brief report

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

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Tuberculosis (TB) is an infectious disease of global impact, killing nearly two million people every year.[1] Efforts to control TB are hampered by expanding human immunodeficiency virus (HIV) infection and its association with active disease and increasing resistance of *Mycobacterium tuberculosis* strains to most effective (first-line) anti-TB drugs.[1] New cases that are resistant to both rifampin (RMP) and isoniazid (INH) (defined as multidrug-resistant TB, MDR-TB) exemplify the problem of drug-resistant TB in a given country since fatality rates for MDR-TB are much higher.[2] Rapid identification of MDR-TB strains is crucial for starting of effective chemotherapy and for initiation of infection control measures.

The resistance of *M tuberculosis* strains to anti-TB drugs develops due to mutations in resistance-conferring genes and MDR-TB strains evolve due to sequential accumulation of these mutations.[3] Mutations in the 81-base pair (bp) (hot-spot) region of the *rpoB* gene, encoding the β-subunit of RNA polymerase, occur in 90% to 95% of RMP-resistant strains.[3-6] In contrast, mutations in several regions of multiple genes can cause INH resistance.[3,7] However, mutations in the *kat*G gene, encoding catalase-peroxidase, occur more frequently and 50% to 95% of INH-resistant strains worldwide contain mutations at the *kat*G codon 315 (*katG315*).[3,7-9] Based on this knowledge, a DNA strip (Genotype MTBDR) assay has been developed for simultaneous detection of resistance of *M tuberculosis* to RMP and INH.[10-12] This PCR-based assay involves hybridization with oligonucleotide probes to detect either wild-type sequences or specific mutations. In this study, Genotype MTBDR assay was evaluated by using 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.

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.[13] Isolation, identification and DST of *M tuberculosis* isolates were performed as described previously.[14,15] 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.[16] 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.[9,13] 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 (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* wild-type (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 514TTC).

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

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	*katG315*
H37Rv	Sensitive	None	WT (susceptible)	WT (susceptible)
S1-S20	Sensitive	None	WT (susceptible)	WT (susceptible)
MDR1,2	Resistant	EMB	ΔWT2,MUT1,D516V	WT
MDR3,5,14,27	Resistant	EMB, SM	ΔWT5,MUT3,S531L	ΔWT,T1,S315T
MDR4	Resistant	EMB	ΔWT1	WT
MDR6,26,35	Resistant	EMB, SM	ΔWT2,MUT1,D516V	ΔWT,T1,S315T
MDR7-9,19,20,32	Resistant	None	ΔWT5,MUT3,S531L	ΔWT,T1,S315T
MDR10,24,29	Resistant	None	ΔWT5,MUT3,S531L	WT
MDR11	Resistant	SM	ΔWT5,MUT3,S531L	ΔWT,T1,S315T
MDR12	Resistant	EMB, SM	ΔWT4,MUT2B,H526D	ΔWT,T1,S315T
MDR13	Resistant	EMB, SM	WT	ΔWT,T1,S315T
MDR15,23,25	Resistant	EMB, SM	ΔWT5,MUT3,S531L	WT
MDR16	Resistant	EMB	ΔWT4,MUT2B,H526D	WT
MDR17	Resistant	SM	ΔWT4	ΔWT,T1,S315T
MDR18	Resistant	SM	ΔWT5,MUT3,S531L	WT
MDR21,22	Resistant	None	ΔWT5,MUT3,S531L	WT
MDR28	Resistant	None	ΔWT1	ΔWT,T1,S315T
MDR30	Resistant	EMB	WT	ΔWT,T1,S315T
MDR31	Resistant	EMB	ΔWT1	ΔWT
MDR33	Resistant	None	ΔWT4	ΔWT,T1,S315T
MDR34	Resistant	EMB	ΔWT5,MUT3,S531L	ΔWT,T1,S315T

RMP, rifampin; INH, isoniazid; EMB, ethambutol; SM, streptomycin; WT, wild-type; ΔWT, absence of hybridization with a wild-type probe

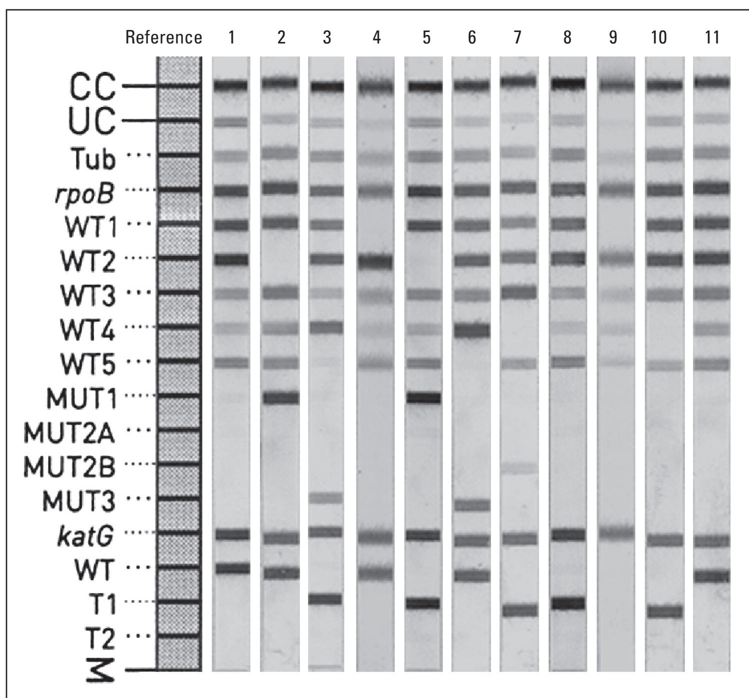

Figure 1. Representative Genotype MTBDR strip patterns obtained with pansusceptible and MDR *M tuberculosis* isolates. The positions of oligonucleotide probes on the strip, their specificity and the targeted genes as shown (from top to bottom) on the left side (Reference lane) are as follows: CC, conjugate 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 (CAC526TTC), H526D (CAC526GAC) and S531L (TCG531TTG) mutation, respectively, at the three most frequently mutated (516, 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* Q513K 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* Q513K and *katG* S315N and MDR33, lane 10, *rpoB* H526R and *katG* S315T) *M tuberculosis* isolates are shown on the right.

specifically detected by *kat*G 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.[17] 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.[2,18] 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.[10-12] 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.[15] 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.[3,10,11] 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.[13,17] 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.[3,5,13]

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 *kat*G T1 (S315T) probe. One isolate (MDR31) was detected as INH-resistant by lack of reaction with the *kat*G WT probe only. This isolate contained the AGC315AAC mutation at *katG315* and no specific probe is present on the strip for detecting this mutation.

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.[3,7] For example, mutations in the *inhA* regulatory region occur in 15% to 35% of INH-resistant *M tuberculosis* strains from some geographical locations.[3,7,12] However, mutations in the *inhA* regulatory region cause low-level of resistance to INH and their detection may have little impact on INH therapy.[7,12] 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.[2,7,12] In conclusion, the Genotype MTBDR assay reliably identifies frequently observed and clinically important mutations associated with resistance of *M tuberculosis* isolates to RMP and INH.

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