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Performance of the MTBDR*sl* assay in Georgia

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SUMMARY

SETTING: The country of Georgia has a high burden of multi- (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB).

OBJECTIVE: To assess the performance of the GenoType® MTBDR*sl* assay in the detection of resistance to kanamycin (KM), capreomycin (CPM) and ofloxacin (OFX), and of XDR-TB.

DESIGN: Consecutive acid-fast bacilli smear-positive sputum specimens identified as MDR-TB using the MTBDR*plus* test were evaluated with the MTBDR*sl* assay and conventional second-line drug susceptibility testing (DST).

RESULTS: Among 159 specimens, amplification was adequate in 154 (97%), including 9 of 9 culture-negative and 2 of 3 contaminated specimens. Second-line DST revealed that 17 (12%) *Mycobacterium tuberculosis* isolates were XDR-TB. Compared to DST, the MTBDR*sl* had 41% sensitivity and 98% specificity in detecting XDR-TB and 81% sensitivity and 99% specificity in detecting OFX resistance. Sensitivity was low in detecting resistance to KM (29%) and CPM (57%), while specificity was respectively 99% and 94%. Median times from sputum collection to second-line DST and MTBDR*sl* results were 70–104 vs. 10 days.

CONCLUSION: Although the MTBDR*sl* assay had a rapid turnaround time, detection of second-line drug resistance was poor compared to DST. Further genetic mutations associated with resistance to second-line drugs should be included in the assay to improve test performance and clinical utility.

KEY WORDS: line-probe assays; tuberculosis; drug resistance

A MAJOR THREAT to tuberculosis (TB) control efforts is the increasing global burden of drug-resistant TB. Inappropriate treatment regimens and poor adherence to treatment are the most common causes of drug-resistant TB and they have led in large part to the development and transmission of both multidrug-resistant TB (MDR-TB; i.e., resistance to isoniazid [INH] and rifampin [RMP]) and extensively-drug resistant TB (XDR-TB; i.e., MDR-TB plus resistance to fluoroquinolones and any injectable agent). The World Health Organization (WHO) has estimated a worldwide prevalence of 660 000 cases of MDR-TB and 150 000 MDR-TB-related deaths annually.[1] Especially worrying is the increasing prevalence of difficult-to-treat XDR-TB, which has been found in 84 countries and is estimated to be present in 9% of patients with MDR-TB.[1] The emergence of XDR-TB has led to the development of virtually untreatable TB in many settings.[2,3]

The highest rates of drug-resistant TB occur in the former Soviet republics, including the country of Georgia, which is one of 27 high-burden MDR-TB countries.[1] Data from the Georgian National Tuberculosis Program (NTP) from 2011 found the prevalence of MDR-TB among newly diagnosed patients to be 10.8%, and 31.4% among previously treated patients; 6.4% of those with MDR-TB had XDR-TB. With the support of the Global Fund to Fight AIDS, Tuberculosis and Malaria, the Georgian NTP has achieved universal access to diagnosis and treatment of MDR- and XDR-TB, and has more recently validated and implemented the commercially available MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) in clinical practice.[4]

The development of commercially available molecular diagnostic tests to detect drug-resistant TB, including the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and MTBDR*plus* assays, have been hailed as significant achievements and provide clinicians with accurate tests for the rapid detection of RMP-resistant and MDR-TB, respectively. In 2009, Hain Lifescience introduced a new line-probe assay (LPA), the MTBDR*sl*, for the rapid detection of mutations associated with resistance to fluoroquinolones, aminoglycosides, cyclic peptides and ethambutol (Hain Lifescience product page, http://www.hain-lifescience.de.en/products/microbiology/mycobacteria/genotype-mtbdrsl.html).

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Investigations on the utility of the MTBDR*sl* assay are limited, and WHO recommendations are based on low-quality evidence.[5] In addition, study results have varied by geographic location, and few have been performed using clinical specimens. MTBDR*sl* implementation projects will help inform current guidelines and set an agenda for future research efforts.

Our primary objective was to assess the performance of the MTBDR*sl* assay compared to conventional culture and drug susceptibility testing (DST) methods when implemented in the workflow of a high-volume National TB Reference Laboratory (NRL).

METHODS

Setting

The study was conducted at the NRL of the Georgian NTP in Tbilisi, Georgia, which processed ~18 000 sputum specimens in 2011. Acid-fast bacilli (AFB) smear-positive sputum specimens from TB suspects throughout Georgia from November 2011 to April 2012 were collected. While data on human immunodeficiency virus (HIV) status were not available, previous research has demonstrated low HIV prevalence (~1%) among TB patients in Georgia.[6]

Approval for this study was received from the Georgian National Center for Tuberculosis and Lung Disease and the Emory University Institutional Review Boards.

Culture and drug susceptibility testing

Two routine sputum specimens were obtained from each patient, and direct smears with Ziehl-Neelsen staining were examined using light microscopy at local microscopy centers in Georgia. One AFB smear-positive sample was sent to the NRL, where it was processed using standard methodologies (decontaminated in a Biosafety Laboratory 2+ area with *N*-acetyl-L-cysteine-sodium hydroxide, centrifuged, and the sediment suspended in 1.5 ml phosphate buffer).[7] The processed specimen was inoculated onto both Löwenstein-Jensen (LJ) based solid medium and the BACTEC™ MGIT™ 960 broth culture system (BD, Sparks, MD, USA). Positive cultures by either method were confirmed as *Mycobacterium tuberculosis* complex using the MTBDR*plus* assay (Hain Lifescience, product page, http://www.hain-lifescience.de.en/products/microbiology/mycobacteria/genotype-mtbdrplus.html). DST against first-line drugs was performed using conventional methods, as previously described.[4,8] DST against second-line drugs was performed using the proportion method on LJ medium with the following drug concentrations: ethionamide 40.0 μg/ml; ofloxacin (OFX) 2.0 μg/ml; para-aminosalicylic acid 0.5 μg/ml, capreomycin (CPM) 40.0 μg/ml and kanamycin (KM) 30.0 μg/ml.[9] The Georgian NRL has undergone external quality assessment by the Antwerp WHO Supranational TB Reference Laboratory (SNRL) annually since 2005. In 2012, SNRL quality assurance certification was given for DST against INH, RMP, KM, CPM and OFX.

Molecular testing

All molecular testing was performed using a portion of the same sputum specimen used for culture. A 500 μl portion of decontaminated sample was used to perform the MTBDR*plus* assay according to the manufacturer's instructions. A portion of extracted DNA was kept refrigerated (+4°C) until the MTBDR*plus* assay results were obtained. If both RMP and INH resistance were detected, the MTBDR*sl* assay was performed. The saved DNA pellet was centrifuged at 13 000× *g* for 5 min and 5 μl of supernatant was removed. The DNA was added to 45 μl amplification mix and amplified using 42 polymerase chain reaction cycles based on the manufacturers' recommendations for clinical specimens, followed by hybridization and test readout steps. Negative controls were used for quality assurance with each run of the MTBDR*sl* assay.

Definitions

New TB cases were patients who had received ⩽30 days of anti-tuberculosis drug treatment; retreatment cases were those with a previous history of anti-tuberculosis treatment for >30 days. A completely interpretable MTBDR*sl* result was defined as a test strip with all control markers positive.

Data analysis

All data were entered into an online REDCap (Research Electronic Data Capture) database (Vanderbilt University, Nashville, TN, USA)[10] and analyzed using SAS, version 9.3 (Statistical Analysis Software Institute, Cary, NC, USA). The sensitivity, specificity, positive predictive value and negative predictive value of the MTBDR*sl* assay in detecting resistance to OFX, CPM and KM were calculated using conventional DST results as the reference standard. Turnaround time was calculated as time between the date of sputum collection and the date of culture, DST and MTBDR*sl* results. The degree of agreement between test results was assessed using the kappa statistic. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 159 patients with a smear-positive AFB sputum specimen and MTBDR*plus* result indicating resistance to RMP and INH were enrolled. Among these, 69 (43%) were new TB cases, and 90 (57%) were retreatment TB cases. Overall, 147 (92.5%) samples were culture-positive for *M. tuberculosis* by either solid or liquid culture, 9 were culture-negative and 3 had contaminated cultures (Figure 1). Of 147 culture-positive patients, 142 (97%) had complete first- and

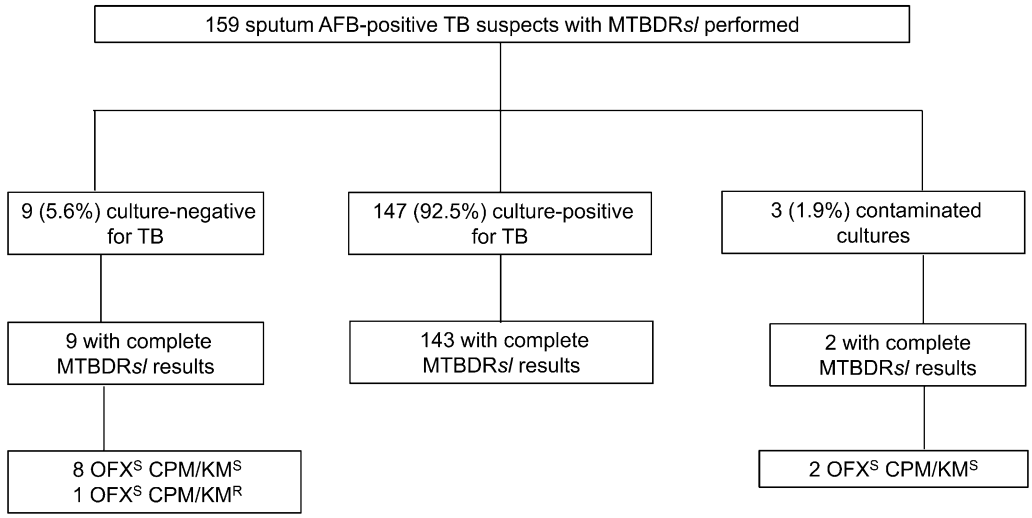

Figure 1 Sputum culture results for all AFB smear-positive TB suspects and corresponding complete MTBDR*sl* assay results. AFB = acid-fast bacilli; TB = tuberculosis; OFX = ofloxacin; S = susceptible; CPM = capreomycin; KM = kanamycin; R = resistant.

second-line DST results. First-line DST of *M. tuberculosis* was performed in liquid (n = 57, 40%) or solid media (n = 85, 60%); all second-line DST was performed using solid media. Excluding four patients with non-interpretable MTBDR*sl* results, conventional second-line DST revealed 60 (43%) *M. tuberculosis* isolates with no OFX, CPM or KM resistance, 17 (12%) with XDR-TB, 9 (7%) with OFX resistance alone and 52 (38%) isolates resistant to CPM and/or KM without OFX resistance (Figure 2).

*MTBDR*sl *assay*

Among the 147 sputum samples with a positive culture for *M. tuberculosis*, 143 (97%) had a completely interpretable MTBDR*sl* assay. The four non-interpretable MTBDR*sl* assay results were due to inadequate amplification. The MTBDR*sl* assay gave interpretable results for most (11/12, 92%) specimens with negative or contaminated sputum cultures (Figure 1). A comparison of resistance patterns generated by the MTBDR*sl* assay and conventional methods among *M. tuberculosis* isolates recovered from the 138 patients with an interpretable MTBDR*sl* assay and second-line DST is shown in Figure 2. Performance parameters of the MTBDR*sl* assay as compared to conventional second-line DST are displayed in Table 1. The sensitivity of the MTBDR*sl* assay in the detection of OFX resistance (80.8%, 95% confidence interval [CI] 65.6–95.9) was moderate, but it was poor for the detection of CPM (56.5%, 95%CI 36.3–76.8)

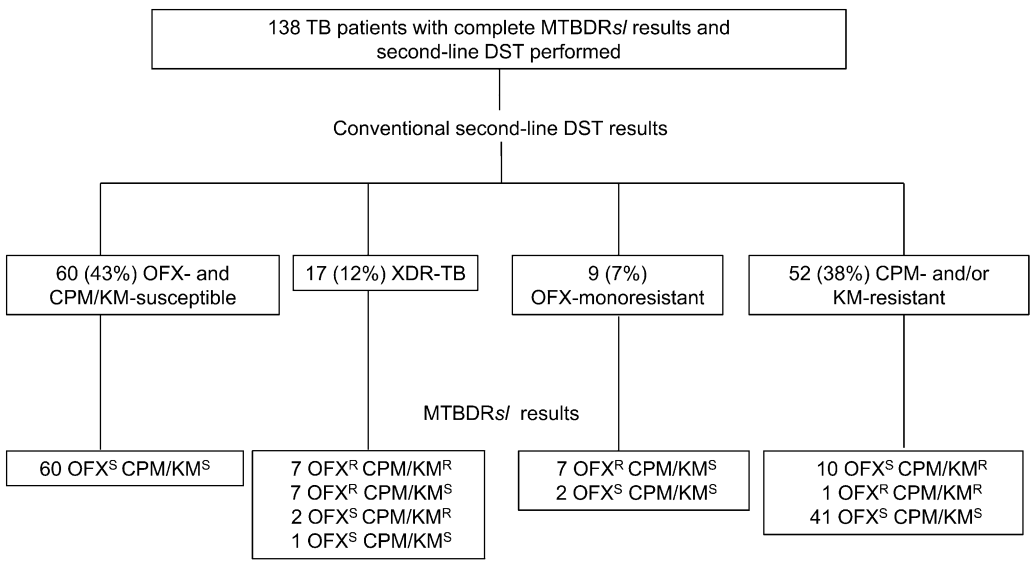

Figure 2 Distribution of MTBDR*sl* assay results according to phenotypic drug resistance patterns using conventional DST. TB = tuberculosis; DST = drug susceptibility testing; OFX = ofloxacin; CPM = capreomycin; KM = kanamycin; XDR-TB = extensively drug-resistant TB; S = susceptible; R = resistant.

Table 1 Performance parameters of the GenoType® MTBDR*sl* assay in detecting any resistance to OFX, CPM, KM and XDR-TB compared to conventional drug susceptibility testing (reference standard; $n = 138$)

	OFX % (95%CI)	CPM % (95%CI)	KM % (95%CI)	XDR-TB % (95%CI)
True-susceptible	111	108	71	119
True-resistant	21	13	19	7
False-susceptible	5	10	47	10
False-resistant	1	7	1	2
Sensitivity	80.8 (65.6–95.9)	56.5 (36.3–76.8)	28.8 (17.9–39.7)	41.2 (17.8–64.6)
Specificity	99.1 (0.97–100)	93.9 (89.5–98.3)	98.6 (95.9–100)	98.3 (96.0–100)
PPV	95.5 (86.8–100)	65.0 (44.1–85.9)	95.0 (85.5–100)	77.8 (50.1–100)
NPV	95.7 (92.0–99.4)	91.5 (86.4–96.5)	59.8 (50.1–68.7)	92.2 (87.5–96.8)
κ	0.85 (0.73–0.96)	0.53 (0.34–0.73)	0.28 (0.16–0.40)	0.49 (0.25–0.74)

OFX = ofloxacin; CPM = capreomycin; KM = kanamycin; XDR-TB = extensively drug-resistant tuberculosis; PPV = positive predictive value; NPV = negative predictive value.

and KM (28.8%, 95%CI 17.9–39.7) resistance as well as detection of XDR-TB (41.2%, 95%CI 17.8–64.6). The specificity of the MTBDR*sl* assay in the detection of OFX (99.1%, 95%CI 97.4–100), CPM (93.9%, 95%CI 89.5–98.3) and KM (98.6%, 95%CI 95.9–100) resistance and XDR-TB (98.3%, 95%CI 96.0–100) was high. There was good agreement between the MTBDR*sl* assay and DST in the detection of OFX resistance ($\kappa = 0.85$, 95%CI 0.73–0.96), and poor agreement in the detection of CPM ($\kappa = 0.53$, 95%CI 0.34–0.73) and KM ($\kappa = 0.28$, 95%CI 0.16–0.40) resistance and XDR-TB ($\kappa = 0.49$, 95%CI 0.25–0.74).

Time to results

Time to detection of drug resistance to OFX, CPM and KM was significantly shorter for the MTBDR*sl* assay as compared to conventional culture methods and DST (Table 2). The median time for detection of resistance using the MTBDR*sl* assay was 10 days, as compared to conventional methods in which first-line DST was performed on liquid media and second-line DST on solid media (70 days) and those that had first- and second-line DST performed on solid media (104 days).

Genetic mutations

The distributions of genetic mutations of drug-resistant *M. tuberculosis* isolates with an interpretable MTBDR*sl* assay ($n = 138$) are shown in Tables 3 and 4. The most common resistance mutation for any OFX resistance was D94G (48%), followed by A90V (29%). Furthermore, a similar percentage had a lack of binding to the *gyr*A wild-type 2 (WT2; 48%) and WT3 (29%) probes. The majority of isolates lacked binding to a WT probe and had a drug resistance mutation (13/21, 62%). The single false-resistant fluoroquinolone isolate lacked binding to the WT2 probe. Almost all of the CPM and KM phenotypic drug-resistant *M. tuberculosis* isolates had an A140G mutation (100% and 84%, respectively), and lacked binding to the WT1 probe (92% and 90%, respectively). Among the 7 false-resistant CPM isolates, 5 had drug resistance mutations and all 7 (100%) were phenotypically resistant to KM. The one false-resistant KM isolate had an A1401G mutation and was phenotypically resistant to CPM. As regards heteroresistance, 6 *M. tuberculosis* isolates (2 to both OFX and KM and/or CPM and 4 only to OFX) had a drug-resistant mutation without lack of binding to the corresponding WT probe (Table 3 and 4).

DISCUSSION

In a country with a high burden of drug-resistant TB, we have demonstrated that the MTBDR*sl* assay can be successfully introduced into the routine workflow of a high-volume NRL, and that results can be provided in a timely fashion; however, the performance of the assay was suboptimal. We found moderate sensitivity for OFX (81%), but poor sensitivity for CPM (57%), KM (29%) and XDR-TB (41%) detection compared to conventional methods, including culture plus DST. Specificity was much higher (≥93%) for all

Table 2 Median time to results in days for detection of tuberculosis and associated drug resistance ($n = 138$)

	Solid medium first-line DST median (25th–75th percentiles)	Liquid medium first-line DST median (25th–75th percentiles)	MTBDR*plus* assay median (25th–75th percentiles)	Liquid medium first-line DST/ solid medium second-line DST median (25th–75th percentiles)	Solid medium first-line and second-line DST median (25th–75th percentiles)	MTBDR*sl* assay median (25th–75th percentiles)
All cases, days	71 (64–81)	21 (18–27)	5 (3–7)	70 (65–76)	104 (97–112)	10 (7–12)
XDR-TB, days	71 (64–76)	23 (21–28)	6 (4–8)	71 (64–71)	102 (96–107)	12 (8–19)

DST = drug susceptibility testing; XDR-TB = extensively drug-resistant tuberculosis.

Table 3 Pattern of genetic mutations in *Mycobacterium tuberculosis* isolates with phenotypic OFX drug resistance and molecular fluoroquinolone drug resistance using the MTBDR*sl* assay ($n = 21$)

*gyr*A gene band	Gene region	OFX-monoresistant ($n = 7$) *n* (%)	XDR-TB ($n = 14$) *n* (%)	Any OFX-resistant ($n = 21$) *n* (%)
ΔWT1 only	85–90	1 (14)	1 (7)	2 (10)
ΔWT2 only	89–93	—	1 (7)	1 (5)
ΔWT2+MUT1	89–93, A90V	2 (29)	2 (14)	4 (19)
ΔWT2+MUT2	89–93, S91P	—	1 (7)	1 (5)
ΔWT2+MUT1+MUT3D	89–93, A90V, D94H	1 (14)	—	1 (5)
ΔWT2+ΔWT3+MUT2	89–93, 92–97, S91P	—	1 (7)	1 (5)
ΔWT3+MUT3A	92–97, D94A	—	1 (7)	1 (5)
ΔWT3+MUT3C	92–97, D94G	1 (14)	4 (29)	5 (24)
MUT1+MUT3B/3C	A90V, D94N/Y, D94G	—	1 (7)	1 (5)
MUT3C only	D94G	2 (29)	2 (14)	4 (19)

OFX = ofloxacin; XDR-TB = extensively drug-resistant tuberculosis; Δ = lack of wild-type band.

categories. Our study is only the third published report evaluating the MTBDR*sl* assay under routine diagnostic conditions, and our results are in agreement with recent meta-analyses finding overall poor performance of the MTBDR*sl* assay.[6,11,12] Improvements in the MTBDR*sl* assay, particularly in detecting KM and CPM resistance, and/or newer technologies, are needed for the rapid and accurate detection of second-line anti-tuberculosis drug resistance.

Our study results provide critical information on the performance of the MTBDR*sl* assay when implemented in the normal workflow using clinical specimens. Five studies have been published evaluating MTBDR*sl* performance using clinical specimens; only two of these used non-frozen clinical specimens and were performed under routine diagnostic conditions.[12–16] One of the studies, carried out in the Western Cape Province, South Africa, found disparate results compared to our findings. Among 516 patients, they found high sensitivity of the MTBDR*sl* in detecting OFX (90.7%), AMK (100%) and XDR-TB (92.3%), and high specificity for all categories (⩾98%).[16] The excellent performance of the MTBDR*sl* assay in their setting may have been related to distinct MDR and XDR-TB *M. tuberculosis* strains circulating in the Western Cape Province and also that they tested for amikacin (AMK) and not KM phenotypic resistance.[17] Mutations in the *rrs* gene have been more commonly found among AMK-resistant vs. KM-resistant strains.[18] The findings of another of the previous studies, conducted in Russia, were closer to our results, including low sensitivity for KM (9.4%) and XDR-TB (13.6%).[12] Our results are in line with those found in recent meta-analyses, including a comprehensive review of published and unpublished studies by a WHO expert group.[5] Among published reports, the sensitivity of the MTBDR*sl* assay in detecting resistance to fluoroquinolones (87%), CPM (82%) and KM (44%) was poor. The WHO report also found that the sensitivity of MTBDR*sl* in detecting XDR-TB varied widely among studies (22.6–100%), but did find overall high specificity (91.8–100%). Our findings provide further evidence supporting WHO recommendations declaring the MTBDR*sl* assay unfit to replace conventional phenotypic DST or for designing individualized MDR or XDR-TB treatment regimens. Given its high specificity, the MTBDR*sl* may have a role 'ruling in' XDR-TB disease among high-risk patients.

The poor sensitivity of the MTBDR*sl* assay reflects our limited knowledge of drug resistance mechanisms and mutations. A recent review of genetic mutations causing resistance to injectable second-line agents evaluated over 1500 *M. tuberculosis* isolates and found that the A1401G mutation could explain only 76% of CPM and 56% of KM resistance and,

Table 4 Pattern of genetic mutations in *Mycobacterium tuberculosis* isolates with phenotypic CPM or KM drug resistance and molecular injectable agent resistance using the GenoType® MTBDR*sl* assay

rrs gene band	Gene region	Any CPM-resistant ($n = 13$) *n* (%)	Any KM-resistant ($n = 19$) *n* (%)	CPM-susceptible, KM-resistant ($n = 7$) *n* (%)	CPM- and KM-resistant ($n = 12$) *n* (%)	XDR-TB ($n = 9$) *n* (%)
ΔWT1 only	1401–2		2 (11)	2 (29)		
ΔWT1+MUT1	1401–2, A1401G	12 (92)	15 (79)	4 (57)	11 (92)	7 (78)
ΔWT2	1484					
MUT1 only	A1401G	1 (8)	1 (5)		1 (8)	1 (11)
MUT2 only	G1484T		1 (5)	1 (14)		1 (11)

CPM = capreomycin; KM = kanamycin; XDR-TB = extensively drug-resistant; Δ = lack of wild-type band.

furthermore, that it was present in 7% of CPM-susceptible isolates.[18] Our results were worse, with mutations in the 1401 region of the *rrs* gene present in respectively 57% and 29% of isolates phenotypically resistant to CPM and KM, and in 9% of CPM-susceptible isolates. Ongoing work has found that additional mutations in the *rrs, eis* promoter, *tly*A and *gid*B genes may be associated with injectable drug resistance and might explain the poor sensitivity of the MTBDR*sl* assay. With regard to fluoroquinolones, we detected mutations in the 90, 91 and 94 codons of the *gyr*A gene in 81% of the OFX-resistant cases, similar to other reports.[11] Mutations in the *gyr*B gene or in genes encoding the *Mfp*A protein may also cause fluoroquinolone resistance and could explain some cases with a normal *gyr*A gene and phenotypic fluoroquinolone resistance. Mutations in the *eis* gene could be responsible for the poor sensitivity of the MTBDR*sl* in detecting KM resistance. Utilizing both the MTBDR*sl* assay and DNA sequencing, Huang et al. found that the sensitivity of MTBDR*sl* in detecting drug resistance to KM could be increased by approximately 27% by adding new alleles of the *eis* promoter into molecular analysis.[19] Additional genomic studies among Beijing strains from Russia, which also have high rates of KM resistance, found that a significant number of CPM- and AMK-susceptible, but KM-resistant strains harbored mutations in the *eis* gene.[20,21] This line of evidence may, in part, explain the poor performance of the MTBDR*sl* assay in Georgia, as the Beijing strain is the most common genotype in the country.[22]

An important finding of our study is the feasibility of implementing an 'add on' rapid molecular test for XDR-TB detection in a busy NRL that already performs LPA for MDR-TB detection. The majority of the MTBDR*sl* assays had sufficient amplification and interpretable results (97%), a rate higher than the percentage of cultures positive for *M. tuberculosis* (92%). We also found that, incorporated into the normal workflow, MTBDR*sl* assay results were available in <2 weeks as compared to 70–104 days using conventional DST methods. If the sensitivity of the MTBDR*sl* or other future LPAs can be improved, this rapid turnaround time could help ensure earlier treatment with effective regimens, which could result in improved treatment outcomes, decreased development of amplified drug resistance and prevention of disease transmission.[23]

Genetic sequencing of *M. tuberculosis* isolates with discordant MTBDR*sl* and DST results would have helped identify non-assay mutations responsible for second-line drug resistance. As a consequence of only testing the initial sputum specimen, we may have had false-negative MTBDR*sl* results and hence lower MTBDR*sl* sensitivity due to heteroresistant bacilli populations. A recent study found 5% and 8% of *M. tuberculosis* isolates with phenotypic drug resistance to respectively OFL and AMK, without molecular markers of drug resistance.[24] Subsequent DNA sequencing of single colonies selected in the presence of OFL and AMK revealed underlying mutations in 78% and 100% of the isolates, thus demonstrating heteroresistance. A further study found that the MTBDR*plus* assay had poor sensitivity in detecting INH resistance in a sample of 1% resistant bacteria.[25] These findings demonstrate the challenges posed by heteroresistant bacilli with regard to genetics-based drug resistance testing. The performance of the MTBDR*sl* was compared to WHO-recommended methods for phenotypic second-line DST; however, these methods have not been standardized internationally, and studies evaluating the reproducibility of DST against second-line anti-tuberculosis drugs are limited.[26,27]

CONCLUSION

We have demonstrated the feasibility of implementing the MTBDR*sl* assay in a 'real world' setting in a country with a high burden of drug-resistant TB, but found that the assay lacks sufficient accuracy to be recommended for clinical use in this setting. For the MTBDR*sl* or other new assays to have a clinical impact on the treatment and transmission of XDR-TB, they need to include additional genetic mutations responsible in particular for second-line drug resistance.

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RÉSUMÉ

CADRE : Dans le pays de Géorgie, le fardeau des tuberculoses à germes multirésistants (TB-MDR) et ultrarésistants (TB-XDR) est élevé.

OBJECTIF : Evaluer les performances du test GenoType® MTBDR*sl* dans la détection de la résistance à la kanamycine (KM), à la capréomycine (CPM) et à l'ofloxacine (OFX) et celle de la TB-XDR.

SCHÉMA : On a évalué des échantillons de crachats consécutifs, avec frottis positifs pour les BAAR et identifiés comme TB-MDR par MTBDR*plus* testing au moyen du test MTBDR*sl* et du test conventionnel de sensibilité (DST) aux médicaments de deuxième ligne.

RÉSULTATS : Sur 159 échantillons, l'amplification a été correcte dans 154 (97%), y compris 9 échantillons à culture négative et 2 des 3 échantillons contaminés. La DST à l'égard des médicaments de deuxième ligne a révélé 17 isolats de *Mycobacterium tuberculosis* (12%) comme TB-XDR. Par comparaison avec la DST, la MTBDR*sl* a une sensibilité de 41% et une spécificité de 98% pour la détection de TB-XDR et une sensibilité de 81% et une spécificité de 99% pour la détection de la résistance à l'OFX. La sensibilité est faible dans la détection de la résistance à KM (29%) et à CPM (57%), alors que la spécificité est respectivement de 99% et de 94%. La durée médiane entre la collecte des crachats et les résultats de la DST de deuxième ligne ont été de 70 à 104 jours vs. 10 jours après MTBDR*sl*.

CONCLUSION : Le test MTBDR*sl* a une durée d'exécution rapide ; toutefois, la détection de la résistance aux médicaments de deuxième ligne est médiocre par comparaison à la DST. Il faudrait inclure dans le test des mutations génétiques complémentaires associées à la résistance aux médicaments de deuxième ligne afin d'améliorer les performances du test et son intérêt clinique.

RESUMEN

MARCO DE REFERENCIA: Georgia es un país con una alta carga de morbilidad por tuberculosis multidrogorresistente (TB-MDR) y extremadamente drogorresistente (TB-XDR).

OBJETIVO: Evaluar el rendimiento diagnóstico de la prueba GenoType® MTBDR*sl* en la detección de la resistencia a kanamicina (KM), capreomicina (CPM) y ofloxacino (OFX) y de la TB-XDR.

MÉTODOS: Se examinaron las muestras de esputo con baciloscopia positiva recibidas consecutivamente y definidas como TB-MDR, mediante confirmación con la prueba MTBDR*sl* y con la prueba corrientes de sensibilidad (DST) a los medicamentos antituberculosos de segunda línea.

RESULTADOS: De las 159 muestras, la amplificación fue adecuada en 154 (97%), incluidas 9 de las 9 muestras con cultivo negativo y 2 de las 3 muestras contaminadas. La DST a los medicamentos antituberculosos de segunda línea revelaron que 17 aislados de *Mycobacterium tuberculosis* eran TB-XDR (12%). En comparación con la DST, la prueba MTBDR*sl* ofreció una sensibilidad de 41% y una especificidad de 98% en la detección de cepas TB-XDR y una sensibilidad de 81% y una especificidad de 99% en la detección de la resistencia a OFX. La prueba exhibió una baja sensibilidad en la detección de la resistencia a KM (29%) y CPM (57%), pero la especificidad fue 99% y 94%, respectivamente. La mediana del lapso entre la recogida del esputo y la obtención de los resultados de la DST osciló entre 70 y 104 días, comparado con 10 días al aplicar la prueba MTBDR*sl*.

CONCLUSIÓN: La prueba MTBDR*sl* ofreció un corto lapso hasta la obtención del resultado analítico; sin embargo, la detección de la resistencia a los medicamentos de segunda línea fue deficiente en comparación con la DST. Es preciso incluir en esta prueba nuevas mutaciones genéticas asociadas con la resistencia a los antituberculosos de segunda línea, a fin de mejorar su rendimiento diagnóstico y su utilidad clínica.