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A Performance Evaluation of MTBDR*plus* (version 2) for the Diagnosis of Multidrug-resistant Tuberculosis

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Summary

OBJECTIVE—To evaluate the performance of a recently updated rapid molecular diagnostic test, MTBDR*plus* version 2 (v2), designed to detect drug resistance in both acid-fast bacilli (AFB) smear negative and positive specimens.

DESIGN—Sputum samples from 1,128 patients at risk for multidrug-resistant tuberculosis (MDR-TB) were tested by MTBDR*plus* v2 and compared to reference standard MGIT 960 drug susceptibility testing. The relationship of participant HIV status, diabetic status, previous treatment, and smear gradation to the likelihood of obtaining an interpretable result was assessed using logistic regression.

RESULTS—MTBDR*plus* v2 sensitivity and specificity for detecting MDR-TB, when compared to a reference standard, were 96.0% (95%CI 93.5–97.6) and 99.2% (95%CI 97.0–99.9) for AFB smear positive specimens and 82.8% (95%CI 63.5–93.5) and 98.3% (95%CI 89.9–99.9) for AFB smear negative specimens, respectively. A dose-response relationship was observed between the proportion of interpretable test results and AFB smear bacterial load after adjusting for age, sex, BMI, HIV status, previous treatment, and diabetic status.

CONCLUSION—While MTBDR*plus* v2 performs well among both AFB smear positive and negative specimens, smear gradation appears to influence both the probability of obtaining an interpretable result and test sensitivity, indicating a significant association between bacillary load and test performance.

Keywords

Diagnostic; Tuberculosis; Multidrug-resistance

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BACKGROUND

Although global incidence rates of tuberculosis (TB) have been decreasing in recent years, multidrug-resistant TB (MDR-TB) or TB that is resistant to both isoniazid (INH) and rifampicin (RIF), the two most commonly used drugs for first-line treatment, threatens to undermine this recent progress. According to World Health Organization (WHO) estimates, in 2013, approximately 3.5% of new and 20.5% of previously-treated cases of TB were multidrug-resistant.[1] These global estimates, however, do not provide a complete picture of the MDR-TB epidemic, as subnational rates of MDR-TB have risen to as high as 35% among new cases and 75% among previously treated cases in several eastern European and central Asian countries.[1]

Historically, phenotypic drug susceptibility testing (DST) *of Mycobacterium tuberculosis (Mtb)* on solid media took months to produce results.[2] The advent of broth-based media reduced the time to results to weeks, and the recent introduction of rapid molecular-based diagnostics has further reduced this time to less than a day.[3–5] Despite these gains, including WHO approval of the GenoType MTBDR*plus* assay (Hain, Nerhen, Germany) and Xpert MTB/RIF assay (Cephid, Sunnyvale, USA) in 2008 and 2010, gaps in MDR-TB diagnosis remain. In 2013, it is estimated that less than half (45%) of MDR-TB cases were detected globally.[1] Differences in diagnostic test characteristics partially explain this gap in MDR-TB diagnosis. For example, Xpert MTB/RIF was developed to detect RIF resistance, but does not assess INH resistance. The MTBDR*plus* version 1 (v1) assay detects resistance to both RIF and INH, however given its performance, WHO recommended its usage among Acid-Fast Bacilli (AFB) smear positive samples only.[6]

In 2012, version 2 (v2) of the MTBDR*plus* assay was released to address performance issues associated with AFB smear status. Several small-scale studies have assessed the performance of this new assay version among homogenous populations, but a large-scale study in a diverse population has yet to be performed.[7–9] Additionally, the impact of AFB smear status, HIV status, body mass index (BMI), and other clinical factors associated with disease progression on the performance of MTBDR*plus* v2 have yet to be assessed.[10–13]

Expanding the rapid testing and detection of MDR-TB cases is one of the five priority actions outlined by WHO in their goals to address the global MDR-TB crisis.[1] Field trials of rapid point-of-care diagnostics provide valuable information for clinical decision making in resource limited settings.[14] In this study we describe the performance of the MTBDR*plus* v2 assay in a large diverse field trial among both AFB smear positive and negative specimens.

METHODS

Study design

This prospective (diagnostic) cohort study was conducted as part of the Global Consortium for Drug-resistant TB Diagnostics (GCDD), a collaboration between UCSD and study sites in India, Moldova, and South Africa. Informed consent was obtained from all patients prior to study enrollment. This study was approved by institutional review boards at the

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University of California, San Diego as well as the participating institutions at the three study sites.

Details of patient recruitment and selection have been reported previously.[15, 16] In brief, between 2012 and 2013, TB patients with risk factors for drug-resistant TB were screened at participating study sites. Patients were eligible if they were at least five years of age and had an AFB smear positive or a positive Xpert result in the previous fourteen days. It addition, patients had to meet at least one of the following MDR suspicion criteria: received >1 month of treatment for a prior TB episode, failing TB treatment with positive sputum smear or culture after 3 months of standard TB treatment, close contact with a known drug-resistant TB case, newly diagnosed with MDR-TB within the previous 30 days, or previously diagnosed with MDR-TB and failed TB treatment with positive sputum smear or culture after 3 months of a standard MDR-TB treatment regimen. Patients were excluded if they were unable to provide at least 7.5 mL combined sputum or had received second line drug susceptibility testing results within the previous three months.

Specimen collection and processing

The initial sputum sample was collected by study staff at the clinic site during enrollment and the second sample by the participant themselves upon waking the following morning. These two samples were pooled and used for multiple diagnostic tests including AFB smear, MTBDR*plus* v2 line probe assay, and MGIT 960 culture (the inoculum for MGIT 960 DST). All tests were performed by local study site laboratories according to manufacturer instructions or per standard procedures.[17, 18] *Mtb* culture positive specimens were tested for drug susceptibility to RIF and INH resistance at concentrations of 1.0 and 0.1 (μg/ml) respectively.[19]

MTBDRplus v2

The updated MTBDR*plus* v2 assay strip contains 27 hybridization probes. Two probes, the conjugate and amplification probes, ensure test function. Four control probes confirm the presence of *Mtb* and identify wild type loci sequences for the *rpoB*, *katG*, and *inhA* promoter gene regions. Eight *rpoB* wild type probes overlap gene regions between codons 505 and 533. Four *rpoB* mutant probes identify the specific mutations D516V, H526Y, H526D, and S531L. One wild type probe is specific for the *katG*315 codon and two mutant probes identify S315T mutations at this codon. Two *inhA* wild type probes cover the *inhA* promoter from position −15 to −8. And, four mutant probes identify specific nucleic acid changes in the *inhA* promotor region (C-15T, A-16G, T-8C, and T-8A). The absence of one or more wild type probes for a given resistance-associated gene region, or the absence of one or more wild type probes in addition to the presence of one or more mutant probes for a given gene region, is considered indicative of resistance. Assay results are classified as indeterminate if either of the test function probes are absent; if the *Mtb* or gene loci probes are absent; or if all wild type probes, in addition to mutation probes, are present.[18]

Data Analysis

Performance of MTBDR*plus* v2 was described by computing sensitivity, specificity, likelihood ratio positive (LR+), and likelihood ratio negative (LR−) for RIF resistance, INH

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resistance, and MDR-TB; confidence intervals for all proportions were calculated using the Wilson Score method.[20] Logistic regression was used to determine if any demographic or clinical data were associated with obtaining valid MTBDR*plus* v2 results. Analyses were performed using Stata 13 (College Station, TX).

RESULTS

A total of 1,128 participants were enrolled in the study. Nine-hundred and fourteen (81%) were *Mtb* culture positive, and of those culture positive specimens 540 (59%) were phenotypically RIF resistant and 592 (65%) were phenotypically INH resistant. Among the 540 phenotypically RIF resistant specimens, 462 (86%) were classified as resistant, 16 (3%) were classified as susceptible, and 62 (11%) were indeterminate by MTBDR*plus* v2. Among the 592 phenotypically INH resistant specimens, 478 (82%) were classified as resistant, 31 (5%) were classified as susceptible, and 83 (14%) were indeterminate by *MTBDRplus* v2 (Table 1). Sub-analysis revealed that among six phenotypically RIF mono-resistant specimens, two were classified as resistant, one as susceptible, and three as indeterminate by MTBDR*plus* v2; and among 58 phenotypically INH mono-resistant specimens, 32 (55%) were classified as resistant, 15 (26%) were classified as susceptible, and 11 (19%) were indeterminate by MTBDR*plus* v2. Culture negative specimens were also assessed using the MTBDR*plus* v2 assay and 42 (20%) specimens were classified as either INH and/or RIF resistant or susceptible.

Overall, sensitivity and specificity of MTBDR*plus* v2 when compared to culture for the detection of *Mtb* were 91.8% (95%CI 89.9–93.5) and 76.2% (95%CI 69.8–81.6). Among specimens with both valid phenotypic drug susceptibility and valid MTBDR*plus* v2 results, sensitivity and specificity of MTBDR*plus* v2 were 96.7% (95%CI 94.5–98.0) and 97.9% (95%CI 95.5–99.1) for detection of RIF resistance; 93.9% (95%CI 91.4–95.8) and 99.6% (95%CI 97.7–100.0) for INH resistance; and 95.1% (95%CI 92.6–96.9) and 99.1% (95%CI 97.1–99.8) for MDR-TB detection. Additionally, performance characteristics were stratified by AFB status (Table 2) and study site (Table 3). Point estimates for both sensitivity and specificity were consistently lower for AFB smear negative specimens than for AFB smear positive specimens. MTBDR*plus* v2 sensitivity and specificity were consistent across study sites with the exception of INH and RIF sensitivity estimates in South Africa, which were significantly lower than estimates in India and Moldova.

Among the 914 *Mtb* culture positive specimens, 102 (11%) were classified as indeterminate for RIF resistance and 121 (13%) were classified as indeterminate for INH resistance by MTBDR*plus* v2 assay. The absence of the *Mtb* control probe was the primary (~70%) cause of indeterminate classification. A majority of the remaining of specimens were classified as indeterminate due to the presence of uninterpretable banding patterns. The proportion of indeterminates did not vary significantly by study site.

Correlates of Test Performance

The distribution of demographic and clinical factors among valid (resistant or susceptible) versus invalid (indeterminate) MTBDR*plus* v2 assay results are presented in Table 4. Univariate analysis was used to assess any significant difference in variable distribution.

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AFB smear grade of +1 or greater was significantly associated with valid results for both RIF and INH. Body mass index (BMI) appeared borderline significantly inversely associated with obtaining a valid result for INH, and significantly inversely associated with obtaining a valid result for RIF, indicating that those with a higher BMI were more likely to have an invalid test result. The likelihood of obtaining a valid test was not significantly associated with age, sex, HIV status, previous treatment, diabetic status, or study site.

Three models were generated to determine the relationship between clinical and demographic factors and the likelihood of a valid or successful test (Table 5). The first model included only AFB smear gradation, the only significant factor associated with valid results for both RIF and INH in the univariate analysis. The second model included BMI to account for any residual association of bacilli load and test performance based on the assumption that individuals with low BMI would likely produce sputum with larger amounts of bacilli due to disease progression. The third model included three more variables, HIV status, a known confounder of test performance, and previous TB treatment and diabetic status, both associated with disease progression. The models were compared using the likelihood ratio test; models two and three were not statistically superior to model one. After controlling for smear status, BMI was no longer significantly associated with valid results in any of the models. HIV status, previous TB treatment status, BMI and diabetic status were not significantly associated with test performance (valid results). However, AFB smear grade was significantly associated with both RIF and INH test performance. Higher amounts of bacteria in the sputum as defined by AFB smear grade were associated with significantly more valid test results. A dose response relationship was evident; interpretable test results increased with each progressive AFB smear grade, culminating in an AFB smear +3 being approximately 10 times more likely to result in an interpretable MTBDR*plus* v2result compared to smear negative specimens.

DISCUSSION

Overall, MTBDR*plus* v2 performed well among both AFB smear positive and negative specimens. However, performance of the test, particularly sensitivity, was affected by AFB smear status. Sensitivity for the detection of MDR-TB among AFB smear positive specimens was 96.0% (95%CI 93.5–97.6) compared to 82.8% (95%CI 63.5–93.5) among AFB smear negative specimens. This difference in sensitivity between AFB smear negative and positive specimens was more pronounced for INH resistance, 81.6 (95%CI 65.1–91.7) versus 94.9% (95%CI 92.4–96.6) than for RIF resistance, 91.4 (95%CI 75.8–97.8) versus 97.1% (95%CI 94.9–98.4). Additionally, the proportion of results that were valid was strongly correlated with AFB smear gradation. After adjusting for BMI, HIV status, diabetic status, and history of previous TB treatment, AFB smear +3 specimens were approximately 10 times more likely to result in a valid result when compared to AFB smear negative specimens.

Among the 914 culture positive specimens, less than 1% of MTBDR*plus* v2 test results were indeterminate due to test failure. Our observed rates of indeterminate assay results (11 to 13%) among culture positive specimens, were similar to those reported by WHO of 10 to 14%.[6] If the study protocol had included repeat testing for indeterminate results, most likely

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indeterminate rates would have been comparable to those reported by Luetkemeyer et al. of 3 to 5% among culture positive specimens.[21]

Mtb was detected, as evidenced by hybridization of the *Mtb* probe, in 92% of all culture positive specimens (both AFB smear negative and positive). This result is slightly higher than two smaller studies evaluating MTBDR*plus* v2 among a mix of AFB smear positive and negative specimens, which reported sensitivities to *Mtb* of 73% and 88%.[7, 8] When comparing performance among only AFB smear negative specimens, the *Mtb* sensitivity for the present study, 74%, was lower than the 79–80% reported by Crudu et al. and higher than the 57% reported by Bernard et al.[7, 8] As a comparison, MTBDR*plus* v1, although not recommended for use on AFB smear negative samples, had previously reported *Mtb* detection rates among exclusively AFB smear negative specimens of 46%, 48% and 65%.[21–23] When compared to Xpert MTB/RIF as reported by the Cochrane Review, the sensitivity of MTBDR*plus* v2 to *Mtb* among AFB smear positive specimens of 92% was lower than the pooled sensitivity of Xpert MTB/RIF of 98%; however the sensitivity of MTBDR*plus* v2 to *Mtb* among AFB smear negative specimens of 74%, was higher than the Xpert MTB/RIF sensitivity of 67%.[24]

The MTBDR*plus* v2 assay also detected *Mtb* among 24% of culture negative specimens in the current study. Probe hybridization in these culture negative cases may have been caused by the presence of non-viable bacteria in the specimen or may indicate that bacteria were present in amounts too small to sustain culture growth.

Direct comparison of assay results by smear status to previously published MTBDR*plus* v2 results was difficult as all previous studies assessed only small numbers of drug resistant specimens. However, the sensitivity and specificity of the test for RIF resistance in this study, stratified by smear status, was similar (within 95% confidence intervals) to those reported by Crudu et al.[7] The only evident difference in INH resistance performance measures between the current study and the Crudu et al. study was the sensitivity and specificity of the test for AFB smear negative specimens. INH sensitivity and specificity among AFB smear negative specimens in this study was 81.6% (95%CI 65.1–91.7) and 98.1% (95%CI 88.6–99.9) respectively, compared to 93.5% and 82.3% reported by Crudu et al. N'guessan et al. reported RIF sensitivity and specificity only among AFB smear positive specimens, and the only evident difference in assay performance was the sensitivity of RIF resistance (73.2%), which was significantly lower than reported in the current study (97.1%, 95%CI 94.9–98.4).[9] The sensitivity and specificity of MTBDR*plus* v2, as reported by Barnard et al., were 100% for both RIF and INH resistance, higher than both estimates from the current study.[8]

Given an absence of large diverse studies assessing the overall performance of MTBDR*plus* v2, sensitivity and specificity for RIF and INH could only be compared to the performance of MTBDR*plus* v1, as reported by the WHO Expert Group Report and a meta-analysis of the *MTBDRplus* v1 by Ling et al.[6, 25] Overall, the sensitivity for RIF resistance found in this study (96.7%, 95%CI 94.5–98.0) was slightly lower than reported by the WHO (98.4%) and by Ling et al. (98.4%).[6, 25] In contrast, overall sensitivity for INH resistance in this study (93.9% 95%C: 91.4–95.8) was slightly higher than reported by the WHO (91.4%) and by

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Ling et al. (88.7%). Specificities of both RIF and INH resistance from the current study were similar to those reported by the WHO and Ling et al.

Smear gradation was the strongest predictor of obtaining valid results (either susceptible or resistant) for either RIF and INH, even after adjusting for age, gender, BMI, previous history of TB treatment, HIV status, and diabetic status. As smear positivity increased, so did the odds of obtaining a valid assay result. This correlates with both the WHO findings for version 1 of the MTBDR*plus* assay and those reported by Singhal et al. for both version 1 and 2 of the MTBDR*plus* assay, where a significant association was found between smear gradation and the proportion of interpretable or valid results.[6, 26]

One notable limitation of this study was the lack of repeat testing of indeterminate MTBDR*plus* v2 assay results. This limitation likely contributed to a higher rate of indeterminate results than may have otherwise been reported if indeterminate MTBDR*plus* v2 assay results had been repeated.

CONCLUSIONS

The MTBDR*plus* v2 assay performed well among both AFB smear positive and negative specimens in the current study, sensitivity and specificity for the detection of RIF resistance, INH resistance, and MDR-TB by MTBDR*plus* v2 were 96.7% (95%CI 94.5–98.0) and 97.9% (95%CI 95.5–99.1), 93.9% (95%CI 91.4–95.8) and 99.6% (95%CI 97.7–100.0), and 95.1%(95%CI 92.6–96.9) and 99.1% (95%CI 97.1–99.8), respectively. Smear gradation appeared to influence both the probability of obtaining an interpretable result and test sensitivity and specificity, indicating a significant association between bacillary load and test performance. MTBDR*plus* v2 does however appear to consistently provide accurate results for both RIF and INH resistance.

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Table 1

Detection of rifampicin and isoniazid resistance by MTBDR*plus* v2 versus MGIT 960

	MGIT Results				
MTBDR*plus* v2	**Resistant**	**Susceptible**	**Culture Negative**	**Null/Not Done**	**Total**
Rifampicin					
Resistant	462	7	9	0	478
Susceptible	16	324	32	3	375
Indeterminate	62	36	173	4	275
Total	540	367	214	7	1128
Isoniazid					
Resistant	478	1	5	0	484
Susceptible	31	280	33	3	347
Indeterminate	83	34	176	4	298
Total	592	315	214	7	1128

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Table 2

Performance parameters of MTBDR*plus* v2 in detecting rifampicin, isoniazid, and multidrug-resistant TB (MDR-TB) compared to conventional DST, stratified by smear status*

Drug Resistance Smear Status	Isolates†	Sensitivity %	Specificity %	LR+	LR−
Mtb	1128	91.8 (89.8, 93.5)	76.2 (69.8, 81.6)	4 (3, 5)	0.11 (0.09, 0.14)
AFB +	826	95.6 (93.8, 96.9)	74.1 (60.7, 84.4)	4 (2, 6)	0.06 (0.04, 0.09)
AFB −	302	71.9 (63.8, 78.9)	76.9 (69.4, 83.1)	3 (2,4)	0.37 (0.28, 0.48)
RIF^R	809	96.7 (94.5, 98.0)	97.9 (95.5, 99.1)	46 (22, 95)	0.03 (0.02, 0.06)
AFB +	712	97.1 (94.9, 98.4)	98.5 (96.0, 99.5)	65 (25, 173)	0.03 (0.02, 0.05)
AFB −	97	91.4 (75.8, 97.8)	95.2 (85.6, 98.7)	19 (6, 57)	0.09 (0.03, 0.27)
INH^R	790	93.9 (91.4, 95.8)	99.6 (97.7, 100.0)	264 (37, 1867)	0.06 (0.04, 0.09)
AFB +	699	94.9 (92.4, 96.6)	100.0 (97.9, 100.0)	∞	0.05 (0.03, 0.08)
AFB −	91	81.6 (65.1, 91.7)	98.1 (88.6, 99.9)	43 (6, 303)	0.19 (0.10, 0.37)
MDR-TB	775	95.1 (92.6, 96.9)	99.1 (97.1, 99.8)	102 (33, 316)	0.05 (0.03, 0.07)
AFB +	686	96.0 (93.5, 97.6)	99.2 (97.0, 99.9)	126 (32, 502)	0.04 (0.03, 0.06)
AFB −	89	82.8 (63.5, 93.5)	98.3 (89.9, 99.9)	50 (7,349)	0.18 (0.08, 0.39)

*Values are percentages with 95% confidence interval in parentheses.

†Samples that did not have both interpretable phenotypic and genotypic results were excluded from analysis.

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Table 3

Performance parameters of MTBDR*plus* v2 by Study Site

	Resistance Smear Status	Isolates	Sensitivity %	Specificity %
India	RIF	438	98.0 (95.8, 99.1)	98.8 (92.5, 99.9)
	AFB+	422	98.0 (95.7, 99.1)	98.7 (91.9, 99.9)
	AFB−	16	100.0 (62.9, 100.0)	100.0 (56.1, 100.0)
	INH	433	97.3 (94.9, 98.6)	100.0 (93.3, 100.0)
	AFB+	418	97.5 (95.1, 98.9)	100.0 (92.9, 100.0)
	AFB−	15	90.9 (57.1, 99.5)	100.0 (39.6, 100.0)
	MDR-TB	426	96.2 (93.4, 97.9)	98.8 (92.5, 99.9)
	AFB+	411	96.4 (93.6, 98.0)	98.7 (92.0, 99.9)
	AFB−	15	88.9 (50.7, 99.4)	100.0 (51.7, 100.0)
Moldova	RIF	204	97.0 (90.9, 99.2)	98.1 (92.5, 99.7)
	AFB+	166	98.7 (92.0, 99.9)	97.8 (91.4, 99.6)
	AFB−	38	91.7 (71.5, 98.5)	100.0 (73.2, 100.0)
	INH	193	92.7 (85.7, 96.6)	100.0 (94.5, 100.0)
	AFB+	161	94.4 (86.8, 97.9)	100.0 (93.7, 100.0)
	AFB−	32	85.7 (62.6, 96.2)	100.0 (67.9, 100.0)
	MDR-TB	190	94.5 (87.1, 98.0)	99.0 (93.7, 99.9)
	AFB+	158	98.6 (91.5, 99.9)	98.8 (92.8, 99.9)
	AFB−	32	78.9 (53.9, 93.0)	100.0 (71.7, 100.0)
South Africa	RIF	167	72.2 (49.6, 88.4)	97.2 (92.6, 99.1)
	AFB+	124	75.0 (50.6, 90.4)	99.0 (94.0, 99.9)
	AFB−	43	50.0 (2.7, 97.3)	92.7 (79.0, 98.1)
	INH	164	61.8 (43.6, 77.3)	99.2 (95.2, 100.0)
	AFB+	120	64.3 (44.1, 80.7)	100.0 (95.0, 100.0)
	AFB−	44	50.0 (13.9, 86.1)	97.4 (84.6, 99.9)
	MDR-TB	159	77.8 (51.9, 92.6)	99.3 (95.5, 100.0)
	AFB+	117	76.5 (49.8, 92.2)	100.0 (95.4, 100.0)
	AFB−	42	100.0 (5.5 100.0)	97.6 (85.6, 99.9)

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Table 4

Distribution of patient demographic and clinical characteristics by validity of MTBDR*plus* v2 assay results for rifampicin (RIF) and isoniazid (INH)[*]

	RIF results			INH results		
	Valid (n=812)	**Invalid (n=102)**	**p-value**	**Valid (n=793)**	**Invalid (n=121)**	**p-value**
Smear gradation						
Negative (ref)	98 (12.1)	48 (47.1)		92 (11.6)	54 (44.6)	
Rare or Scanty	58 (7.1)	17 (16.7)	0.12	55 (6.9)	20 (16.5)	0.13
Few (+1)	171 (21.1)	14 (13.7)	<0.001	166 (20.9)	19 (15.7)	<0.001
Many (+2)	168 (20.7)	7 (6.9)	<0.001	165 (20.8)	10 (8.3)	<0.001
TNTC (+3)	317 (39.0)	16 (15.7)	<0.001	315 (39.7)	18 (14.9)	<0.001
Age (continuous)	33 (24,45)	33 (24, 47)	0.39	33 (24,45)	33 (24, 46)	0.63
Male	525 (64.7)	61 (59.8)	0.34	514 (64.8)	72 (59.5)	0.26
HIV Positive (vs. Negative/Unknown)	99 (12.2)	14 (13.7)	0.66	97 (12.2)	16 (13.2)	0.75
Previous treatment	629 (77.5)	78 (76.5)	0.82	619 (78.1)	88 (72.7)	0.20
BMI[†] (continuous)	18.0 (16.0, 20.4)	19.6 (17.3, 21.8)	0.005	18.0 (16.0, 20.4)	18.8 (16.9, 21.3)	0.06
Diabetes	42 (5.2)	7 (6.9)	0.49	43 (5.4)	6 (5.0)	0.83
Site						
India (ref)	439 (53.8)	53 (52.0)		433 (54.6)	59 (48.8)	
Moldova	204 (25.1)	22 (21.6)	0.67	193 (24.3)	33 (27.3)	0.33
South Africa	169 (21.4)	27 (26.5)	0.27	167 (21.1)	29 (24.0)	0.32

[*] Categorical data are reported as percentages; continuous data as medians with interquartile ranges (Q1, Q3)

[†] Weight (kg)/height $(m)^2$

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Table 5

Logistic regression of demographic and clinical characteristics and the odds of obtaining a valid (resistant or susceptible) versus invalid (indeterminate) MTBDR*plus* v2 result

Characteristics	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
Rifampicin Resistance			
Smear gradation			
Negative (ref)			
Rare	1.7 (0.9, 3.2)	1.6 (0.9, 3.1)	1.8 (0.9, 3.4)
Few	6.0 (3.1, 11.4) *	6.6 (3.0, 11.1) *	6.6 (3.4, 12.7) *
Many	11.8 (5.1, 27.0 *	11.6 (5.1, 26.7) *	12.4 (5.4, 28.8) *
TNTC	9.7 (5.3, 17.8) *	8.9 (4.8, 16.6) *	10.6 (5.6, 20.1) *
BMI		0.9 (0.9, 1.0)	0.9 (0.9, 1.0)
Previously Treated			0.6 (0.4, 1.1)
HIV status			1.3 (0.7, 2.7)
Diabetic status			0.7 (0.3, 1.7)
Isoniazid Resistance			
Smear gradation			
Negative (ref)			
Rare	1.6 (0.9, 3.0)	1.6 (0.9, 3.0)	1.7 (0.9, 3.2)
Few	5.1 (2.9, 9.2) *	5.1 (2.8, 9.1) *	5.3 (2.9, 9.6) *
Many	9.7(4.7, 20.0) *	9.6 (4.7, 19.8) *	10.0 (4.8, 20.6) *
TNTC	10.3 (5.7, 18.4) *	10.0 (5.5, 17.9) *	10.6 (5.8, 19.5) *
BMI		1.0 (0.9, 1.0)	0.9 (0.9, 1.0)
Previously Treated			0.9 (0.5, 1.4)
HIV status			1.3 (0.7, 2.5)
Diabetic status			1.0 (0.4, 2.5)

* *P* values meeting significance criterion with an alpha < 0.05

OR= odds ratio; CI = confidence interval