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Xpert *Mycobacterium tuberculosis*/Rifampicin–Detected Rifampicin Resistance is a Suboptimal Surrogate for Multidrug-resistant Tuberculosis in Eastern Democratic Republic of the Congo: Diagnostic and Clinical Implications

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Background. Rifampicin (RIF) resistance is highly correlated with isoniazid (INH) resistance and used as proxy for multidrug-resistant tuberculosis (MDR-TB). Using MTBDR*plus* as a comparator, we evaluated the predictive value of Xpert MTB/RIF (Xpert)–detected RIF resistance for MDR-TB in eastern Democratic Republic of the Congo (DRC).

Methods. We conducted a cross-sectional study involving data from new or retreatment pulmonary adult TB cases evaluated between July 2013 and December 2016. Separate, paired sputa for smear microscopy and MTBDR*plus* were collected. Xpert testing was performed subject to the availability of Xpert cartridges on sample remnants after microscopy.

Results. Among 353 patients, 193 (54.7%) were previously treated and 224 (63.5%) were MTBDR*plus* TB positive. Of the 224, 43 (19.2%) were RIF monoresistant, 11 (4.9%) were INH monoresistant, 53 (23.7%) had MDR-TB, and 117 (52.2%) were RIF and INH susceptible. Overall, among the 96 samples detected by MTBDR*plus* as RIF resistant, 53 (55.2%) had MDR-TB. Xpert testing was performed in 179 (50.7%) specimens; among these, 163 (91.1%) were TB positive and 73 (44.8%) RIF resistant. Only 45/73 (61.6%) Xpert-identified RIF-resistant isolates had concomitant MTBDR*plus*-detected INH resistance. Xpert had a sensitivity of 100.0% (95% CI, 92.1–100.0) for detecting RIF resistance but a positive-predictive value of only 61.6% (95% CI, 49.5–72.8) for MDR-TB. The most frequent mutations associated with RIF and INH resistance were S531L and S315T1, respectively.

Conclusions. In this high-risk MDR-TB study population, Xpert had low positive-predictive value for the presence of MDR-TB. Comprehensive resistance testing for both INH and RIF should be performed in this setting.

Keywords. GenoType MTBDR*plus* assay; drug resistance; *rpoB* mutations; *inhA* mutations; DRC.

Multidrug-resistant tuberculosis (MDR-TB), defined as TB disease with *Mycobacterium tuberculosis* (MTB) strains resistant to at least rifampicin (RIF) and isoniazid (INH), threatens the global TB response, particularly in low- and middle-income countries (LMICs) [1]. It is commonly assumed that RIF resistance occurs when INH resistance is present. Rifampicin-resistant TB has hence been used as a proxy for MDR-TB and is treated with second-line regimens that omit INH [2, 3].

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However, the validity of this assumption may be setting specific. Indeed, testing only for RIF resistance might unnecessarily deny access to INH for patients with low-level INH resistance or no INH resistance (ie, RIF-monoresistant TB). Conversely, INH-monoresistant, RIF-susceptible TB that is, on the basis of an Xpert RIF–susceptible result, wrongly assumed to be fully susceptible can lead to mismanagement, since patients that receive the standard first-line TB regimen will have higher risks of treatment failure, relapse, and MDR-TB acquisition [4, 5].

The Democratic Republic of the Congo (DRC), home to an estimated 81 million people, is 1 of 14 countries on the World Health Organization (WHO) list of countries with high TB, TB/human immunodeficiency virus (HIV), and MDR-TB burdens [1]. In 2018, the estimated TB incidence rate was 322 per 100 000 with 60 000 TB-related deaths [1]. The estimated prevalence of MDR/RIF-resistant TB in the DRC was 1.7% and 9.5% in new and previously treated TB cases [1], respectively, but the accuracy of these estimates is limited by low laboratory coverage in many areas for the performance of MTB culture and drug susceptibility testing (DST). In contrast, an analysis of DRC surveillance between 2007 and 2016 reported an MDR-TB prevalence of 42.8% (95% confidence interval [CI], 38.4–47.8%) among high-risk patients with MDR-TB [6].

Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA) development has been a game changer for improving the diagnosis of MTB and detecting RIF resistance globally. Xpert is a rapid (2-hour), fully automated, real-time nucleic acid amplification technology that requires minimal staff training but does not test for INH resistance [7, 8]. In 2012, postconflict South Kivu province in eastern DRC was the first province to roll out Xpert at 10 urban and rural community sites through the Stop TB Partnership's TB REACH initiative [9]. Subsequently, in 2013, the line probe assay (LPA) GenoType MTBDR*plus* (MTBDR*plus*; Hain Lifescience GmbH, Nehren, Germany) was made available, but only at the referral laboratory in Bukavu, the capital city of South Kivu. MTBDR*plus* is a molecular LPA containing probes specific for the MTB complex as well as common mutations conferring RIF and INH resistance [10, 11].

We aimed to compare the diagnostic accuracy of Xpert and MTBDR*plus* for MDR-TB detection and evaluate the frequency of INH- and RIF-associated mutations in eastern DRC.

METHODS

Study Design, Patients, and Setting

We conducted a cross-sectional study at 10 urban and rural TB diagnostic and treatment centers (French acronym: CSDTs) as well as from military camps and artisanal mining sites between July 2013 and December 2016 in the post–armed-conflict South Kivu province of eastern DRC. Our study inclusion criteria were adult patients aged 18 years or older with newly diagnosed pulmonary TB or retreatment pulmonary TB cases (relapses, failures, and return after loss to follow-up with documented TB treatment exposure). Presumptive TB cases were found and identified either passively (by referral) or actively (via symptom screening by CSDT staff or community health workers).

Microscopy, Xpert, and MTBDR*plus*

All sputum specimens included in this study were analyzed using microscopy as per routine clinical care, whereas MTBDR*plus* was performed for study purposes and Xpert testing was performed subject to availability of cartridges and as indicated by DRC national TB program guidelines (eg, high-risk for MDR-TB based on TB treatment history) on remnant microscopy samples (Figure 1A and 1B). From 2015, specimens were preserved in 90% ethanol (dilution, 1:1) prior to transportation to preserve the quality of the DNA and decrease the biohazard risk. The first sputum specimen was provided in a sterile screw-cap universal disposable container. Ziehl-Nielsen slides were examined at CSDTs by bright-field microscopy (×1000 magnification). The second sputum specimen (for MTBDR*plus* assay) was provided in a sterile screw-cap universal disposable container and transported to a centralized laboratory in Bukavu city. DNA was extracted using the GenoLyse kit (Hain Lifesciences GmbH, Nehren, Germany). Multiplex polymerase chain reaction (PCR), reverse hybridization, and results interpretation were performed per the manufacturer's instructions. Demographic data and clinical information were abstracted from laboratory request forms.

Statistical Analysis

Data were summarized using proportions and means (± standard deviations) for categorical and continuous variables, respectively. Pearson's chi-square and Student's *t* tests were applied for tests of association, where appropriate. Sensitivity, specificity, and positive- and negative-predictive values and their 95% CIs were calculated to determine the diagnostic accuracy characteristics of Xpert compared to MTBDR*plus* for diagnosis of MDR-TB. Using logistic regression models, we investigated the unadjusted association between baseline patient characteristics and the presence of MDR-TB as determined by MTBDR*plus*. We estimated adjusted associations by including all baseline covariates a priori in a multivariable model. Starting with a full model, we then used a backward elimination procedure, excluding predictor variables with a P value less than .1, and compared the estimated reduced model adjusted odds ratios (aORs) and associated 95% CIs with the full multivariable model estimates. Results for which P values were less than .05 were considered statistically significant. Statistical analyses were performed using STATA version 12.1 (StataCorp, College Station, TX).

Research Ethics Approval

This study was approved by the Institutional Ethics Committee of the Université Catholique de Bukavu (reference number UCB/CIE/NC/07/2015).

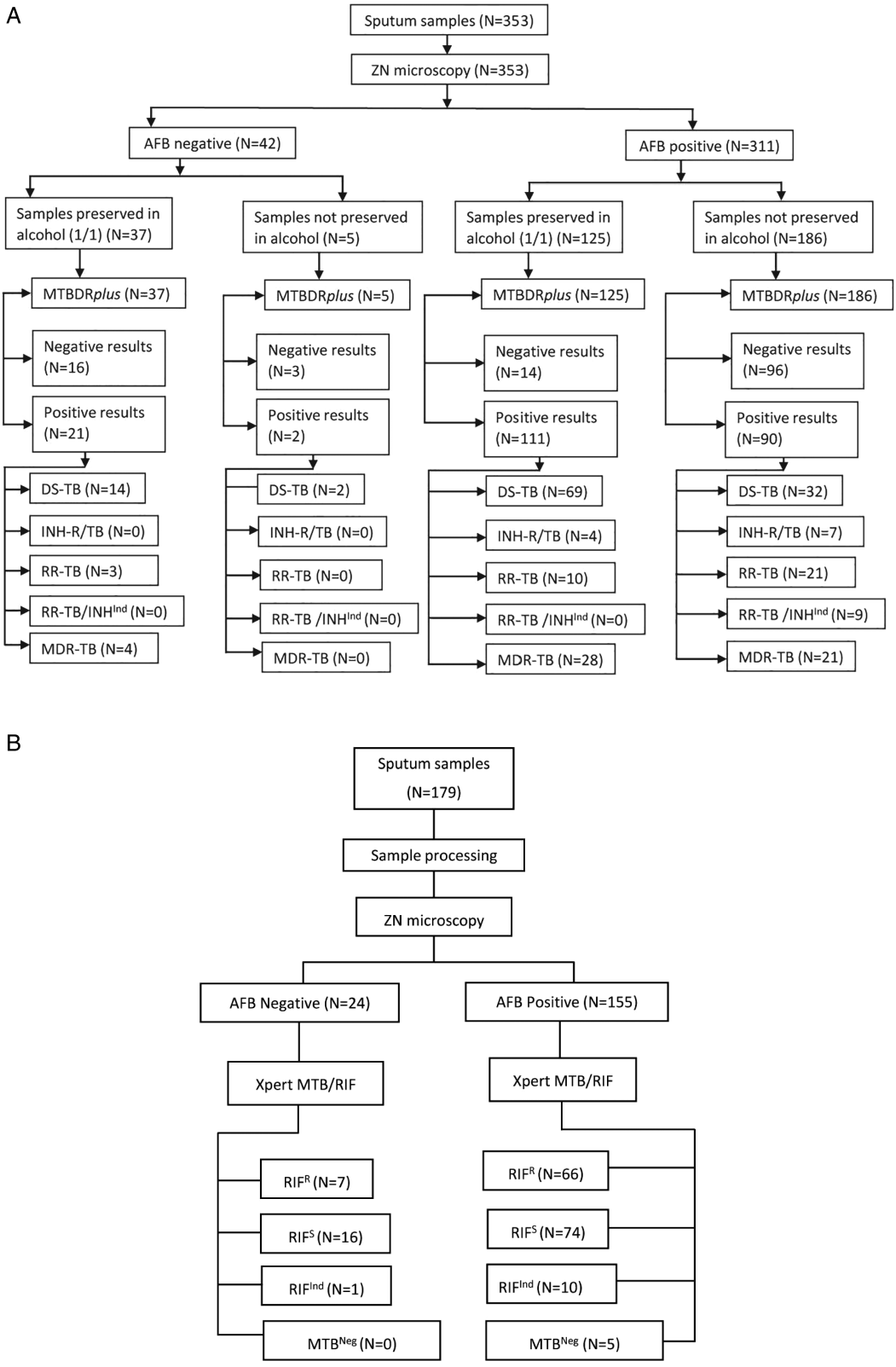

Figure 1. *A*, Flow diagram of MTBDR*plus* diagnostic assay done on sputum specimens included in the study. *B*, Flow diagram of Xpert MTB/RIF diagnostic assay done on sputum specimens included in the study. Abbreviations: AFB, acid-fast bacilli; DS-TB, drug-susceptible tuberculosis; INH^{Ind}, isoniazid indeterminate; INH-R, isoniazid resistance; MDR-TB, multidrug-resistant tuberculosis; MTB, *Mycobacterium tuberculosis*; MTB^{Neg}, *Mycobacterium tuberculosis* negative; RIF, rifampicin; RIF^{Ind}, rifampicin indeterminate; RIF^{R}, rifampicin resistant; RIF^{S}, rifampicin susceptible; RR-TB, rifampicin-resistant tuberculosis; ZN, Ziehl Nielsen.

RESULTS

Sociodemographic and Clinical Characteristics

As shown in Table 1, the mean age of individuals in the sample population was 37.6 years. Seventy-five percent were males, and two-thirds resided in rural areas. Approximately 30% had HIV testing results available; one-third of those tested were HIV positive. Most patients (193, 54.7%) had a previous TB treatment history and were more likely to be tested by Xpert (54.7% vs 34.2%; $P < .001$) (Supplementary Table 1). Forty-two (12%) were acid-fast bacilli (AFB) smear negative (Figure 1A and 1B).

MTBDR*plus* Assay Results

Among the 353 sputum samples tested by MTBDR*plus*, 193 (54.7%) had TB treatment history and 224 (63.5%) were MTB positive. Of these, 43 (19.2%) had RIF-monoresistant TB, 11 (4.9%) had INH-monoresistant TB, 53 (23.7%) had MDR-TB, and 117 (52.2%) were susceptible to both INH and RIF. Among the 96 samples with RIF resistance detected by MTBDR*plus* (43 RIF-monoresistant TB + 53 MDR-TB), only 53 (55%) had MDR-TB (Table 2). Among the 38 patients with HIV, 23 (60.5%) of whom were newly diagnosed with TB, 13 (34.2%) had MDR-TB, 5 (13.1%) had RIF-monoresistant TB, 3 (8%) had INH-monoresistant TB, 12 (31.6%) were TB susceptible, and 5 (13.1%) tested negative for MTB. In contrast, among 94 specimens from individuals without HIV, 16 (17%) had MDR-TB, 12 (12.8%) had RIF-monoresistant TB, 1 (1.1%) had INH-monoresistant TB, 38 (40.4%) were TB susceptible, and 27 (28.7%) were MTB negative. In multivariable logistic regression model, we found that HIV-positive status was independently associated with increased risk of RIF resistance and MDR-TB by Xpert and MTBDR*plus* (aOR [95% CI], 3.07 [1.14–8.27] [$P = .026$] and 3.3 [1.37–7.95] [$P = .008$], respectively) (Supplementary Tables 2 and 3), while only history of previous TB treatment was independently associated with an increased risk of MDR-TB (aOR, 3.00; 95% CI, 1.41–6.37; $P = .004$) (Supplementary Table 3).

Xpert Results and Concordance With MTBDR*plus*

As shown in Table 3, among 179 samples tested by both Xpert and MTBDR*plus*, 163 (91.1%) were MTB positive by the LPA, 11 (6.1%) were identified as MTB positive by Xpert but not LPA, and 5 (2.8%) were non-MTB by both Xpert and MTBDR*plus*; these 5 non-MTB cases were excluded from the analysis of drug-resistance concordance. Among the 163 positive samples by both MTBDR*plus* and Xpert, MTBDR*plus* identified 22 (13.5%) as RIF monoresistant, 11 (7%) as INH monoresistant, 45 (28%) as MDR-TB, and 83 (51%) as susceptible to both INH and RIF. Of note, among the 45 MDR-TB samples (identified by MTBDR*plus*) tested by Xpert, all 45 (100%) were positive for RIF resistance, and all 22 (100%) RIF-monoresistant samples (by MTBDR*plus*) were positive for RIF resistance by Xpert. However, overall, only 45 of 73 (61.6%) cases identified as RIF resistant by Xpert had concomitant INH resistance detected by MTBDR*plus* (ie, were identified as MDR-TB). Therefore, Xpert had sensitivity, specificity, and positive- and negative-predictive values of 100.0% (95% CI, 92.1–100.0%), 79.1% (95%

Table 1. Sociodemographic Characteristics of Participants (Tested With MTBDR*plus*)

Variables	All Patients (N = 353)	MTB Positive (n = 224)	MTB Negative (n = 129)	*P*
Age, mean ± SD, years	37.6 ± 14	36.5 ± 15.7	38.3 ± 15	.810
Gender n (%)				.464
Male	263 (74.5)	162 (72.3)	101 (78.3)	
Female	90 (25.5)	62 (27.7)	28 (21.7)	
Residence, n (%)				.958
Urban	130 (36.8)	80 (35.7)	50 (38.8)	
Rural	223 (63.2)	144 (64.3)	79 (61.2)	
Occupation, n (%)				.046
Miners	45 (12.7)	39 (17.4)	6 (4.7)	
Military	48 (13.6)	29 (13)	19 (14.7)	
Other	260 (73.7)	156 (69.6)	104 (80.6)	
HIV status, n (%)				.150
Positive	38 (10.8)	33 (14.7)	5 (3.9)	
Negative	94 (26.6)	66 (29.5)	28 (21.7)	
Past TB treatment, n (%)				.011
Yes	193 (54.7)	141 (63.%)	52 (40.3)	
No	149 (42.2)	77 (34.3%)	72 (55.8)	
Smear status, n (%)				.258
Negative	34 (9.6)	15 (6.7)	19 (14.7)	
Smear +1	101 (28.6)	47 (21)	54 (41.9)	
Smear +2	125 (35.4)	89 (39.7)	36 (27.9)	
Smear +3	93 (26.4)	73 (32.6)	20 (15.5)	

Abbreviations: HIV, human immunodeficiency virus; MTB, *Mycobacterium tuberculosis*.

Table 2. MTBDR *plus* Assay Results

	All (N = 353)	New PTB Patients (n = 149) (42.2%)	Previously Treated PTB Patients (n = 193) (54.7%)	Patients With Unknown PTB History (n = 11) (3.2%)	Smear-positive Patient Specimens	Smear-negative Patient Specimens
Specimens with positive MTBDR*plus* results						
RIF^S-INH^S	117 (33.1)	46 (30.9)	65 (33.7)	6 (54.5)	101 (86.3)	16 (13.7)
RIF^S-INH^R	11 (3.1)	6 (4)	5 (2.6)	0	11 (100)	0
RIF^R-INH^S	34 (9.6)	15 (10.1)	19 (9.8)	0	31 (91.2)	3 (8.8)
RIF^R-INH^{Ind}	9 (2.6)	3 (2)	6 (3.1)	0	9 (100)	0
RIF^R-INH^R	53 (15)	12 (8.1)	41 (21.2)	0	49 (92.5)	4 (7.5)
Subtotal	224 (63.5)	82 (36.6)	136 (60.7)	6 (2.7)	201 (89.7)	23 (10.3)
Specimens with negative, invalid, or indeterminate MTBDR*plus* results						
Negative	99 (76.7)	50 (50.5)	34 (34.3)	15 (15.2)	81 (81.8)	18 (18.2)
Invalid	22 (17)	6 (27.3)	16 (72.7)	0	21 (95.5)	1 (4.5)
Indeterminate	8 (6.3)	1 (12.5)	7 (87.5)	0	8 (100)	0
Subtotal	129 (36.5)	57 (44.2)	57 (44.2)	15 (11.6)	110 (85.3)	19 (14.7)

Data are presented as n (%).
Abbreviations: INH^{Ind}, isoniazid indeterminate; INH^R, isoniazid resistant; INH^S, isoniazid susceptible; PTB, pulmonary tuberculosis; RIF^R, rifampicin resistant; RIF^S, rifampicin susceptible.

CI, 71.2–85.6%), 61.6% (95% CI, 49.5–72.8%), and 100% (95% CI, 96.6–100%), respectively, for correct diagnosis of MDR-TB (Table 4). Of note, 4 (5.5%) samples identified as RIF resistant by Xpert were found to be RIF susceptible by MTBDR*plus*.

Isoniazid and Rifampicin Resistance–Conferring Mutations

Of 96 patient specimens identified as RIF resistant with MTBDR*plus*, 77 (86.5%) were missing the wild-type 8 (WT8) band that covers codons 530 to 533 of the *rpoB* gene and 51 (53.1%) had mutation S531L in the *rpoB* gene according to hybridization with the *rpoB* gene MUT3 band (Table 5). Of 64 specimens identified as INH resistant with MTBDR*plus,* the most frequent resistance-conferring mutation was *katG* S315T1 (MUT1) (60; 93.8%), while only 3 (4.7%) specimens had *inhA* promoter C-15T mutations (MUT1). Concurrent *katG* S315T1 (MUT1) and *inhA* promoter C-15T (MUT1) mutations were present in only 1 (1.5%) patient specimen.

DISCUSSION

Our findings suggest that comprehensive testing of patients with TB for susceptibility to both RIF and INH is necessary in eastern DRC. When tested with MTBDR*plus,* only 55% of isolates with RIF resistance had concomitant INH resistance and thus were MDR-TB. We further demonstrated that, in a subgroup of samples tested by both Xpert and MTBDR*plus*, only 61.6% of Xpert-detected RIF-resistant samples had MDR-TB. Furthermore, we found that 4.9% of these patients had INH monoresistance. These results strongly suggest that Xpert-detected RIF resistance is a suboptimal marker for MDR-TB in eastern DRC; this testing platform also missed clinically significant INH monoresistant cases that would presumably be treated as drug-susceptible TB if relying on Xpert testing alone. Therefore, previous assumptions that RIF and INH monoresistance are rare may not apply in all settings. As also shown in our study, there have been previous reports of increased prevalence of RIF monoresistance or MDR-TB and its association with HIV positivity [12–14]. However, the latter association should be interpreted with caution, given that most of our study population had unknown HIV status and there have been conflicting reports on this issue [15–17].

We also documented a relatively high prevalence (23.7%) of MDR-TB by LPA in our study sample, which purposively included a high proportion of high-risk patients with MDR-TB. Importantly, 47.8% of the MTB-positive patients

Table 3. Xpert MTB/RIF Results and Concordance With MTBDR*plus*

	MTBDR*plus*, n (%)							
Xpert MTB/RIF	RIF^R-INH^R	RIF^R-INH^S	RIF^S-INH^S	RIF^R-INH^{Nv}	RIF^S-INH^R	Non-MTB	Neg	Total
MTB-pos/RIF^R	45 (61.6)	22 (30.1)	4 (5.5)	2 (2.7)	0	0	0	73 (100)
MTB-pos/RIF^S	0	0	79 (98.7)	0	11 (1.3)	0	0	90 (100)
MTB-Neg	0	0	0	0	0	5	0	5 (100)
MTB-pos/RIF^{Ind}	0	0	0	0	0	3 (27.3)	8 (72.7)	11 (100)
Total	45 (25.1)	22 (12.3)	83 (46.4)	2 (1.1)	11 (0.6)	8 (10.1)	8 (4.5)	179 (100)

Abbreviations: INH^{Nv}, not valid results for INH (there was not clear evidence that these specimens were INH resistant since the *katG* and *inhA* bands were extremely faint); INH^R, isoniazid resistant; INH^S, isoniazid susceptible; MTB, *Mycobacterium tuberculosis*; Neg, negative; pos, positive; RIF, rifampicin; RIF^{Ind}, rifampicin indeterminate; RIF^R, rifampicin resistant; RIF^S, rifampicin susceptible.

Table 4. Performance of Xpert Against the Reference Standard of MTBDR*plus* (Line Probe Assay) for Multidrug-resistant Tuberculosis Detection

		MDR-TB by LPA	
Xpert RIF Resistant	Total	Positive	Negative
Results			
Positive	73	45 (a)	28 (b)
Negative	90	0 (c)	90 (d)[a]
Total	163	45	118
Sensitivity, % (95% CI)	…	100.0 (92.1–100)	
Specificity, % (95% CI)	…	76.3 (71.2–85.6)	
Positive-predictive value, % (95% CI)	…	61.6 (49.5–72.8)	
Negative-predictive value, % (95% CI)	…	100.0 (96.6–100)	

Sensitivity = a/a + c; Specificity = d/b + d; positive-predictive value = a/a + b; negative-predictive value = d/d + c.

[a]The 2 invalid isoniazid results by LPA were assumed to be susceptible (ie, did not detect MDR-TB) and included in the denominator for the calculation of specificity.

Abbreviations: CI, confidence interval; LPA, line probe assay; MDR-TB, multidrug-resistant tuberculosis; RIF, rifampicin.

had at least 1 form of TB drug resistance (MDR-TB, RIF- or INH-monoresistant TB). Our convenience study sampling may partially account for the higher than average community-based MDR-TB prevalence in DRC [18]. However, our data are in line with the 2007–2010 DRC TB drug-resistance surveillance, which reported a prevalence of 42.8% (95% CI, 38.4–47.8%) among high-risk patients with MDR-TB [6]. A study by Dube-Mandishora et al [19] also reported a high prevalence (42%) of MDR-TB among Zimbabwean patients with known risk factors for MDR-TB, albeit from a sample size (n = 69) much smaller than ours. The majority of RIF resistance in our study sample was conferred by the *rpo*B S531L mutation, which is among the most prevalent RIF resistance–associated mutations and has been previously described in sub-Saharan Africa, including in rural western DRC [20–22].

In our study, a high proportion of RIF-resistant sputum specimens failed to hybridize with the wild-type (WT8) probe by MTBDR*plus*. These specimens, lacking WT8 and MUT3 hybridization, could reflect a technical problem or a new, previously unreported mutation. Seifert et al [23] suggested that this type of result is likely due to the failure of the mutant to hybridize with the mutation probe and not the presence of a rare or new mutation. The absence of the *rpoB* WT8 is associated with L533P and S531W mutations in the setting of low RIF resistance [24–26]. Unfortunately, DNA sequencing could not be performed on these specimens to confirm or identify the mutations. Nevertheless, the codon 531 mutation is considered the most prevalent RIF resistance–associated mutation among such specimens in the South Kivu province.

Table 5. Mutations Associated With Rifampicin- and Isoniazid-resistant Tuberculosis as Detected by MTBDR*plus*

Rifampicin Resistance			Isoniazid Resistance						
rpoB Gene			*katG* Gene			*inhA* Gene			
WT Absent	MUT Present	*rpoB* Mutation	WT Absent	MUT Present	*katG* Mutation	WT Absent	MUT Present	*inhA* Promoter Mutation	Frequency
WT1	…	…	…	…	…	…	…	…	2 (2.2%)
WT2	…	…	…	…	…	…	…	…	3 (3.3%)
WT3/4	…	…	…	…	…	…	…	…	3 (3.3%)
WT3/4	MUT1	D516V	…	…	…	…	…	…	2 (2.2%)
WT3/4	MUT1	D516V	*katG*WT	*katG*MUT1	S315T1	…	…	…	1 (1.1%)
WT7	MUT2A	H526Y	*katG*WT	*katG*MUT1	S315T1	*inhA*WT1	*inhA*MUT1	C-15T	1 (1.1%)
WT7	MUT2A	H526Y	*katG*WT	*katG*MUT1	S315T1	…	…	…	3 (3.3%)
WT7	MUT2A	H526Y	*katG*WT	*katG*MUT1	S315T1	…	…	…	1 (1.1%)
WT8	MUT3	S531L	*katG*WT	*katG*MUT1	S315T1	…	…	…	30 (33.3%)
WT8	MUT3	S531L	…	…	…	*inhA*WT1	*inhA*MUT1	C-15T	1 (1.1%)
WT8	…	S531L	*katG*WT	…	…	*inhA*WT1	*inhA*MUT1	C-15T	1 (1.1%)
WT8	…	…	…	…	…	*inhA*WT2	…	…	1 (1.1%)
WT8	MUT3	S531L	…	*katG*MUT1	S315T1	…	…	…	8 (8.9%)
WT8	…	…	*katG*WT	*katG*MUT1	S315T1	…	…	…	1 (1.1%)
WT8	MUT3	S531L	…	…	…	…	…	…	13 (14.4%)
WT8	…	…	…	…	…	…	…	…	19 (21.1%)

Abbreviation: WT, wild-type.

Only 3 patient isolates contained a mutation in the *inhA* promoter gene. The other 61 patients with INH resistance had the S315T *katG* gene mutation corresponding to the AGC-ACC modification at codon 315. Results of previous studies suggest that mutations in *katG* and *inhA* account for the majority of INH-resistant strains of TB [27–29]. While the *katG* gene is associated with a high level of INH resistance, studies have suggested that mutations in the *inhA* promoter have limited impact on INH resistance as it is also present in many INH-susceptible strains [30]. Patients with *inhA* promoter mutations may benefit from high-dose INH but not require inclusion of ethionamide in their treatment regimens.

All discrepancies (n = 4) found in this study between Xpert and MTBDR*plus* were due to samples being characterized as sensitive by MTBDR*plus* but resistant by Xpert. This finding is consistent with studies in LMICs, where the numbers of discrepancies between Xpert and MTBDR*plus* were even higher [19, 24, 31, 32]. A study by Rahman et al [31] noted that Xpert performed more accurately than MTBDR*plus* in detection of mutations associated with RIF resistance, as MTBDR*plus* failed to detect mutations that occurred in regions 530-533, 513-519, and S522P.

Our findings have important clinical, diagnostic, and treatment guideline implications. Indeed, reliance on molecular assays that test for RIF resistance in isolation, without ascertainment of INH resistance, can lead to suboptimal treatment of INH- or RIF-monoresistant TB. The 2019 WHO guideline recommends that patients with INH-resistant and RIF-susceptible TB be treated with a 6-month regimen composed of RIF, ethambutol (EMB), pyrazinamide (PZA), and levofloxacin [3]. Patients with INH-monoresistant TB who are treated with a 6-month first-line TB regimen (2-month INH-RIF-EMB-PZA/4-month INH-RIF) have higher risks of treatment failure, relapse, and acquiring additional resistance than those with drug-susceptible TB [5]. Conversely, patients with confirmed low-level or no INH resistance (RIF-monoresistant TB) will benefit from the inclusion of INH in their treatment regimens. Surprisingly, the 2018 WHO MDR-TB treatment guidelines no longer included high-dose INH, one of the key drugs in the short-course MDR-TB regimen that achieved success in approximately 80% of patients from observational studies in Bangladesh [33] and Africa [34] as well as in stage 1 of the Standardized Treatment Regimen of Anti-TB drugs for patients with MDR-TB (STREAM) trial [35]. Because of data from our study and others, the 2019 WHO consolidated guidelines now recommend that INH again be used in patients with confirmed INH susceptibility or the presence of mutations that do not usually confer complete resistance to INH, as indicated by specific *inhA* promoter mutations in the absence of *katG* mutations [3].

Furthermore, in this high-risk MDR-TB study population, our results showed that Xpert had a low positive-predictive value (61.6%) for MDR-TB. This finding suggests that approximately 40% of cases, if tested only with Xpert and not LPA, could be false positives and assumed to be MDR-TB, but in fact could be INH susceptible. Our data suggest that, if Xpert is used in a population with an even lower prevalence of RIF-resistant TB (ie, for TB diagnosis in a general population), the positive-predictive value for MDR-TB will decrease since predictive values are a function of disease prevalence in a population. Therefore, our study underscores the importance of and continuing need for the development of near-care/point-of-care technologies that provide more comprehensive and cost-efficient DST to guide individualized treatment regimens using WHO's "target product profiles" for new diagnostics [36]. In light of the data we present here and that of others [4, 5], the DRC National TB Program revised its MDR-TB diagnostic guidelines in 2019 and now recommends that all patients with Xpert-identified RIF resistance have a second sputum sample collected for INH susceptibility testing by LPA at the regional reference laboratory [37]. However, LPA has its own limitations since it only captures about 85% of INH mutations, has a 4- to 6-hour turnaround (precluding same-day therapeutic decision making), and is confined to reference laboratories that meet infrastructure and assay training requirements [10–12]. One alternative to LPA is a novel investigational cartridge for use with the Xpert platform to rapidly detect resistance to INH, fluoroquinolones, and aminoglycosides, which can be used as complementary testing on all patients with documented Xpert-identified RIF-resistant TB strains [38]. Another alternative include platform such as the BD MAX MDR-TB assay (Becton Dickinson Diagnostics, Franklin Lakes, NJ), which provides INH and RIF resistance information and is relatively fast and automated; however, this technology has workflow limitations and is likely best situated in reference laboratories [39].

Our study has several limitations. First, logistical and cost constraints prevented the use of the standard references of culture and conventional phenotypic sensitivity testing. However, we believe our approach is acceptable because the main objective of our study was to determine the frequency of unrecognized concomitant INH and RIF resistance in our setting, where Xpert MTB/RIF is the primary test for drug susceptibility. Second, the method of preservation and transportation of LPA samples was improved 1 year before the end of our study, which increased the yield of the MTBDR*plus* testing, consequently underestimating LPA performance. Finally, Xpert testing based on cartridge availability and convenience sampling, which enriched our study with individuals more likely to have MDR-TB, may limit the generalizability of our findings to all DRC. Despite the above limitations, we believe our study is timely and adds value to the field. We provided a direct, comparative evaluation of 2 molecular diagnostic tests for TB diagnosis and genotypic DST in a real-life programmatic setting with important clinical, diagnostic, and treatment implications for LMICs.

In conclusion, in this high-risk MDR-TB population, Xpert-identified RIF resistance has poor positive-predictive value as a proxy for the presence of MDR-TB. Isoniazid, as part of an MDR-TB regimen, is likely to be an effective therapy for 2 out of 5 individuals with Xpert-diagnosed RIF resistance. The most frequent mutations associated with RIF and INH resistance were S531L and S315T1, respectively. Our findings highlight the urgency for continuing the development of near-care technologies to provide more comprehensive and cost-efficient DST to guide individualized treatment regimens in LMICs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. B. C. B. contributed to the protocol development, study implementation, data collection, statistical preliminary analysis, interpretation of results, and drafting and revisions of the manuscript. R. M. W., G. T., J. Z. M., M. S., A. H. D., M. Y., and N. A. S.-A. assisted with the data interpretation, revisions, and writing of the manuscript. J. B. N. assisted with study design, statistical analyses, interpretation of data, manuscript writing, and revisions. E. B. performed statistical analyses and contributed to interpretation of results, manuscript writing, and revisions. P. D. M. C. K. and A.N. H. B. contributed to data analysis, manuscript writing, and revisions. Z. M. K. and S. C. supervised the clinical and laboratory aspects of the study, respectively, as well as data interpretation and manuscript writing. E. M., J.-P. C., F. M. B., and R. N. managed programmatic aspects of the study. S. B. led the laboratory MTBDR*plus* and Xpert MTB/RIF assays under the supervision of B. C. B. All authors reviewed the paper for additional data interpretation and revisions and approved the final version of the manuscript.

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