Performance Assessment of the GenoType MTBDR*plus* Test and DNA Sequencing in Detection of Multidrug-Resistant *Mycobacterium tuberculosis*[▽]

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To facilitate the management of multidrug-resistant (MDR) tuberculosis, two nucleic acid sequence-based methods, the GenoType MTBDR*plus* test and DNA sequencing, were assessed for the rapid detection of drug-resistant *Mycobacterium tuberculosis* for the first time in the Asia-Pacific region. The performances of these two assays in detecting the presence of rifampin (rifampicin) (RIF) and isoniazid (INH) resistance-associated mutations in the *rpoB*, *katG*, *inhA* regulatory region, *inhA*, and *oxyR-ahpC* genes were compared to that of a conventional agar proportion drug susceptibility test. A total of 242 MDR and 30 pansusceptible *M. tuberculosis* isolates were evaluated in this study. The sensitivities obtained for RIF-resistant detection by the GenoType MTBDR*plus* test and by resistance gene sequencing were 95.5% and 97.9%, respectively. The sensitivities for INH resistance detection by the GenoType MTBDR*plus* test and by resistance gene sequencing were 81.8% and 93.4%, respectively. Together, the sensitivity for MDR tuberculosis detection was 78.5% with the GenoType MTBDR*plus* test and 91.3% by resistance gene sequencing. The specificity for RIF resistance, INH resistance, and MDR detection was 100% by both methods. The GenoType MTBDR*plus* test has the advantage of a short turnaround time for drug-resistant *M. tuberculosis* detection. Overall, the two assays performed equally well in detecting RIF resistance ($P = 0.13$). However, DNA sequencing demonstrated superior performance in detecting INH resistance ($P < 0.001$) and MDR tuberculosis ($P < 0.001$). We suggest that new alleles of INH resistance genes should be evaluated to improve the sensitivity of the GenoType MTBDR*plus* test, especially for different geographic areas with genetically diverse *M. tuberculosis* strains.

The emergence of multidrug-resistant tuberculosis (MDR-TB), defined as infection with a *Mycobacterium tuberculosis* complex isolate resistant to at least isoniazid (INH) and rifampin (rifampicin) (RIF), is a public health concern and threatens global TB control programs (22). In Taiwan, approximately 15,000 new TB cases are diagnosed annually, of which an estimated 4% are MDR-TB (12). Therefore, the Taiwan Centers for Disease Control (CDC) not only has strengthened directly observed treatment in the management of TB as of 2006, to prevent MDR generation, but also has implemented a DOTS-Plus (directly observed treatment, short-course) strategy for the management of MDR-TB patients as of 2007 (8). However, this program can be hampered by delayed laboratory diagnosis. The completion of diagnosis by conventional methods and drug susceptibility testing (DST) of *M. tuberculosis* normally take months.

The World Health Organization and partners have endorsed the use of the molecular test GenoType MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) for rapid detection of high-risk MDR-TB cases, even directly from certain clinical specimens (1, 4, 6, 10, 15, 21). The GenoType MTBDR*plus* test is a PCR-based amplification and reverse blotting assay that employs specific probes hybridized to nitrocellulose strips to detect RIF and INH resistance. The assay detects mutations in the *rpoB* gene for RIF resistance, in the *katG* gene for high-level INH resistance, and in the *inhA* regulatory region gene for low-level INH resistance. To evaluate the reliability of the assay, DNA sequencing analyses of *rpoB* for RIF and *katG*, the *inhA* regulatory region gene, *inhA*, or *oxyR-ahpC* for INH were conducted in parallel.

Our previous study demonstrated the genetic diversity of MDR *M. tuberculosis* isolates with novel alleles in the *rpoB* gene in Taiwan (11). Likewise, the distribution of *M. tuberculosis* isolates differs in different geographic regions (5, 11). The GenoType MTBDR*plus* test has been assessed in Europe (6, 10, 15, 21), South Africa (4), and the Caribbean (1), but not in the Asia-Pacific region, where there is a high prevalence of Beijing family *M. tuberculosis* isolates. Here we report the performance of the revised GenoType MTBDR*plus* test compared to that of DNA sequencing using a culture-based phenotypic DST, which is considered the gold standard for routine clinical practice.

MATERIALS AND METHODS

***Mycobacterium tuberculosis* isolates.** One bacteriologically (cultured in Löwenstein-Jensen or MGIT medium) and biochemically (nitrate and niacin tests) identified *M. tuberculosis* complex isolate was collected from each of 272 TB cases in hospitals throughout Taiwan between May 2007 and April 2008. Of the 272 isolates, 242 isolates were MDR and 30 were fully susceptible, as determined by conventional DST. No INH-monoresistant isolate was used in this study. Of the 242 MDR isolates, 133 (55%) belonged to the Beijing family and 109 (45%) were non-Beijing isolates.

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TABLE 1. Genotype MTBDR*plus* assay and sequencing results for 242 MDR *Mycobacterium tuberculosis* isolates

Result by the GenoType MTBDR*plus* test	Result by sequencing	No. (%) with the following result by conventional DST:		
		RIF resistant	INH resistant	MDR
Resistant	Resistant	229 (94.6)	198 (81.8)	188 (77.7)
Resistant	Susceptible	2 (0.8)	0 (0)	2 (0.8)
Susceptible	Resistant	8 (3.3)	28 (11.6)	33 (13.6)
Susceptible	Susceptible	3 (1.2)	16 (6.6)	19 (7.9)

DST. (i) Conventional methods. Test methods, media, and drug concentrations were used as previously described (7). Most laboratories in Taiwan have adopted the agar proportion method using either Middlebrook 7H10 or 7H11 medium (Creative Microbiologicals or Sancordon, Taipei, Taiwan). A few laboratories have employed BACTEC MGIT 960 SIRE kits (Becton Dickinson Diagnostic Systems, Sparks, MD) with a liquid culture system (2). Growth on a control medium was compared to growth on a drug-containing medium in order to determine susceptibility or resistance. The DST results were classified as resistant or susceptible. Tests were validated by the susceptibility of *M. tuberculosis* H37Ra, included in the same test. Although conventional DST was performed by clinical laboratories using either an agar proportion method or an MGIT method, all MDR-TB isolates used in this study were retested by both methods at the Reference Laboratory of Mycobacteriology in the Taiwan CDC.

(ii) Molecular methods. The two genotypic assays were evaluated blindly by two medical technologists in parallel. Cell lysates, prepared according to the protocol provided by the manufacturer of the GenoType MTBDR*plus* kit, were also used for sequencing.

GenoType MTBDR*plus* test. The GenoType MTBDR*plus* assay was performed according to the instructions provided by the manufacturer (Hain Lifescience GmbH, Nehren, Germany). Briefly, the amplification mixture contained 35 µl of the primer nucleotide mix, 5 µl of 10× polymerase incubation buffer, 5 µl of 25 mM MgCl$_2$, 1 µl of AmpliTaq Gold polymerase (5 U/µl; Applied Biosystems), and 5 µl of the supernatant of the cell lysate, for a final volume of 50 µl. The amplification protocol consisted of 5 min of denaturation at 95°C, followed by 10 cycles comprising 30 s at 95°C and 2 min at 58°C, an additional 20 cycles comprising 25 s at 95°C, 40 s at 53°C, and 40 s at 70°C, and then a final extension at 70°C for 8 min. Hybridization and detection were performed with a TwinCubator (Hain Lifescience GmbH, Nehren, Germany). The hybridization procedure included the following steps: chemical denaturation of the amplification products at room temperature for 5 min, hybridization of the single-stranded biotin-labeled amplicons to membrane-bound probes at 45°C for 30 min, stringent washes, addition of a streptavidin–alkaline phosphatase (AP) conjugate at room temperature for 30 min, and an AP staining reaction to detect colorimetric bands. To detect RIF resistance, eight wild-type (WT) *rpoB* probes encoding amino acids 505 to 533 and four probes for common mutations were utilized. Probes used for INH resistance detection were designed to recognize a WT S315 region, with two mutant probes for the highly resistant *katG* gene and two probes specific for WT regions, as well as four mutant probes for the *inhA* gene, which demonstrates low-level resistance. When all WT probes showed positive staining for an isolate and mutant probes demonstrated no staining, the isolate was considered susceptible. In contrast, the isolate was considered resistant when either any one of the WT probes was absent or any one of the mutant probes was present.

DNA sequencing of the *katG*, *inhA*, *inhA* locus, *oxyR-ahpC*, and *rpoB* genes. Mutations in *katG*, *inhA*, the *inhA* locus (*inhA* regulatory region), the *oxyR-ahpC* intergenic region (*ahpC*), and *rpoB* were analyzed by PCR amplification, and then were sequenced with the respective oligonucleotide primers: *katG*-F (5′-GTC ACA CTT TCG GTA AGA C-3′) and *katG*-R (5′-TTG TCG CTA CCA CGG AAC G-3′); *inhA* 1713-F (5′-CCG AGG ATG CGA GCT ATA TC-3′) and *inhA* 1713-R (5′-GGC TCG GGT CGA AGT CCA TG-3′); *inhA* 2194-F (5′-AGG CGC TGC TGC CGA TCA TG-3′) and *inhA* 2194-R (5′-CCG AAC GAC AGC AGC AGG AC-3′); *inhA* locus-F (5′-AAT GCG CGT CAGT TCC AC-3′) and *inhA* locus-R (5′-GTC GGT GAC GTC ACA TTC GA-3′); *ahpC*-F (5′-GCT TGA TGT CGG AGA GCA TCG-3′) and *ahpC*-R (5′-GGT CGC GTA GGC AGT GCC CC-3′); *rpoB*-F (5′-TCG GCG GTC CCA TCACGT CG-3′) and *rpoB*-R (5′-GCG TAC ACC GAC AGC GAG CC-3′). PCRs were performed as follows: 33 cycles at 96°C for 1 min; annealing for 1 min at 64°C for *rpoB*, 55°C for *katG*, and 60°C for *inhA* 1713, *inhA* 2194, the *inhA*

TABLE 2. Gene mutations in 231 RIF-resistant and 198 INH-resistant *Mycobacterium tuberculosis* isolates using the GenoType MTBDR*plus* test

Gene(s) and mutation probe(s)	Mutation(s) or codon(s) analyzed	No. (%) of isolates
rpoB		
MUT1	D516V	6 (2.6)
MUT2A	H526Y	23 (10)
MUT2B	H526D	8 (3.5)
MUT3	S531L	146 (63.2)
Missing WT	505–533	48 (20.8)
katG		
MUT1	S315T1	96 (48.5)
MUT1,2	S315T1, S315T2	1 (0.5)
MUT2	S315T2	0 (0)
Missing WT	315	10 (5.1)
inhA		
MUT1	C15T	60 (30.3)
MUT2	A16G	0 (0)
MUT3A	T8C	5 (2.5)
MUT3B	T8A	0 (0)
katG and *inhA*		
katG MUT1, *inhA* MUT1	S315T1, C15T	19 (9.6)
katG MUT1, *inhA* MUT3A	S315T1, T8C	2 (1.0)
katG MUT1, *inhA* MUT3B	S315T1, T8A	3 (1.5)
katG MUT1, missing *inhA* WT	S315T1, (−15/−16/−8)	1 (0.5)
Missing *katG* WT, *inhA* MUT1	315, C15T	1 (0.5)

regulatory region, and *ahpC*; and elongation at 72°C for 1 min. Thereafter, the PCR products were analyzed with an ABI 3730 automated sequencer (Applied Biosystems), and the sequence data were assembled and edited using Sequencing Analysis software (version 5.2.0; Applied Biosystems).

Data analysis. The performances of the GenoType MTBDR*plus* test and DNA sequencing were compared to that of a conventional DST for the detection of RIF and INH resistance. The sensitivity, specificity, and accuracy of the molecular assays were calculated. Data were analyzed using Epi Info 6 (Centers for Disease Control and Prevention, Atlanta, GA). Comparisons were performed using chi-square analysis and Fisher's exact test. Statistical significance was defined as a P value of <0.05.

RESULTS

All 30 fully drug susceptible *M. tuberculosis* isolates demonstrated no mutations, as determined using both molecular assays.

GenoType MTBDR*plus* test and sequencing results conferring RIF resistance. Of the 242 MDR *M. tuberculosis* isolates, 231 (95.4%) and 237 (97.9%) isolates revealed mutations conferring RIF resistance by the GenoType MTBDR*plus* test and DNA sequencing, respectively (Table 1). The frequencies of gene mutations in RIF-resistant isolates identified by the GenoType MTBDR*plus* test are shown in Table 2. Predominant mutations identified as RIF resistance mutations were *rpoB* S531L (63.2%), a missing *rpoB* WT pattern (20.8%), and *rpoB* H526Y (10%). Among the 48 RIF-resistant isolates with at least one missing *rpoB* WT pattern, 9 displayed a sole H526R mutation and 5 had a single L533P mutation. Both H526R and L533P were not included in the GenoType MTBDR*plus* test. Nevertheless, of the 11 (4.5%) isolates classified
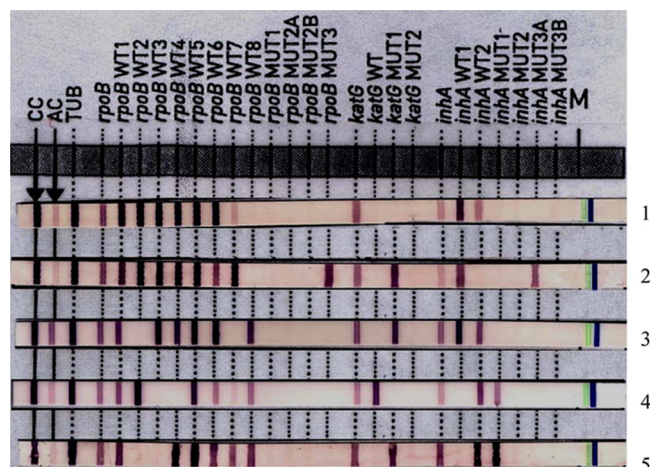
FIG. 1. Representative patterns obtained by the GenoType MTBDR-*plus* test for isolates with dual mutations in the *rpoB* gene based on sequencing. Lane 1, ΔWT8 (sequenced mutations in *rpoB*, H526N and L533P); lane 2, ΔWT8 and MUT3 (*rpoB* D444V and S531L); lane 3, ΔWT2 and ΔWT7 (*rpoB* S511P and H526N); lane 4, ΔWT3 and ΔWT4 (*rpoB* D516Y and T525I); lane 5, ΔWT2 and ΔWT3 (*rpoB* Q513E and M558K).

by the GenoType MTBDR*plus* test as RIF susceptible, 8 showed resistant mutations at L533P based on sequencing. Overall, the L533P mutation was identified in 13 (5.4%) isolates among 242 MDR isolates. In contrast, two MDR isolates having all eight positive WT stains and possessing additionally either the S531L (MUT3) or the H526Y (MUT2A) mutation, as identified by the GenoType MTBDR*plus* test, displayed the *rpoB* WT pattern by DNA sequencing (Table 1). These two isolates were judged to be resistant by the GenoType MTBDR*plus* test. The coexistence of a major WT peak and a minor mutation peak was found by reading the raw sequencing data visually for each sample. However, after data were analyzed using the Sequencing software, the two samples were judged drug susceptible. Finally, the results were later confirmed as drug resistant using conventional DST methods.

Sequencing data revealed that five MDR isolates each had two mutated codons, as shown in Fig. 1. Of the mutated *rpoB* codons identified, D444V and M558K were not within the hot spot region of the *rpoB* gene. In addition, two MDR isolates were identified by the GenoType MTBDR*plus* test. Those two isolates with discordant results were identified by sequencing as harboring either an *rpoB* H526N (Fig. 1, lane 1) or an *rpoB* T525I (lane 4) mutation; they stained WT7 positive by the GenoType MTBDR*plus* test (Fig. 1). Three MDR Beijing genotype isolates confirmed by conventional DST could not be identified as RIF resistant by either genotypic assay.

GenoType MTBDR*plus* test and sequencing results for mutations conferring INH resistance. Of the 242 MDR *M. tuberculosis* isolates, 198 (81.8%) and 226 (93.4%) isolates were detected by the GenoType MTBDR*plus* test and DNA sequencing, respectively, as INH resistant (Table 1). By the GenoType MTBDR*plus* test, 54.0% (107/198) demonstrated mutations in *katG* alone, 32.8% (65/198) showed mutations in *inhA* alone, and 13.1% (26/198) displayed mutations in both *katG* and *inhA*. The predominant mutation patterns identified as conferring INH resistance included *katG* S315T1 (48.5% of

isolates [96/198]), *inhA* C15T (30.3% [60/198]), and the *katG* S315T1 *inhA* C15T double mutation (9.6% [19/198]). None of the single *katG* MUT2, *inhA* MUT2, or *inhA* MUT3B mutations were identified in our study population (Table 2).

Discordant results by sequencing compared to the Geno-Type MTBDR*plus* test were observed for 28 MDR isolates (Table 3). Based on DNA sequencing, 7.4% (18/242) of the MDR isolates had a mutation in the *ahpC* gene. Of those 18 isolates, 15 harbored a sole *ahpC* mutation. Overall, 16 (6.6%) MDR isolates still showed WT patterns in the *katG*, *inhA*, *inhA* regulatory region, and *ahpC* genes sequenced (Table 1). Of the 16 MDR isolates, 12 had Beijing family genotypes.

Performances of the GenotypeMTBDR*plus* test and DNA sequencing. For the detection of RIF resistance, the sensitivity, specificity, and accuracy of the GenoType MTBDR*plus* test were 95.4%, 100%, and 96.0%, respectively, and the corresponding values for DNA sequencing were 97.9%, 100%, and 98.2%, respectively. In contrast, for the detection of INH resistance, the sensitivity, specificity, and accuracy of the Geno-Type MTBDR*plus* test were 81.8%, 100%, and 83.8%, respectively, and the corresponding values for DNA sequencing were 93.4%, 100%, and 94.1%, respectively. Furthermore, for MDR-TB detection, the sensitivity, specificity, and accuracy of the GenoType MTBDR*plus* test were 78.5%, 100%, and 80.9%, respectively, and the corresponding values for resistance gene sequencing were 91.3%, 100%, and 92.3%, respectively. Overall, the two assays performed equally well in detecting RIF resistance ($P = 0.13$). However, DNA sequencing demonstrated superior performance for the detection of INH resistance ($P < 0.001$) and MDR-TB ($P < 0.001$).

TABLE 3. Discordance between results obtained by the GenoType MTBDR*plus* test and those obtained by DNA sequencing in the detection of INH resistance for 28 MDR[a] *M. tuberculosis* isolates

INH result[b] of Genotype MTBDR*plus* test for isolates with the WT *katG/inhA* regulatory region pattern	DNA sequencing		No. of isolates
	Mutated codon(s) (*katG, inhA, ahpC*)	INH result	
S	*ahpC* C−10T	R	6
S	*ahpC* G−66A	R	2
S	*ahpC* G−9A	R	2
S	*katG* Y337C	R	2
S	*katG* D419H	R	1
S	*katG* Y413H	R	1
S	*katG* del401C	R	1
S	*katG* A379V	R	1
S	*katG* L378P, *inhA* E217D	R	1
S	*katG* del364T, *ahpC* C−12T	R	1
S	*katG* A362D	R	1
S	*katG* del310A, *ahpC* C−10T	R	1
S	*katG* G299A, *ahpC* C−10T	R	1
S	*inhA* E217D	R	1
S	*inhA* R202G	R	1
S	*ahpC* C−39T	R	1
S	*ahpC* −4/−5 insert A	R	1
S	*ahpC* G−5A, *ahpC* G−6A	R	1
S	*ahpC* C−15T	R	1
S	*ahpC* C−32T	R	1

[a] Resistance was confirmed by conventional DST.
[b] S, susceptible; R, resistant.

DISCUSSION

We evaluated a revised version of the GenoType MTBDR*plus* test, including three extra WT *rpoB* probes for RIF, two extra WT *inhA* regulatory region probes, and four extra mutated probes for INH, enhancing the accuracy of the detection of resistance to RIF and INH. This test is useful due to its accuracy and specificity, as well as its short turnaround time and ease of use. Previous studies have demonstrated that the GenoType MTBDR*plus* test had outstanding performance: 91.7% to 100% accuracy in detecting RIF resistance, 34.6% to 94.6% accuracy in detecting INH resistance, and 92% to 100% accuracy in detecting MDR (1, 4, 6, 10, 15, 21). In comparison, the GenoType MTBDR*plus* test identified 95.4% of RIF-resistant isolates, 81.8% of INH-resistant isolates, and 78.5% of MDR isolates in our study. The overall performance for MDR detection was acceptable in our setting. However, resistance gene sequencing had to be employed as a supplement.

Based on DNA sequencing, all RIF-resistant isolates showed mutations within the hot spot region between codon 507 and codon 533. The frequency of the major mutation S531L (63.2%) is consistent with those found in other studies (50% and 86%) (1, 4, 6, 10, 15, 21). Our results were consistent despite reports that the D516V mutation was commonly found (38%) in Hungarian strains (5), while it was not common in our MDR isolates (2%) (data not shown). In addition, rare mutations in either D444V or M558K were considered associated with low-level RIF resistance (15), while dual mutations might correlate with high-level RIF resistance (23). Of the five isolates shown in Fig. 1, four were of the Beijing family genotype. Our previous study showed that the majority of our MDR isolates (60%) were of the Beijing family genotype (11). In addition, some RIF-resistant *M. tuberculosis* strains isolated either in Cape Town, South Africa (6%) (20), or in Hungary (10%) (5) did not possess an *rpoB* mutation, while 1.2% did not have the *rpoB* mutation in our study. This finding may be due to rare mutations in other regions of the *rpoB* gene, such as the V146F mutation, reported for a Hungarian population (5), or the V176F mutation, reported for a German population (9).

The frequency of INH-resistant isolates harboring a single C15T mutation in the *inhA* regulatory region gene in our study (30.3%) was higher than that found in a German population (3%) (10) and comparable to that found in a South African study (27%) (4). The high genetic diversity of INH resistance in our MDR isolates is shown in Table 2. A previous study reported a silent mutation that might result in the absence of one of the WT probes in the GenoType MTBDR*plus* test (1); however, no silent mutations were identified in our MDR isolates. Since 6.6% of our INH-resistant isolates demonstrated WT patterns, they might potentially have mutations in other genomic regions, such as the *kasA, iniA, iniB, iniC, efpA, furA,* and *ndh* genes (17, 24).

The association between mutations in codon 533 of the *rpoB* gene and RIF resistance remains controversial (10). Of the 13 (5.4%) MDR isolates carrying the L533P mutation, both resistant and susceptible results were obtained by the GenoType MTBDR*plus* test. Eight of these isolates were determined to be susceptible (the WT8 position showed weakly positive staining) by the GenoType MTBDR*plus* test. The coexistence of a major mutation peak and a minor WT peak was found by reading the raw sequencing data visually. However, after analysis of data using the Sequencing software, all eight samples were judged drug resistant. According to the manufacturer's instructions, conventional DST results must be obtained before a final report can be provided. Therefore, we suggest the inclusion of an L533P mutant probe to improve the accuracy and turnaround time of detection of RIF resistance. In addition, although mutations in the *oxyR-ahpC* intergenic region have been shown to be associated with INH resistance (3, 13, 14, 16, 18, 19, 25), detection of this region was not generally included in molecular methods. Of the 18 MDR isolates possessing mutations in the *ahpC* gene, 8 harbored the *ahpC* C−10T mutation, which has been described relative to the *ahpC* mRNA initiation site (14). Concurrent mutations in the *ahpC* and *katG* genes were identified in 3 of our MDR isolates and in 10 (12.6%) isolates in the study of Kiepiela et al. (14). Sixteen (88.9%) of our 18 isolates carried mutations in the *ahpC* promoter region between −45 and −4 relative to the *ahpC* mRNA initiation site (26). Since a single *ahpC* gene mutation plays an important role in detecting MDR isolates, it might be useful to include an *ahpC* mutation codon to improve the detection of INH-resistance.

In conclusion, the present study demonstrated that the GenoType MTBDR*plus* test is applicable for the rapid detection of RIF and INH resistance. The accuracy of the GenoType MTBDR*plus* test for the detection of RIF resistance was found to be comparable to that of DST using conventional culture-based methods, but the GenoType MTBDR*plus* test was less accurate for the detection of INH resistance. Although molecular techniques could be used for rapid DST, we were unable to replace conventional DST. Furthermore, the GenoType MTBDR*plus* test may need to include new alleles of RIF- and INH-resistant genes in order to improve sensitivity for genetically diverse *M. tuberculosis* isolates recovered from different geographic areas.

REFERENCES

1. **Akpaka, P. E., S. Baboolal, D. Clarke, L. Francis, and N. Rastogi.** 2008. Evaluation of methods for rapid detection of resistance to isoniazid and rifampin in *Mycobacterium tuberculosis* isolates collected in the Caribbean. J. Clin. Microbiol. **46:**3426–3428.
2. **Ardito, F., B. Posteraro, M. Sanguinetti, S. Zanetti, and G. Fadda.** 2001. Evaluation of Bactec Mycobacteria Growth Indicator Tube (MGIT 960) automated system for drug susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **39:**4440–4444.
3. **Baker, L. V., T. J. Brown, O. Maxwell, A. L. Gibson, Z. Fang, M. D. Yates, and F. A. Drobniewski.** 2005. Molecular analysis of isoniazid-resistant *Mycobacterium tuberculosis* isolates from England and Wales reveals the phylogenetic significance of the *ahpC* −46A polymorphism. Antimicrob. Agents Chemother. **49:**1455–1464.
4. **Barnard, M., H. Albert, G. Coetzee, R. O'Brien, and M. E. Bosman.** 2008. Rapid molecular screening for multidrug resistant tuberculosis in a high-volume public health laboratory in South Africa. Am. J. Respir. Crit. Care Med. **177:**787–792.
5. **Bártfai, Z., A. Somoskövi, C. Ködmön, N. Szabó, E. Puskás, L. Kosztolányi, E. Faragó, J. Mester, L. M. Parsons, and M. Salfinger.** 2001. Molecular characterization of rifampin-resistant isolates of *Mycobacterium tuberculosis* from Hungary by DNA sequencing and the line probe assay. J. Clin. Microbiol. **39:**3736–3739.

6. **Causse, M., P. Ruiz, J. B. Gutierrez, J. Zerolo, and M. Casal.** 2008. Evaluation of new GenoType MTBDR*plus* for detection of resistance in cultures and direct specimens of *Mycobacterium tuberculosis*. Int. J. Tuberc. Lung Dis. **12:**1456–1460.

7. **Centers for Disease Control, Department of Health, Executive Yuan, Taiwan.** 2004. A manual of mycobacteriology, 2nd ed. Centers for Disease Control, Taipei, Taiwan.

8. **Centers for Disease Control, Department of Health, Executive Yuan, Taiwan.** 2008. Annual report. http://www.cdc.gov.tw/public/data/8871524971 .pdf.

9. **Heep, M., U. Rieger, D. Beck, and N. Lehn.** 2000. Mutations in the beginning of the *rpoB* gene can induce resistance to rifamycins in both *Helicobacter pylori* and *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **44:** 1075–1077.

10. **Hillemann, D., S. Rüsch-Gerdes, and E. Richter.** 2007. Evaluation of the GenoType MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J. Clin. Microbiol. **45:**2635–2640.

11. **Jou, R., H. Y. Chen, C. Y. Chiang, M. C. Yu, and I. J. Su.** 2005. Genetic diversity of multidrug-resistant *Mycobacterium tuberculosis* isolates and identification of 11 novel *rpoB* alleles in Taiwan. J. Clin. Microbiol. **43:**1390–1394.

12. **Jou, R., P. C. Chung, Y. S. Wu, J. J. Yang, and K. T. Luh.** 2006. Drug-resistant *Mycobacterium tuberculosis*, Taiwan. Emerg. Infect. Dis. **12:**871– 872.

13. **Kelley, C. L., D. A. Rouse, and S. L. Morris.** 1997. Analysis of *ahpC* gene mutations in isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **41:**2057–2058.

14. **Kiepiela, P., K. S. Bishop, A. N. Smith, L. Roux, and D. F. York.** 2000. Genomic mutations in the *katG*, *inhA* and *aphC* genes are useful for the prediction of isoniazid resistance in *Mycobacterium tuberculosis* isolates from Kwazulu Natal, South Africa. Tuber. Lung Dis. **80:**47–56.

15. **Lacoma, A., N. Garcia-Sierra, C. Prat, J. Ruiz-Manzano, L. Haba, S. Rosés, J. Maldonado, and J. Domínguez.** 2008. GenoType MTBDR*plus* assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* strains and clinical samples. J. Clin. Microbiol. **46:**3660–3667.

16. **Ramaswamy, S., and J. M. Musser.** 1998. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber. Lung Dis. **79:**3–29.

17. **Ramaswamy, S. V., R. Reich, S. J. Dou, L. Jasperse, X. Pan, A. Wanger, T. Quitugua, and E. A. Graviss.** 2003. Single nucleotide polymorphisms in genes associated with isoniazid resistance in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **47:**1241–1250.

18. **Sreevatsan, S., X. Pan, Y. Zhang, V. Deretic, and J. M. Musser.** 1997. Analysis of the *oxyR-ahpC* region in isoniazid-resistant and -susceptible *Mycobacterium tuberculosis* complex organisms recovered from diseased humans and animals in diverse localities. Antimicrob. Agents Chemother. **41:** 600–606.

19. **Telenti, A., N. Honore, C. Bernasconi, J. March, A. Ortega, B. Heym, H. E. Takiff, and S. T. Cole.** 1997. Genotypic assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: a blind study at reference laboratory level. J. Clin. Microbiol. **35:**719–723.

20. **Van Rie, A., R. Warren, I. Mshanga, A. M. Jordaan, G. D. van der Spuy, M. Richardson, J. Simpson, R. P. Gie, D. A. Enarson, N. Beyers, P. D. van Helden, and T. C. Victor.** 2001. Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. J. Clin. Microbiol. **39:**636–641.

21. **Vijdea, R., M. Stegger, A. Sosnovskaja, Å. B. Andersen, V. Ø. Thomsen, and D. Bang.** 2008. Multidrug-resistant tuberculosis: rapid detection of resistance to rifampin and high or low levels of isoniazid in clinical specimens and isolates. Eur. J. Clin. Microbiol. Infect. Dis. **27:**1079–1086.

22. **World Health Organization.** 2008. Anti-tuberculosis drug resistance in the world. Report WHO/HTM/TB/2008.394. World Health Organization, Geneva, Switzerland.

23. **Zakerbostanabad, S.** 2008. Multiple mutations in *rpoB* gene of *M. tuberculosis* correlate with high level of resistance to rifampicin, abstr. S-51. Abstr. 39th World Conf. on Lung Health of the International Union Against Tuberculosis and Lung Disease.

24. **Zhang, M., J. Yue, Y. P. Yang, H. M. Zhang, J. Q. Lei, R. L. Jin, X. L. Zhang, and H. H. Wang.** 2005. Detection of mutations associated with isoniazid resistance in *Mycobacterium tuberculosis* isolates from China. J. Clin. Microbiol. **43:**5477–5482.

25. **Zhang, S. L., J. G. Shen, P. H. Xu, D. X. Li, Z. Q. Sun, L. Li, Z. R. Yang, and Q. Sun.** 2007. A novel genotypic test for rapid detection of multidrug-resistant *Mycobacterium tuberculosis* isolates by a multiplex probe array. J. Appl. Microbiol. **103:**1262–1271.

26. **Zhang, Y., S. Dhandayuthapani, and V. Deretic.** 1996. Molecular basis for the exquisite sensitivity of *Mycobacterium tuberculosis* to isoniazid. Proc. Natl. Acad. Sci. USA **93:**13212–13216.