Original Article

Diagnostic Performance of the GenoType MTBDR*plus* and MTBDR*sl* Assays to Identify Tuberculosis Drug Resistance in Eastern China

Qiao Liu[1], Guo-Li Li[1], Cheng Chen[1], Jian-Ming Wang[2], Leonardo Martinez[3,4], Wei Lu[1], Li-Mei Zhu[1]

[1]Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, Nanjing, Jiangsu 210009, China
[2]Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu 211166, China
[3]Department of Epidemiology and Biostatistics, University of Georgia School of Public Health, Athens 21401, Georgia, USA
[4]Center for Global Health, University of Georgia School of Public Health, Athens 21401, Georgia, USA

Abstract

Background: The WHO recently has recommended the GenoType MTBDR*plus* version 1.0 and MTBDR*sl* version 1.0 assays for widespread use in countries endemic with drug-resistant tuberculosis. Despite this, these assays have rarely been evaluated in China, where the burden of drug-resistant tuberculosis is among the highest globally.

Methods: *Mycobacterium tuberculosis* clinical isolates were obtained between January 2008 and December 2008. Isolates were tested for drug resistance against rifampicin (RFP) and isoniazid (INH) using the GenoType MTBDR*plus* assay and drug resistance against ethambutol (EMB), ofloxacin (OFX), and kanamycin (KM) using the Genotype MTBDR*sl* assay. These results were compared with conventional drug-susceptibility testing (DST).

Results: Readable results were obtained from 235 strains by GenoType MTBDR*plus* assay. Compared to DST, the sensitivity of GenoType MTBDR*plus* assay to detect RFP, INH, and multidrug resistance was 97.7%, 69.9%, and 69.8%, respectively, whereas the specificity for detecting RFP, INH, and multidrug resistance was 66.7%, 69.2%, and 76.8%, respectively. The sensitivity and specificity of the GenoType MTBDR*sl* assay were 90.9% and 95.2% for OFX, 77.8% and 99.5% for KM, 63.7% and 86.4% for EMB, respectively. Mutations in codon S531L of the *rpoB* gene and codon S315T1 of *KatG* gene were dominated in multidrug-resistant tuberculosis (MDR-TB) strains.

Conclusions: In combination with DST, application of the GenoType MTBDR*plus* and MTBDR*sl* assays may be a useful supplementary tool to allow a rapid and safe diagnosis of multidrug resistance and extensively drug-resistant tuberculosis.

Key words: GenoType MTBDR*plus* Assay; GenoType MTBDR*sl* Assay; Multidrug-resistant Tuberculosis; Rapid Diagnosis

Introduction

The emergence of drug-resistant tuberculosis is a major public health concern and threatens global progress toward reaching the World Health Organization's (WHO) post-2015 new End TB Strategy goal of tuberculosis elimination.[1] China has the third highest burden of new tuberculosis. Globally, 3.9% of new cases and 21% of previously treated cases have multidrug-resistant tuberculosis (MDR-TB) and more than half of these patients are located in India, China, and the Russian Federation.[1,2] In a nationwide survey across China in 2007, the prevalence of MDR-TB was 10.2%. Estimates of MDR-TB prevalence were 5.7% and 25.6% among new and previously treated cases, respectively. Approximately 8% of MDR-TB patients had extensively drug-resistant (XDR) tuberculosis.[3]

Although laboratories in many of these countries can perform sputum smear microscopy, a shortage of laboratories capable of performing accurate, rapid culture and drug-susceptibility

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.208248

Address for correspondence: Dr. Li-Mei Zhu, Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, Nanjing, Jiangsu 210009, China
E-Mail: jsjkmck@163.com

Received: 10-01-2017 **Edited by:** Li-Min Chen
How to cite this article: Liu Q, Li GL, Chen C, Wang JM, Martinez L, Lu W, Zhu LM. Diagnostic Performance of the GenoType MTBDR*plus* and MTBDR*sl* Assays to Identify Tuberculosis Drug Resistance in Eastern China. Chin Med J 2017;130:1521-8.

testing (DST) still exists. Due to this, the time to obtain a bacteriological culture-based diagnosis may range from weeks to months for many patients.[4,5] Furthermore, many cases with low bacillary loads are misdiagnosed, underdiagnosed, or poorly treated.[6] Among the nearly half a million estimated cases of MDR-TB that occurred globally in 2014, about one in four were detected. Comparatively, China detects only 11% of estimated MDR-TB cases.[1]

To enlarge the capacity for the detection of drug resistance, the WHO recommends the use of a line-probe assay, the GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany), which can identify the *Mycobacterium tuberculosis* (MTB) complex as well as resistance to rifampicin (RFP) and isoniazid (INH) drugs.[7] The assay detects mutations in the *rpoB* gene for RFP resistance, *katG* gene for INH resistance, and *inhA* regulatory region gene for low-level INH resistance.[8] Subsequently, a new DNA strip assay, GenoType MTBDR*sl* version 1.0 (Hain Lifescience GmbH, Nehren, Germany), was developed to detect resistance to ethambutol (EMB), fluoroquinolones, and injectable aminoglycosides/cyclic peptides allowing diagnosis of XDR-TB among MDR-TB patients.

Several evaluation studies of GenoType MTBDR*plus* and MTBDR*sl* assays have been conducted in different countries,[9-11] including in China where the burden of drug-resistant tuberculosis has reached epidemic levels and programmatic detection is poor.[8,12,13] The objective of the present study was to evaluate the diagnostic performance of the GenoType MTBDR*plus* and MTBDR*sl* assays in a high-burden Chinese population using a culture-based phenotypic DST as a gold standard.

Methods

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Jiangsu Province Centre for Disease Control and Prevention. The study was conducted in accordance with approved guidelines, and written informed consent was obtained from all eligible TB patients.

Study population and isolates

The study design has been described previously.[14] Briefly, MTB isolates were collected from Jiangsu province in 2008. In all, 235 isolates were evaluated, including 192 MDR-TB, 25 RFP monoresistant, four INH monoresistant, and 14 fully susceptible isolates.

An extensive investigation of treatment history of chemotherapy was undertaken by trained field workers and nurses using a structured questionnaire. Other demographic information collected from participants included age, gender, smoking status, drinking status, occupation, and family contact with tuberculosis.

Isolate identification and drug-susceptibility testing

Sputum samples were cultured and isolated on Lowenstein-Jensen (LJ) culture media. Culture-positive isolates were used for isolate identification and DST. Identification of MTB was completed using p-nitrobenzoic acid (PNB) method. Growth in LJ medium containing PNB indicated that the bacilli were not an MTB complex. Species other than MTB were excluded from all final analyses.

LJ medium impregnated one antituberculosis drug was used for DST and the critical drug concentrations were 0.2 μg/ml for INH, 40 μg/ml for RFP, 2 μg/ml for EMB, 30 μg/ml for kanamycin (KM), and 2 μg/ml for ofloxacin (OFX). Growth on the control medium was compared with growth on a drug-containing medium to determine susceptibility. DST results were categorized as resistant or susceptible. For internal quality assurance of DST, a standard H37Rv isolate was included with each new batch of LJ medium.

Genomic DNA preparation

Mycobacterial genomic DNA was extracted from mycobacterial colonies growing on LJ medium by resuspending one loop of mycobacterial colonies in 200 μl TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA) and was incubated at 85°C for 30 min to obtain genomic DNA. After centrifugation of the suspension, the supernatant fluid containing DNA was removed and stored at −20°C until further use.[15,16] Laboratory isolate H37Rv was used as a control for all microbiological and genetic procedures.

Molecular methods

GenoType MTBDR*plus* and GenoType MTBDR*sl* assays were performed according to the manufacturer's instructions. Genotypic assays were evaluated blindly by two medical technologists independently. In addition, the presence of wild-type sequence along with the corresponding mutant probe indicated the sample carrying heteroresistance isolate.

Statistical analysis

Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with 95% confidence intervals (*CI*) of the GenoType MTBDR*plus* and MTBDR*sl* assay results were calculated. A value of $P < 0.05$ was considered statistically significant. SPSS (version 13.0, SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses.

Results

A total of 235 patients were included in this study. The majority of patients (168/235, 71.5%) were male. The participants' median age was 49.6 years (interquartile range, 35.8–60.0 years). Of the 235 participants, 88 (37.4%) were new cases and 147 (62.6%) were previously treated cases. There was a higher rate of MDR-TB among patients with a prior history of TB treatment compared to persons never treated (65.6% vs. 48.8%, $P = 0.04$) [Table 1].

When DST was performed on participants, 81.7% (192/235) were MDR-TB, 92.3% (217/235) displayed any RFP resistance, and 10.6% (25/235) demonstrated monoresistant specimens to RFP. Furthermore, 83.4% (196/235) displayed any form of INH resistance, 1.7% (4/235) were INH monoresistant,

Table 1: Characteristics in patients with differing drug-susceptibility patterns

Characteristic	Number of patients	Drug-susceptibility pattern		χ^2	*P*
		MDR (%)	Non-MDR (%)		
Age (years)					
≤40	80	71 (37.0)	9 (20.9)	5.152	0.076
41–57	80	65 (33.9)	15 (34.9)		
≥58	75	56 (29.2)	19 (44.2)		
Gender					
Male	168	134 (69.8)	34 (79.1)	1.484	0.223
Female	67	58 (30.2)	9 (20.9)		
Treatment history					
New cases	88	66 (34.4)	22 (51.2)	4.227	0.040
Previously treated cases	147	126 (65.6)	21 (48.8)		
Occupation					
Farmer	139	111 (57.8)	28 (65.1)	0.776	0.378
Nonfarmer	96	81 (42.2)	15 (34.9)		
Alcohol use					
No	116	96 (50.0)	20 (46.5)	0.577	0.750
Occasionally	79	65 (33.9)	14 (32.6)		
Often	40	31 (16.1)	9 (20.9)		
Smoking status					
No	103	83 (43.2)	20 (46.5)	0.289	0.866
Previous smoker	96	80 (41.7)	16 (37.2)		
Yes	36	29 (15.1)	7 (16.3)		
Family contact					
Yes	14	13 (6.8)	1 (2.3)		0.476*
No	221	179 (93.2)	42 (97.7)		

MDR: Multidrug resistance. *Fisher's Exact Test.

50.6% (119/235) were any EMB resistant, and 22.1% (52/235) were EMB monoresistant. Among the 235 clinical isolates with a positive culture for MTB, 97.9% (230/235) displayed results to OFX and KM. 34.8% (80/230) isolates showed any OFX resistance, and 9.79% (23/230) isolates were OFX monoresistant. 7.8% (18/230) were any KM resistant and 2 (0.85) were KM monoresistant. Only 6.25% (12/192) of the MDR isolates were XDR.

Genetic mutations

In the GenoType MTBDR*plus* assay, RFP resistance was detected using probes from the *rpoB* gene. Among 74 RFP monoresistant isolates, 62.2% (46/74) had *rpoB* MUT3, 8.1% (6/74) had *rpoB* MUT1, and 4.1% (3/74) had *rpoB* MUT2A. All RFP monoresistant isolates had *rpoB* WT1 band present, 73 (98.6%) had WT2, WT5, and WT6 bands, 91.9% (68/74) had WT3 and WT4 band, 83.8% (64/74) had WT7 band, and 27.0% (20/74) had WT8 band. 54.9% (79/144) of MDR-TB isolates had *rpoB* MUT3, 11.8% (17/144) had *rpoB* MUT2A, 9.7% (14/144) had *rpoB* MUT2B, and 3.5% (5/144) had *rpoB* MUT1.

In the GenoType MTBDR*plus* assay, INH resistance was detected using probes of the *katG* and *inhA* genes. In the case of INH monoresistant isolates, the corresponding *katG* MUT1 was observed in 20% (1/5) of INH monoresistant isolates and in 66.7% (96/144) of MDR-TB isolates. The *katG* MUT2 was observed in 1.4% (2/144) of MDR-TB isolates. In the case of the *inhA* gene, the *inhA* MUT1 was observed in 80% (4/5) of INH monoresistant isolates and in 18.1% (26/144) of MDR-TB isolates [Table 2].

Among the 235 clinical isolates with a positive culture for MTB, 223 (94.9%) had a completely interpretable MTBDR*sl* assay. The distributions of genetic mutations of drug-resistant MTB isolates with an interpretable MTBDR*sl* assay are shown in Table 3. The predominant mutations of the GenoType MTBDR*sl* assay identified as conferring OFX resistance was MUT1 (44/77, 57.1%) followed by MUT3C (25/77, 32.5%). In addition, a similar proportion of isolates demonstrated a lack of binding to the *gyrA* WT3 (34/77, 44.2%) probe. All KM drug-resistant MTB isolates had an MUT1 mutation (15/15, 100%) and 53.3% (8/15) did not bind to the WT1 probe. EMB resistance was detected in 87 isolates of which the MUT1B gene was the most prevalent (50.6%, 44/87) followed by the MUT1A exchange in seven cases (36.8%, 32/87).

The distribution of gene mutations in the 31 OFX-monoresistant isolates identified by the GenoType MTBDR*sl* assay is shown in Table 3. The most prevalent mutation of OFX monoresistant was MUT1 (64.5%, 20/31). MUT1 (2/2) was the most prevalent mutation of KM-monoresistant isolates and for EMB-monoresistant isolates was missing WT1 (95.4%,42/44) followed by MUT1B (56.8%, 25/44). All XDR-TB isolates had *rrs* MUT1 mutation while 5 were missing *gryA* WT3 mutation and 4, 3, 2, and 2 had the *gryA*

Table 2: Band patterns of drug-resistant MTB isolates using the GenoType MTBDR*plus* assay

Gene	Band	Gene region/mutation	RFP monoresistant (*n* = 74), *n* (%)	INH monoresistant (*n* = 5), *n/N*	MDR-TB (*n* = 144), *n* (%)
rpoB	WT1	506–509	74 (100.0)	5/5	142 (98.6)
	WT2	510–513	73 (98.6)	5/5	137 (95.1)
	WT3	513–517	68 (91.9)	5/5	136 (94.4)
	WT4	516–519	68 (91.9)	5/5	140 (97.2)
	WT5	518–522	73 (98.6)	5/5	142 (98.6)
	WT6	521–525	73 (98.6)	5/5	141 (97.9)
	WT7	526–529	64 (83.8)	5/5	104 (72.2)
	WT8	530–533	20 (27.0)	5/5	54 (37.5)
	MUT1	D516 V	6 (8.1)	0	5 (3.5)
	MUT2A	H526 Y	3 (4.1)	0	17 (11.8)
	MUT2B	H526 D	0	0	14 (9.7)
	MUT3	S531 L	46 (62.2)	0	79 (54.9)
katG	WT	315	74 (100.0)	4/5	26 (18.1)
	MUT1	S315 T1	0	1/5	96 (66.7)
	MUT2	S315 T2	0	0	2 (1.4)
inhA	WT1	−15/−16	74 (100.0)	1/5	121 (84.0)
	WT2	−8	74 (100.0)	5/5	140 (97.2)
	MUT1	C15T	0	4/5	26 (18.1)
	MUT2	A16G	0	0	0
	MUT3A	T8C	0	0	0
	MUT3B	T8A	0	0	0

RFP: Rifampicin; INH: Isoniazid; MDR: Multidrug resistant; TB: Tuberculosis; MTB: *Mycobacterium tuberculosis*.

Table 3: Patterns of gene mutations in resistant MTB isolates using the GenoType MTBDR*sl* assay

Gene	Band	Gene region/ mutation	OFX resistant (*n* = 77), *n* (%)	OFX monoresistant (*n* = 31), *n* (%)	KM resistant (*n* = 15), *n/N*	KM monoresistant (*n* = 2), *n/N*	EMB resistant (*n* = 87), *n* (%)	EMB monoresistant (*n* = 44), *n* (%)	XDR-TB (*n* = 6), *n/N*
gyrA	WT1	85–90	77 (100.0)	31 (100.0)	15/15	2/2	87 (100.0)	44 (100.0)	6/6
	WT2	89–93	65 (84.4)	29 (93.5)	14/15	2/2	78 (89.7)	44 (100.0)	6/6
	WT3	92–97	43 (55.8)	19 (61.3)	8/15	2/2	67 (77.01)	44 (100.0)	1/6
	MUT1	A90V	44 (57.1)	20 (64.5)	6/15	0	21 (24.1)	0	3/6
	MUT2	S91P	1 (1.3)	1 (3.2)	6/15	0	0	0	0
	MUT3A	D94A	15 (19.5)	5 (1.61)	1/15	0	9 (10.3)	0	0
	MUT3B	D94N/Y	6 (7.8)	6 (1.94)	0	0	0	0	0
	MUT3C	D94G	25 (32.5)	11 (3.55)	5/15	0	12 (13.8)	0	4/6
	MUT3D	D94H	2 (2.6)	1 (3.2)	1/15	0	1 (1.1)	0	1/6
rrs	WT1	1401–1402	70 (90.9)	31 (100.0)	7/15	2/2	83 (95.4)	44 (100.0)	2/6
	WT2	1484	76 (98.7)	31 (100.0)	14/15	2/2	86 (98.9)	44 (100.0)	5/6
	MUT1	A1401G, C1402T	11 (14.3)	0	15/15	2/2	8 (9.2)	0	6/6
	MUT2	G1484T	0	0	0	0	0	0	0
embB	WT1	306	38 (49.4)	31 (100.0)	8/15	2/2	4 (4.6)	2 (4.5)	2/6
	MUT1A	M306I	19 (24.7)	0	5/15	0	32 (36.8)	11 (25.0)	2/6
	MUT1B	M306V	19 (24.7)	0	3/15	0	44 (50.6)	25 (56.8)	2/6

OFX: Ofloxacin; KM: Kanamycin; EMB: Ethambutol; XDR-TB: Extensively drug-resistant tuberculosis; MTB: *Mycobacterium tuberculosis*.

MUT3C, *gryA* MUT1, *embB* MUT1A, and *embB* MUT1B mutations, respectively.

Performance of GenoType MTBDR*plus* and GenoType MTBDR*sl* assays

Compared with the DST, the GenoType MTBDR*plus* assay had a sensitivity and specificity of 97.7% and 66.7% for detection of RFP resistance, 69.9% and 69.2% for INH resistance, and 69.8% and 76.8% for MDR-TB resistance, respectively. The GenoType MTBDR*sl* assay had a sensitivity and specificity of 90.9% and 95.2% for detection of OFX resistance, 77.8% and 99.5% for detection of KM resistance, 63.7% and 86.4% for detection of EMB resistance, and 46.2% and 100.0% for detection of XDR-TB resistance, respectively. The PPV ranged from 82.8% (EMB) to 100.0% (XDR-TB);

the NPV was lowest for INH (31.4%) and highest for XDR-TB (96.3%) [Table 4].

Discussion

In this study, we determined the diagnostic accuracy of the GenoType MTBDR*plus* and MTBDR*sl* assays to detect resistance to antituberculosis drugs in a setting with endemic tuberculosis drug resistance. With respect to culture isolates, the sensitivities of the MTBDR*plus* assay for the detection of RFP resistance were recently reported to be in the range of 95–99%.[17,18] This is in concordance with the high sensitivity of the MTBDR*plus* assay measured in our study (97.7%). In our study, the specificity for RFP (66.7%) and INH (69.2%) and the sensitivity for INH (69.9%) were much lower than other studies.[19,20] The sensitivity (69.8%) and specificity (76.8%) for the detection of MDR-TB in the present study were also lower than previous reports.[18]

More specifically, 95% of these RFP resistance-causing mutations are located within an 81 bp hotspot region of *rpoB* spanning codons 507–533, known as the RFP resistant determining region.[21] Mutations in codons 516, 526, and 531 of *rpoB* are most commonly associated with high-level RFP resistance.[20,22] Our results showed that the S531L mutation in *rpoB* was most frequent (125/218, 57.3%), followed by mutations in codon 526 (34/218, 15.6%). In 144 (66.1%) isolates resistant to RFP isolates, a missing WT8 band was observed. This correlates well with a recent study;[18] however, the observed distribution varies by geographic location.

Some authors cited the low sensitivity to detect INH resistance as a main limitation of the GenoType MTBDR*plus* assay.[23,24] Mutations that cause INH resistance are located in several genes and regions. Between 50% and 95% of INH-resistant isolates have been found to contain mutations in codon 315 of the *katG* gene[25,26] and an additional 10–15% of INH-resistant isolates had mutations in the *ahpC-oxyR* intergenic region.[26,27] In the study, a mutation at codon 315 of the gene *katG* was present in 66.4% of INH-resistant isolates.

Although the most common mutations predictive of drug resistance are well known for some antituberculosis drugs, these mutations are sometimes silent and are not always related to the acquisition of resistance. In addition, the exact ratio of resistant to susceptible bacilli that results in phenotypic resistance is unclear. This means that in practice, a molecular assay result can differ from the one obtained by a susceptibility proportion method.[20]

Table 4: Performance of GenoType MTBDR*plus* assay and GenoType MTBDR*sl* assay compared to phenotypic DST

Molecular methods	Phenotypic DST result						
	Resistant (*n*)	Susceptible (*n*)	Invalid (*n*)	Sensitivity, % (95% *CI*)	Specificity, % (95% *CI*)	PPV, % (95% *CI*)	NPV, % (95% *CI*)
RFP							
Resistant	212	6	0	97.7 (94.7–99.2)	66.7 (41.0–86.7)	97.2 (94.1–99.0)	70.6 (44.0–89.7)
Susceptible	5	12	0				
INH							
Resistant	137	12	0	69.9 (63.0–76.2)	69.2 (52.4–83.0)	91.9 (86.4–95.8)	31.4 (21.8–42.3)
Susceptible	59	27	0				
MDR							
Resistant	134	10	0	69.8 (62.8–76.2)	76.8 (61.4–88.2)	93.1 (87.6–96.6)	36.3 (26.4–47.0)
Susceptible	58	33	0				
OFX							
Resistant	70	7	0	90.9 (82.2–96.3)	95.2 (90.4–98.1)	90.9 (82.2–96.3)	95.2 (90.4–98.1)
Susceptible	7	139	0				
Invalid	3	4	5				
KM							
Resistant	14	1	0	77.8 (52.4–93.6)	99.5 (97.3–100.0)	93.3 (68.1–99.8)	98.1 (95.1–99.5)
Susceptible	4	204	0				
Invalid	0	7	5				
EMB							
Resistant	72	15	0	63.7 (54.1–72.6)	86.4 (78.5–92.2)	82.8 (73.2–90.0)	69.9 (61.4–77.4)
Susceptible	41	95	0				
Invalid	6	6	0				
XDR							
Resistant	6	0	0	46.2 (19.2–74.9)	100 (98.0–100.0)	100 (54.1–100.0)	96.3 (92.5–98.5)
Susceptible	7	181	29				
Invalid	0	7	5				

RFP: Rifampicin; INH: Isoniazid; MDR: Multidrug resistant; OFX: Ofloxacin; KM: Kanamycin; EMB: Ethambutol; XDR: Extensively drug resistant; *CI*: Confidence interval; DST: Drug-susceptibility testing; PPV: Positive predictive value; NPV: Negative predictive value.

A previous study displayed that the sensitivity of GenoType MTBDR*plus* for detection of MTB increased as the smear grade increased, reflecting an association between assay sensitivity and sputum bacillary burden.[28] Several studies have shown that the sensitivities and specificities of drug resistance detection in culture samples are slightly higher than for those conducted in sputum-positive samples.[18,29] Previous study showed that most invalid results were in sputum specimens with a lower bacillary load (1+) or culture-negative samples. More results were interpretable on sputum samples with higher bacillary load while in samples containing less bacillary load the performance of the assays was poorer.[28] The assays are also not useful in sputum specimens with lower bacillary load and paucibacillary extrapulmonary TB specimens. The sensitivity to detect INH resistance increased from 67.3% to 89.4% when most isolates were highly drug resistant.[19] Similarly, in Cavusoglu *et al*.,[20] sensitivity rose from 72.9% to 87.1% when only highly resistant isolates were tested. The low detection rate of INH resistance by the GenoType MTBDR*plus* method in the present study might be because this study population comprises a relatively high number of low-level INH resistance or that these isolates harbor resistant mutations at other *katG* gene regions or in other loci.

Heteroresistance has also been reported as an important factor potentially affecting the accuracy and reliability of DST results by line probe assays and impacting double patterns on GenoType MTBDR*plus* membranes.[30] We assume that heteroresistance is more likely to occur in high TB burden settings and in cultures isolated from chronic patients because these patients are more likely infected with various populations of mycobacteria.[31] Drug-susceptible isolates contaminated with resistant DNA isolates might also induce false-positive results.[32]

Previous studies have shown that the sensitivity of GenoType MTBDR*sl* assay to be between 75.6% and 90.6% for detecting fluoroquinolone resistance, 77–100% for detecting KM resistance, and 57–69.2% for detecting EMB resistance.[33-35] In the study, the GenoType MTBDR*sl* assay identified 90.9% of OFX-resistant isolates, 77.8% of KM-resistant isolates, and 63.7% of EMB-resistant isolates. We found that GenoType MTBDR*sl* assay had excellent accuracy for detecting phenotypic resistance to OFX, modest accuracy for detecting resistance to KM, but poor accuracy for detecting resistance to EMB, showed similar results to previous study.[36] We also found that GenoType MTBDR*sl* was specific for the diagnosis of XDR-TB, although there is room for improvement regarding sensitivity.

We observed that the most prevalent mutation was *gyrA* MUT1/A90V (44/77, 57.1%) followed by the *gyrA* MUT3C/D94G (25/77, 32.5%) mutation from OFX-resistant isolates conflicting with previous studies.[13,34] Furthermore, heteroresistant isolates might result from the coexistence of wild type and mutant alleles of the *gyrA* gene at the preliminary stage of full-drug resistance.[37] High rates of heteroresistance to fluoroquinolone-resistant isolates were found in the study (40.3%), higher than other studies reporting between 4.2% and 21.9%.[33,34,37]

Specifically, the A1401G mutation in the *rrs* gene is associated with resistance to KM and AM and in this present study the A1401G mutation appeared in all KM isolates with 99.5% specificity and 93.3% PPV using the GenoType MTBDR*sl* assay. The nucleotide changes in the region from positions 1400 to 1500 of the *rrs* gene indicated that the assay performs well in detecting the presence of these mutations. The sensitivity and specificity of the GenoType MTBDR*sl* assay was 77.8% and 99.5% for KM, respectively, similar to a previous study.[33] KM resistance may be caused by a mutation in other genes, such as the eis promoter region.[8]

We noticed that the predominant mutation was *embB* M306V (50.6%), which presented a close analogy to a Taipei study in which *embB*-M306V accounted for 59.3% EMB mutations[38] and another study which *embB*-M306V accounted for 60.0%.[36] This suggests that the significance of mutations in this codon is limited.

A recent meta-analysis by Cheng *et al*.[39] showed a similar sensitivity and specificity with the MTBDR*sl* assay for detecting EMB resistance (55% and 71%). This poor performance of the MTBDR*sl* assay is likely caused by the inherent difficulties in phenotypic DST for EMB and by the fact that only mutations at position 306 are screened with this assay. Given the poor performance of the MTBDR*sl* assay, this assay can be used neither for detecting nor for ruling out EMB resistance accurately and clinicians should await the results of phenotypic DST before deciding on changes in treatment regimens.

The Genotype MTBDR*plus* version 1.0 assay prompted a 21.6% increase in the direct detection of INH resistance due to the incorporation of the *inhA* gene conferring low-level INH resistance.[20,40] GenoType MTBDR*plus* version 1.0 assay has been limited for the use on smear-positive patient material.[41] GenoType MTBDR*sl* version 1.0 assay only targets selected mutations involving gyrA (fluoroquinolone) and *rrs* (second-line injectable drugs [SLID]) gene loci, mutations encoding resistance to fluoroquinolone, and SLID that occur outside these regions would be missed by the assay.[42] GenoType MTBDR*sl* version 2.0 assay is redesigned based on version 1.0 assay and accommodates additional mutations for the molecular detection of resistance to fluoroquinolone involving gyrA and gyrB and SLID resistance covering both *rrs* and *eis* genes.[43,44]

In conclusion, rapid diagnosis of MDR and XDR-TB is critically important for clinical and epidemiological reasons. These assays can inform clinicians about MTB resistance patterns of tuberculosis patients within 1 day. However, since discordance still exists between conventional and molecular approaches in resistance testing, we suggest including more target genes, such as the *gyrB* and *eis* genes, to improve the sensitivity of this assay and allow for more effective programmatic application. We recommend

that the GenoType assay might serve as an early guide for tuberculosis disease therapy until phenotypic DST results can be administered.

Financial support and sponsorship

This study was supported by a grant from the National Natural Science Foundation of China (No. 81302480).

Conflicts of interest

There are no conflicts of interest.

References

1. WHO. WHO Treatment Guidelines for Drug-Resistant Tuberculosis, 2016 Update. Geneva: WHO; 2016.
2. WHO. Global Tuberculosis Report 2016. Geneva: WHO; 2016.
3. Zhao Y, Xu S, Wang L, Chin DP, Wang S, Jiang G, *et al.* National survey of drug-resistant tuberculosis in China. N Engl J Med 2012;366:2161-70. doi: 10.1056/NEJMoa1108789.
4. Richter E, Rüsch-Gerdes S, Hillemann D. Drug-susceptibility testing in TB: Current status and future prospects. Expert Rev Respir Med 2014;3:497-510. doi: 10.1586/ers.09.45.
5. Sreeramareddy CT, Panduru KV, Menten J, Van den Ende J. Time delays in diagnosis of pulmonary tuberculosis: A systematic review of literature. BMC Infect Dis 2009;9:91. doi: 10.1186/1471-2334-9-91.
6. Yang CY, Guo HR, Cheng YY, Huang RM. Factors associated with misdiagnosis of smear-negative tuberculosis: An experience in Taiwan. Respir Care 2012;57:753-7. doi: 10.4187/respcare.01454.
7. WHO. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): Policy statement. Geneva, Switzerland: WHO; 2008. Available from: http://www.who.int/tb/dots/laboratory/lpa_policy. [Last accessed on 2017 Jan 08].
8. Huang WL, Chen HY, Kuo YM, Jou R. Performance assessment of the GenoType MTBDRplus test and DNA sequencing in detection of multidrug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol 2009;47:2520-4. doi: 10.1128/JCM.02499-08.
9. Feng Y, Liu S, Wang Q, Wang L, Tang S, Wang J, *et al.* Rapid diagnosis of drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol using genotype MTBDRsl assay: A meta-analysis. PLoS One 2013;8:e55292. doi: 10.1371/journal.pone.0055292.
10. Bai Y, Wang Y, Shao C, Hao Y, Jin Y. GenoType MTBDRplus assay for rapid detection of multidrug resistance in *Mycobacterium tuberculosis*: A meta-analysis. PLoS One 2016;11:e0150321. doi: 10.1371/journal.pone.0150321.
11. Mao X, Ke Z, Shi X, Liu S, Tang B, Wang J, *et al*. Diagnosis of drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol with genotype MTBDRsl assay: A meta-analysis. Ann Clin Lab Sci 2015;45:533-44.
12. Lu W, Feng Y, Wang J, Zhu L. Evaluation of MTBDRplus and MTBDRsl in detecting drug-resistant tuberculosis in a Chinese population. Dis Markers 2016;2016:2064765. doi: 10.1155/2016/2064765.
13. Zeng X, Jing W, Zhang Y, Duan H, Lu Y, Dong Q, *et al.* Performance of the MTBDRsl line probe assay for rapid detection of resistance to second-line anti-tuberculosis drugs and ethambutol in China. Diagn Microbiol Infect Dis 2016. pii: S0732-889330171-7. doi: 10.1016/j.diagmicrobio.2016.06.011.
14. Shao Y, Yang D, Xu W, Lu W, Song H, Dai Y, *et al.* Epidemiology of anti-tuberculosis drug resistance in a Chinese population: Current situation and challenges ahead. BMC Public Health 2011;11:110. doi: 10.1186/1471-2458-11-110.
15. Dou HY, Tseng FC, Lin CW, Chang JR, Sun JR, Tsai WS, *et al.* Molecular epidemiology and evolutionary genetics of *Mycobacterium tuberculosis* in Taipei. BMC Infect Dis 2008;8:170. doi: 10.1186/1471-2334-8-170.
16. Qiao L, Yang D, Xu W, Wang J, Bing LV, Yan S, *et al*. Molecular typing of *Mycobacterium tuberculosis* isolates circulating in Jiangsu Province, China. BMC Infect Dis 2011;11:1-10. doi: 10.1186/1471-2334-11-288.
17. Asante-Poku A, Otchere ID, Danso E, Mensah DD, Bonsu F, Gagneux S, *et al.* Evaluation of GenoType MTBDRplus for the rapid detection of drug-resistant tuberculosis in Ghana. Int J Tuberc Lung Dis 2015;19:954-9. doi: 10.5588/ijtld.14.0864.
18. Yadav RN, Singh BK, Sharma SK, Sharma R, Soneja M, Sreenivas V, *et al*. Comparative evaluation of GenoType MTBDR plus line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. PLoS One 2013;8:e72036. doi: 10.1371/journal.pone.0072036.
19. Lacoma A, Garcia-Sierra N, Prat C, Ruiz-Manzano J, Haba L, Rosés S, *et al.* GenoType MTBDRplus assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* strains and clinical samples. J Clin Microbiol 2008;46:3660-7. doi: 10.1128/JCM.00618-08.
20. Cavusoglu C, Turhan A, Akinci P, Soyler I. Evaluation of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* isolates. J Clin Microbiol 2006;44:2338-42.
21. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis 2010;202:3-29.
22. Rigouts L, Nolasco O, de Rijk P, Nduwamahoro E, Van Deun A, Ramsay A, *et al*. Newly developed primers for comprehensive amplification of the rpoB gene and detection of rifampin resistance in *Mycobacterium tuberculosis*. J Clin Microbiol 2007;45:252-4.
23. Akpaka PE, Baboolal S, Clarke D, Francis L, Rastogi N. Evaluation of methods for rapid detection of resistance to isoniazid and rifampin in *Mycobacterium tuberculosis* isolates collected in the Caribbean. J Clin Microbiol 2008;46:3426-8. doi: 10.1128/JCM.01455-08.
24. Bazira J, Asiimwe BB, Joloba ML, Bwanga F, Matee MI. Use of the GenoType (R) MTBDRplus assay to assess drug resistance of *Mycobacterium tuberculosis* isolates from patients in rural Uganda. BMC Clin Pathol 2010;10:5. doi: 10.1186/1472-6890-10-5.
25. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of KatG Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. Antimicrob Agents Chemother 2002;46:1417-24.
26. Telenti A, Honore N, Bernasconi C, March J, Ortega A, Heym B, *et al*. Genotypic assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: A blind study at reference laboratory level. J Clin Microbiol 1997;35:719.
27. Piatek AS, Telenti A, Murray MR, El-Hajj H, Jacobs WR Jr., Kramer FR, *et al*. Genotypic analysis of *Mycobacterium tuberculosis* in two distinct populations using molecular beacons: Implications for rapid susceptibility testing. Antimicrob Agents Chemother 2000;44:103-10.
28. Dorman SE, Chihota VN, Lewis JJ, van der Meulen M, Mathema B, Beylis N, *et al.* Genotype MTBDRplus for direct detection of *Mycobacterium tuberculosis* and drug resistance in strains from gold miners in South Africa. J Clin Microbiol 2012;50:1189-94. doi: 10.1128/JCM.05723-11.
29. Mironova S, Pimkina E, Kontsevaya I, Nikolayevskyy V, Balabanova Y, Skenders G, *et al.* Performance of the GenoType® MTBDRPlus assay in routine settings: A multicenter study. Eur J Clin Microbiol Infect Dis 2012;31:1381-7. doi: 10.1007/s10096-011-1453-1.
30. Miotto P, Piana F, Cirillo DM, Migliori GB. Genotype MTBDRplus: A further step toward rapid identification of drug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol 2008;46:393-4. doi: 10.1128/JCM.01066-07.
31. Baldeviano-Vidalón GC, Quispe-Torres N, Bonilla-Asalde C, Gastiaburú-Rodriguez D, Pro-Cuba JE, Llanos-Zavalaga F. Multiple infection with resistant and sensitive *M. tuberculosis* strains during treatment of pulmonary tuberculosis patients. Int J Tuberc Lung Dis 2005;9:1155-60.
32. Chen C, Kong W, Zhu L, Zhou Y, Peng H, Shao Y, *et al.* Evaluation of the GenoType® MTBDRplus line probe assay on sputum-positive samples in routine settings in China. Int J Tuberc Lung Dis 2014;18:1034-9. doi: 10.5588/ijtld.13.0857.
33. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection

by GenoType MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant *Mycobacterium tuberculosis* complex isolates. J Clin Microbiol 2010;48:1683-9. doi: 10.1128/JCM.01947-09.
34. Hillemann D, Rüschgerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol 2009;47:567-72. doi: 10.1128/JCM.00081-09.
35. Kiet VS, Lan NT, An DD, Dung NH, Hoa DV, van Vinh Chau N, *et al.* Evaluation of the MTBDRsl test for detection of second-line-drug resistance in *Mycobacterium tuberculosis*. J Clin Microbiol 2010;48:2934-9. doi: 10.1128/JCM.00201-10.
36. Simons SO, van der Laan T, de Zwaan R, Kamst M, van Ingen J, Dekhuijzen PN, *et al.* Molecular drug susceptibility testing in the Netherlands: Performance of the MTBDRplus and MTBDRsl assays. Int J Tuberc Lung Dis 2015;19:828-33. doi: 10.5588/ijtld.15.0043.
37. Mokrousov I, Otten T, Manicheva O, Potapova Y, Vishnevsky B, Narvskaya O, *et al.* Molecular characterization of ofloxacin-resistant *Mycobacterium tuberculosis* strains from Russia. Antimicrob Agents Chemother 2008;52:2937-9. doi: 10.1128/AAC.00036-08.
38. Huang WL, Chi TL, Wu MH, Jou R. Performance assessment of the GenoType MTBDRsl test and DNA sequencing for detection of second-line and ethambutol drug resistance among patients infected with multidrug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol 2011;49:2502-8. doi: 10.1128/JCM.00197-11.
39. Cheng S, Cui Z, Li Y, Hu Z. Diagnostic accuracy of a molecular drug susceptibility testing method for the antituberculosis drug ethambutol: A systematic review and meta-analysis. J Clin Microbiol 2014;52:2913-24. doi: 10.1128/JCM.00560-14.
40. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: A meta-analysis. Eur Respir J 2008;32:1165. doi: 10.1183/09031936.00061808.
41. Matabane MM, Ismail F, Strydom KA, Onwuegbuna O, Omar SV, Ismail N. Performance evaluation of three commercial molecular assays for the detection of *Mycobacterium tuberculosis* from clinical specimens in a high TB-HIV-burden setting. BMC Infect Dis 2015;15:1-7. doi: 10.1186/s12879-015-1229-9.
42. Theron G, Peter J, Richardson M, Warren R, Dheda K, Steingart KR. GenoType® MTBDRsl assay for resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev. 2016;9:CD01070510.1002/14651858.CD010705.pub3.
43. Gardee Y, Dreyer AW, Koornhof HJ, Omar SV, da Silva P, Bhyat Z, *et al.* Evaluation of the GenoType MTBDRsl Version 2.0 assay for second-line drug resistance detection of *Mycobacterium tuberculosis* isolates in South Africa. J Clin Microbiol 2017;55:791-800. doi: 10.1128/JCM.01865-16.
44. Ajileye A, Alvarez N, Merker M, Walker TM, Akter S, Brown K, *et al.* Some synonymous and nonsynonymous gyrA mutations in *Mycobacterium tuberculosis* lead to systematic false-positive fluoroquinolone resistance results with the Hain GenoType MTBDRsl assays. Antimicrob Agents Chemother 2017;61. pii: E02169-16. doi: 10.1128/AAC.02169-16.