GenoType MTBDR*sl* Line Probe Assay Shortens Time to Diagnosis of Extensively Drug-Resistant Tuberculosis in a High-Throughput Diagnostic Laboratory

Marinus Barnard[1,2], Rob Warren[2], Nico Gey Van Pittius[2], Paul van Helden[2], Marlein Bosman[1], Elizabeth Streicher[2], Gerrit Coetzee[3], and Richard O'Brien[4]

[1]National Health Laboratory Service (NHLS), Tuberculosis Reference Laboratory, Green Point, Cape Town, South Africa; [2]Department of Science and Technology/National Research Foundation Centre of Excellence in Biomedical Tuberculosis Research, University of Stellenbosch/Medical Research Council Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa; [3]National TB Reference Laboratory, NHLS, Sandringham, Johannesburg, South Africa; and [4]Foundation for Innovative New Diagnostics, Geneva, Switzerland

***Rationale*: Conventional culture-based drug susceptibility testing (DST) for the second-line antituberculosis drugs is slow, leading to diagnostic delay with associated exacerbation of transmission, amplification of resistance, and increased mortality.**

***Objectives*: To assess the diagnostic performance of the GenoType MTBDR*sl* line probe assay (LPA) for the rapid detection of mutations conferring resistance to ofloxacin (OFX), amikacin (AMK), and ethambutol and to determine the impact of implementation on the turnaround time in a high-throughput diagnostic laboratory.**

***Methods*: Six hundred and fifty-seven direct patient acid-fast bacilli smear-positive specimens resistant to isoniazid, rifampin, or both according to the GenoType MTBDR*plus* assay were consecutively tested, using the GenoType MTBDR*sl* LPA. The diagnostic performance was assessed relative to the "gold standard" culture-based method, and the laboratory turnaround times for both methods were determined.**

***Measurements and Main Results*: A total of 516 of 657 patient specimens had valid results for both tests. The sensitivity for detecting OFX, AMK, and extensive drug resistance, using the GenoType MTBDR*sl* LPA, was 90.7% (95% confidence interval [CI], 80.1–96.0%), 100% (95% CI, 91.8–100%), and 92.3% (95% CI, 75.9–97.9%), respectively, and the specificity for detection was 98.1% (95% CI, 96.3–99.0%), 99.4% (95% CI, 98.2–99.8%), and 99.6% (95% CI, 98.5–99.9%), respectively. Implementation of this test significantly reduced the turnaround time by 93.3% ($P < 0.001$), calculated from the date that the specimen was received at the laboratory to reporting second-line results. In addition, a significant increase in diagnostic yield of 20.1% and 19.3% ($P < 0.001$) for OFX and AMK resistance, respectively, was obtained for isolates that were either contaminated or had lost viability.**

***Conclusions*: The GenoType MTBDR*sl* LPA is a rapid and reliable DST that can be easily incorporated into the diagnostic algorithm. This assay significantly improved diagnostic yield ($P < 0.001$) while simultaneously decreasing diagnostic delay for reporting second-line DST. The rapid dissemination of second-line DST results will guide initiation of appropriate treatment, thereby reducing transmission and improving treatment outcome.**

Keywords: *Mycobacterium tuberculosis*; GenoType MTBDR*plus*; GenoType MTBDR*sl*; line probe assay; XDR-TB

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The MTBDR*sl* test is a molecular line probe assay for the rapid detection of mutations associated with resistance to fluoroquinolone and aminoglycoside antibiotics and cycloserine, drugs used to treat multidrug-resistant tuberculosis. Published studies have shown that the assay performs well when using culture isolates. However, until this study there have been few data on direct testing of sputum specimens.

What This Study Adds to the Field

This study showed that MTBDR*sl* testing on acid-fast bacilli smear-positive respiratory specimens gave highly accurate results and a greater number of valid results compared to culture and drug susceptibility testing. Moreover, the turnaround time was over 90% shorter than that for phenotypic testing.

The increasing burden of drug-resistant tuberculosis (TB) poses an escalating threat to national and global TB control programs. It is estimated that there were 8.8 million new and retreatment cases of TB in 2010 and 1.45 million deaths (1). The estimated number of multidrug-resistant TB (MDR-TB) cases (resistant to at least isoniazid [INH] and rifampin [RIF]) has risen sharply from 440,000 in 2008 (2) to 650,000 in 2010 (1). Alarmingly, only 16% of these MDR-TB cases were reported to be enrolled for treatment (1). This can be ascribed in part to the fact that only 5% of TB cases are tested for MDR-TB (1). The emergence of extensively drug-resistant TB (XDR-TB; defined as MDR-TB with additional resistance to a fluoroquinolone, e.g., ofloxacin [OFX], and one of three injectable second-line drugs, i.e., amikacin [AMK], capreomycin [CM], or kanamycin [KM]) (3, 4) further exacerbates resistance amplification, with more than 40,000 cases reported in more than 58 countries in 2010 (5). By 2011 XDR-TB had been reported from 77 countries and the World Health Organization (WHO, Geneva, Switzerland) estimated that there were more than 58,000 prevalent cases (6). However, the true burden of XDR-TB cannot be accurately estimated on a global scale because of the absence of laboratory infrastructure in many parts of the developing world (7–9).

The WHO Stop TB Department has emphasized the need to strengthen diagnostic services and highlighted the need to develop rapid diagnostics (1). At present, the "gold standard" drug susceptibility testing (DST) method measures phenotypic resistance by the culture-based indirect proportion method, which has been standardized for solid and liquid media for the detection of OFX and AMK resistance (7, 10). Using these platforms, a DST may take between 10 days and 6 weeks (7), and even

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Correspondence and requests for reprints should be addressed to Richard O'Brien, M.D., Foundation for Innovative New Diagnostics, avenue de Bude 16, 1202 Geneva, Switzerland. E-mail: rjobrien1@gmail.com

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longer if a contaminant is present. This delay contributes to ongoing transmission of drug-resistant strains within the community and amplification of resistance with associated higher mortality rates among infected patients (11).

According to the development pipeline for new diagnostics as set out in the WHO report on Global Tuberculosis Control 2011, line probe assays (LPAs) for XDR-TB at the reference laboratory level are categorized as being in the late stages of development (1). To achieve the objectives of the WHO development pipeline for new diagnostics, diagnostic assays must be standardized, efficient, and rapid to diagnose, guide treatment regimens, and stop the transmission of drug-resistant strains of *Mycobacterium tuberculosis*. Furthermore, there is a need to reduce the costs of implementing these late-stage diagnostic tests. In addition, national tuberculosis control programs must consider the ease with which these new assays can be incorporated into already existing diagnostic algorithms used in the routine diagnostic laboratory.

LPAs have been approved by the WHO for the detection of mutations conferring resistance to INH and RIF (12). LPAs targeting resistance to second-line anti-TB drugs are under evaluation (13). These assays detect mutations in the *gyrA* gene (fluoroquinolone resistance), *rrs* gene (KM, AMK, and CM resistance), and *embB* gene (ethambutol [EMB] resistance) and are intended to complement the Hain GenoType MTBDR*plus* LPA for the detection of XDR-TB. The sensitivity of these tests as well as Hain GenoType MTBDR*plus* LPA depends on the inclusion of all mutations conferring resistance and the analysis of the frequency at which these mutations are found in TB strains in various geographical settings to establish their predictive value. Performance evaluations of the newly developed GenoType MTBDR*sl* LPA have suggested that the sensitivity for detecting fluoroquinolone resistance is between 75.6 and 90.6%, and the sensitivity for aminoglycoside/cyclic peptide resistance is between 77 and 100% (13–20). Two studies, however, found that the sensitivity for detecting KM resistance was between 25.0 and 42.7% (15, 16), and the sensitivity for detecting EMB resistance was between 56.0 and 70% (13–15, 17–20). However, most of these studies have evaluated the GenoType MTBDR*sl* LPA on cultured isolates (14–16, 19, 20). Only 3 studies have reported an evaluation using direct patient material that, when combined, totaled only 177 specimens from 139 patients (13, 17, 18). Thus there is a need to establish the reliability of this test in a routine diagnostic laboratory and to establish the "diagnostic fit" of the assay into the existing diagnostic algorithm.

In this study we have evaluated the GenoType MTBDR*sl* LPA (Hain Lifescience, Nehren, Germany) on patient specimens as an adjunct to the GenoType MTBDR*plus* LPA for the rapid diagnosis of OFX and AMK resistance through the detection of mutations in the *gyrA* and *rrs* genes, respectively.

METHODS

Setting

The study was approved by the Health Research Ethics Committee at Stellenbosch University (Cape Town, South Africa) and was conducted at the National Health Laboratory Service (NHLS) Tuberculosis Reference Laboratory in Green Point, South Africa. This is a high-volume public health laboratory that receives specimens from clinics and hospitals throughout Western Cape Province. Approximately 750 patient specimens are processed on a daily basis by decontamination with *N*-acetyl-L-cysteine–sodium hydroxide (NALC–NaOH) with a final concentration of 1%, as per published guidelines (21). This is followed by acid-fast bacilli (AFB) smear microscopy and inoculation into BBL MGIT (mycobacteria growth indicator tube, BD Diagnostics Systems, Sparks, MD) 960 medium. In this setting, residual sample material from smear-positive specimens is analyzed, using the GenoType MTBDR*plus* LPA (12, 22), for the detection of mutations conferring resistance to INH and RIF. On Day 0, the smear-positive specimens are selected and DNA is extracted according to the manufacturer's instructions for use after the addition of a small volume of 0.01% Tween 80–saline. On Day 2, the PCR and reverse hybridization are completed and the results are entered into the Laboratory Information Management System (LIMS) via interfaced networks, after which the results are manually reviewed. If INH- or RIF-monoresistant or MDR-TB is found, the results are faxed to the respective clinics. All LPA tests are done according to ISO 15189 standards (23). The laboratory performing the LPA tests participates in External Quality Assurance programs from the NHLS Quality Assurance Division, the South Africa National Tuberculosis Reference Laboratory, and the South Africa Medical Research Council. Since 2008, all results reported have had 100% concordance with the External Quality Assurance specimens. All controls were successful, indicating that the DNA extraction area and the PCR master-mix area were free of contaminants and amplicons and that the extraction procedure was performed correctly.

Routinely, second-line DST for OFX and AMK is immediately performed on all MGIT culture isolates that are positive for *M. tuberculosis* complex as well as resistant to INH, RIF, or both drugs by the GenoType MTBDR*plus* LPA test performed either directly on smear-positive decontaminated specimens or on smear-negative, culture-positive isolates. For this evaluation, however, only smear-positive decontaminated specimens were used. Second-line DST is performed on 7H11 agar slants containing OFX (2 μg/ml) and AMK (4 μg/ml), using the indirect proportion method (24). An additional DST for INH (0.1 μg/ml) is done for all isolates that are RIF monoresistant by the GenoType MTBDR*plus* LPA. Routine DST for EMB was stopped in 2010 because of frequent ambivalent resistance patterns observed within the laboratory, which was supported by literature review (25).

Selection Criteria for the GenoType MTBDR*sl* LPA Procedure

During the period from March 23, 2010 to September 5, 2011 a total of 736 smear-positive specimens, which exhibited resistance to INH, RIF, or both by the GenoType MTBDR*plus* LPA, were concurrently tested by the GenoType MTBDR*sl* LPA from the same DNA extract that was used for the GenoType MTBDR*plus* LPA. The selection contained both pulmonary and extrapulmonary specimens, all decontaminated as previously described (26). Routine practice allows for the decontamination of extrapulmonary specimens such as fine-needle aspirates and cerebrospinal fluid if the volume is more than 10 ml (26). The PCR for the GenoType MTBDR*sl* LPA was done on Day 2 and the reverse hybridization of the amplified products of the GenoType MTBDR*sl* LPA was done the next day in accordance with the manufacturer's instructions for use. The presence of a mutant band or the absence of a wild-type band for a specific gene on the strip was indicative of resistance to the drug associated with the specific gene, whereas the presence of all the wild-type probes as well as a mutant probe was considered indicative of heteroresistance. The possibility of silent mutations resulting in no change to the amino acid was also assessed.

Time Difference in Diagnosis

The difference in diagnosis time between the two tests was calculated from the NHLS LIMS.

Statistical Analysis

Statistical analyses were done with EP Evaluator, EPI INFO (27), and STATA 10.0 software (Statacorp LP, College Station, TX). The sensitivity, specificity, and positive and negative predictive values were calculated for each drug and compared with culture-based DST as the gold standard. The κ value and overall correct classification were taken as measures of agreement, where a κ value of 75% or more is considered "high" agreement, and an overall correct classification value close to 100% is considered good. The data were not normally distributed, so medians and

interquartile ranges were calculated. Differences in medians between the total turnaround time (TAT) of the two assays were calculated by Mann-Whitney *U* test for TAT and the increased yield in diagnostic result for cultures that had lost viability and were contaminated. Results were considered statistically significant at $P < 0.05$.

Genetic Sequencing

Sequencing of the *gyrA*, *rrs*, and *embB* genes was performed on a representative sample of isolates with discrepant LPA and DST results (n = 12) and XDR isolates (n = 11), as well as on randomly selected OFX-monoresistant isolates (n = 4), AMK-monoresistant isolates (n = 4), and OFX- and AMK-susceptible isolates (n = 16). The methodology for the sequencing has been published elsewhere (28).

RESULTS

During the period March 23, 2010 to September 5, 2011 a total of 736 smear-positive specimens that were resistant to INH, RIF, or both, according to the GenoType MTBDR*plus* LPA, were analyzed by GenoType MTBDR*sl* LPA. After exclusion of serial specimens, the first available specimens from 657 patients were included in the performance evaluation. These included 56.5% (371 of 657) MDR, 27.7% (182 of 657) INH-monoresistant, and 15.8% (104 of 657) RIF-monoresistant specimens. Interpretable GenoType MTBDR*sl* LPA results for OFX, AMK, and EMB were 98.13% (632 of 657), 97.20% (626 of 657), and 97.82% (630 of 657), respectively. The overall interpretability was 97.7% (SD, 0.389).

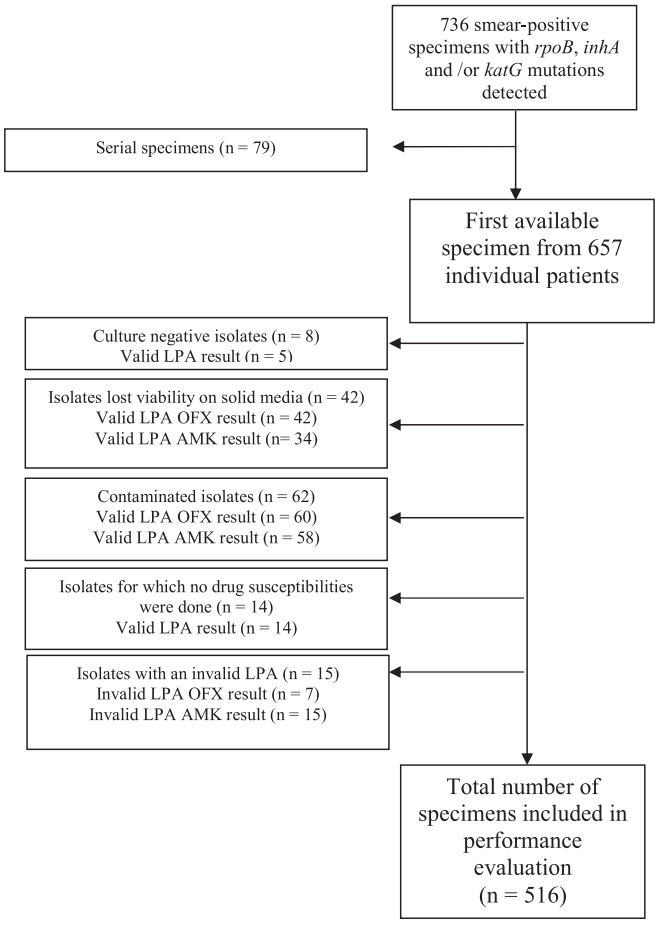

Figure 1. Flow diagram of specimens included in the study. AMK = amikacin; LPA = line probe assay; OFX = ofloxacin.

TABLE 1. PERFORMANCE OF GENOTYPE MTBDR*sl* LINE PROBE ASSAY IN DETECTING OFLOXACIN AND AMIKACIN RESISTANCE AND EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS

	OFX % (95% CI)	AMK % (95% CI)	XDR-TB % (95% CI)
Sensitivity	90.7 (80.1–96.0)	100 (91.8–100)	92.3 (75.9–97.9)
Specificity	98.1 (96.3–99.0)	99.4 (98.2–99.8)	99.6 (98.5–99.9)
PPV	84.5 (73.1–91.6)	93.5 (82.5–97.8)	92.3 (75.9–97.9)
NPV	98.9 (97.5–99.5)	100 (99.2–100)	99.6 (98.5–99.9)
OCC	97.3 (95.5–98.4)	99.4 (98.3–99.8)	99.2 (98.0–99.7)
κ	86.0 (77.4–94.6)	96.3 (87.7–100)	91.9 (83.3–100)

Definition of abbreviations: AMK = amikacin; CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value; OCC = overall correct classification; OFX = ofloxacin; XDR-TB = extensively drug-resistant tuberculosis.

To assess the performance of the GenoType MTBDR*sl* LPA test, isolates that lost viability, were culture negative or contaminated, or had an invalid GenoType MTBDR*sl* LPA result were excluded (Figure 1). A total of 516 (78.5%) isolates were included in the final analysis, of which 57.5% (297 of 516) were MDR, 27.3% (141 of 516) were INH monoresistant, and 15.1% (78 of 516) were RIF monoresistant. Table 1 summarizes the performance of the GenoType MTBDR*sl* LPA for the detection of mutations conferring OFX and AMK resistance as well as detection of XDR-TB relative to phenotypic DST on culture isolates. Test sensitivity for detecting ofloxacin, amikacin, and extensive drug resistance was 90.7% (95% CI, 80.1–96.0%), 100% (95% CI, 91.8–100%), and 92.3% (95% CI, 75.9–97.9%), respectively, and the specificity for detection was 98.1% (95% CI, 96.3–99.0%), 99.4% (95% CI, 98.2–99.8%), and 99.6% (95% CI, 98.5–99.9%), respectively. Tables 2 and 3 show the results of OFX and AMK resistance by GenoType MTBDR*sl* compared with MGIT culture and DST, respectively.

Of the 516 isolates tested, 54 were phenotypically resistant to OFX and 43 were resistant to AMK. Only 8.7% (26 of 297) of the MDR specimens were XDR, and none of the non-MDR specimens exhibited resistance to both OFX and AMK; thus resistance to OFX and AMK was strongly associated with MDR-TB (Fisher's exact test, $P = 0.0001$).

Even though there is no gold standard against which the performance characteristics for EMB could be measured, it was found that 35.3% (182 of 516) were resistant to EMB based on the GenoType MTBDR*sl* LPA. Of these, 92.3% (24 of 26) exhibited resistance in the XDR strains, 56.3% (18 of 32) in the OFX-monoresistant strains, 85% (17 of 20) in the AMK-monoresistant strains, and 28.1% (123 of 438) in the OFX/AMK-susceptible strains.

Table 4 shows the distribution of different banding patterns in drug-resistant specimens, including XDR, OFX-monoresistant, and AMK-monoresistant strains and OFX/AMK-susceptible strains. Only 2 of the 516 OFX mutation patterns had the *gyrA* WT1 absent with no corresponding MUT band. The amino acid changes for these were cysteine → serine and alanine → threonine. The correlation between phenotypic DST and the GenoType MTBDR*sl* LPA and OFX resistance was 100%. There were no silent mutations observed for AMK, whereas 23 specimens showed the absence of the WT probe without a corresponding MUT band for EMB, where the amino acid change was from methionine → isoleucine. Because there was no phenotypic DST available, no comparisons could be made.

Table 5 shows the results of genetic sequencing of the *gyrA*, *rrs*, and *embB* genes for selected isolates. The results support the accuracy of the LPA results over the phenotypic DST results. All 12 isolates with discordant LPA and DST results had sequencing results supporting the LPA.

TABLE 2. SUMMARY OF RESULTS OF OFLOXACIN RESISTANCE BY GENOTYPE MTBDR*sl* COMPARED WITH MGIT CULTURE AND DRUG SUSCEPTIBILITY TESTING

OFL LPA	CONTAM	CUL NEG	LV	N/D	N/S	R	S	Grand Total
R	4	0	11	1	0	49	9	74
S	56	5	31	12	1	5	453	563
INV	2	3	0	0	0	0	7	12
AMK INV	0	0	0	0	0	0	8	8
Grand total	62	8	42	13	1	54	477	657

Definition of abbreviations: AMK INV = deleted from dataset because of invalid amikacin data; CONTAM = not done because of contaminated culture; CUL NEG = not done because of negative culture; INV = invalid (no bands on strip); LV = lost viability during drug susceptibility testing (DST); N/D = not done; N/S = not done because DST not requested; OFL LPA = ofloxacin line probe assay; R = resistant; S = susceptible.

The specimens consisted of 4.4% (29 of 657) extrapulmonary specimens, of which 1 (1 of 29) gave an uninterpretable result on the GenoType MTBDR*sl* LPA, and 9 (9 of 29) had no DST because of contamination and loss of viability. One of the XDR results that correlated 100% between the two methods was a fine needle aspirate, suggesting that the GenoType MTBDR*plus* LPA and the GenoType MTBDR*sl* LPA can be used on extrapulmonary specimens.

To determine whether the use of the GenoType MTBDR*sl* LPA could reduce the TAT for reporting resistance to the second-line drugs OFX and AMK, laboratory reporting records documented on the NHLS LIMS were analyzed. This analysis showed that the TAT from receipt of specimens in the laboratory to the reporting of smear results was achieved within 24 hours. On the same day, the smear-positive specimens were selected and screened for genetic-based DST. The median TAT from receipt of specimens to reporting INH and RIF resistance, using the GenoType MTBDR*plus* LPA, was 2 days (range, 1–4 d), and the median TAT for reporting the GenoType MTBDR*sl* LPA was one additional day. Thus the total TAT for the genetic diagnosis of resistance to OFX and AMK was 3 days (range, 2–5 d). This was significantly shorter than for culture-based DST. In this routine diagnostic laboratory, the average time for the MGIT to become positive for AFB growth detection was 12 days (range, 6–47 d) for the isolates tested. In addition, the TAT for reporting second-line DST results was 31 days (range, 13–82 d), and the overall culture-based TAT (from the date that the specimen was received at the laboratory to reporting second-line results) was 45 days (range, 27–122 d). Thus, the implementation of the GenoType MTBDR*sl* LPA significantly reduced the TAT to 3 days (93.3%; Mann-Whitney *U* two-tailed test, $P < 0.001$).

DISCUSSION

This is the first reported study to assess the performance of the GenoType MTBDR*sl* LPA under routine diagnostic conditions in a high-throughput TB laboratory using decontaminated smear-positive specimens that were resistant to INH, RIF, or both by the GenoType MTBDR*plus* LPA. In this study, the correlation between the GenoType MTBDR*sl* LPA results and conventional culture-based DST for OFX and AMK was good. The sensitivity for detection of OFX and AMK resistance and XDR-TB was 90.7, 100, and 92.3%, respectively. Similarly, the specificity for detecting OFX and AMK resistance, and XDR-TB was 98.1, 99.4, and 99.6%, respectively.

To date, the only comparative DST proficiency testing studies in which the indirect proportion method can be assessed are those for the first-line drugs (29). However, the European Union Tuberculosis Reference Laboratory Network has made progress in testing a range of antituberculosis drugs, including the fluoroquinolones and the injectables, in their quest to standardize second-line DST and to strengthen laboratories (30).

Of the three injectable drugs, only AMK can be missed when comparing the conventional DST result with that of the rapid GenoType MTBDR*sl* LPA result, because the absence of the *rrs* WT1 probe is not associated with phenotypic resistance to AMK (31). The possibility that XDR isolates might have been missed that were phenotypically susceptible to AMK but could potentially have been phenotypically resistant to kanamycin or capreomycin might be plausible because the wild-type and mutant probes on the GenoType MTBDR*sl* LPA associated with phenotypic resistance to kanamycin and capreomycin fall within the *rrs* gene. However, treatment with KM results in the acquisition of mutations in three other genes (*tlyA*, *eis* promoter, and *gidB*) associated with injectable drug resistance and may result in phenotypic resistance (32). Furthermore, there might also be rare mutations outside the *rrs* gene region, and thus it is suggested that all strains found to be FLQ monoresistant on the GenoType MTBDR*sl* LPA be subjected to phenotypic AMK/KM DST to "rule in" XDR-TB. This is similar to what is being done to rule in MDR-TB for other INH resistance–associated mutations when strains are found to be RIF monoresistant on the GenoType MTBDR*plus* LPA.

The percentage of interpretable results for the GenoType MTBDR*sl* LPA was comparable to that found for the GenoType MTBDR*plus* LPA (22). These results are comparable with previous performance evaluation studies using direct patient material (13, 17, 19). To date, only one study has reported poor performance for the assay, which was ascribed to the possibility that a proportion of the *M. tuberculosis* strains circulating in that setting had evolved resistance through mutations not included in the assay (15).

The low percentage of EMB resistance (35.3%) observed in strains exhibiting drug resistance to INH, RIF, or both is notable, because the majority of XDR strains (92.3%) and AMK-monoresistant strains (85%) were resistant to EMB, based on the GenoType MTBDR*sl* LPA. Published studies indicate that the sensitivity for EMB when comparing the GenoType MTBDR*sl* LPA with DST on solid media ranges from 56.2 to 64.2% (13–15, 19) and ranges from 67.6 to 86% in liquid media (16–18). Another study also described the difference in sensitivity for EMB between solid media (27.3%) and liquid media (81.7%) on the same set of isolates (31) and thus strengthens the argument that conventional EMB DST is fraught with problems (33). Considering this observation, it appears that liquid DST for EMB gives greater sensitivity when compared with the GenoType MTBDR*sl* LPA.

In this study, the lower sensitivity for OFX resistance relative to AMK resistance may be explained by resistance that can occur

TABLE 3. SUMMARY OF RESULTS OF AMIKACIN RESISTANCE BY GENOTYPE MTBDR*sl* COMPARED WITH MGIT CULTURE AND DRUG SUSCEPTIBILITY TESTING

AMK LPA	CONTAM	CUL NEG	LV	N/D	N/S	R	S	Grand Total
R	9	0	4	3	0	43	3	62
S	49	5	30	10	1	0	470	565
INV	4	3	1	0	0	1	14	23
OFL INV	0	0	0	0	0	0	7	7
Grand total	62	8	35	13	1	44	494	657

Definition of abbreviations: AMK LPA = amikacin line probe assay; CONTAM = not done because of contaminated culture; CUL NEG = not done because of negative culture; INV = invalid (no bands on strip); LV = lost viability during drug susceptibility testing (DST); N/D = not done; N/S = not done because DST not requested; OFL INV = deleted from dataset because of invalid ofloxacin data; R = resistant; S = susceptible.

TABLE 4. PATTERNS OF GENE MUTATIONS IN RESISTANT *MYCOBACTERIUM TUBERCULOSIS* STRAINS, USING THE GENOTYPE MTBDR*sl* ASSAY

Gene	Band(s)	Nucleotide/Codon Analyzed	Gene Region or Mutation	XDR (*n* = 26)	FLQ Monoresistant (*n* = 32)	AG/CP Monoresistant (*n* = 20)	FLQ/AG/CP Susceptible, EMB Resistant (*n* = 438)
gyrA	*gyrA* WT1	85–90	C88S, A88T	24 (92.3)	32 (100)	20 (100)	0
	gyrA WT2	89–93		21 (80.8)	25 (78.1)	20 (100)	0
	gyrA WT3	92–97		10 (38.5)	16 (80.0)	20 (100)	0
	gyrA MUT1		A90V	7 (26.9)	10 (31.2)	0	0
	gyrA MUT2		S91P	0	1 (3.1)	0	0
	gyrA MUT3A		D94A	6 (23.1)	3 (9.4)	0	0
	gyrA MUT3B		D94N, D94Y	3 (11.5)	8 (25)	0	0
	gyrA MUT3C		D94G	9 (34.6)	12 (37.5)	0	0
	gyrA MUT3D		D94H	0	1 (3.1)	0	0
	gyrA WT1-3, MUT3C	85–97	D94G	3 (11.5)	4 (12.5)	0	0
	gyrA WT1-3, MUT1, MUT3C	85–97	A90V, D94G	0	1 (3.1)	0	0
	gyrA WT1-3, MUT3A	85–97	D94A	0	2 (6.25)	0	0
	gyrA WT1-3, MUT3B	85–97	D94N, D94Y	0	2 (6.25)	0	0
rrs	*rrs* WT1	1401, 1402		0	32 (100)	0	0
	rrs WT2	1484		26 (100)	32 (100)	20 (100)	0
	rrs MUT1		A1401G, C1402T	26 (100)	0	20 (100)	0
	rrs MUT2		G1484T	0	0	0	0
	rrs WT1-2, MUT1	1401–2, 1484	1401G, C1402T	1 (3.8)	0	0	0
embB	*embB* WT1	306		2 (7.7)	14 (44.8)	3 (15)	324 (73.8)
	embB MUT1A		M306I (ATG→ATA)	11 (42.3)	7 (21.9)	7 (35)	23 (5.25)
	embB MUT1B		M306V	5 (19.2)	10 (31.2)	4 (20)	92 (21)
	Absent WT1		M306I (ATG→ATC/ATT)	8 (30.8)	1 (3.1)	6 (30)	8 (1.8)
	WT1, MUT1A			0	0	0	1 (0.2)
	WT1, MUT1B			0	0	0	8 (1.8)

Definition of abbreviations: AG/CP = aminoglycoside/cyclic peptide; EMB = ethambutol; FLQ = fluoroquinolone; XDR = multidrug-resistant tuberculosis with additional resistance to an FLQ and one of three injectable second-line drugs: amikacin (AMK), capreomycin (CM), or kanamycin (KM).

Values represent numbers, with percentages in parentheses.

through mutations in the *gyrB* gene (34), which are not included in the GenoType MTBDR*sl* LPA. Furthermore, one study showed that heteroresistance accounted for approximately 25% of OFX-resistant isolates that showed resistance in the absence of *gyrA* mutations (35). Should the heteroresistant specimens have been excluded from the performance analysis, the sensitivity, specificity, and κ value would have been 84.2% (95% CI, 71.2–92.1%), 98.9% (95% CI, 97.3–99.6%), and 85.7%, respectively, resulting in lower performance characteristics. On repeat LPA and DST on culture isolates of the heteroresistant specimens, both wild-type and mutant probes were again seen, and after an incubation period of greater than 2 weeks the DST proved to be resistant. This illustrates the need for standardized DST, as reported in even well-established laboratories (36). The advantage of the GenoType MTBDR*sl* LPA is that it is able to detect in a standardized fashion heteroresistant cases, the phenomenon of which is ascribed to superinfections and strain segregation that occur over time (37). Accordingly, it has been suggested that genetically based DST should be used as a "rule in" test for OFX and AMK resistance and that repeating the test during the course of therapy for initially drug–susceptible patients may be essential to "rule in" drug resistance that emerges during treatment (28).

Contamination of the primary MGIT culture used for second-line DST, and the loss of viability of the culture during the DST, are two "diagnostic delay drivers." According to the current South African guidelines, in such instances a new specimen must be obtained from the patient, thereby delaying diagnosis and the initiation of appropriate treatment. This in turn may exacerbate transmission to close contacts and impact on treatment outcome. Of the 104 specimens whose cultures were either contaminated or had lost viability, 102 (98%) gave a valid LPA result for OFX, and 98 (94%) gave a valid LPA result for AMK. This resulted in a significant increase in the yield of DST results of 20.1% for OFX ($P < 0.001$) and 19.3% for AMK ($P < 0.001$). This in turn will have significant cost-saving implications by reducing the number of tests required and will reduce diagnostic delay associated with culture contamination or loss of viability.

The use of molecular tools enables a reduction in TAT (33) and, in this study, the GenoType MTBDR*sl* LPA in conjunction with the GenoType MTBDR*plus* LPA proved to significantly reduce the TAT for second-line DST compared with conventional culture-based DST. On average, the TAT to reporting second-line DST was reduced by 42 days, where the current TAT is 45 days (range, 27–122 d) ($P < 0.001$). The rapid diagnosis of resistance to either OFX or AMK has the potential to impact on treatment outcome, transmission, and amplification of resistance. This is a significant benefit given the high degree of mortality associated with XDR-TB in HIV-coinfected patients (38, 39).

At present the GenoType MTBDR*sl* LPA is designed for the direct analysis of smear-positive specimens. However, in settings where TB–HIV coinfection is high, because of the high proportion of smear-negative cases (40), its usefulness will not have the same impact. Despite this limitation, the reduction in TAT of 31 days (range, 13–82 d) can still be achieved when using the primary culture from smear-negative cases. This is routine practice in laboratories that rely on the GenoType MTBDR*plus* LPA for diagnosis of INH and RIF resistance. Rapid second-line DST results will enable appropriate patient treatment where heteroresistance is observed, because it has been observed that the population structure of clones may change during treatment, resulting in emerging resistance (35). The heteroresistant patterns observed in the GenoType MTBDR*sl* LPA are thus an early indication of emerging resistance.

The high proportion of RIF-monoresistant specimens (15.8%) in this study further supports the described increase in RIF-monoresistant strains in the Western Cape (41), where it was found that the most significant percentages were from the Beijing strain family (27.9%), family 11 (7.7%), and the low copy

TABLE 5. SEQUENCE ANALYSIS OF REPRESENTATIVE SAMPLE SET OF ISOLATES WITH DISCREPANT LINE PROBE ASSAY AND DRUG SUSCEPTIBILITY TESTING RESULTS AND EXTENSIVELY DRUG-RESISTANT, OFLOXACIN-MONORESISTANT, AMIKACIN-MONORESISTANT, AND SUSCEPTIBLE ISOLATES

			Sequencing					
					Amino Acid Change (Nucleotide Changes)			
Results Group ($n = 47$)	Conventional DST	GenoType MTBDR*s*l LPA	Valid	Invalid	*gyrA*	*rrs*	*embB*	Frequency n (%)
XDR (OFL:AMK)								
XDR	11	11	10	1	A90V	A1401G	M306I	2 (18.2)
					D94A	A1401G	M306I	1 (9.10)
					D94A	A1401G	INV	1 (9.10)
					D94G	A1401G	M306I	4 (36.7)
					D94G	A1401G	WT	1 (9.10)
					S91P	A1401G	M306V	1 (9.10)
					D89N	INV	INV	1 (9.10)
Discordant $DST^{R:R}/LPA^{S:R}/Seq^{S:R}$ *	2	2	2	0	WT	A1401G	M306I	2 (100)
OFL								
Monoresistant	4	4	4	0	D94G	WT	WT	2 (50)
					S91P	WT	WT	1 (25)
					A90V	WT	WT	1 (25)
Discordant $DST^{R}/LPA^{S}/Seq^{S}$	3	3	3	0	WT	WT	M306V	1 (20)
					WT	WT	WT	1 (20)
					WT	WT	WT	1 (20)
					WT	A1401G	M306I	2 (40)*
Discordant $DST^{S}/LPA^{R}/Seq^{R}$	5	5	5	0	A90V	WT	M306V	1 (20)
					A90V	WT	WT	1 (20)
					A90V	INV	M306I	1 (20)
					D94G	WT	M306V	1 (20)
					D94Y	WT	M306V	1 (20)
AMK (n = 4)								
Monoresistant	4	4	4	0	WT	A1401G	M306I	2 (50)
					WT	A1401G	M306V	1 (25)
					WT	A1401G	WT	1 (25)
Discordant $DST^{R}/LPA^{S}/Seq^{S}$	0	0	0	0	—	—	—	0
Discordant $DST^{S}/LPA^{R}/Seq^{R}$	1	1	1	0	D94N	A1401G	M306I	1 (100)
Discordant $DST^{S}/LPA^{XDR}/Seq^{R}$	1	1	1	0	A90V	A1401G	M306I	1 (100)
OFL/AMK susceptible								
Susceptible	12	12	12	0	WT	WT	WT	7 (58.3)
					WT	WT	M306V	5 (41.7)
Discordant $DST^{S:S}/LPA^{S:S}/Seq^{R:R}$	0	0	1	0	A90V	A1401G	WT	1 (100)
Discordant $DST^{S:S}/LPA^{S:S}/Seq^{S:R}$	0	0	1	0	WT	A1401G	M306V	1 (100)
Discordant $DST^{S:S}/LPA^{S:S}/Seq^{R:S}$	0	0	2	0	A90V	WT	M306V	1 (50)
					D94G	WT	M306V	1 (50)
EMB (n = 47)								
LPA^{R}/Seq^{R}	ND	27	27	2	WT	WT	M306V	6 (22.2)
					WT	A1401G	M306I	4 (14.8)
					WT	A1401G	M306V	2 (7.4)
					A90V	INV	M306I	1 (3.7)
					A90V	A1401G	M306I	2 (7.4)
					A90V	WT	M306V	2 (7.4)
					D89N	INV	INV	1 (3.7)
					D94A	A1401G	M306I	1 (3.7)
					D94A	A1401G	INV	1 (3.7)
					D94G	A1401G	M306I	4 (14.8)
					D94G	WT	M306V	2 (7.4)
					D94N	A1401G	M306I	1 (3.7)
					D94Y	WT	M306V	1 (3.7)
					S91P	A1401G	M306V	1 (3.7)
LPA^{R}/Seq^{S}	ND	0	0	0	—	—	—	0
LPA^{S}/Seq^{R}	ND	1	1	0	A90V	A1401G	M306I	1 (100)
LPA^{S}/Seq^{S}	ND	17	17	0	WT	WT	WT	9 (52.9)
					WT	A1401G	WT	1 (5.88)
					S91P	WT	WT	1 (5.88)
					D94G	A1401G	WT	1 (5.88)
					D94G	WT	WT	2 (11.8)
					A90V	A1401G	WT	1 (5.88)
					A90V	WT	WT	2 (11.8)

Definition of abbreviations: AMK = amikacin; DST = conventional drug susceptibility testing; EMB = ethambutol; INV = invalid; LPA = line probe assay; ND = not done; OFL = ofloxacin; R = resistant; S = susceptible; Seq = sequencing; XDR = extensively drug resistant.

*Count included in discordant XDR group.

clade (LCC) family (6.7%). Furthermore, none of the RIF-monoresistant strains that were submitted for INH DST exhibited resistance toward the drug, even though 6–7% of all RIF-monoresistant strains are resistant to INH at 0.1 μg/ml (unpublished data from the NHLS LIMS), indicating that the genes responsible for INH resistance other than *katG* and *inhA* must also be screened for.

In summary, the results obtained in this study suggest that the GenoType MTBDR*sl* LPA is a rapid and reliable DST for OFX and AMK. Because the GenoType MTBDR*sl* LPA makes use of the same equipment and standard operating procedures as the GenoType MTBDR*plus* LPA, this allows for "diagnostic fit" within the diagnostic algorithm (42), thereby drastically decreasing the diagnostic delay for second-line drug resistance results. The availability of this information will guide initiation of appropriate treatment regimens, thereby reducing transmission and improving treatment outcome. The implementation of this assay will also reduce laboratory costs, thereby alleviating the financial burden of the national tuberculosis control programs.

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