**Performance of the Genotype MTBDR assay for molecular detection of multidrug-resistant strains of Mycobacterium tuberculosis**

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**METHODS**

Thirty-five MDR (MDR1 to MDR35) and 20 pansusceptible (S1 to S20) *M. tuberculosis* strains isolated in Kuwait were used. The *rpoB* mutations in some MDR-TB strains were previously detected by another strip (INNO-LiPA Rif. TB, Innogenetics, Ghent, Belgium) assay. Isolation, identification and DST of *M. tuberculosis* isolates were performed as described previously. The isolates were defined as MDR-TB strains when bacterial growth occurred in the presence of 2 mg/L RMP and 0.1 mg/L INH, added separately. Resistance to ethambutol (EMB, 2.5 mg/L) and streptomycin (SM, 2 mg/L) was also determined (Table 1).

The *M. tuberculosis* reference strain H37Rv was used as a control in the Genotype MTBDR assay (Hain Life Sciences, Nehren, Germany). Genomic DNA from *M. tuberculosis* H37Rv and BACTEC cultures of MDR and pansusceptible *M. tuberculosis* isolates was prepared as described previously. The Genotype MTBDR assay was performed as recommended by the manufacturer. Briefly, multiplex PCR was performed with extracted DNA and the biotinylated amplicons were hybridized with oligonucleotides immobilized on nitrocellulose strips. Hybridized amplicons were detected by the addition of streptavidin-alkaline phosphatase conjugate followed by color development with a chromogenic substrate. Assay results were interpreted according to manufacturer’s instructions and were confirmed by DNA sequencing. The sequencing of the *rpoB* hot-spot region and the *katG315* DNA region was performed as described previously. The sensitivity of detecting RMP-resistant, INH-resistant and MDR-TB strains by the Genotype MTBDR assay was calculated using phenotypic DST as the gold standard.

**RESULTS**

The multiplex PCR yielded expected amplicons from all 35 MDR and 20 pansusceptible *M. tuberculosis* strains isolated in Kuwait. The results were compared to conventional drug susceptibility testing (DST) performed on each isolate.
strains (data not shown). The amplicons were hybridized to probes (organized on membrane strips as shown in Figure 1, lane reference) under controlled conditions. The UC and Tub probes as well as rpoB and katG probes were positive for all pansusceptible and MDR M. tuberculosis strains, as expected. All pansusceptible strains (banding patterns for S1 and S5 are shown in Figure 1, lanes 1 and 11) reacted with all five rpoB wildtype (WT1 to WT5) and katG WT probes but not with any rpoB and katG mutant probes, as expected.

The 35 MDR-TB strains exhibited 11 different hybridization patterns (Table 1). RMP resistance was detected in 33 of 35 (94%) isolates by lack of reaction with an rpoB WT probe and with 28 of 33 (85%) isolates also reacting with an rpoB MUT (mutant) probe (banding patterns for selected isolates are shown in Figure 1, lanes 2-10). Two isolates reacted with all rpoB WT and none of the rpoB MUT probes (RMP susceptible pattern, Table 1). The majority (21 of 35, 60%) of MDR-TB strains reacted with rpoB MUT3 (S531L) with concomitant lack of hybridization with rpoB WT5 probe (Table 1). DNA sequencing of the rpoB gene confirmed the strip results for 34 of 35 MDR-TB strains. One isolate (MDR13) identified as RMP susceptible by the Genotype MTBDR assay contained an insertion mutation at rpoB codon 514 (insertion 514TCT).

As for INH resistance, a mutation at katG315 was detected in 22 of 35 (63%) MDR-TB strains by absence of a signal with a katG WT probe (Table 1) with 21 of 22 isolates also reacting with a katG T1 (S315T) probe (as shown in Figure 1, lane 3, 5, 7, 8 or 10). DNA sequencing of the katG315 DNA region confirmed these results and also identified the AGC315AAC (S315N) mutation in one isolate (MDR31) that could not be

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**Table 1.** Genotype MTBDR assay results for detecting RMP and INH resistance in 20 pansusceptible (S) and 35 multidrug-resistant (MDR) M. tuberculosis isolates and comparison with conventional drug susceptibility data.

| M. tuberculosis strains | Susceptibility to RMP and INH | Other resistance | Genotype MTBDR assay patterns for rpoB hot-spot region | Genotype MTBDR assay patterns for katG315 |
|------------------------|-------------------------------|------------------|-------------------------------------------------------|----------------------------------------|
| H37Rv                  | Sensitive None                 | WT (susceptible) | WT (susceptible)                                      |
| S1-S20                 | Sensitive None                 | WT (susceptible) | WT (susceptible)                                      |
| MDR1,2                 | Resistant EMB                  | WT5,MUT3,S531L   | ΔWT1, S315T                                           |
| MDR3,5,14,27           | Resistant EMB, SM              | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR4                   | Resistant EMB                  | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR6,26,35             | Resistant EMB, SM              | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR7,9,19,20,32        | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR10,24,29            | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR11                  | Resistant SM                   | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR12                  | Resistant EMB, SM              | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR13                  | Resistant EMB, SM              | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR15,23,25            | Resistant EMB, SM              | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR16                  | Resistant EMB                  | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR17                  | Resistant SM                   | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR18                  | Resistant SM                   | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR21,22               | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR23                  | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR28                  | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR30                  | Resistant EMB                  | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR31                  | Resistant EMB                  | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR33                  | Resistant None                 | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |
| MDR34                  | Resistant EMB                  | ΔWT5,MUT3,S531L  | ΔWT1, S315T                                           |

RMP, rifampin; INH, isoniazid; EMB, ethambutol; SM, streptomycin; WT, wild-type; ΔWT, absence of hybridization with a wild-type probe.
specifically detected by katG mutant probes (Figure 1, lane 9).

**DISCUSSION**

The conventional DST of *M. tuberculosis* isolates is time consuming. A simple strip assay (INNO-LiPA Rif. TB) was developed in late 1990s for rapid detection of *M. tuberculosis* isolates resistant to RMP, a surrogate marker for MDR-TB. However, detection of RMP-resistant *M. tuberculosis* isolates may not reflect infection with MDR-TB strains as monoresistance to RMP develops frequently during HIV infection and some other underlying conditions.

The Genotype MTBDR strip assay combines probes targeting the rpoB hot-spot region with katG315 probes for simultaneous detection of the majority of *M. tuberculosis* isolates resistant to both RMP and INH. Although the Genotype MTBDR assay may be directly applied to smear-positive respiratory specimens, it is not economical for countries with a high incidence of extrapulmonary TB and low rates of MDR-TB such as Kuwait. Application of the test on isolates from suspected patients is more practical.

All of the 20 pansusceptible *M. tuberculosis* strains were correctly identified as susceptible to RMP and INH. Resistance to RMP was correctly identified in 33 of 35 (94%) MDR-TB strains by the Genotype MTBDR assay (Table 1). The DNA sequencing data confirmed the results and specific mutations identified by the strip assay. The S531L mutation was most common (21 of 35, 60%). A high frequency of S531L mutation in the rpoB gene in MDR-TB strains has also been reported from several other countries.

Two isolates (MDR13 and MDR30) were identified as RMP susceptible. DNA sequencing identified insertion of the 514TTC mutation in the rpoB gene in one isolate (MDR13). Another strip assay (INNO-LiPA Rif. TB) also failed to detect *M. tuberculosis* isolates containing the insertion 514TTC mutation as RMP-resistant. Thus, the rates of concordance of the Genotype MTBDR assay results for detecting RMP resistance with conventional DST and rpoB gene sequencing were 94% and 97%, respectively. One isolate (MDR30) contained a wild-type sequence in the rpoB hot-spot region. The molecular basis of resistance in this isolate either involves mutations in other regions of the rpoB gene or in other genes mediating resistance of *M. tuberculosis* to RMP.

The Genotype MTBDR assay identified resistance to both RMP and INH in 20 of 35 (57%) MDR-TB strains. This is mainly because the assay identified INH resistance in only 22 of 35 (63%) MDR-TB strains. Nearly all the isolates with the katG315 mutation (21 of 22, 95%) were detected by katG T1 (S315T) probe. One isolate (MDR31) was detected as INH-resistant by lack of reaction with the katG WT probe only. This isolate contained the AGC315AAC mutation at katG315 and no specific probe is present on the strip for detecting this mutation.

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**Figure 1.** Representative Genotype MTBDR strip patterns obtained with pansusceptible and MDR *M. tuberculosis* isolates. The positions of oligonucleotide probes on the strip, their specificity and the targeted genes are shown (from top to bottom) on the left side (Reference lane) as follows: CC, conjugate control (for test of kit components); UC, amplification control for high G+C gram-positive bacteria; Tub, *M. tuberculosis* complex-specific control; rpoB, control for rpoB amplification; WT1 to WT5, controls for presence of wild-type sequences in 81-bp hot-spot region of rpoB gene; MUT1, MUT2A, MUT2B and MUT3, probes for D516V (GAC516GTC), H526Y (CAC526YTC), H526D (CAC526GAC) and S531L (TCG531TTC) mutations, respectively, at the three most frequently mutated (S16, 526 and 531) rpoB codons; katG, control for katG gene amplification; WT, control for presence of wild-type sequence at katG315. T1 and T2, probes for S315T (AGC315ACC) and S315T (AGC315ACA) mutation, respectively, at katG315. Near the bottom is a marker line (marked by horizontal M) for alignment of strips with the key (reference lane) provided with the kit. Representative patterns on strips from pansusceptible (S1, lane 1 and S2, lane 11) and MDR (MDR1, lane 2, rpoB D516V and katG S315; MDR3, lane 3, rpoB S531L and katG S315T; MDR4, lane 4, rpoB D513K and katG S315; MDR6, lane 5, rpoB D516V and katG S315T; MDR10, lane 6, rpoB S531L and katG S315; MDR12, lane 7, rpoB H526D and katG S315T; MDR13, lane 8, rpoB insertion 514TTC and katG S315T; MDR31, lane 9, rpoB D513K and katG S315N and MDR33, lane 10, rpoB H526R and katG S315T) *M. tuberculosis* isolates are shown on the right.
The low (63%) sensitivity of the Genotype MTBDR assay for detection of INH resistance is a limitation of this assay. It is likely due to the fact that the assay targets only katG315 mutations while INH resistance in M. tuberculosis strains could also involve mutations in other katG gene regions or in other loci. For example, mutations in the inhA regulatory region occur in 15% to 35% of INH-resistant M. tuberculosis strains from some geographical locations. However, mutations in the inhA regulatory region cause low-level of resistance to INH and their detection may have little impact on INH therapy. Since mutations at katG315 in M. tuberculosis strains cause a high-level of resistance to INH, their detection by the Genotype MTBDR assay is valuable in clinical settings and will have highest impact on INH therapy.

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