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## CASE REPORT

# Case Report of False Rifampin Resistance with Xpert® MTB/RIF from an HIV Infected Patient

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### SUMMARY

***Background:*** **Tuberculosis is one of the main infectious diseases threatening human health, especially in HIV co-infected patients. Xpert® MTB/RIF assay amplifies the *rpoB* gene of MTB was recommended by the World Health Organization as the initial diagnostic test in cases of suspected infections with Mycobacterium tuberculosis (MTB) or HIV-coinfected TB.**
***Methods:*** **A 44-year-old male HIV-positive patient co-infected with MTB presented with low-grade fever for 3 months. Rifampicin (RIF) resistance was detected in the celiac pus but not in the pleural effusion using Xpert® MTB/RIF assay. The same samples were then sequenced by next-generation sequencing (NGS) and in-house PCR for *rpoB* gene.**
***Results:*** **The results of NGS and in-house PCR, however, were paradoxical in the same samples with low or no mutation sequences of RIF resistance. The patient's tuberculosis (TB) therapy was optimized based on first-line anti-TB drugs and antiretroviral treatment. The patient improved with this therapy.**
***Conclusions:*** **Even with high specificity, false positive results remain possible and RIF resistance detection by Xpert must be considered for clinical interpretation.**
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**KEY WORDS**

*Mycobacterium tuberculosis*, AIDS, Xpert® MTB/RIF assay, false positive

### LIST OF ABBREVIATIONS

TB - Tuberculosis
MDR-TB - multidrug-resistant *mycobacterium tuberculosis*
RIF - rifampicin
NGS - next-generation sequencing
WHO - World Health Organization
CT - computed tomography
BAL fluid - broncho-alveolar lavage fluid

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## INTRODUCTION

Tuberculosis is one of the main infectious diseases threatening human health worldwide and one of the most common opportunistic infections and causes of death in HIV co-infected patients [1]. Detections of MDR-TB have considerable clinical implications, since these strains should be treated with appropriate therapy [2]. In 2010, Xpert® MTB/RIF assay was introduced. The test amplifies the *rpoB* gene of MTB and was recommended by the World Health Organization (WHO) as the initial diagnostic test in cases of suspected infections with MDR-TB or HIV-coinfected TB [3]. Compared with traditional diagnosis over sputum smear test and culture, Xpert offered both increased accuracy and speed as well as the ability of detecting RIF resistance [3,4].

A 44-year old male who was diagnosed co-infected with HIV-HBV. Based on the initial test of the Xpert MTB/RIF assay, RIF resistance was shown in the celiac pus but not in his pleural effusion. Conformed by sequencing the *rpoB* gene associated with RIF drug resistance, there was no RIF resistance mutations sequences. A standard therapeutic regimen was directed for his pulmonary and extrapulmonary TB.

## CASE PRESENTATION

In January 2018, a 44-year-old male presented to the Beijing Ditan Hospital, a tertiary care hospital for HIV/AIDS patients. He presented with low-grade fever for 3 months, mainly in later afternoon, and fatigue. In addition, the patient was HBV co-infected, and the HBV load was 3,970 copies/ML. The recent HIV viral load was 448,404 copies/mL with a CD4+ cell count of 4 cells/μL. Computed tomographic (CT) scan of the thorax demonstrated with diffuse small pulmonary nodules, pulmonary effusion, enlargement of hilar and paratracheal lymph nodes (Figure 1A). In addition, enlargement of spleen with multiple low-density nodules and low-density cystic shadow out of the liver was observed in the CT scan of abdomen (Figure 1B). With that evidence, reactivation pulmonary TB and splenic TB was suspected.

At admission, based on the characteristics of CT scan, the celiac pus from spleen was analyzed using Xpert® MTB/RIF assay G4, version 5 and detected positive for MTB with RIF resistance (Supplement Table 1). In discordance, MTB was detected with RIF susceptible in the pleural effusion (Supplement Table 2). Since a phenotypic susceptibility test is not available in our hospital, this discordance could not be confirmed by a drug-susceptibility test. Based on the 81-bp core region (condons 507-533) of *rpoB* gene associated with RIF drug resistance, we amplified this region using NGS and in-house PCR; however, none of sequences found matched alterations with samples of pleural effusion and celiac pus. The result of NGS showed that less than 0.44 percent reads (4103/940227 reads) identified as mutant including codons 516, 526, and 531. Sequencing was performed via the standard operating procedure of the Illumina Hiseq 2500 platform (Supplement Table 3). Meanwhile, in those two samples, the in-house PCR based on 81-bp core region also demonstrated no alterations in sequences of codons 516, 526, and 531 (Supplement Figure 1). For the traditional test, Ziehl Neelsen in the pleural effusion and celiac pus were all positive, but negative in broncho-alveolar lavage (BAL) fluid. There was only positive culture in celiac pus. The patient was started on isoniazid, ethambutol, moxifloxacin, and amikacin against TB at the beginning of the treatment. Considering the abnormal liver function, rifampicin and pyrazinamide were added when liver function recovered. Based on the HBV load, telbivudine directed against HBV was added with treatment against TB in the meantime. After two weeks, the patient was started an antiretroviral treatment including tenofovir, lamivudine, and efavirenz when symptoms improved. With clinical remission and radiographic improvement, he was discharged on day 21 and, via clinical follow-up, complete improvement was achieve in the sixth month of treatment. This study was approved by the ethics committee of Beijing Ditan Hospital.

## DISCUSSION AND CONCLUSION

In this presentation, we report a different test result of RIF resistance in two parts of one patient by Xpert; however, sequencing of the *rpoB* gene confirmed no resistance mutations to RIF. MDR-TB are associated with HIV-infected patients. The last data on MDR-TB in HIV-infected hospitalized patients from Beijing are from 2018. The overall rates of MDR-TB were 11.9% [5]. It looked like that this patient was associated with high risk for MDR-TB because of HIV-TB co-infection and RIF resistance detected by Xpert. However, this was his prior TB infection so a standard therapeutic regimen was directed for his pulmonary and extrapulmonary TB before we received the results of sequencing. Six months later with the standard therapeutic treatment, complete clinical recovery and radiographic improvement was reached.

For MTB and RIF resistance detection, the multiplex real-time PCR technique has improved the sensitivity and turn-around time. In detecting RIF resistance with Xpert® MTB/RIF assay G4, a positive predictive value of 99.5% has been shown by Osman et al., but they have one false positive result for RIP resistance [6]. False positive cases were also reported by Huh et al. and Claessens et al. The sequence of the *ropB* gene showed that a silent mutation induced the false positive result. In the presentation of Claessens et al., there was no mutation sequence [7,8]. In this case, sequencing of NGS and in-house PCR of the *ropB* gene did not find any mutation. A false positive result may be caused by various reasons. Low bacillary burden of MTB has an

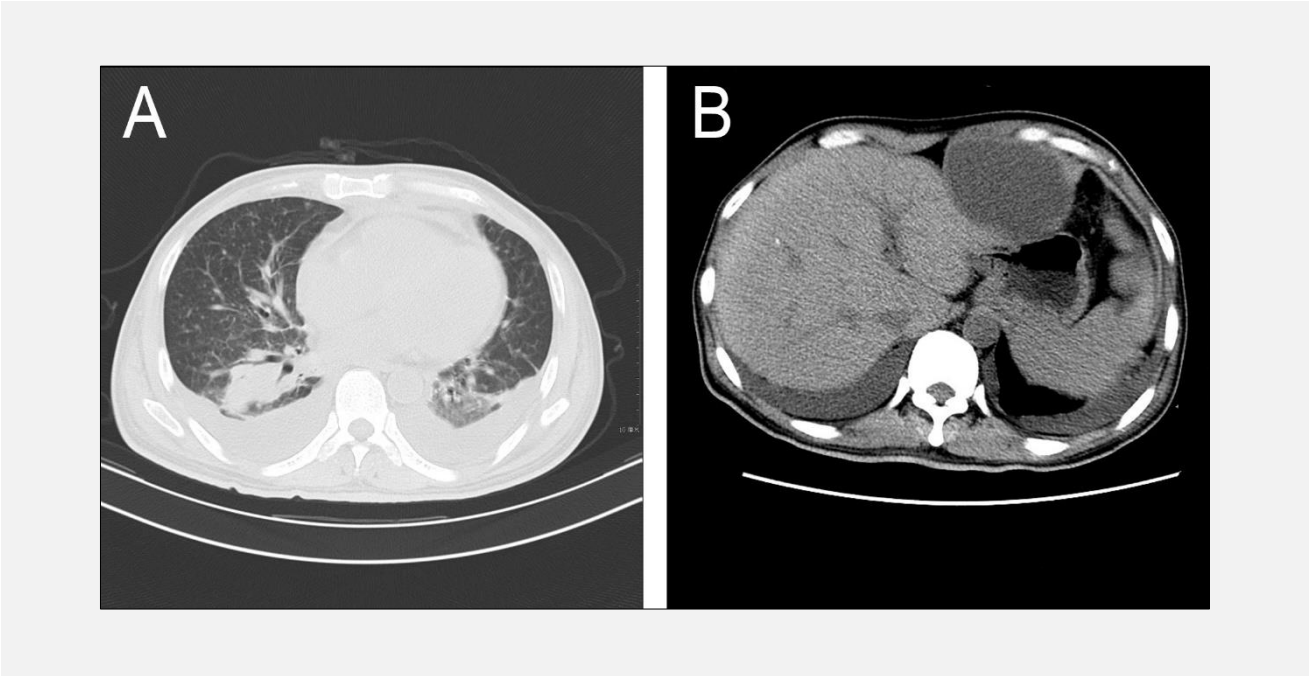


**Figure 1. A. CT scan of the thorax shown with diffuse small pulmonary nodules, pulmonary effusion. B. CT scan of abdomen with enlargement of spleen with multiple low-density nodules.**

influence on the detecting of RIF resistance; however, the sample of celiac pus with RIF resistance had a high level of MTB detected, so the false positive result of RIF resistance could not be caused by low bacillary burden [9]. Because the patient was treated in a separate ward and no MDR-TB patients admitted, infection from MDR-TB patients or samples could not been the reason for resistance result.

HIV infection is an important risk factor for the progress of MDR-TB, and patients in whom prior treatment has failed are more likely associated with MDR-TB [10]. In Beijing, HIV co-infection has a higher prevalence of MDR-TB and a single Xpert test could been meaningful to confirm RIF resistance [5,11]. However, it is clear that this patient had recovered from pleural and splenic TB after the treatment of a standard therapeutic regimen for TB. If the discordance in test of RIP resistance by Xpert is true, this could be another interesting fact. The bacillary burden in the celiac pus was enough to induce genotypic drug resistance of RIF in the detection of molecular test, but standard therapeutic regimen still worked in the patient. The gold standard for MTB drug resistance is a phenotypic drug susceptibility test, compared to the genotypic drug susceptibility test. The consistency between these two methods needs more research.

In conclusion, although Xpert® MTB/RIF assay G4 is reported with high specificity, a phenotypic drug susceptibility test is needed.

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**Contributions of each Author:**
YSY and WLH performed all conception and design of the study; WF, YD, and GGJ organized the database and had useful suggestions on clinical significance of this case report; WLS, LD, and XDH contributed the imaging diagnosis. YSY contributed the molecular diagnostic experiments and wrote the first draft of the manuscript; WLH revised the discussion. All authors have read and approved the manuscript.

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**Availability of Data and Materials:**
No applicable. All major data generated or analyzed during this study were included in this article.

**Ethics Approval and Consent to Participate:**
This study was approved by the ethics committee of Beijing Ditan Hospital with the reference 2017-040-01.

**Consent for Publication:**
Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

**Declaration of Interest:**
The authors declare that they have no competing interests.

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