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Discordance of Rifampin Resistance Result by GeneXpert MTB/RIF, Phenotypic and Molecular Methods in A TB Patient: Case Report

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Abstract

GeneXpert MTB/RIF can quickly detect Mycobacterium tuberculosis and whether it is a drug-resistant strain to provide doctors with accurate medication treatment and prevention. This case presented to the doctor due to urogenital discomfort. The chest radiography(CXR) during hospitalization revealed an abnormality. The sputum acid fast staining in the laboratory was positive, and the sputum and urine samples were sent for GeneXpert MTB/RIF. It is positive for MTB and resistant to Rifampin (RIF). But it is found that Rifampin (RIF) is sensitive through gold standard LJ proportion drug susceptibility (LJ-DST). After further genetic analysis, it was found that the gene *rpoB* had silent mutation P520P (silent mutation P520P), so the susceptibility of rifampin was judged to be sensitive. This similar genomic mutation cases are really rare and there were only 8 cases reported in Taiwan. (J Intern Med Taiwan 2021; 32: 356-362)

Key Words: Mycobacterium tuberculosis, GeneXpert MTB/RIF, Silent mutation P520P

Introduction

GeneXpert MTB/RIF(Cepheid, Sunnyvale, California, USA) is a rapid detection tool for MTB and Rifampin drug-resistant gene recommended by the World Health Organization (WHO) in 2010. It has high sensitivity and distinguishing features such as simple operation and short inspection time[1]. According to the Huadong District TB Prevention and Control Plan, if the residents in Taitung area are screened by X-ray and found to be abnormal or a medical institution detects a positive case of acid-fast staining, the district health center or hospital will immediately send sputum specimen to Ministry of Health and welfare Taitung Hospital Laboratory Department for GeneXpert MTB and Rifampin(RIF) rapid testing.

GeneXpert MTB/RIF is a rapid molecular test for the diagnosis of *Mycobacterium tuberculosis* (MTB) and the mutations that confer Rifampicin resistance (RR).This assay has revolutionized the diagnosis of TB by simultaneously detecting the bacteria and RR[2,3], which is a surrogate marker for MDR-TB[2,4]. RR is determined based on mutations in

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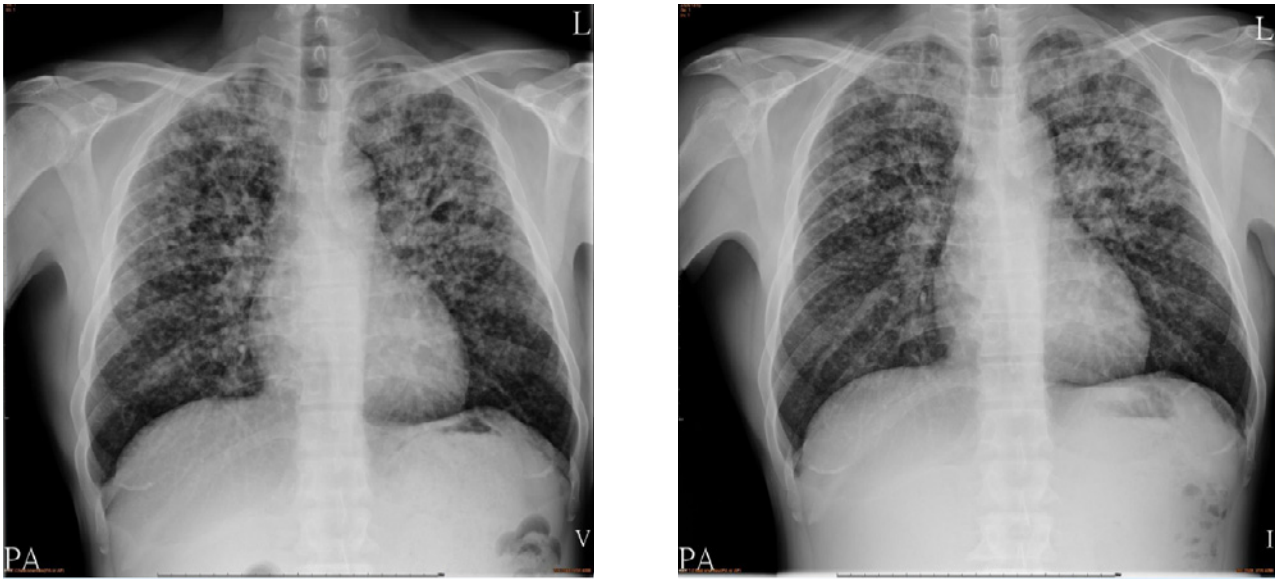
the 81 bp (codons 507–533) regions of the β-subunit of the RNA polymerase enzyme (*rpoB*) gene using five overlapping probes[3,5]. These probes are named as Probe A (codons 507–511), Probe B (codons 511–518), Probe C (codons 518–523), Probe D (codons 523–529) and Probe E (codons 529–533)[3,5]. A mutation in these regions accounts for more than 95% of RR[3,4].

In recent years, the sensitivity and specificity of GeneXpert MTB/RIF is close to 90-95%[3], and the molecular examination can be fast and only needs 2 - 4 hours to reveal its drug resistance status and provide clinicians with the basis for immediate medication treatment. The sensitivities of GeneXpert MTB/RIF for pulmonary tuberculosis were 98% in those who were positive by sputum smear microscopy but only 67% in those who were negative by sputum smear microscopy. This limits the usefulness of GeneXpert MTB/RIF in patients with sputum smear-negative or extrapulmonary tuberculosis. For detection of rifampicin resistance, Xpert can give a false-positive result for strains that carry phenotypically silent mutations or if the bacillary burden is very low, although this is rare[5]. Therefore, it is necessary to combine the patient's clinical symptoms and use it with multiple laboratory tests to improve the accuracy of tuberculosis diagnosis.

Anti-tuberculosis drugs can be divided into first-line and second-line drugs. When the first-line drugs are resistant, the second-line drugs must be used.

Case report

This patient was seen in a teaching hospital on June 25, 2020. He had a history of diabetes, high blood pressure and hyperlipidemia. The main complaint was that the testicles were red, swollen and painful. At the end of June, he started to show dizziness, poor physical strength, fatigue and weight loss in a moth. (From 82 kg to 72 kg). There were no respiratory symptoms and only slight runny nose was complained. The chest radiography(CXR) revealed diffused nodular densities in the bilateral lung (Figure 1). The sputum was found to be positive for acid-fast staining. GeneXpert MTB/RIF detection and the report showed positive MTB and rifampin drug resistance. From July 31 to August 4, 2020, the various preliminary tuberculosis exami-



A (Before therapy) B(Afetr therapy)

Figure 1. Chest X-ray (CXR) on July 30, 2020(A) and January 18, 2021(B).

nations were: 3 sets of acid-fast bacteria stained positive, GeneXpert MTB/RIF tested positive for MTB and RIF resistance, and further sent to the contract laboratory to confirm the same with Genotype MTB positive and RIF resistant (Table 1). The grade of sputum AFB smear are 3+. He was diagnosed with left tuberculous epididymitis. The patient did not have any contact history of MDRTB or special travel history. The patient had HIV-Negative and history of diabetes (HbA1C: 10.2% on August 8,2020; HbA1C: 6.9% on September 24, 2020).

Because his main complaint was testicular pain, he sent his urine for acid-fast stain, culture and GeneXpert MTB/RIF test again on September 3. The grade of urine AFB smear was Negative. But it also tested positive for MTB and RIF drug resistance, so he can be judged to have extrapulmonary tuberculosis.

On September 16, the contract laboratory tested the traditional tuberculosis drug susceptibility test and found that the first-line tuberculosis drugs (Isoniazid, Rifampin, Streptomycin, Ethambutol) were all sensitive including RIF was sensitive. Because the molecular test showed that the RIF resistance was inconsistent with the results of the traditional drug susceptibility test, the strain was sent to the research center for further genetic analysis. The research center detects that its *rpoB* hot-spot region is Silent mutation P520P (silent mutation P520P).

According to Table 2, the results of drug susceptibility testing of tuberculosis in a number of laboratories showed susceptibility to both first-line and second-line drugs.

This case was notified of a new tuberculosis case on August 3, 2020. The treatment with Isoniazid (INH), RINA, Ethambutol (E), Pyrazinamide (Z), Moxifloxacin (MOXI), Kanamycin (KM) was started on August 8. The patient had binaural high-frequency hearing loss and pause KM on August 14. On August 27 Genotype test was RIF resistant and cancel RMP. Drug susceptibility testing of tuberculosis was all sensitive and cancel 40 doses of KM

Table 1. Sputum and urine GeneXpert probe types and DNA amounts report

GeneXpert Probe (Ct)	Probe A	Probe B	Probe C	Probe D	Probe E
Sputum (MTB :HIGH)	13.3	15.7	0.0	13.7	15.4
Urine (MTB :VERY LOW)	31.2	31.9	0.0	30.2	32.9

Table 2. Antimicrobial susceptibility reports of three inspection units

Drugs testing and Laboratories	INH	RIF	E	S	Z	KM AM Cm	FQ	Method
Ministry of Health and Welfare Taitung Hospital Medical Laboratory		R						GeneXpert
Tris-Service General Hospital	S	R			S	S	S	Genotype
Hualien Tzu Chi Hospital	S	S	S	S				DST

Drugs testing and Laboratories	Z	KM AM Cm	PAS CS	MOXI LFX	Pto Eto	CFZ LZD	RBT	BDQ
Research Center	S	S	S	S	S	S	S	S

DST: The traditional drug susceptibility test, INH: Isoniazid, RIF: Rifampin, Z: Pyrazinamide, E: Ethambutol, S: Streptomycin, Km: Kanamycin, Am: Amikacin, Cm: Capreomycin, FQ: Fluoroquinolone, PAS: Para-Aminosalicylate, Cs: Cycloserine, MOXI: Moxifloxacin, LFX: Levofloxacin, Pto: Prothionamide, Eto: Ethionamide, CFZ: Clofazimine, LZD: Linezolid, RBT: Rifabutin, Bdq: Bedaquiline.

on October 5. The detailed medication process has been adjusted according to the test report as shown in Table 3.

On November 25, the sputum AFS and TB culture was tested again and were all negative. The chest radiography(CXR) revealed better than before (Figure 1). The patient's condition is stable and gradually improves. The course of treatment is expected to last until August, 2021. The follow-up medication effect is worth further observation.

Results and discussion

In order to quickly detect drug-resistant tuberculosis and provide appropriate quarantine and treatment, all countries are committed to the development of molecular testing. The molecular tests currently recommended by WHO include: LPA, GeneXpert MTB/RIF, GenoType MTBDRplus, etc[6],. GeneXpert MTB/RIF uses the specific gene sequence of MTBC and the corresponding probe of the RIF resistance gene *rpoB* to perform sample processing, nucleic acid amplification, and target sequence determination through real-time PCR and reverse transcription RCR, and fully automated testing and integration inspection result. The system requires the use of a disposable GeneXpert MTB/RIF detection cartridge to contain PCR reagents and control the PCR process. Since the detection cassette is independent, it can eliminate cross-contamination between samples and perform rapid detection of Mycobacterium tuberculosis and RIF drug resistance. GenoType MTBDRplus uses nucleic acid amplification technology and special probe principles to qualitatively detect mutation sites of drug resistance genes. After nucleic acid amplification, it undergoes a heterozygous reaction, and is detected by *rpoB*, *KatG*, and *InhA* probes to identify wild type and mutant type sites to identify the drug resistance genes of the bacteria[7]. It can simultaneously detect the Mycobacterium tuberculosis group and the resistance of Isoniazid and Rifampin, providing rapid diagnosis of multi-drug resistant tuberculosis.

Through this case discussion, it was found that GeneXpert detected RIF-resistant patients but did not match the traditional drug sensitivity test with RIF as a sensitivity report. The research

Table 3. Medication process

2020	8/8	8/14	8/19	8/22 (Hospitalized)	8/27	9/3	9/24	10/5	10/12
Rina(300mg)	2	2	2	2	DC	2	2	2	2
E(400mg)	3	3	3	3	3	3	3	3	3
Z(500mg)	3	3	3	3	3	3	3	3	3
INH(100mg)					3	DC			
Moxi(400mg)	1	1	1	1	1	1	1	1	1
KM(gm)	1	hold	1	1	1	1	1	DC	

2020	10/19	10/19	11/23	12/21	2021	1/18	2/8
Rina(300mg)	2	2	2	2	Rina(300mg)	2	2
E(400mg)	3	3	3	3	E(400mg)	DC	
Z(500mg)	3	3	3	3	Z(500mg)	3	3
INH(100mg)					INH(100mg)		
Moxi(400mg)	1	1	1	1	Moxi(400mg)	1	1

Rina: rifampin 300mg+isoniazide 150mg.

center detects that its *ropB* hot-spot region is Silent mutation P520P (silent mutation P520P), and its nucleotide may be mutated from CCG to ACG at position 520, but the amino acid (Proline) is still no change (Proline). This mutation means that the code (Coden) is changed but the corresponding amino acid remains unchanged, and it does not change the sensitivity of the bacteria to drugs. Therefore, the traditional drug susceptibility test shows RIF sensitivity. So far, 8 case of active TB with silent mutation in rpoB P520P were reported. It mainly distributed in 4 counties and cities (Taitung, Hualien, Keelung, Xinbei). The same Silent mutation P520P gene mutation cases are really rare, and need to further explore the route of infection and the source of infection.

Discordance across phenotypic and molecular methods for MTB drug susceptibility testing may have gene dispute mutation and silence mutation[8]. Disputed rpoB mutations often cause "borderline" or subcritical levels of RIF resistance, which are easily missed in phenotypic susceptibility assays, particularly using the automated MGIT 960 system (BD, Sparks, MD, USA)[9]. Disputed *rpoB* mutations may be responsible for the majority of rifampicin (RMP) resistance and lead to adverse outcomes of first-line treatment. Their minimum inhibitory concentrations (MICs) can be below the conventional critical concentration. Their low MICs and in our own experience often also pronounced loss make them difficult to grow and thus to detect in phenotypic DST. Molecular RMP DST could greatly simplify resistance surveillance, in addition to offering the best prospects for early and accurate individual diagnosis.

Rifampin resistance is mainly caused by mutations in the *ropB* RIF- resistance - determining region (RRDR) gene fragment[10], accounting for about 90%[11,12]. When the proportions of MTBC mutant (drug resistant) and wild type (sensitive) in different samples are different, different results of RIF interpretation will appear[13,14]. The current Xpert reagent detection of RIF is based on the unbound or delayed binding of Probe. If the DNA in the sample is insufficient, it will cause the lack of binding or delayed binding of the specific probe, which is not a real drug resistance[15,16,17]. Silent mutations can be detected by molecular assays but do not modify the aa and the protein structure, and they are not relevant for drug resistance. Silent mutations may cause false positivity in molecular tests and overtreatment. Silent mutations treatment is based on traditional drug susceptibility testing. Silent mutations affect molecular genotyping based on probe binding[18,19]. The molecular test is inconsistent with traditional drug sensitivity, further genetic analysis and comprehensive determination of drug sensitivity are required[20].

Referenaces

1. WHO. Global Tuberculosis Report. Geneva: World Health Organization; 2018.
2. Mboowa G, Namaganda C, Sengooba W. Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: a retrospective study. BMC Infection Diseases 2014; 14:481-9.
3. K. al zahrani, H. al jahdali, L. poirier, P. rené, M. l. gennaro, and D. menzies. Accuracy and Utility of Commercially Available Amplification and Serologic Tests for the Diagnosis of Minimal Pulmonary Tuberculosis. Am J Respir Crit Care Med 2002; 162: 1323-9.
4. Yew WW, and Leung CC. Management of multidrug-resistant tuberculosis: Update 2007. Respirology 2008;13: 21-46.
5. Ocheretina O, Byrt E, Mabou MM. False-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load. Diagn Microbiol Infect Dis. 2016; 85:53-5.
6. Hossein Kazemian, Jalil KY, Abbas Bahador, et al. Efficacy of Line Probe Assay In Detection Of Drug-Resistant Pulmonary Tuberculosis In Comparison With GeneXpert And Phenotypic Methods In Iran And Genetic Analysis Of Isolates By MIRU-VNTR. Infect Drug Resist 2019;12: 3585-93.
7. A. Lacoma, N. Garcia-Sierra, C. Prat, J. Ruiz-Manzano, L. Haba, S. Rosés, J. Maldonado, and J. Domínguez. GenoType MTBDRplus Assay for Molecular Detection of Rifampin and Isoniazid Resistance in Mycobacterium tuberculosis Strains and Clinical Samples. Journal of Clinical Microbiology 2008; 46(11):3660-7.
8. Huo FM, Ma YF, Liu RM, Ma LP, Li SS. Interpretation of Discordant Rifampicin SusceptibilityTest Results Obtained Using GeneXpert vs PhenotypicDrug Susceptibility Testing.

Open Forum Infectious Diseases 2020; 7(8) :1-7.

9. Gonzalo X, Claxton P, Brown T, et al.True rifampicin resistance missed by the MGIT: prevalence of this pheno/genotype in the UK and Ireland after 18 month surveillance. Clin Microbiol Infect 2017. 23:260-3.
10. Mboowa G, Namaganda C, Sengooba W. Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: a retrospective study. BMC Infect Dis 2014; 14:481-9.
11. Kaur R, Jindal N, Arora S, Kataria S. Epidemiology of rifampicin-resistant tuberculosis and common mutations inrpoB gene of Mycobacterium tuberculosis: a retrospectivestudy from six districts of Punjab (India) using Xpert MTB/RIF assay. Journal of Laboratory Physicians 2016; 8:96-100.
12. Ullah I, Shah AA, Basit A, et al.Rifampicin resistance mutations in the81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert MTB/RIF in Khyber Pakhtunkhwa, Pakistan: a retrospectivestudy. BMC Infect Dis 2018; 16:413-25.
13. Yelis Tanriverdi Cayci and Kemal Bilgin. An evaluation of false –positive rifampin resistance on the Xpert MTB/RIF. Mem. Inst. Oswaldo Cruz 2017, 112:756-9.
14. Chakravorty S, Kothari H, Aladegbami B, Jin Cho E, Lee JS, Roh SS. Rapid, high-throughput detection of rifampicin resistance and heteroresistance in Mycobacterium tuberculosis by use of sloppy molecular beacon melting temperature coding. J Clin Microbiol 2012; 50(7): 2194-202.
15. Jean Claude, Semuto Ngabonziza, Armand Van Deun, et al. Prevalence and divers of false-positive rifampin resistance Xpert MTB/RIFT results: a prospective observational study in Rwanda. The Lancet 2020;1(2):74-83.
16. Asegedom SW, Teweldemedhin M, Gebreyesus H. Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review. Hindawi J Pathog 2018; Article ID 7104921, 8.
17. Derbie A, Worku S, Mekonnen D, et al. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and its Rifampicin resistanceat Felege Hiwot and Debre Tabor Hospitals, Northwest Ethiopia: A preliminary implementation research. Ethiopian Journal of Health Development 2016; 30(2):60-65.
18. Maria Alonso, Juan Jose Palacios, Marta Herranz, et al. Isolation of Mycobacterium tuberculosis Strains with Silent Mutation in ropB Leading to Potential Misassignment of Resistance Category. J Clin Microbiol 2011; 20:2688-90.
19. Barnard M, H Albert, G. Coetzee, R. O'Brien, and M. E. Bosman. Rapid molecular screening for multidrug-resistant tuberculosis in a high volume public health laboratory in South Africa. Am. J. Respir. Crit 2008; 177:787-92.
20. Nusrath Unissa A and Hanna LE. Molecular mechanisms of action, resistance, detection to the first-line anti tuberculosis drugs: rifampicin and pyrazinamide in the post whole genome sequencing era. Tuberculosis 2017; 105:96-107.

結核分生 (GeneXpert MTB/RIF) 檢測出 Rifampin 抗藥性結果與傳統結核藥敏報告不符合之探討：案例報告

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摘 要

GeneXpert MTB/RIF 能快速檢測結核桿菌及其是否為抗藥性菌株，以提供醫師正確之用藥治療及防治隔離是具有其相當重要性。此個案因泌尿生殖系統不適而就診，於住院時的胸部光檢查（CXR）發現異常，經由實驗室痰液抗酸性染色為陽性，而送驗痰液及尿液檢體做 GeneXpert MTB/RIF 皆呈現 MTB 陽性及 Rifampin(RIF) 抗藥，但經由傳統瓊指藥敏試驗卻發現 Rifampin(RIF) 是不具抗藥性而是具有敏感性的。再經由進一步基因分析下發現其基因 rpoB 發生 Silent mutation P520P（沉默的突變 P520P），故仍以 Rifampin 表現敏感性為最終判定。這種相似的基因組突變病例確實很罕見，目前台灣約有 8 例。