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MAJOR ARTICLE

Programmatic Diagnostic Accuracy and Clinical Utility of Xpert MTB/XDR in Patients With Rifampicin-Resistant Tuberculosis in Georgia

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Background. Xpert MTB/XDR (Cepheid) is recommended by the World Health Organization for drug susceptibility testing in patients with tuberculosis, with potential for rapid detection of isoniazid and fluoroquinolone resistance. However, diagnostic accuracy and clinical utility in a programmatic setting are unknown.

Methods. We evaluated the accuracy and clinical utility of Xpert MTB/XDR in patients with rifampicin-resistant pulmonary tuberculosis during programmatic implementation in Georgia between July 2022 and August 2024, using phenotypic drug susceptibility testing (DST) as a reference standard.

Results. An overall 140 patients were tested with Xpert MTB/XDR and phenotypic DST, and 94.9% and 33.8% had isoniazid and fluoroquinolone resistance by phenotypic DST, respectively. Xpert MTB/XDR showed 99.2% sensitivity (95% CI, 95.5%–100%) and 100% specificity (95% CI, 54.1%–100%) for isoniazid resistance. Sensitivity and specificity for fluoroquinolone resistance were 88.4% (95% CI, 74.9%–96.1%) and 100% (95% CI, 95.6%–100%). When indeterminate/invalid Xpert MTB/XDR results were included, 17.4% (8/46) and 6.2% (8/129) of patients with phenotypic fluoroquinolone and isoniazid resistance were missed. Median turnaround time for Xpert MTB/XDR was 1 day (IQR, 1–3) and median time to treatment was 4 days (IQR, 1–7). Phenotypic DST results took a median 43 days (IQR, 29–63) longer than Xpert MTB/XDR results. Finally, 95% (115/121; 95% CI, 89.5%–98.2%) of patients had fluoroquinolones appropriately prescribed based on Xpert MTB/XDR results.

Conclusions. Programmatic data confirm the high accuracy of Xpert MTB/XDR, despite being below the World Health Organization target product profile targets for fluoroquinolones, with significantly faster time to results than phenotypic DST.

Drug-resistant tuberculosis (DR-TB) remains a global health concern with an estimated 410 000 cases and up to 85 000 deaths attributed annually [1, 2]. Diagnosis remains a key barrier, with only 43% of those with drug resistance starting appropriate treatment, of whom only 63% achieve favorable treatment outcomes [1]. Furthermore, selecting effective treatment requires identifying resistance to key drugs, including rifampicin, isoniazid, and fluoroquinolones. Implementation of culture-based drug susceptibility testing (DST) for TB is challenging due to long turnaround times and the need for expensive laboratory infrastructure [3]. The development and scale-up of rapid tests for resistance detection in correspondence to new treatment regimens is a priority for the World Health Organization (WHO) [4].

In 2021, the WHO recommended Xpert MTB/XDR (henceforth, Xpert XDR; Cepheid), a rapid low-complexity molecular assay, as a follow-on test for detection of isoniazid and fluoroquinolone resistance in patients with bacteriologically confirmed pulmonary TB [5]. Additionally, the test is recommended for detecting resistance to ethionamide and second-line injectable drugs in patients with rifampicin-resistant TB. Xpert XDR demonstrated high diagnostic accuracy for resistance to isoniazid (sensitivity, 94.2%; specificity, 98.5%) and fluoroquinolone (sensitivity, 93.2%; specificity, 98.0%) in multicenter diagnostic accuracy studies [6–9].

Different programmatic use cases have been suggested for Xpert XDR. In patients with presumptive TB and high risk for resistance, it was suggested as a test for TB diagnosis and resistance detection. Additionally, in patients with rifampicin-resistant TB

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and those not responding to treatment, it could facilitate detection of resistance to first- and second-line drugs [7]. While the relevance of detecting second-line injectable drug resistance has decreased following the scale-up of all-oral treatment regimens for DR-TB, Xpert XDR remains valuable as the only rapid test capable of detecting resistance to isoniazid and fluoroquinolones at lower levels of care. However, its diagnostic accuracy in a programmatic setting, as well as its utility in clinical treatment of patients with DR-TB, is not well established. We sought to evaluate the accuracy and clinical utility of Xpert XDR when implemented within routine programmatic care in a setting with a high proportion of DR-TB among new and retreated patients with TB [1, 10].

METHODS

Study Design, Setting, and Participants

We evaluated the diagnostic accuracy of routine Xpert XDR testing at the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia, between 4 July 2022 and 1 August 2024. Results from programmatic testing of sputum—including Xpert MTB/RIF Ultra, Xpert XDR, mycobacterial culture, and phenotypic DST—were prospectively collected through routine care records for consecutive patients with rifampicin-resistant TB enrolled in the Rapid Research in Diagnostics Development (R2D2) TB Network study on rapid DST assays for DR-TB [11]. Eligibility criteria for the R2D2 DR-TB study were rifampicin-resistant pulmonary TB, age ≥18 years, provision of study sputum samples, and not undergoing a WHO-recommended DR-TB treatment regimen for >72 hours [12]. Patients without a positive result for *Mycobacterium tuberculosis* (MTB) on at least 1 sputum culture were excluded. Patients without a valid Xpert XDR result were excluded from programmatic diagnostic accuracy analysis, although they were included for clinical utility analysis. Eligible participants were identified upon presentation to the NCTLD outpatient and inpatient therapeutic departments for treatment initiation.

The R2D2 study, under which data for this analysis were collected, was approved by research ethics committees at the University of California San Francisco (20-32670) and the Medical Faculty of the University of Heidelberg (S-519/2023), as well as the local ethics committee of NCTLD (FWA00020831). All participants provided written informed consent. The study's reporting conforms to STARD guidelines (Standards for Reporting of Diagnostic Accuracy Studies; see STARD checklist in Supplementary Table 1).

Programmatic Testing and Clinical Management

Xpert XDR implementation for all positive Xpert MTB/RIF Ultra and confirmed rifampicin-resistant TB cases started from July 2022 at the NCTLD in Georgia, followed by decentralized country-wide implementation from September 2022. Testing was done by trained laboratory technicians or health care workers in primary care centers and regional public sector laboratories. Xpert XDR results were recorded as MTB detected, MTB not detected, or invalid and resistance detection per drug as resistance detected, not detected, indeterminate, or invalid. If Xpert XDR failed to detect MTB in a sample, the resistance detection results were reported as invalid. Indeterminate results indicated that resistance could not definitively be assessed by the assay algorithm, whereas an invalid result was indicating that the sample processing control failed. Repeat testing was not performed systematically; therefore, any repeat test results were not recorded. All eligible consented patients provided sputum at enrollment, which underwent culture inoculation in a mycobacterium growth indicator tube in the Bactec 960 system (MGIT; Becton Dickinson) and phenotypic DST, including rifampicin, isoniazid, moxifloxacin, levofloxacin, and amikacin based on WHO-recommended critical concentrations [13]. Patients with rifampicin-resistant TB were treated by clinicians through routine programmatic care, according to the Georgia national TB treatment guidelines following the last update of the WHO treatment guideline [12]. The R2D2 study team was not involved in decisions on treatment initiation and drug regimens.

Study Procedures

All clinical information, including medical history and treatment profile, was captured on electronic case report forms, while results from TB testing, including Xpert XDR, mycobacterial culture, and DST, were extracted from the NCTLD laboratory information system. DNA extraction from positive culture isolates and whole genome sequencing was conducted under the R2D2 protocol for a subset of participants in this study. Details on microbiological testing and molecular testing can be found in the supplementary material.

Outcomes

The primary outcome was diagnostic accuracy of routine programmatic Xpert XDR testing for detection of isoniazid and fluoroquinolone resistance as compared with a phenotypic DST reference standard. Secondary outcomes were as follows: (1) indeterminate and invalid results, including diagnostic accuracy of Xpert XDR by intention-to-diagnose analysis; (2) diagnostic accuracy of Xpert XDR when compared with a composite genotypic and phenotypic reference standard; (3) diagnostic accuracy for low-level fluoroquinolone resistance detection, based on phenotypic DST at the WHO-recommended clinical breakpoint [13]; (4) time to DR-TB treatment initiation after Xpert XDR testing; and (5) proportion of patients who started appropriate DR-TB treatment regimens based on Xpert XDR results. Post hoc analysis of diagnostic accuracy for amikacin resistance was added in response to a reviewer.

Statistical Analysis

We calculated sensitivity, specificity, positive predictive value, and negative predictive value in respect to the reference standard with binomial 95% CIs. Observations with indeterminate and/or invalid results of the index and reference standard tests were excluded from primary diagnostic accuracy analyses. Indeterminate rates for the index test were calculated, and we performed an intention-to-diagnose analysis in which we considered indeterminate/invalid index test results as negative for resistance to ensure that sensitivity and specificity were not overestimated [14, 15]. We performed diagnostic accuracy analyses using a composite reference standard of phenotypic DST and whole genome sequencing for a subset of participants with available whole genome sequencing data (supplementary methods). We modeled predictive values for fluoroquinolone resistance detection across a range of resistance prevalences (5%–30%) relevant to settings with high DR-TB burden. Time to treatment was calculated as the interval in days between Xpert XDR testing and treatment initiation and was reported by median and IQR. Turnaround time for Xpert XDR was calculated as the time between sputum collection and Xpert XDR result. We used the date of the initial Xpert Ultra testing as a proxy for the date of sputum collection. The proportion of participants who initiated appropriate treatment was based on the appropriate use of fluoroquinolone—specifically, inclusion of fluoroquinolone in treatment regimen if Xpert XDR demonstrated fluoroquinolone sensitivity and exclusion of fluoroquinolone from the regimen if Xpert XDR showed resistance. An alluvial diagram was used to visualize the relation between index and reference test results and initiated fluoroquinolone treatment. Analyses were conducted with Stata/SE version 18.0 (StataCorp) and R version 4.3.2 (R Foundation for Statistical Computing).

RESULTS

Baseline Characteristics

An overall 201 patients were screened for eligibility for the R2D2 TB Network DR-TB study: 28 (13.9%) were excluded due to negative or contaminated sputum culture results and 3 were missing culture at the time of analysis (Figure 1). In total, 140 participants were included in the accuracy analysis, with 128 and 125 in the analysis for isoniazid and fluoroquinolone resistance detection, respectively. Of the 140 participants, 38 were female; the median age was 45 years; and 30 (21.4%) and 15 (10.7%) stated that they had previously been treated for drug-susceptible TB and DR-TB. Of 136 participants, 129 (94.9%) had isoniazid resistance and 46 (33.8%) were resistant to fluoroquinolone by the primary reference standard. Thirty additional participants, who were excluded from accuracy analyses due to missing Xpert XDR testing (30/173, 17.3%), were included in clinical utility analyses (Table 1).

Xpert XDR

Xpert XDR sensitivity for isoniazid resistance was 99.2% (95% CI, 95.5%–100.0%; n = 128) and specificity was 100% (95% CI, 54.1%–100%) as compared with phenotypic DST. Sensitivity and specificity for fluoroquinolone resistance were 88.4% (95% CI, 74.9%–96.1%; n = 125) and 100.0% (95% CI, 95.6%–100%; Table 2), respectively. Diagnostic accuracy estimates did not differ by sex or Xpert Ultra semiquantitative grades, although fluoroquinolone sensitivity was lower in those with high or medium semiquantitative grade (77.3% for medium/high vs 100% for low/very low, $P = .02$; Supplementary Table 2). Accuracy was also similar in secondary analysis with the composite reference standard (Supplementary Table 3).

No invalid results were observed for MTB detection. Invalid results for resistance detection occurred only in samples where no MTB was detected by Xpert XDR. No indeterminate results were registered for isoniazid resistance detection. For fluoroquinolone resistance, 3.8% (5/131) of results were indeterminate, occurring in samples with high and low bacillary load (Supplementary Table 4). Intention-to-diagnose analysis found a lower sensitivity for detection of isoniazid resistance (93.8%) and fluoroquinolone resistance (82.6%) as compared with the primary analysis (Table 2). When indeterminate and invalid results were included, 17.4% (8/46) of patients with phenotypic fluoroquinolone resistance and 6.2% (8/129) of patients with phenotypic isoniazid resistance were missed by Xpert XDR (Supplementary Tables 5 and 6). Xpert XDR showed consistently high positive and negative predictive values for isoniazid and fluoroquinolone resistance detection across a range of resistance prevalence rates (Supplementary Table 7).

Xpert XDR detected MTB in 80.0% (12/15) of samples that had negative or contaminated cultures; of these, 58.4% (7/12) showed isoniazid resistance and 8.3% (1/12) showed fluoroquinolone resistance. Xpert XDR failed to detect MTB in 9 (6.4%) of 140 culture-positive samples, all of which had low or very low semiquantitative grades on Xpert Ultra; furthermore, 77.8% (7/9) were isoniazid resistant by phenotypic DST and 22.2% (2/9) were fluoroquinolone resistant by phenotypic DST.

Xpert XDR sensitivity for amikacin resistance was 92.3% (95% CI, 64.0%–99.8%; n = 121) and specificity was 96.3% (95% CI, 90.8%–99.0%) as compared with phenotypic DST, and the intention-to-diagnose analysis found lower sensitivity at 75.0% (Supplementary Table 8).

Clinical Utility

The median turnaround time for Xpert XDR was 1 day (IQR, 1–3) and the median time from Xpert XDR result to treatment was 4 days (IQR, 1–7). The median time between sputum collection and phenotypic DST results was 40 days (IQR, 27–59). Data on fluoroquinolone treatment were available for 135 of 140 participants with Xpert XDR results. Of 121 participants,

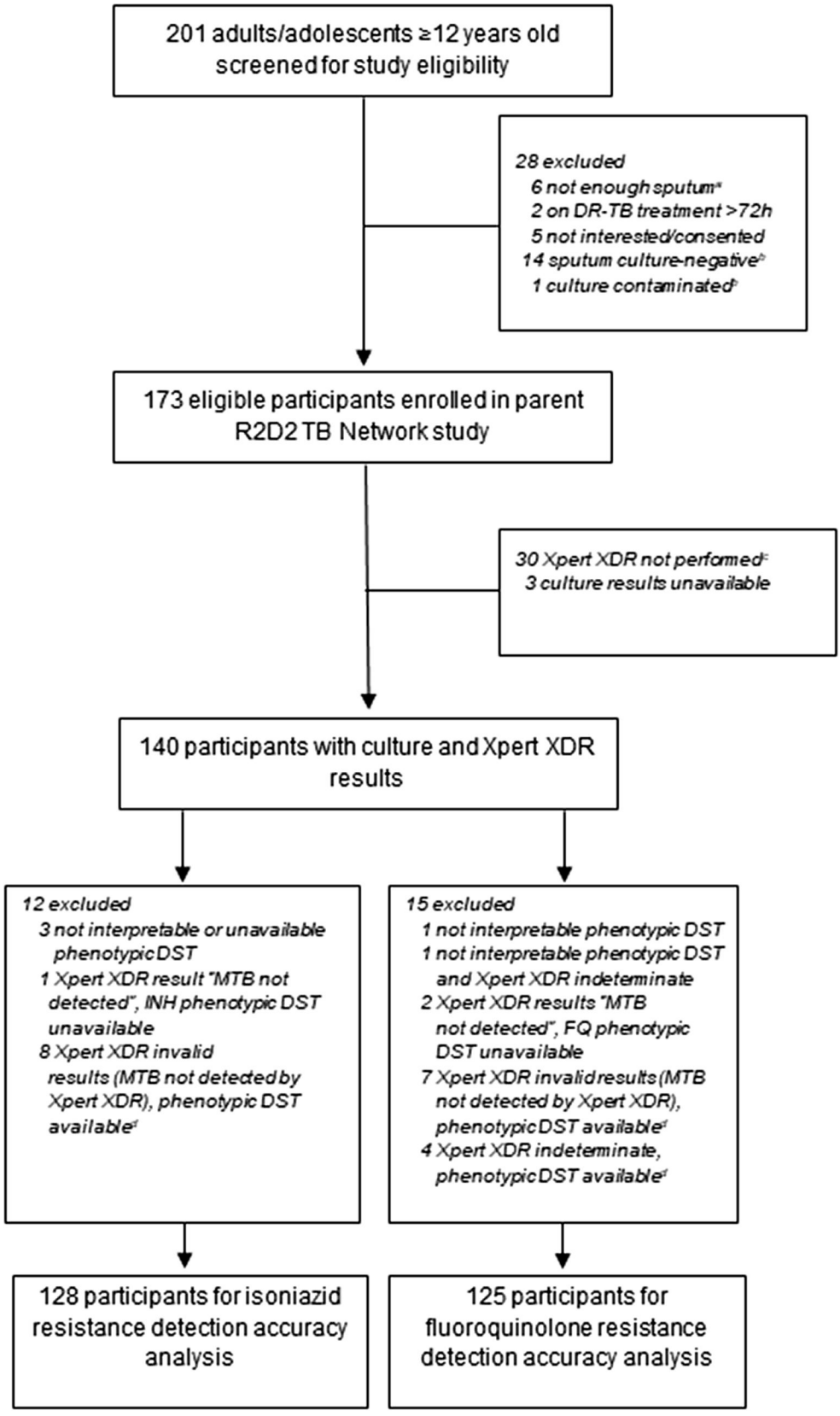

Figure 1. Participant flow. [a] A minimum of 6 mL of sputum was required for enrollment in the Rapid Research in Diagnostics Development (R2D2) TB Network study. [b] Late exclusions based on reference standard results. [c] Excluded from accuracy analyses but included in clinical utility analyses. [d] Included in the intention-to-diagnose analysis. DR-TB, drug-resistant tuberculosis; DST, drug susceptibility testing; FQ, fluoroquinolone; INH, isoniazid; Xpert XDR, Xpert MTB/XDR.

Table 1. Baseline Characteristics

	Participants, No. (%)		
	Total[a] (n = 140)	Fluoroquinolone-Resistant TB (n = 46)	No Xpert MTB/XDR[b] (n = 30)
Sex			
Male	102 (72.9)	36 (78.3)	27 (90.0)
Female	38 (27.1)	10 (21.7)	3 (10.0)
Age, y, median (IQR)	45 (33–58)	41 (32–53)	52 (39–58)
Body mass index, median (IQR)	20.4 (18.4–23.1)	20.6 (19.1–22.6)	21.1 (18.1–23.4)
Comorbidities: self-report			
People with HIV[c]	5 (3.6)	2 (4.3)	2 (6.7)
CD4 count, mean (SD)	271 (223.6)	65 (18.4)	74 (0.0)
Diabetes mellitus[d]	8 (5.7)	1 (2.2)	3 (10.0)
Symptoms in the past 30 d			
Cough	132 (94.3)	44 (95.7)	28 (93.3)
Night sweats	123 (87.9)	42 (91.3)	21 (70.0)
Weight loss	101 (72.1)	32 (69.6)	17 (56.7)
Fever	96 (68.6)	32 (69.6)	17 (56.7)
DR-TB contact			
No	73 (52.1)	25 (54.3)	13 (43.3)
Yes	30 (21.4)	10 (21.7)	7 (23.3)
Unsure	37 (26.4)	11 (23.9)	10 (33.3)
Previous TB treatment	41 (29.3)	12 (29.3)	9 (30.0)
DR-TB[e]	15 (36.6)	3 (25.0)	2 (22.3)
DS-TB[f]	30 (73.2)	10 (83.3)	6 (66.7)
Semiquantitative grade of Xpert Ultra			
Very low/low	69 (49.3)	24 (52.2)	9 (30.0)
Medium/high	71 (50.7)	22 (47.8)	9 (30.0)
TB regimen started	135 (96.4)	43 (93.5)	29 (96.7)
BPaLM	57 (42.2)	8 (18.6)	9 (31.0)
BPaL	29 (21.5)	28 (65.1)	4 (13.8)
Short all-oral regimen[g]	42 (31.1)	5 (11.6)	13 (44.8)
Other regimen	7 (5.2)	2 (4.7)	3 (10.3)
Resistance prevalence[h]			
Isoniazid	129 (94.9)	45 (97.8)	23 (92.0)
Fluoroquinolone	46 (33.8)	…	8 (32.0)

Data are presented as No. (%) unless noted otherwise.

Abbreviations: BPaL, bedaquiline, pretomanid, and linezolid; BPaLM, bedaquiline, pretomanid, linezolid, and moxifloxacin; DR-TB, drug-resistant tuberculosis; DS-TB drug-sensitive tuberculosis; TB, tuberculosis.

[a]Diagnostic accuracy analysis.

[b]Clinical utility analysis (in addition to total).

[c]3 missing.

[d]1 missing.

[e]2 missing.

[f]4 missing.

[g]Bedaquiline, fluoroquinolone, linezolid, clofazimine, and cycloserine.

[h]3 exclusions (unavailable phenotypic drug susceptibility testing).

115 (95.0%; 95% CI, 89.5%–98.2%) had fluoroquinolones appropriately prescribed based on their Xpert XDR result. Of those with fluoroquinolone-sensitive Xpert XDR results, 98.8% (85/86; 95% CI, 93.7%–100.0%) had fluoroquinolones prescribed, while 85.7% (30/35; 95% CI, 69.7%–95.2%) of those with fluoroquinolone resistance per Xpert XDR had no fluoroquinolone prescribed. In addition, 91.7% (110/120; 95% CI, 85.2%–95.9%) of participants with valid Xpert XDR and phenotypic DST results had fluoroquinolones appropriately prescribed based on reference standard results, as compared with 82.9% (29/35; 95% CI, 66.4%–93.4%; $P = .13$) of participants without valid Xpert XDR results. Among participants who initiated DR-TB treatment, 4.1% (5/121) started fluoroquinolone treatment despite Xpert XDR indicating resistance, all of whom showed phenotypic resistance to fluoroquinolone. For 3 of 5 patients, fluoroquinolones were removed from the regimen before phenotypic DST results became available. Figure 2 shows the relationship among Xpert XDR results, initiation of a fluoroquinolone treatment, and phenotypic resistance for fluoroquinolone in patients with and without valid

Table 2. Diagnostic Accuracy of Xpert MTB/XDR vs Phenotypic DST

	Intention to Diagnose				Low-Level Resistance Intention to Diagnose Moxifloxacin (n = 136)
	Isoniazid (n = 128)	Fluoroquinolone (n = 125)	Low-Level Resistance Moxifloxacin (n = 125)	Isoniazid (n = 136)	Fluoroquinolone (n = 136)	
% (95% CI)						
Sensitivity	99.2 (95.5–100.0)	88.4 (74.9–96.1)	95.5 (77.2–99.9)	93.8 (88.1–97.3)	82.6 (68.6–92.2)	87.5 (67.6–97.3)
Specificity	100.0 (54.1–100.0)	100.0 (95.6–100.0)	83.5 (74.9–90.1)	100.0 (59.0–100.0)	100.0 (96.0–100.0)	84.8 (76.8–90.9)
No.						
TP	121	38	21	121	38	21
FP	0	0	17	0	0	17
FN	1	5	1	8	8	3
TN	6	82	89	7	90	95
% (95% CI)						
PPV	100.0 (97.0–100.0)	100.0 (90.7–100.0)	55.3 (38.3–71.4)	100.0 (97.0–100.0)	100.0 (90.7–100.0)	55.3 (38.3–71.4)
NPV	85.7 (42.1–99.6)	94.3 (87.1–98.1)	98.9 (93.8–100.0)	46.7 (21.3–73.4)	91.8 (84.5–96.4)	96.9 (91.3–99.4)

For 15 patients included in our analysis, sputum for culture and phenotypic DST was collected up to 3 days after Xpert MTB/XDR testing, and for 2 patients, positive sputum culture results for phenotypical DST could be obtained up to 2 months after Xpert MTB/XDR testing.

Abbreviations: DST, drug susceptibility testing; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative TP, true positive.

Xpert XDR results. Xpert XDR detected MTB in 80% (12/15) of samples from participants who were excluded from accuracy analyses due to negative or contaminated cultures.

DISCUSSION

In this pragmatic diagnostic accuracy and clinical utility evaluation, we found that Xpert XDR has high sensitivity and specificity for isoniazid and fluoroquinolone resistance when implemented in a programmatic setting. The positive and negative predictive values for resistance detection of both drugs were consistently high across a range of prevalences. Indeterminate resistance results from Xpert XDR were uncommon (4% for fluoroquinolones) and mostly associated with low sample mycobacterial load. The median time to treatment after

Figure 2. Relation among Xpert MTB/XDR results, fluoroquinolone treatment, and phenotypic drug susceptibility testing (DST) results in patients (*A*) with and (*B*) without valid Xpert MTB/XDR results. FQ, fluoroquinolone; Ind, indeterminate; Inv, invalid; N no; NA, not available (missing, not done); R, resistant; S sensitive; Y, yes.

Table 3. Clinical Use Cases of Xpert MTB/XDR Across Settings

Use case	Guide choice of WHO BDQ-Containing 6-mo Regimen (BPaLM and BPaL)	Guide drug choice of 9-mo All-Oral Regimen	Guide drug choice of Longer Individualized DR-TB Regimen	Detection of INH Monoresistance	Guide Treatment / Replace Programmatic Phenotypic DST[a]
Xpert MTB/XDR result	FQ resistance	• INH resistance • Low-level INH resistance • FQ resistance • Low-level FQ resistance	• INH resistance • FQ resistance • Low-level FQ resistance • ETO resistance • AMK resistance	• INH resistance • FQ resistance • Low-level INH resistance	• INH resistance • FQ resistance
Outcome based on Xpert MTB/XDR result	Exclude MFX/FQ (ie, BPaL instead of BPaLM)	• Exclude INH • Consider high-dose INH • Exclude fluoroquinolone • Consider high-dose moxifloxacin and confirmatory phenotypic DST at 1.0 mg/L	• Exclude INH • Exclude FQ • Consider high-dose MFX and confirmatory phenotypic DST at 1.0 mg/L • Exclude ETO • Exclude AMK	• Need for addition of FQ to regimen • Exclusion of FQ in regimen • Consideration of high-dose isoniazid	Selection of DS-TB/ DR-TB regimens
Advantages	+ Avoid toxicity and costs of ineffective drug treatment + Avoid increased risk of acquired drug resistance [21]	+ Avoid toxicity and costs of ineffective drug treatment + Could reduce need for phenotypic DST for (eg, INH, FQ) + Rapid treatment initiation + Avoid increased risk of acquired drug resistance [21]	+ Avoid toxicity and costs of ineffective drug treatment + Could reduce need for phenotypic DST for (eg, INH, FQ) + Rapid treatment initiation with confidence around susceptibility / resistance to INH, FQ, ETO, and injectables	+ Avoid ineffective drug treatment	+ Enable resistance testing and informed treatment decisions for patients without culture + Cost saving
Disadvantages	– ~10% started FQ inappropriately due to missed FQ resistance – Lack of information on BDQ and LZD susceptibility			– Expensive to use for all TB; will depend on background INH monoresistance rate	– Missing some INH and FQ resistance – Reduced surveillance for new mutations

Abbreviations: AMK, amikacin; BDQ, bedaquiline; BPaL, bedaquiline, pretomanid, and linezolid; BPaLM, bedaquiline, pretomanid, linezolid, and moxifloxacin; DR-TB, drug-resistant tuberculosis; DST, drug susceptibility testing; DS-TB, drug-sensitive tuberculosis; ETO, ethionamide; FQ, fluoroquinolones; INH, isoniazid; LZD, linezolid; MFX, moxifloxacin; TB, tuberculosis; WHO, World Health Organization.

[a]Guide treatment decisions for patients without viable culture / replace programmatic phenotypic DST for INH and FQ.

Xpert XDR testing was 4 days. Phenotypic DST took on average >5 weeks longer than Xpert XDR. The use of fluoroquinolone was largely consistent with Xpert XDR results. While a small subset of sensitive results (1%) for fluoroquinolone were later determined resistant on phenotypic DST, there were no false-positive resistance results on Xpert XDR. Nevertheless, some participants initiated a fluoroquinolone-containing regimen despite Xpert XDR indicating fluoroquinolone resistance.

Xpert XDR demonstrated similar diagnostic accuracy for isoniazid and fluoroquinolone resistance in routine care when compared with early accuracy clinical studies [6, 7, 16, 17]. However, our results of sensitivity for fluoroquinolone resistance detection (88%; 83% in intention-to-diagnose analysis) fall just below the recent WHO target product profile (TPP) targets (>90%) [18] and are slightly lower than estimates from testing programmatic specimens in South Africa [19]. Surprisingly, we observed lower sensitivity for fluoroquinolone resistance in samples with higher bacillary burden based on Xpert Ultra semiquantitative grade. Previous accuracy studies have reported no difference in sensitivity by smear status [6], and further data on this, including genotypic DST results, will be useful given our relatively small sample size. As previously demonstrated in research settings, Xpert XDR also exhibits a low indeterminate rate for isoniazid resistance detection with programmatic use [16, 20]. In this real-world setting, Xpert XDR enabled early informed treatment decisions for patients with rifampicin-resistant TB, with treatment initiation within a median 4 days of testing, substantially surpassing the turnaround time of phenotypic DST by weeks. Although missing approximately 10% of fluoroquinolone resistance, Xpert XDR still has significant utility to assess eligibility for inclusion of moxifloxacin in the WHO-recommended BPaLM regimen (bedaquiline, pretomanid, linezolid, and moxifloxacin), based on its high specificity for fluoroquinolone resistance and fast

turn-around time, as well as given the limitations of culture-based fluoroquinolone resistance testing [12].

Prior to implementation and scale-up of a new rapid DST, it is essential to clearly define the appropriate clinical use cases and how results should be interpreted by clinicians to inform patient treatment. Use cases proposed for Xpert XDR as a rapid molecular DST for isoniazid and fluoroquinolones include it as a reflex test following detection of rifampicin resistance [7, 17]. On the basis of current WHO treatment guidelines, previous literature, and our results of programmatic accuracy and utility, we propose several potential use cases for Xpert XDR (Table 3) [7, 12, 17, 19]. Of note for patients with rifampicin-resistant TB, the appropriate use of moxifloxacin in the BPaL (bedaquiline, pretomanid, and linezolid) or BPaLM regimen is important not only for avoiding unnecessary and potentially life-threatening side effects (eg, QT prolongation) caused by a non-effective drug combination [22] but also when considering the risk of acquiring further drug resistance on some regimens in the context of undiagnosed fluoroquinolone resistance [21]. There are also concerns about bedaquiline resistance and the use of the BPaL regimen. However, the detection of fluoroquinolone resistance through Xpert XDR could prompt expedited testing for bedaquiline resistance through targeted next-generation sequencing and/or phenotypic DST, if available [17]. Additionally, in our study, 80% of culture-negative samples had positive XDR results, yielding important information on drug resistance that would otherwise not be available.

Among patients in this analysis, 5% started a treatment regimen that included ineffective fluoroquinolone, and clinical data showed that treatment was changed for most when phenotypic results became available. This could be due to several factors, such as unfamiliarity with Xpert XDR or the diagnostic algorithm incorporating its use or suboptimal communication of results.

While the assay has demonstrated high diagnostic accuracy for isoniazid and fluoroquinolone resistance detection in research as well as routine care settings, there are limitations to its use in DR-TB due to implementation of newer, bedaquiline-based regimens. Xpert XDR's resistance panel, while useful for determining fluoroquinolone resistance prior to starting BPaL/BPaLM and for deciding on the use of ethionamide and amikacin in the longer regimens, does not align well with the current DR-TB treatment recommendations, most importantly by lacking resistance targets for bedaquiline [12]. According to the recent WHO TPP, rapid molecular DSTs should at least include targets for rifampicin, isoniazid, fluoroquinolone, as well as bedaquiline, to help protect acquisition of resistance to newer second-line DR-TB drugs [18].

Another limitation to the widespread use and the assay's potential clinical impact is the cost of the cartridge, currently around US $15 for the Global Fund, Stop TB Partnership, USAID, and Cepheid Global Access Program. Considering that an additional test is needed to determine resistance to rifampicin, the cost of Xpert XDR does not meet minimal TPP price requirements for DST [18]. Also the 10-color GeneXpert platform required to run Xpert XDR may not be available across settings. The potential of the test when used in a real-world programmatic setting, as demonstrated in our analysis, supports the calls for an affordable pricing of the cartridge to take down the costs of comprehensive DST. However, Xpert XDR may be a cost-effective replacement or alternative for phenotypic DST in some settings, given the high running costs of biosafety level 3 laboratories needed for mycobacterial culture.

The main strengths of this study are its embeddedness in a real-world programmatic setting with robust data collection and quality control for microbiological testing within a large diagnostic accuracy trial platform and the availability of clinical patient-level data to relate testing results to treatment decision making. We have also performed an intention-to-diagnose analysis to accurately demonstrate diagnostic performance in a real-world setting without overestimating sensitivity and specificity. However, the programmatic nature of our data comes with some limitations, including some missing data on date of sputum collection and a small number of patients with delays between sputum samples used for Xpert XDR and those used for phenotypic DST. Last, isoniazid resistance prevalence is extremely high in the patient population; thus, our sample size for specificity of isoniazid resistance detection analyses is small, resulting in wide confidence intervals for specificity estimates.

CONCLUSION

The high accuracy of Xpert XDR for detection of isoniazid and fluoroquinolone resistance in a programmatic setting is in line with estimates from diagnostic accuracy studies, although below WHO TPP targets, especially when accounting for indeterminate/invalid results. In a setting with available infrastructure and resources for phenotypic DST, the added benefit of Xpert XDR testing for clinical decision making lies mainly in a substantially shorter turnaround time. However, a key use case for the assay might be in settings without access to reliable phenotypic DST. While Xpert XDR remains useful as one of the few endorsed rapid, low-complexity molecular tests for isoniazid and fluoroquinolone resistance, there is a strong need for rapid DST that includes bedaquiline and other newer TB drugs, as well as the need for assessment of impact against diagnostic guidelines and algorithms.

Supplementary Data

Supplementary materials are available at *Open Forum 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. A. G.-W., N. T., and T. P. conceptualized and designed the study. Funding for the R2D2 project under which data collection for this study was conducted was acquired by C. M. D. and N. T. N. T., N. M., A. T., M. G., N. B., and T. P. conducted data collection. A. G.-W., N. T., and T. P. coordinated the research activity. C. M. D., S. Y., A. G.-W., and N. T. provided scientific support. T. P. and A. G.-W. did the formal data analysis. T. P., A. G.-W., and N. T. wrote the manuscript draft. All authors had full access to all data in the study, contributed to the interpretation of results, reviewed and edited the manuscript, approved the final version, and agreed to the submission for publication.

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Potential conflicts of interest. C. M. D. was working for FIND until 2019 and, through this, was involved in the development and evaluation of Xpert MTB/XDR. All other authors report no potential conflicts.

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