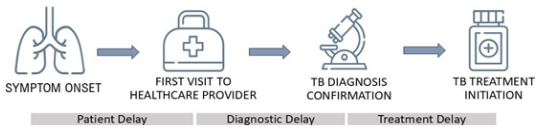

Figure 3: Types of delay in TB management. Adapted from Mohamad et al. (2022)

While patient delay remains beyond our control, the Xpert Ultra assay was able to mitigate both diagnostic and treatment delays. By making it accessible as part of initial diagnostic testing, Mtb detection can be hastened, shortening the diagnostic interval. Rifampicin-resistant TB can also be recognised through the assay, accentuating timely diagnosis in populations at risk of MDR-TB. A targeted, effective regimen for MDR-TB can be administered, shortening the treatment interval.

In comparison to drug-susceptible TB, smear microscopy is not the front-line tool used for MDR-TB diagnosis. The national Clinical Practice Guidelines (CPG) for the management of drug-resistant TB recommend using the Xpert Ultra alongside microscopy, culture, and drug susceptibility testing as the initial diagnostic tool for adults and children suspected of having MDR-TB or HIV-associated TB (Kannan et al., 2016). This explains why the clinicians were quick to resort to Xpert Ultra for a multidrug-resistant tuberculosis work-up when there was a delay in sputum smear conversion in this case. Their prompt decision-making is commendable, reflecting vigilance in managing potentially resistant TB cases.

Rational case-finding strategies are imperative and recommended by the national CPG for early identification of populations at risk for MDR-TB. Risk factors include failure of retreatment regimens with first-line anti-TB drugs (i.e., chronic TB cases), failure of new TB regimens, exposure to a known drug-resistant TB case, persistent smear-positive sputum after two months of an anti-TB drug regimen, loss to follow-up, residence or exposure in institutions with high drug-resistant TB prevalence, and comorbidities such as HIV and malabsorption (Kannan et al., 2016). In this patient's case, he was tested with Xpert Ultra due to his persistent smear-positive sputum despite completing the intensive phase of DOTS.

Monitoring adult TB patients using sputum direct smear for AFB at the first, second, fifth, and sixth months of treatment is recommended by the national clinical practice guidelines (CPG) to assess treatment compliance and effectiveness. AFB smear microscopy has the disadvantage of being unable to differentiate between the M. tuberculosis complex and atypical mycobacteria, as demonstrated in our case (Goon et al., 2021). Persistent AFB positivity during treatment is most commonly due to drug resistance. However, other contributing factors, such as nonadherence to tuberculosis therapy and reduced intestinal drug absorption, should be ruled out before confirming actual treatment failure. In cases where treatment failure is unlikely, alternative explanations—such as the presence of nonviable tuberculous bacilli or, as in our case, coinfection with another acid-fast bacilli–positive organism—should be considered (Franco-paredes & Ray, 2012). This patient underwent Xpert Ultra testing due to persistent AFB positivity, which was later

identified as M. chelonae, a ubiquitous non-tuberculous mycobacterium that is generally not clinically significant.

With 99.7% specificity and 92.7% sensitivity of rifampicin resistance detection, more occurrences of false-negative rifampicin resistance have been detected compared to false positivity (Zetola et al., 2014). Moreover, Zetola and colleagues have shown a more pronounced reduction of sensitivity of 80.0% in mixed rifampicin-susceptible and rifampicin-resistant Mtb infections, especially in isolates consisting of less than 90% rifampicin-resistant strains (Zetola et al., 2014). Rufai et al also demonstrated a false negative rifampicin resistance detection of 35.5%, higher than the false positive rate of 5.1% (Rufai et al., 2014). These forementioned studies, however, use the Xpert MTB/RIF assay instead of our currently used Xpert Ultra.

Although rare, false-positive rifampicin resistance has been reported. A New York–based study reported a 60% false-positive rate for rifampicin resistance with the previous Xpert MTB/RIF assay (Ocheretina et al., 2016). Regarding the Xpert Ultra assay, false rifampicin resistance has occurred in an HIV-associated extrapulmonary TB. Ng et al. (2020) reported rifampicin resistance in a lymph node aspirate from a recurrent supraclavicular TB abscess with an rpoB3 mutant melting temperature, as tested using the Xpert Ultra assay, similar to our case. With phenotypic drug susceptibility testing and other molecular tests, including targeted deep sequencing, Ng attributed the false rifampicin resistance to a rare matrix effect in the reaction tube that distorted the melt curves (Ng et al., 2020). Although Cepheid does not fully disclose the details of its nested PCR cartridge technology, it is generally known that interference from the sample—such as the presence of bubbles or issues with sample consistency and viscosity—may negatively impact the amplification process and disrupt accurate fluorescence signal interpretation, potentially mimicking resistance-associated mutations. This may also explain the finding in our case.

An algorithm was recommended in the national Clinical Practice Guideline (CPG) for interpreting genotypic drug susceptibility testing. For groups with a high risk of MDR-TB, clinicians are advised to initiate a WHO-recommended MDR-TB regimen. For low-risk groups, a repeat molecular test is recommended, with the result of the second test used for clinical decision-making (Kannan et al., 2016). Although it serves as a safety net to prevent overcalling rifampicin resistance detected by Xpert Ultra, many institutions in the country still do not implement this practice due to limited financial resources to support repeat testing. The authors believe that, had the clinicians taken into account the patient's reasonable response to DOTS—as evidenced by improving clinical and radiological findings—and the prior Mtb culture showing a drug-susceptible strain, the decision to switch to more toxic second-line anti-TB agents could have been postponed until confirmatory testing was performed. This case highlights the importance of repeat testing when incidental Xpert Ultra findings indicate rifampicin resistance, especially in patients with low risk factors for MDR-TB.

The benefit of this repeat testing practice is demonstrated by a Ugandan cross-sectional study, in which TB patients with rifampicin resistance detected via Xpert were retested (Ssengooba et al., 2019). Upon repeated testing with the same assay, 15.6% of results (15/96) were found to be discordant, of which approximately two-thirds were false rifampicin resistance, as confirmed by other molecular methods (Ssengooba et al., 2019). Although the study applies to the Xpert MTB-RIF assay, rather than our currently used Xpert MTB/RIF Ultra, it reveals that false positivity is evident, and countermeasures need to be addressed.

We attempted similar measures for

this possible false rifampicin resistance, in which we assessed the patient with clinical improvement, performed Xpert Ultra repeat testing, and tested with alternative methods such as culture direct susceptibility testing and line probe assay from the reference laboratory – all of which failed to support the first Xpert Ultra result, leading to the patient to be managed as per drug-susceptible TB.

CONCLUSION

False-positive rifampicin resistance is a rare but possible occurrence with the highly sensitive Xpert Ultra test. In patients with low risk of MDR-TB, rifampicin resistance detected from Xpert Ultra should be retested after clinical correlation and parallel testing with other available testing alternatives, prior to switching to second-line anti-TB treatment. This approach minimises patients' risk of unnecessary exposure to toxic medications associated with second-line therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONSENTS

The patient has given written consent for publication

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