

# Molecular tests for rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis*

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## KEY MESSAGES

1. In patients suspected to have tuberculosis, a valid molecular test result was obtained in 94.3% and 88.3% of patients for the *rpoB* and *katG* loci, respectively, for prediction of drug susceptibility.
2. Molecular testing based on *katG* and *rpoB* enabled rapid detection of *Mycobacterium tuberculosis* resistance in 46.8% and 87.5% of patients infected with isoniazid- and rifampicin-resistant strains, respectively. The same number of patients infected with resistant strains with mutations in *katG* and *rpoB* were detected using the MTBDRplus line probe assay. The combined use of *katG* and *inhA* regulatory region in the MTBDRplus line probe assay increased the proportion of patients identified with INH-resistant MTB strains to 72.3%.

3. The rapid tests improved patient management by enabling earlier admission, more timely use of World Health Organization category IV anti-tuberculosis regimens and discontinuation of ineffective drugs.

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## Introduction

Multidrug-resistant tuberculosis (MDR-TB) often entails prolonged treatment and the use of more toxic drugs. It is also associated with higher rates of treatment failure and death. The World Health Organization category I regimens for the management of MDR-TB are inadequate. In British Medical Research Council studies, only three of the 11 patients with MDR-TB were cured when prescribed standard treatment. Early recognition of resistance and administration of two or more drugs have improved patient outcome. It is important to reduce the turn-around time for reporting anti-tuberculosis drug susceptibility. Cultures are the gold standard for susceptibility testing, but they are limited by the pre-requisite for a pure growth from clinical specimens. As the mechanisms for rifampicin (RIF) and isoniazid (INH) resistance in many isolates of *Mycobacterium tuberculosis* (MTB) are predictable, molecular methods are used for direct detection of RIF and INH resistance. This study evaluated genotypic tests to detect RIF and INH resistance in MTB in clinical specimens. The potential impact of implementing molecular tests to detect RIF and INH resistance in MTB was also assessed.

## Methods

This study was conducted from April 2006 to June

2009. Patients were prospectively identified from 15 participating centres at the tuberculosis and chest unit of the Department of Health, the Grantham Hospital, the Kowloon Hospital, and the Haven of Hope Hospital. Adult patients suspected to have active tuberculosis and were at risk of infection with INH- and/or RIF-resistant strains were enrolled. Clinical specimens (sputum or other appropriate samples) were tested by polymerase chain reaction (PCR) assays specific for IS6110, *rpoB*, and *katG*. The amplicons were sequenced to detect resistance mutations. PCR and sequencing were conducted on a weekly basis. Specimens with resistant strains were also tested by the MTBDRplus and the INNO-LiPA Rif.TB line probe assays. Acid-fast bacillus smear and mycobacterial culture were performed by conventional methods.

## Results

### Patient demographics and sensitivity of the PCR assays

A total of 820 specimens (>90% respiratory) were obtained from 720 patients. In 596 (82.7%) patients, the culture was positive for MTB. Using the culture and susceptibility testing result as reference, the overall sensitivity of the IS6110, *rpoB*, and *katG* PCR assays was 98.9%, 95.9%, and 88.7%, respectively (Table 1). 94.3% (562/596) and 88.3% (526/596) of

patients had valid molecular test results for the *rpoB* and *katG* loci, respectively.

Susceptibility testing revealed that 50 patients were infected with strains resistant to INH and/or RIF, including MDR-TB (INH-R/RIF-R) [n=13], INH-R/RIF-S (n=34), and INH-S/RIF-R (n=3). The remaining 542 MTB isolates were susceptible to both INH and RIF.

### Correlation between susceptibility testing and *rpoB* mutations

Among 562 valid molecular *rpoB* test results, the concordance rate with susceptibility testing was 99.5% (559/562). Mutations were found in codons 531, 533, 526, and 516 within the *rpoB* hotspot region. The discordant results involved three patients with RIF-susceptible isolates and one of two mutations (L533P and H526N). All *rpoB* mutations, including the three discordant results, were confirmed by testing the purified isolates. Fourteen (87.5%) of 16 patients infected with RIF-resistant strains were detected by the PCR-sequencing method. The detection rate for *rpoB* mutation was the same using the GenoType MTBDRplus test.

Among 527 valid molecular *katG* test results, the concordance rate with susceptibility testing was 97.2% (512/527). Our *katG* PCR assay could

rapidly detect resistance in only 22 (46.8%) of the 47 patients infected with INH-resistant strains. The same number (ie 22) of *katG* mutations was detected using the GenoType MTBDRplus test, which also detected an additional 12 patients with mutations in the promoter region of *inhA*. Therefore, INH resistance was correctly predicted by the GenoType MTBDRplus test in 34 (72.3%) of the 47 patients.

### Impact on patient management

Overall, 50 patients had a positive culture for MTB isolates resistant to INH and/or RIF. Susceptibility testing revealed that 34 isolates were resistant to INH alone, three to RIF alone, and 13 to both INH and RIF (ie MDR-TB). 92% and 80% of the 50 patients had a valid result for *rpoB* and *katG* mutations, respectively. The genotypic results according to PCR-sequencing correctly predicted *rpoB* resistance mutations in 15 specimens and *katG* resistance mutations in 22 specimens. These mutations involved 32 patients and the correlation with the susceptibility result is summarised in Table 2. The mean±standard deviation (SD) turnaround time was 10.2±5 days for *rpoB* and 9.5±4.8 days for *katG*. This was significantly shorter than the interval between first sputum specimen and susceptibility test reporting (15.4±5.4 weeks). This enables earlier

TABLE 1. Comparative sensitivity of three polymerase chain reaction (PCR) assays for detection of *Mycobacterium tuberculosis* (MTB)

Parameter	No. (%) of patients								
	Positive results			<i>rpoB</i> mutation			<i>katG</i> Thr315		
	IS6110 PCR	<i>rpoB</i> PCR	<i>katG</i> PCR	Present	Absent	Indeterminate	Present	Absent	Indeterminate
MTB culture-positive (n=596)	590 (98.9)	572 (95.9)	529 (88.8)	17	545	10	21	505	3
Smear-positive (n=538)	538 (100)	532 (98.8)	503 (93.5)	16	512	4	19	482	2
Smear-negative (n=58)	52 (89.6)	40 (68.9)	26 (44.8)	1	33	6	2	23	1
MTB culture-negative (n=124)*	66	54	41	0	53	1	0	41	0
<b>Total</b>	<b>656 (91.1)</b>	<b>626 (86.9)</b>	<b>570 (79.2)</b>	<b>17</b>	<b>598</b>	<b>11</b>	<b>21</b>	<b>546</b>	<b>3</b>

\* In 13 patients, the culture was positive for non-tuberculous mycobacteria

TABLE 2. Correlation between polymerase chain reaction–sequencing for *rpoB* and *katG* mutations and susceptibility testing in 32 patients infected with *Mycobacterium tuberculosis* resistant to isoniazid and/or rifampicin

Genotypic result obtained by testing of respiratory specimens		No. of phenotypic result obtained by susceptibility testing		
<i>rpoB</i>	<i>katG</i>	Isoniazid-resistant (n=17)	Rifampicin-resistant (n=3)	Multidrug-resistant (n=12)
Mutated	Mutated	-	-	4
Mutated	Wild type	-	3	4
Indeterminate	Mutated	-	-	1
Mutated	Indeterminate	-	-	3
Wild type	Mutated	17	-	-

TABLE 3. Potential impact of molecular testing for *Mycobacterium tuberculosis* resistance mutations on patient management for seven representative cases

Case No.	Sex/age (years)	History of tuberculosis	Risk factors for resistant tuberculosis	Genotypic test on sputum				Anti-tuberculosis treatment		Remarks
				IS6110	rpoB	katG	gyrA	At testing	After testing	
26	M/30	Yes	Multiple treatment in China	Positive	S531L	S315T	Mutated	None	Category IV	Multidrug-resistant tuberculosis (MDR-TB); individualised category IV treatment started 1 week after patient presentation
11	M/66	No	Persistent smear positivity	Positive	WT	S315T	-	Category I	Stop isoniazid	Test result contributed to individualised regimen decision
14	F/48	Yes	Previous treatment in China	Positive	D516A	S315N	-	Category II	Category IV	MDR-TB; test result contributed to individualised regimen decision
28	F/50	No	-	Positive	S531L	WT	-	Category I	Category IV	Polydrug resistant tuberculosis; test result contributed to individualised regimen decision
29	M/30	No	Extensive disease	Positive	S531L	Indeterminate	-	Category I	Category IV	MDR-TB; patient admitted for individualised treatment because of test result
45	F/37	Yes	Failed category I regimen	Positive	S531L	WT	Mutated	Category IV	Category IV	Extensively drug-resistant tuberculosis; test result contributed to individualised regimen decision
50	M/70	Yes	Failed category I regimen	Positive	D516V	Indeterminate	-	Category I	Category IV	MDR-TB; culture and sensitivity testing was delayed because of mixed growth of <i>M tuberculosis</i> and <i>M gordonae</i> ; test result contributed to individualised regimen decision

patient isolation, admission for treatment, and discontinuation of inactive drugs (Table 3).

## Discussion

In Hong Kong, diagnostic PCR is useful to detect MTB in clinical specimens.<sup>1</sup> This study extended this by inclusion of two PCR assays specific for rpoB and katG genes to determine whether there were mutations associated with resistance to RIF and INH. Using culture and drug susceptibility results as reference, 94.3% and 88.3% of patients had valid molecular test results for the rpoB and katG loci, respectively. The result for both rpoB and katG was highly concordant with the conventional susceptibility result.<sup>2</sup>

As RIF and INH resistance could occur as a result of mutations in multiple gene loci, the specificity of molecular assays (as compared with susceptibility testing) would not be 100%. For RIF, a high degree of concordance between mutations in the rpoB hotspot region (codon 507-533) and phenotypic resistance was found. A rapid diagnosis was made for 87.5% of patients infected with RIF-resistant strains, as more than 96% of RIF-resistant strains have mutations in this region.<sup>3</sup> In another three patients infected with phenotypic RIF-susceptible strains, the rapid test identified rpoB mutations (L533P or H526N), which were uncommon. The molecular assay results were reliable because identical mutations were confirmed by testing the isolates. These infrequent

rpoB mutations may cause weak resistance to RIF and have the potential to cause highly discordant RIF susceptibility results, even when tested under proficiency evaluation settings by the Supra-National Tuberculosis Reference Laboratories.<sup>4</sup> The MTBDRplus insert also indicated that the L533P mutation may be RIF-susceptible in susceptibility testing. Nonetheless, both L522P and H526N have been reported to cause high-level RIF resistance and to occur in MDR-TB strains.<sup>5</sup> Further studies to assess the clinical significance of these low-level resistant isolates are necessary.

Unlike RIF resistance, the katG PCR assay could provide rapid diagnosis in only 22 (46.8%) of the 47 patients infected with INH-resistant strains. The same number of katG mutations was detected by the MTBDRplus assay. The inhA promoter region was included in the MTBDRplus assay as an additional target. This enabled detection of an additional 12 patients with INH-resistant infection. Thus, the MTBDRplus assay could rapidly detect INH resistance in 34 (72.3%) of the 47 patients. 38 of the 47 INH-resistant isolates were found to have mutations in the katG gene and/or the inhA regulatory region. Thus, even if the PCR assay was 100% sensitive in detecting mutations in katG and the promoter of inhA in clinical specimens, direct testing would at best identify 80.9% (38/47) of INH-resistant infections. To improve the sensitivity further, it is necessary to include other gene target in molecular assays. A tuberculosis drug resistance

database has been established.<sup>5</sup> The INH-resistant strains with wild type *katG* and promoter region in *inhA* may have mutations in the *katG* outside the fragment that was sequenced, within the *inhA*, intergenic region of *oxyR-ahpC* or other genes.

## Conclusions

The molecular test result is valid in most patients with suspected and confirmed tuberculosis. The test result for *rpoB* and *katG* is reliable and highly concordant with results using culture isolates. Molecular testing enables better management of patients infected with drug-resistant tuberculosis.

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