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Original Article

Comparison of genotype MTBDR*plus* results between TB patients with different levels of drug resistance: a retrospective study in China

Xinfeng Wang[1], Haimei Qin[2], Chunfang Wang[2], Maoshui Wang[1], Junli Wang[2]

[1]*Department of Laboratory Medicine, Shandong Provincial Chest Hospital, Jinan, China;* [2]*Center of Clinical Laboratory, Affiliated Hospital of Youjiang Medical College for Nationalities, Baise, China*

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Abstract: *Background and aim:* To evaluate and compare the performance of GenoType MTBDR*plus* for the detection of isoniazid (INH) and rifampicin (RIF) resistance at two different levels of drug-resistant tuberculosis (TB) in China, a retrospective study was conducted. *Methods:* Between May 2012 and September 2016, 253 INH- or RIF-resistant TB patients were enrolled in the study. Phenotypic drug susceptibility test (DST) was performed using the absolute concentration method. The MTBDR*plus* assay was done on culture specimens. Comparisons of the positivity were made using Fisher's exact test. *Results:* Positivity of MTBDR*plus* for detecting resistance in low-level INH-resistant TB was 84.0% (78.1%, 88.6%) and according to resistance to high-level INH, the groups were divided as follows: 1) resistant subgroup (+), the positivity was 63.6% (43.0%, 80.3%); 2) sensitive subgroup (-), the positivity was 86.8% (80.8%, 91.1%). For RIF, positivity of MTBDR*plus* in low-level RIF-resistant TB was 91.4% (86.6%, 94.7%). According to resistance to high-level INH (+ and -), positivity was 97.2% (93.0%, 98.9%) and 73.3% (59.0%, 84.0%), respectively. The statistical analysis indicated significant differences in positivity between subgroups with different levels of drug-resistant TB isolates (INH or RIF-resistant, all P<0.05). *Conclusions*: Although MTBDR*plus* assay accurately predicts drug resistance in most TB isolates in China, conventional DST remains necessary to confirm MDR-TB. It was demonstrated that MTBDR*plus* assay has significant differences in positivity between low- and high-level drug-resistant groups. The exact mechanism behind the phenomenon is still unclear, and warrants further study, since high-dose drugs may have a role in MDR-TB therapy.

Keywords: GenoType MTBDR*plus* assay, isoniazid, rifampicin, discordance

Introduction

Despite the availability of effective chemotherapy, tuberculosis (TB) remains one of the world's deadliest diseases. In 2012, TB killed 1.3 million people [1]. Drug resistance develops as a result of inadequate treatment creating a selection pressure on spontaneously occurring mutants and thus complicates management of TB. Multidrug-resistant tuberculosis (MDR-TB) represents one of the most important threats in the control of TB worldwide. MDR-TB is defined as resistance to two of the most potent first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF) with or without resistance to other drugs [2]. According to a WHO estimate, there were approximately 300,000 new cases of MDR-TB and around 190,000 fatalities from TB worldwide only in 2014 [3].

Solid and liquid culture methods for drug susceptibility test (DST) of *Mycobacterium tuberculosis* (*M.TB*) are time consuming requiring weeks to months in providing the results [4, 5]. Genotypic (molecular) methods for DSTs that target MDR-TB make it possible for the patients to be detected earlier, thus improving outcomes. GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) which is used for the rapid detection of *M.TB* complex and resistance to INH and/or RIF was endorsed by WHO [6]. The molecular line probe assay detects mutations associated with the *rpoB* gene for RIF resistance, *katG* genes and *inhA* regulatory region gene for INH resistance [7].

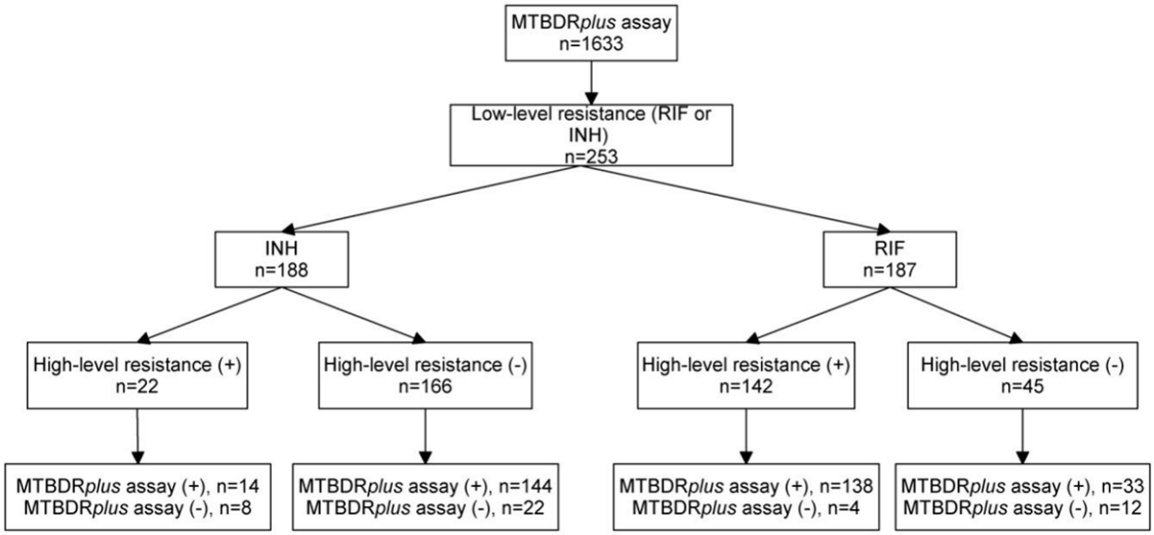

Figure 1. Flow chart showing enrollment process. INH, isoniazid; RIF, rifampicin.

Three previously published meta-analyses found that MTBDR*plus* assay had good accuracy for rapid detection of drug resistance to INH and/or RIF of *M.TB* [8-10].

However, although greater than 95% of RIF resistance is associated with mutations in an 81 base pair section of the *rpoB* gene, INH resistance appears more complex and has been associated with multiple genes [11], like *katG*, *inhA*, oxy*R*-aph*C*, *kasA,* and *ndh* [12-14]. In addition, the two mutations, *katG*315 and *inhA*-15, combined with ten of the most commonly occurring mutations in the *inhA* promoter and the *ahpC-oxyR* intergenic region, explain 84% of global phenotypic INH resistance [11]. Therefore, the accuracy of MTBDR*plus* is associated with prevalence of antibiotic resistance genes and its mutant locus. Mutation loci on these genes (such *rpoB*, *k*at*G*) are associated with a wide range of INH or RIF minimum inhibitory concentrations [15-17]. Cessation of INH and RIF are generally recommended in the confirmation of MDR-TB. However, high doses might have a role [18, 19]. This implied that: 1) it is necessary to perform DST at different levels; 2) these mutations may have effect on the choice of anti-MDR-TB therapy; 3) The composition of mutations related with antibiotic resistance should be different between TB isolates with different levels of drug resistance.

In this retrospective study, we aimed to evaluate and directly compare the performance of MTBDR*plus* for the detection of INH and RIF resistance at two different levels of drug-resistant TB in China.

Materials and methods

This study was approved by the Human Research Ethics Committees of Shandong Provincial Chest Hospital (SPCH). Because of the retrospective nature, written consent was waived.

Subjects

Between May, 2012 and September, 2016, 253 INH- or RIF-resistant TB patients were enrolled in the study. Culture and phenotypic DST were performed on all cases. Cases were divided according to whether high-level resistance to INH (or RIF) was present. Their clinicopathological characteristics were reviewed and analyzed.

Methods

The absolute concentration method (INH: 1 and 10 μg/mL, RIF: 50 and 250 μg/mL) on Löwenstein-Jensen medium was used to screen *M.TB* isolates [20]. The MTBDR*plus* assay was done on culture specimens. There were three steps: i) DNA extraction from processed sputum specimen, ii) amplification of target region by PCR, and iii) Hybridization of PCR product to the specific oligo-nucleotide probes, immobilized on the strip. All three steps were carried

Table 1. Positivity of GenoType MTBDRplus assay in patients with different levels resistance to isoniazid or rifampicine

	Resistance	Number	Positivity (95% CI)
Isoniazid (High-level resistance)	+	22	63.6% (43.0%, 80.3%)
	-	166	86.8% (80.8%, 91.1%)
	Total	188	84.0% (78.1%, 88.6%)
Rifampicine (High-level resistance)	+	142	97.2% (93.0%, 98.9%)
	-	45	73.3% (59.0%, 84.0%)
	Total	187	91.4% (86.6%, 94.7%)

out according to manufacturer's instructions. Drug resistance was expressed as the absence of wild-type band, presence of mutation band or both.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 16.0. Continuous data are summarized as mean with standard deviation, all calculations were estimated at a 95% confidence interval (95% CI). Binary data are presented as percentages. Comparisons of the percentages were made using a two-tailed Fisher's exact test. P<0.05 was taken as statistically significant.

Results

From May, 2012 to September, 2016, the MTBDR*plus* assay was performed on 1633 samples. In total, 253 low-level INH- or RIF-resistant TB patients were enrolled in the study. The average age was 40.1 ± 16.9 years (range 5 to 61 years), with 70.4% (178/253) male. All (223/223) were HIV-negative. The flow chart used to evaluate the performance of MTBDR*plus* is shown in **Figure 1**.

As shown in **Table 1**, positivity of MTBDR*plus* for detecting antibiotic resistance in low-level INH-resistant TB was 84.0% (78.1%, 88.6%); According to resistance to high-level INH, the group was divided into the: 1) resistant subgroup (+), the positivity was 63.6% (43.0%, 80.3%); 2) sensitive subgroup (-), the positivity was 86.8% (80.8%, 91.1%).

For RIF, the positivity of MTBDR*plus* for detecting antibiotic resistance in low-level RIF-resistant TB was 91.4% (86.6%, 94.7%); According to resistance to high-level INH, the group was divided into the: 1) resistant subgroup (+), the positivity was 97.2% (93.0%, 98.9%); 2) sensitive subgroup (-), the positivity was 73.3% (59.0%, 84.0%).

Fisher's exact test was used to compare the positivity of MTBDR*plus* assay for detecting INH or RIF resistance. The results (**Table 2**) indicated: 1) significant difference between subgroups with different types (+ and -) of high-level resistance to INH (or RIF) (all P<0.05); 2) significant difference between groups with high-level resistance to INH and RIF (P<0.05).

Discussion

While the number of cases of TB has been steadily declining over the past decade, the prevalence of drug-resistant disease threatens to reverse these declines [3]. Recognition of drug resistance and the timely initiation of effective therapy are essential for treating and preventing transmission of MDR-TB. Lots of studies have evaluated the diagnostic performance of the MTBDR*plus* assay. However, there are little data comparing the performance of MTBDR*plus* for the detection of INH and RIF resistance in TB isolates with two different levels of drug resistance. Our study demonstrates that MTBDR*plus* assay for detection of INH and RIF resistance has significantly higher sensitivity in low-level drug-resistant group than that in high-level resistant group. Moreover, the sensitivity of MTBDR*plus* assay for detection of INH resistance was lower than that of RIF resistance.

In this study, the total sensitivity for INH and RIF resistance was 91.4% (86.6%, 94.7%) and 84.0% (78.1%, 88.6%), respectively. The sensitivities were lower than that was reported in a meta-analysis (INH: 91% (88%, 94%); RIF: 96% (95%, 97%)) conducted in 2016. This may be partially attributed to limitations of the molecular methods for detection of first line drug resistance. Prevalence of mutations in the *rpoB*, *katG* and *inhA* genes seems to vary widely in different geographic locations as reported in various studies conducted in different countries [21-23]. In another study conducted by

Table 2. Comparison of GenoType MTBDRplus assay results between patients with different levels resistance to isoniazid or rifampicine

	Resistance	Isoniazid (High-level resistance)			Rifampicine (High-level resistance)	
		+	-	Total	+	-
Isoniazid (High-level resistance)	+					
	-	P<0.05				
	Total	P<0.05	P>0.05			
Rifampicine (High-level resistance)	+	P<0.05	P<0.05	P<0.05		
	-	P>0.05	P>0.05	P>0.05	P<0.05	
	Total	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Brossier F et al., the assay can detect 89% of the INH-resistant strains with a high level of resistance, but only 17% of the strains are characterized by a low level of INH resistance. The results are inconsistent with findings of our study, although this may be contributed to the different concentrations of INH used in the DSTs (0.1, 0.2 and ≥1 *vs.* 1 and 10 μg/mL).

Although the molecular basis of these discordant results between different-levels resistant TB has not been fully elucidated, we can speculate as to the mechanisms behind test discordance. First, for example, alterations in multiple genes [11], like *kat*G, *inh*A, *oxy*R-*aph*C, *kas*A, and *ndh* [12-14], are associated with INH resistance. As is known, the MTBDR*plus* assay for the detection of INH resistance is designed to detect only one mutation in *katG* and three in *inhA*. The limited numbers of probes in MTBDR*plus* restricted its detection of all mutation loci, which might also have decreased its sensitivity. Moreover, the types of mutations and the types of resistant genes are associated with different-levels resistance [24]. So other mutations may play role in the discordant results. An alternative explanation for the discordant results may be the presence of mixed populations of bacteria, consisting of both susceptible and resistant strains, and hetero-resistance [25, 26].

Molecular methods for detecting resistance in *M.TB* have some limitations. These assays have significantly increased MDR-TB detection. However, no test has yet replaced phenotypic DST as the gold standard for MDR-TB diagnosis. The MTBDR*plus* assay is designed to detect the more frequent mutations related to INH and RIF resistance, not to detect the whole mutations. Therefore, this would decrease the sensitivity in detection of drug resistance, especially of INH resistance. Although common mutations confer drug resistance are well known for some drugs, in some cases the mutations are silent and are not always related to the resistance acquisition. Unfortunately, we cannot reveal exact mechanisms behind the observed discordance. Future studies will conduct research to detail the full mechanism.

In conclusion, although the MTBDR*plus* assay accurately predicts drug resistance in most of TB isolates in China, conventional DST remains necessary to confirm MDR-TB. It was demonstrated that MTBDR*plus* assay for detection of INH and RIF resistance has significantly higher sensitivity in low-level resistant group than that in high-level group. This implied that there is difference in the MTBDR*plus* results between TB isolates with low- and high-level drug resistance. The exact mechanism behind the phenomenon is still unclear, and warrants further study, since high-dose drugs may have a role in MDR-TB therapy [18, 19].

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Disclosure of conflict of interest

None.

Address correspondence to: Maoshui Wang, Department of Lab Medicine, Shandong Provincial Chest Hospital, 46 Lishan Road, Jinan, China. Tel: +86 531-86568107; Fax: +86 531-86956760; E-mail: wangmaoshui@gmail.com; Junli Wang, Center of Clinical Laboratory, Affiliated Hospital of Youjiang Medical College for Nationalities, Baise, China. E-mail: 13907768146@163.com

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