INT J TUBERC LUNG DIS 19(12):1528–1535

http://dx.doi.org/10.5588/ijtld.14.0956

Systematic screening for drug-resistant tuberculosis with Xpert® MTB/RIF in a referral hospital in Cambodia

N. Lorent,*† C. Kong,* T. Kim,‡ S. Sam,§ S. Thai,* R. Colebunders,†¶ L. Rigouts,#** L. Lynen†

*Infectious Diseases Department, Sihanouk Hospital Centre of HOPE, Phnom Penh, Cambodia; †Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; ‡Mycobacteriology Laboratory, Sihanouk Hospital Centre of HOPE, Phnom Penh, §Cambodian Health Committee, Phnom Penh, Cambodia; ¶Epidemiology and Social Medicine, University of Antwerp, Antwerp, #Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, **Department of Biomedical Sciences, University of Antwerp, Belgium

SUMMARY

SETTING: Limited access to drug susceptibility testing (DST) in referral hospitals contributes to delayed detection of multidrug-resistant tuberculosis (MDR-TB).

OBJECTIVE: To document the impact of identifying rifampicin (RMP) resistance using Xpert® MTB/RIF on time to diagnosis and time to treatment, and evaluate its performance under programmatic conditions.

METHODS: Using a prospective observational study, we screened presumptive MDR-TB cases with Xpert and solid culture/conventional DST. RMP resistance was confirmed using a line-probe assay (LPA). We recorded diagnostic and treatment delays. We performed *rpo*B gene sequencing post hoc to resolve discordant RMP susceptibilities.

RESULTS: We screened 299 of 345 presumptive MDR-TB individuals, and identified 44 Xpert RMP-resistant cases: 16/165 (10%) were new and 28/136 (20%) retreated. The median time to diagnosis was 2 days (Xpert) vs. an additional 6 with LPA; the median time to treatment was 14 days. Confirmatory LPA on 39/44 revealed 27 concordant, 6 discordant and 6 invalid results. Xpert RMP resistance was confirmed in respectively 24/30 (80%) and 21/23 (91%) by phenotypic DST and *rpo*B sequencing.

CONCLUSION: Screening presumptive MDR-TB patients with Xpert enabled rapid diagnosis and treatment of MDR-TB. Xpert performed well, provided appropriate risk assessment was done. Rapid confirmatory testing added little to clinical decision making. Our findings support the latest World Health Organization guidelines to abandon confirmatory LPA in favour of repeat Xpert when in clinical doubt, pending phenotypic DST.

KEY WORDS: rapid drug susceptibility testing; rifampicin resistance; positive predictive value

WITH ONLY 45% OF THE ESTIMATED 480 000 new multidrug-resistant (MDR) tuberculosis (TB) cases detected in 2013 and an overall success rate for second-line treatment of <50%, drug-resistant TB (DR-TB) is jeopardising worldwide TB control efforts.[1] Although considerable progress has been made globally, the response is far from sufficient. Expanding access to drug susceptibility testing (DST) and reducing the time to diagnosis and treatment are key strategies to prevent the transmission of DR-TB.[2] Until recently, diagnosing DR-TB relied mainly on conventional culture and phenotypic DST—a costly, highly specialised technique, requiring sophisticated laboratory infrastructure not readily available in low-income settings.[3] Furthermore, results took 1–4 months, which was too slow to have a meaningful impact on patient management.[4]

The development of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA)—a rapid nucleic acid amplification test—enabled accurate and timely diagnosis of TB and detection of rifampicin (RMP) resistance.[5] In the light of these advantages, the World Health Organization (WHO) rapidly endorsed Xpert as the initial diagnostic test for the screening of individuals with presumptive MDR-TB and human immunodeficiency virus (HIV) infection to allow rapid initiation of appropriate treatment and to reduce disease transmission.[6]

In its initial guidelines, the WHO recommended that RMP resistance identified by Xpert be confirmed by conventional DST or line-probe assay (LPA) in settings with <15% MDR-TB prevalence,[6] citing the poor positive predictive value (PPV) and acknowledging the major implications of diagnosing RMP resistance, a surrogate for MDR-TB.[7] Challenges to accessing these tests in many settings complicated the implementation of the proposed algorithm and limited the impact of Xpert.[8,9] Furthermore, as the

Correspondence to: Natalie Lorent, Pulmonology Department, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium. Tel: (+32) 3 247 66 36. Fax: (+32) 3 247 66 36. e-mail: natalie.lorent@uzleuven.be
Article submitted 17 December 2014. Final version accepted 22 June 2015.

complexity of interpreting various (sometimes discordant) test reports could result in unnecessary confusion and delay appropriate management,[10–13] some authors suggested omitting rapid confirmatory testing, provided adequate patient selection is applied ensuring a high pre-test probability.[14] This strategy has been adopted by the WHO in its most recent Xpert policy.[15] However, how this will improve clinicians' confidence in making appropriate management decisions requires further evaluation.

Publications on the performance of Xpert in the detection of RMP resistance are relatively abundant.[5,16,17] Apart from two observational studies from South Africa, however, few studies have evaluated the implementation and impact of screening for drug resistance using Xpert under field conditions.[18,19] Compared with conventional DST, Xpert increases case detection,[18] and reduces the time to diagnose MDR-TB and initiate appropriate treatment.[18,19] Evidence of impact on individual patient outcomes[19] and disease transmission[20] are encouraging, although these require further research.

The purpose of the present study was to document the impact of rapid molecular DST methods such as Xpert and LPA when used for the systematic screening of individuals with presumptive MDR-TB in terms of MDR-TB case detection and management in an urban Cambodian setting. We also evaluated Xpert performance in the identification of RMP resistance under programmatic conditions.

METHODS

In this prospective observational study conducted from February 2012 to March 2014, we systematically screened all consecutive patients aged ⩾15 years presenting to our TB clinic either at the start of or on first-line treatment to determine their risk of DR-TB.

Study setting

Cambodia has the second highest TB prevalence rate in the world, and 6.3% of TB patients are HIV-infected.[1] According to the latest 2006 national drug resistance survey, MDR-TB prevalence was respectively 1.4% and 10.5% among new and previously treated cases (vs. 0% and 3.1% in 2001).[21,22] In 2011, only 11% of the estimated MDR-TB cases were detected,[23] which prompted the National TB Programme (NTP) to prioritise MDR-TB detection and invest in better diagnosis.

The present study was conducted at the Sihanouk Hospital Centre of HOPE (SHCH), a referral hospital providing free of charge medical care to the poor in Phnom Penh, Cambodia. The SHCH operates an HIV treatment centre caring for over 3000 HIV patients, and manages a TB clinic under the NTP network. The mycobacteriology laboratory is equipped with fluorescence microscopy, conventional culture, DST and Xpert testing. In 2011, the year before our intervention, 1786 patients were screened for TB using routine culture, of whom 221 were positive. Of the 168 patients for whom DST was requested at the physician's discretion, 7 were MDR-TB.

Presumptive MDR-TB screening algorithm at SHCH

Criteria for presumptive (M)DR-TB included 1) previously treated patients (failure, relapse, return after default), 2) symptomatic close contacts of known MDR-TB cases, 3) new TB with delayed smear conversion at month 2/3 of first-line treatment, and 4) all HIV-infected patients, regardless of smear results. Except for the latter (the NTP recommends screening of smear-positive HIV patients only), we followed national guidelines (Figure).

All eligible individuals with presumptive MDR-TB were asked to submit two spot sputum specimens at the first clinic encounter and another early morning sample the next day. In addition to routine smear examination by fluorescence microscopy (iLED Primostar, Zeiss, Germany), a single Xpert test was performed on a random spot or morning sputum specimen. Solid conventional culture was performed on the remaining specimen. If Xpert revealed RMP resistance, the patient was contacted to return for further work-up. An experienced clinician then interviewed and examined the patient, and requested a chest radiography (CXR) to evaluate the extent of lung involvement and a confirmatory LPA (on the same sample as Xpert, provided sufficient material was available) to rapidly confirm RMP and isoniazid (INH) resistance. At the time of the study, national guidelines recommended awaiting confirmatory DST results before referring patients for second-line treatment. Based on emerging evidence[14] and accumulating numbers of inconclusive LPA results, second-line treatment referrals from August 2013 onwards were based on Xpert and clinical risk assessment, awaiting conventional indirect DST.

Patients with RMP resistance were discussed with the national coordinator of the MDR-TB programme on the same day. Referral to a designated MDR-TB treatment site was organised as soon as bed capacity and health provider availability allowed. MDR-TB treatment in Cambodia was directly observed in line with 2011 WHO recommendations. A standard regimen comprised at least 20 months of levofloxacin (or moxifloxacin), ethionamide (or prothionamide), cycloserine (or p-aminosalicylic acid), pyrazinamide and ethambutol (if no resistance was detected), supplemented with kanamycin injections in the minimum 8-month initial phase. Second-line treatment was modified, where required, based on final DST results. Most patients starting second-line anti-tuberculosis treatment were admitted to hospital, and, when indicated, continued on ambulatory care. Second-line anti-tuberculosis drugs were quality

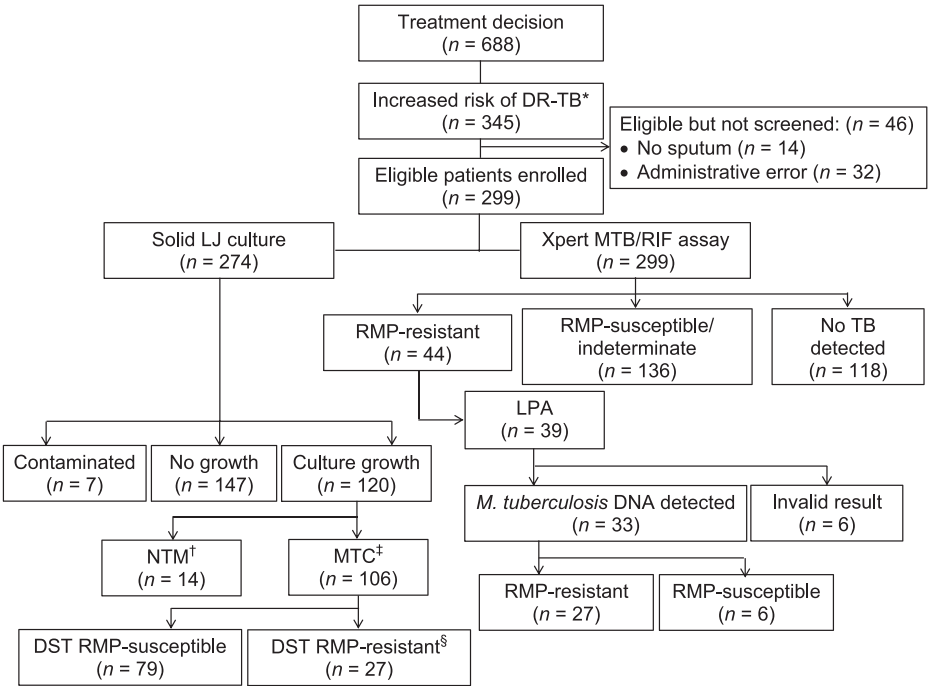

Figure Screening algorithm of individuals at increased risk for DR-TB. *Indications for Xpert testing were 1) a history of anti-tuberculosis treatment, 2) delayed smear conversion (at month 2 or 3) on first-line drugs, 3) symptomatic contact of a known MDR-TB case, and 4) HIV positivity regardless of smear status. Testing criteria were not mutually exclusive. †All 14 NTM grew from Xpert-negative (*M. tuberculosis* not detected) specimens. ‡MTC was isolated in 106 (88.3%) patients: 4 from Xpert '*M. tuberculosis* not detected' and 102 from Xpert '*M. tuberculosis* detected' specimens. §Includes 24/30 RMP-resistant, 2 RMP-susceptible and 1 with indeterminate RMP susceptibility on Xpert. DR-TB = drug-resistant TB; LJ = Löwenstein-Jensen; RMP = rifampicin; TB = tuberculosis; LPA = line probe assay; NTM = non-tuberculous mycobacteria; MTC = *Mycobacterium tuberculosis* complex; DST = drug susceptibility testing.

assured through the Green Light Committee and provided free of charge, as were all ancillary drugs, nutritional support and transport.

All TB patients were offered HIV testing. If positive, antiretroviral therapy was started within 2 weeks of MDR-TB treatment initiation if the patient's condition allowed.

Definitions

We used the revised WHO definitions for case definitions of drug-susceptible and DR-TB.[24] The time to treatment initiation was calculated from the time of sputum collection to the time of second-line treatment initiation. This comprised 1) the diagnostic delay (time from sputum collection to Xpert result); 2) referral delay (time from obtaining Xpert result to referral to a designated MDR-TB treatment site); and 3) second-line treatment initiation delay (time from patient referral to actual start of treatment at the referral site). We also calculated the turnaround time for LPA results separately, as it did not affect time to treatment for all patients.

Laboratory procedures

Xpert testing was performed directly on sputum according to the manufacturer's instructions. Conventional culture was performed on solid medium (Löwenstein-Jensen) following the standard operating procedures of the laboratory. All the above procedures were performed at the SHCH Mycobacteriology laboratory. An MTBDR*plus* assay (Hain Lifesciences, Nehren, Germany), directly on sputum, was performed at the Institut Pasteur du Cambodge in Phnom Penh. From 1 March 2012, the MTBDR*plus* version 2 was used on both smear-positive and smear-negative sputum.

All laboratories were certified through external quality assurance. To resolve discordances in LPA and/or phenotypic DST results, we performed post hoc Sanger sequencing of the *rpo*B gene at the Institute of Tropical Medicine (ITM), Antwerp, Belgium, as described elsewhere,[25] on *Mycobacterium tuberculosis* strains of Xpert RMP-resistant culture-positive cases stored at –70°C.

Data collection and management

Eligible patients were given a unique identifier code. The clinician completed a data collection form for each patient, including demographic, clinical, laboratory and radiological data. All data were entered into an Access database (MicroSoft, Redmond, WA,

Table 1 Baseline characteristics of 299 presumptive MDR-TB patients screened with Xpert® MTB/RIF assay

Patient characteristics	Xpert RMP-resistant *n* (%)	Xpert RMP-susceptible *n* (%)	No *M. tuberculosis* detected on Xpert *n* (%)	All *n* (%)	*P* value	Unadjusted OR* (95%CI)
Total	44	136	119	299		
Age, years, median [IQR]	40 [33–55]	43 [33.5–50]	43 [35–53]	43 [34–52]		
Age group, years						
⩾45	18 (40.9)	56 (41.2)	53 (46.2)	127 (42.5)	0.683	
15–44	26 (59.1)	80 (58.8)	64 (53.8)	170 (56.9)		1.01 (0.51–2.02)
Sex						
Female	27 (61.4)	51 (37.5)	61 (51.3)	139 (46.5)	0.009	2.65 (1.31–5.32)
Male	17 (38.6)	85 (62.5)	58 (48.7)	160 (53.5)		
Anti-tuberculosis treatment history						
No	16 (36.4)	89 (65.4)	58 (48.7)	163 (54.5)	0.001	
Yes	28 (63.6)	47 (34.6)	61 (51.3)	136 (45.5)		3.31 (1.63–6.73)
Smear-positive at month 2†						
No	0	1 (2.9)	1 (14.3)	2 (4.3)	0.347	
Yes	5 (100)	34 (97.1)	6 (85.7)	45 (95.7)		NA
Close contact with MDR-TB‡						
No	11 (57.9)	20 (95.2)	22 (100)	53	<0.0001	
Yes	8 (42.1)	1 (2.3)	0	9 (14.5)		14.5 (1.6–131.9)
HIV seroprevalence§						
Negative	26 (61.9)	45 (33.8)	33 (28.0)	104 (35.5)	<0.0001	
Positive	16 (38.1)	88 (66.2)	85 (72.0)	189 (64.5)		0.31 (0.15–0.65)
Smear status at diagnosis¶						
Negative	9 (20.1)	18 (13.3)	63 (53.4)	90 (30.4)	<0.0001	
Positive	34 (79.1)	117 (86.7)	55 (46.6)	206 (69.6)		0.58 (0.24–1.41)
Smoking						
No	33 (75.0)	73 (53.7)	86 (72.3)	192 (64.2)	0.002	
Yes	11 (25.0)	63 (46.3)	33 (27.7)	107 (35.8)		0.39 (0.18–0.83)
Diabetes (reported)						
No	37 (84.1)	129 (94.8)	113 (95.0)	279 (93.3)	0.030	
Yes	7 (15.9)	7 (5.2)	6 (5.0)	20 (6.7)		3.49 (1.15–10.57)
Bilateral lung involvement#						
No	16 (42.1)	50 (59.5)	61 (61)	127 (57.2)	0.116	
Yes	22 (57.9)	34 (40.5)	39 (39)	95 (42.8)		2.02 (0.93–4.40)
Cavities on CXR¶						
No	26 (68.4)	76 (90.5)	98 (98.0)	200	<0.0001	
Yes	12 (31.6)	8 (9.5)	2 (2.0)	22 (9.9)		4.38 (1.61–11.91)

* OR comparing Xpert RMP-resistant vs. RMP-susceptible.
† Only assessed among the 47 patients currently on anti-tuberculosis treatment.
‡ $n = 62$; very few patients knew whether they had been in contact with a case of confirmed MDR-TB.
§ $n = 293$; 6 missing HIV seroprevalence results (2 RMP-resistant, 3 RMP-susceptible, 1 no TB).
¶ $n = 296$; 3 missing smear results (1 RMP-resistant, 1 RMP-susceptible, 1 no TB).
\# $n = 222$; 75 CXR not performed (6 RMP-resistant, 52 RMP-susceptible, 1 no TB).
MDR-TB = multidrug-resistant TB; RMP = rifampicin; OR = odds ratio; CI = confidence interval; IQR = interquartile range; HIV = human immunodeficiency virus; CXR = chest radiography; TB = tuberculosis.

USA). Data were analysed using STATA® software version 10.0 (StataCorp, College Station, TX, USA).

Statistical analysis

We used descriptive statistics to report frequencies and proportions. The χ^2 test was used for comparisons of categorical variables. A two-sided *P* value of <0.05 was considered significant. We calculated sensitivity, specificity, positive (PPV) and negative predictive values (NPV), with 95% confidence intervals (CIs), to identify RMP resistance using Xpert compared with conventional DST and *rpo*B gene sequencing as gold standard.

Ethical considerations

The study was approved by the National Ethics Committee for Health Research (Phnom Penh, Cambodia), and the ITM Institutional Review Board and the Ethics Committee of the University Hospital in Antwerp, Belgium. Patients provided written or thumb-printed informed consent to participate.

RESULTS

Of the 688 TB patients requiring a treatment decision, 345 were considered at increased risk for DR-TB, fulfilling one or more of the four criteria above; of these, 299 (in order of priority, 136 with TB treatment history, 45 delayed converters, 9 MDR-TB contacts, 189 HIV-infected) underwent RMP susceptibility screening with Xpert, while 46 eligible patients were not screened: 32 were administrative errors and 14 were unable to expectorate sputum.

Xpert identified RMP resistance in 44/299 (14.7%)

Table 2 Time to second-line anti-tuberculosis treatment initiation and availability of the different resistance results for patients diagnosed with rifampicin-resistant tuberculosis detected on Xpert® MTB/RIF assay

Time (in days) between	Median [IQR]
Sputum collection* and Xpert result in clinic ($n = 44$)	2 [1–4]
Sample for LPA and LPA result in clinic ($n = 39$)	6 [4–7]
Sputum collection and conventional DST result in clinic ($n = 29$)	97 [78–126]
Sputum collection* and referral for treatment ($n = 41$)	6 [2–12]
Referral for second-line drugs and actual treatment start ($n = 39$)	7 [2–13]
Sputum collection* and treatment start ($n = 39$)	14 [9–25]

* Sputum was often collected one to a few days before study enrolment, depending on the smear reading workload in the laboratory.
IQR = interquartile range; LPA = line-probe assay; DST = drug susceptibility testing.

eligible patients (Figure): 20.6% (28/136) among previously treated patients and 9.7% (16/165) among new patients. Previous TB history, contact with MDR-TB, having diabetes, cavities on CXR and female sex were associated with higher odds of RMP resistance. Significantly fewer smokers and HIV-infected patients were RMP-resistant (Table 1).

Xpert results were available in the clinic after a median of 2 days vs. 97 for phenotypic DST; awaiting LPA results added 6 days (median). The median time to treatment initiation was 14 days (Table 2).

Table 3 shows the correlation in terms of RMP resistance of the different tests performed on all Xpert RMP-resistant cases. Confirmatory LPA performed on 39/44 Xpert RMP-resistant specimens (5 had insufficient sample) identified RMP resistance in 27/39 (69.2%). LPA results were invalid for 6/39 (15.4%) (5 smear-negative, 1 confirmed RMP resistance on phenotypic DST). LPA failed to detect an *rpo*B mutation in six, of which three were later confirmed to be RMP-resistant on phenotypic DST and sequencing (Table 4). In 15/39 (38.5%) patients, LPA identified additional INH resistance-conferring mutations (*inh*A and/or *kat*G), i.e., MDR-TB.

For 274/299 (91.6%) presumptive MDR-TB cases, a specimen was cultured: 120 cultures were positive (106 *M. tuberculosis* complex [MTC]), 147 negative and 7 contaminated (Appendix Table A.1).* Of the 44 Xpert-positive RMP-resistant cases, 30 (68.2%) cultures yielded MTC, 11 were negative, 1 was contaminated and 2 were not done. Phenotypic DST confirmed RMP resistance in 24/30 (PPV 80.0%, 95%CI 61.4–91.3), 18 of which were MDR-TB. One Xpert RMP-indeterminate and two RMP-susceptible cases were RMP-resistant on conventional DST (Table 5).

To explore discordances between molecular and/or phenotypic DST results, post hoc *rpo*B gene sequencing was performed on 28/44 (63.6%) Xpert RMP-resistant cases, with interpretable results for 23/44 (52.3%). Sequencing confirmed the presence of an *rpo*B gene mutation in 21/23 (PPV 91.3%, 95%CI 72.0–98.9) Xpert RMP-resistant strains (Table 5). Two Xpert RMP-resistant cases with very low bacillary load turned out to have a wild-type *rpo*B gene (one retreatment, one new case). Both tests presented a partial inhibition of amplification with a delta cycling threshold (ΔCT) of respectively 5 and

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2015/00000019/00000012/art00021

Table 3 Overview in terms of confirmatory tests performed, validity of results and RMP resistance correlation by HIV status for Xpert RMP-resistant cases

Tests	HIV-positive ($n = 16$) n (%)	HIV-negative ($n = 26$) n (%)	HIV unknown ($n = 2$) n (%)	Total ($n = 44$) n (%)
MTBDR*plus* line-probe assay	12 (75)*	25 (96.1)	2 (100)	39 (88.6)
Valid result	11 (68.7)	21 (80.1)	1 (50)	33 (75.0)
*rpo*B gene mutation	10 (62.5)	17 (65.4)	1 (50)	27 (61.4)
Conventional culture	15 (93.7)	25 (96.1)	2 (100)	42 (95.4)
MTC growth	12 (75)	17 (65.4)	1 (50)	30 (68.2)
Phenotypic DST	12 (75)	17 (65.4)	1 (50)	30 (68.2)
RMP-resistant	10 (62.5)	14 (53.8)	0	24 (54.5)
*rpo*B gene sequencing	10 (62.5)†	17 (65.4)	1 (50)	28 (63.6)
Valid result	9 (52.2)	13 (50)	1 (50)	23 (52.3)
*rpo*B gene mutation	9 (52.2)	12 (46.1)	0	21 (47.7)

* Not performed in 4 patients: 1 smear-negative, 3 administrative errors.
† Not performed in 6 patients: negative culture ($n = 3$), lack of stored sample (because no resistance, $n = 1$), culture not done ($n = 1$), administrative error ($n = 1$).
RMP = rifampicin; HIV = human immunodeficiency virus; MTC = *Mycobacterium tuberculosis* complex; DST = drug susceptibility testing.

Table 4 GenoType® MTBDR*plus* LPA results with regard to detection of *rpo*B mutations for Xpert rifampicin-resistant cases

MTBDR*plus* LPA result	Smear-negative	Smear-positive	All
Total done	8	31	39
No valid result	5	1	6
No *rpo*B mutation	2	4	6
*rpo*B mutation	1	26	27
Not done	2	3	5

LPA = line-probe assay.

4.3 for both probes D and E, and probe D. For both, LPA was invalid and phenotypic DST indicated RMP susceptibility. An overview of the different genotypic and phenotypic results for all Xpert RMP-resistant cases can be found in Appendix Table A.2.

DISCUSSION

In this operational study, systematic screening of presumptive MDR-TB cases with Xpert reduced the time to diagnosis from 97 to 2 days compared with phenotypic DST, and enabled initiation of second-line treatment within 2 weeks, in line with previous reports from South Africa.[18]

Citing the poor PPV of Xpert in low MDR-TB prevalence settings, until recently international policy guidance insisted on confirming RMP resistance before starting MDR-TB treatment.[6,15] Although this is essential to identify additional types of resistance, awaiting the results of conventional culture and DST before MDR-TB referral would undo all the advantages of Xpert. The only other WHO-endorsed rapid molecular DST is the LPA. The GenoType® MTBDR*plus* version 2 (a newer version intended for use irrespective of smear status) was recommended to complement screening by Xpert; however, concerns were raised about its applicability on smear-negative specimens.[26,27] With 15% discordant and invalid results, LPA was of limited additional value in our setting and risked delaying treatment decisions. In addition to procedural issues with LPA, occasional failure to detect the Leu533Pro mutation has been reported,[28] but should no longer occur with recent batches (>batch 57). Failure to detect an *rpo*B mutation in Xpert RMP-resistant samples caused confusion. Mutations located outside the RMP resistance-determining region, but associated with phenotypic RMP resistance,[29,30] are missed by all current commercial assays.

Our findings suggest acceptable performance of Xpert for the detection of RMP resistance, with a high NPV (compared with phenotypic DST), in line with Korean data,[31] although a less convincing PPV than in other programmatic evaluations.[32] The explanation can be two-fold. Recent evidence suggests that conventional DST may incorrectly indicate RMP susceptibility in some low-level but clinically relevant RMP-resistant isolates.[33,34] Concerns have also been raised about false-positive RMP resistance resulting from the detection of non-viable but intact mycobacterial DNA in previously treated patients,[10] silent mutations[35,36] or (more commonly) registration errors and laboratory contamination.[15] A low bacillary load also seems to affect Xpert's performance in the identification of RMP resistance.[11,13]

Compared with sequencing, the 91% PPV of Xpert in detecting RMP resistance was more reassuring, although it remains disputable whether treatment initiation can be based on a single Xpert result in our setting, as sequencing results were only available for 52% of RMP-resistant cases.

In its latest policy guidelines,[15] the WHO addressed the challenges of confirmatory testing by simplifying the algorithm for Xpert as follows: assess

Table 5 Performance of Xpert in predicting RMP resistance compared with proportion method DST and *rpo*B gene sequencing

	Löwenstein-Jensen culture				
Xpert	Positive		Negative	Contaminated	Not done
M. tuberculosis detection	MTC	NTM			
M. tuberculosis detected	102	0	57	5	16
M. tuberculosis not detected	4	14	90	2	9
Total	106	14	147	7	25
	Conventional proportion method DST				
RMP resistance detection	RMP-resistant	RMP-susceptible	Total		
RMP-resistant	24	6*	30		
RMP-susceptible	3	69	72		
Total	27	75	102		
	Sanger sequencing of *rpo*B gene				
Xpert	*rpo*B mutation	Wild type	Total		
RMP-resistant	21	2	23		

* RMP-susceptible on conventional DST includes two wild-type strains identified by sequencing and one disputed mutation (Leu511Pro, Thr508Ser); no sequencing performed for the remaining three.
RMP = rifampicin; DST = drug susceptibility testing; MTC = *Mycobacterium tuberculosis* complex; NTM = non-tuberculous mycobacteria.

clinical risk to guide the interpretation of results, repeat Xpert for 'low-risk' individuals, no need for rapid molecular confirmation. This might be a workable strategy for Cambodia.

Our data reflect true programmatic conditions, which have inherent limitations. The small numbers, the considerable amount of missing data and the wide CIs call for cautious interpretation of the findings. The high HIV-TB co-infection rate is typical for an antiretroviral treatment centre, and not uncommon for TB-endemic countries. Despite their limited generalisability, we believe our findings can be informative for many high TB burden countries implementing systematic screening for MDR-TB with Xpert.

In conclusion, in this observational study, Xpert proved effective for the systematic screening of presumptive MDR-TB patients: Xpert facilitated screening, reduced the time to diagnosis and enabled rapid treatment initiation, provided Xpert screening was guided by careful clinical judgment. Rapid confirmatory testing had little additional value: it complicated clinical decision making and risked delaying appropriate management. Our findings support the new WHO policy to abandon confirmatory LPA in favour of repeating Xpert where RMP resistance is unexpectedly identified, and starting second-line treatment while awaiting conventional DST; however, more data are needed. As the unravelling of MTC resistance mechanisms continues, improved molecular tests will be developed. It will be challenging but critical to translate the complexities of molecular diagnosis into readily interpretable diagnostic tools that can be used in low-resource settings.

Acknowledgements

The authors thank the TB clinic staff, in particular N Seng and R Pe, and the Cambodian Health Committee (Phnom Penh, Cambodia) team members for their excellent collaboration and care for our patients. The project was financially supported by grants from the Belgian Directorate General of Development Cooperation, Brussels, through the Institute of Tropical Medicine, Antwerp and the Research Foundation – Flanders, Brussels, Belgium.

Conflicts of interest: none declared.

References

1 World Health Organization. Global tuberculosis report, 2014. WHO/HTM/TB/2014 08 2014. Geneva, Switzerland: WHO, 2014.
2 Falzon D, Jaramillo E, Schunemann H J, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respir J 2011; 38: 516–528.
3 Khann S, Mao E T, Rajendra Y P, Satyanarayana S, Nagaraja S B, Kumar A M. Linkage of presumptive multidrug resistant tuberculosis (MDR-TB) patients to diagnostic and treatment services in Cambodia. PLOS ONE 2013; 8: e59903.
4 Stall N, Rubin T, Michael J S, et al. Does solid culture for tuberculosis influence clinical decision making in India? Int J Tuberc Lung Dis 2011; 15: 641–646.
5 Boehme C C, Nicol M P, Nabeta P et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011; 377: 1495–1505.
6 World Health Organization. Rapid implementation of Xpert MTB/RIF diagnostic test. Technical and operational 'how to'. Practical considerations. WHO/HTM/TB/2011 2 2011. Geneva, Switzerland: WHO, 2011.
7 Ling D I, Zwerling A A, Pai M. Rapid diagnosis of drug-resistant TB using line-probe assays: from evidence to policy. Expert Rev Respir Med 2008; 2: 583–588.
8 Qin Z Z, Pai M, Van Gemert W, Sahu S, Ghiasi M, Creswell J. How is Xpert MTB/RIF being implemented in 22 high tuberculosis burden countries? Eur Respir J 2015; 45: 549–554.
9 Scott L, de Lima Y, da Silva M P, et al. Management of rifampicin resistant tuberculosis in the Xpert MTB/RIF era: experiences from South Africa. Int J Tuberc Lung Dis 2014; 18: S494.
10 Boyles T H, Hughes J, Cox V, Burton R, Meintjes G, Mendelson M. False-positive Xpert® MTB/RIF assays in previously treated patients: need for caution in interpreting results. Int J Tuberc Lung Dis 2014; 18: 876–878.
11 Marlowe E M, Novak-Weekley S M, Cumpio J, et al. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J Clin Microbiol 2011; 49: 1621–1623.
12 Van Deun A., Martin A, Palomino J C. Diagnosis of drug-resistant tuberculosis: reliability and rapidity of detection. Int J Tuberc Lung Dis 2010; 14: 131–140.
13 Van Rie A, Mellet K, John M A et al. False-positive rifampicin resistance on Xpert® MTB/RIF: case report and clinical implications. Int J Tuberc Lung Dis 2012; 16: 206–208.
14 Chiang C Y, Van Deun A. Rapid diagnosis of rifampicin resistance: who needs confirmation? Int J Tuberc Lung Dis 2013; 17: 2.
15 World Health Organization. Xpert MTB/RIF implementation manual. Technical and operational 'how to': practical considerations. WHO/HTM/TB/2014 01 2014. Geneva, Switzerland: WHO, 2014.
16 Chang K, Lu W, Wang J, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis. J Infect 2012; 64: 580–588.
17 Steingart K R, Schiller I, Horne D J, Pai M, Boehme C C, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014; 1: CD009593.
18 Naidoo P, du Toit E, Dunbar R, et al. A comparison of multidrug-resistant tuberculosis treatment commencement times in MDRTB*Plus* line-probe assay and Xpert® MTB/RIF-based algorithms in a routine operational setting in Cape Town. PLOS ONE 2014; 9: e103328.
19 Van Rie A, Da Silva P, Voss-De Lima Y, et al. Impact of rapid diagnosis of rifampicin resistance by Xpert MTB/RIF on mortality among patients with rifampicin-resistant tuberculosis. Int J Tuberc Lung Dis 2014; 18: S176.
20 Dharmadhikari A S, Mphahlele M, Venter K, et al. Rapid impact of effective treatment on transmission of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2014; 18: 1019–1025.
21 National Centre for Tuberculosis and Leprosy Control, Ministry of Health. Second national tuberculosis prevalence survey. Phnom Penh, Cambodia: Ministry of Health, 2011.
22 Yamada N, Saorith K, Yamakami K, et al. The national tuberculosis drug resistance survey in Cambodia, 2000–2001. Int J Tuberc Lung Dis 2007; 11: 1321–1327.

23 World Health Organization. Global tuberculosis report, 2012. WHO/HTM/TB/2012 6 2013.Geneva, Switzerland: WHO, 2012.
24 World Health Organization. Definitions and reporting framework for tuberculosis-2013 revision. WHO/HTM/TB/2013 2 2013. Geneva, Switzerland: WHO, 2013.
25 Rigouts L, Nolasco O, de Rijk P, et al. Newly developed primers for comprehensive amplification of the *rpo*B gene and detection of rifampin resistance in *Mycobacterium tuberculosis*. J Clin Microbiol 2007; 45: 252–254.
26 Barnard M, Gey van Pittius N C, van Helden P D, Bosman M, Coetzee G, Warren R M. The diagnostic performance of the GenoType MTBDR*plus* version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. J Clin Microbiol 2012; 50: 3712–3716.
27 Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. J Clin Microbiol 2012; 50: 1264–1269.
28 Huang W L, Chen H Y, Kuo Y M, Jou R. Performance assessment of the GenoType MTBDR*plus* test and DNA sequencing in detection of multidrug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol 2009; 47: 2520–2524.
29 Siu G K, Zhang Y, Lau T C, et al. Mutations outside the rifampicin resistance-determining region associated with rifampicin resistance in *Mycobacterium tuberculosis*. J Antimicrob Chemother 2011; 66: 730–733.
30 Van Deun A, Barrera L, Bastian I, et al. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. J Clin Microbiol 2009; 47: 3501–3506.
31 Kwak N, Choi S M, Lee J, et al. Diagnostic accuracy and turnaround time of the Xpert MTB/RIF assay in routine clinical practice. PLOS ONE 2013; 8: e77456.
32 Durovni B, Saraceni V, van den Hof S, et al. Impact of replacing smear microscopy with Xpert MTB/RIF for diagnosing tuberculosis in Brazil: a stepped-wedge cluster-randomized trial. PLOS MED 2014; 11: e1001766.
33 Rigouts L, Gumusboga M, de Rijk W B, et al. Rifampin resistance missed in automated liquid culture system for *Mycobacterium tuberculosis* isolates with specific *rpo*B mutations. J Clin Microbiol 2013; 51: 2641–2645.
34 Van Deun A., Aung K J, Bola V, et al. Rifampin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol 2013; 51: 2633–2640.
35 Ocheretina O, Escuyer V E, Mabou M M, et al. Correlation between genotypic and phenotypic testing for resistance to rifampin in *Mycobacterium tuberculosis* clinical isolates in Haiti: investigation of cases with discrepant susceptibility results. PLOS ONE 2014; 9: e90569.
36 Williamson D A, Basu I, Bower J, Freeman J T, Henderson G, Roberts S A. An evaluation of the Xpert MTB/RIF assay and detection of false-positive rifampicin resistance in *Mycobacterium tuberculosis*. Diagn Microbiol Infect Dis 2012; 74: 207–209.

APPENDIX

Table A.1 Comparison of performance of Xpert® MTB/RIF assay vs. conventional Löwenstein-Jensen culture

	Culture, *n* (%)				
Xpert	Positive	Negative	Contaminated	Total	Not done
Error	0	1	0	1	0
M. tuberculosis not detected/RMP not detected	18*	89	2	109	9
M. tuberculosis detected/RMP not detected	69	44	3	116	14
M. tuberculosis detected/RMP-indeterminate	3	2	1	6	0
M. tuberculosis detected/RMP detected	30	11	1	42	2
Total	120 (43.8)	147 (53.6)	7 (2.6)	274	25

* 14 grew non-tuberculous mycobacteria; all other positive cultures were *M. tuberculosis* complex.
RMP = rifampicin (resistance).

Table A.2 Overview of the different genotypic and phenotypic test results for all Xpert rifampicin-resistant cases

Study code	Age years	Sex	New vs. retreatment	HIV status	Smear result	Genotypic DST		Phenotypic DST	*rpo*B gene sequencing	Treatment
						Xpert MTBRIF	GenoType MTBDR*plus* mutations	Proportion method		
MDR_006	38	M	New	+	+	MTB+/RMP+	Not done	HRE	Not done	Start Category IV
MDR_010	27	F	Retreatment	–	+	MTB+/RMP+	*rpo*B	HRSEKm	Tyr516(TAC)	Start Category IV
MDR_012	60	F	Retreatment	–	+	MTB+/RMP+	No mutation	R	Arg526(CGC)	Refused
MDR_061	52	F	Retreatment	+	+	MTB+/RMP+	*rpo*B, *inh*A	HR	Tyr516(TAC)	Start Category IV
MDR_101	20	M	New	–	+	MTB+/RMP+	*rpo*B, *kat*G	HRSE	Leu531(TTG)	Start Category IV
MDR_106	24	F	Retreatment	–	+	MTB+/RMP+	*rpo*B	H(0.2)R	Leu531(TTG)	Start Category IV
MDR_114	32	F	Retreatment	+	+	MTB+/RMP+	*rpo*B	R	Tyr526(TAC)	Start Category IV
MDR_124	63	F	Retreatment	–	+	MTB+/RMP+	*rpo*B, *kat*G	HR	Tyr516(TAC)	Start Category IV
MDR_146	33	F	New	+	+	MTB+/RMP+	Not done	Not done	Not done	Died
MDR_152	42	F	Retreatment	+	+	MTB+/RMP+	*rpo*B	Culture-negative	Not done	Start Category IV
MDR_153	28	M	Retreatment	–	–	MTB+/RMP+	No mutation	Culture-negative	Not done	Start Category IV
MDR_159	58	M	Retreatment	+	+	MTB+/RMP+	*rpo*B, *kat*G	Culture-negative	Not done	Start Category IV
MDR_192	41	M	Retreatment	+	+	MTB+/RMP+	*rpo*B, *kat*G	HRSE	Leu531(TTG)	Start Category IV
MDR_205	29	M	Retreatment	+	+	MTB+/RMP+	*rpo*B	No resistance	Not done	Start Category IV
MDR_207	37	M	New	–	+	MTB+/RMP+	*rpo*B, *kat*G	HRSE	Leu531(TTG)	Died
MDR_215	75	F	New	–	+	MTB+/RMP+	*rpo*B, *kat*G	HRSEOfx	Tyr516(TAC), Arg511(CGG)	Start Category IV
MDR_220	38	F	Retreatment	+	+	MTB+/RMP+	*rpo*B, *inh*A unint	R	Tyr526(TAC)	Start Category IV
MDR_221	41	M	Retreatment	–	+	MTB+/RMP+	*rpo*B, *inh*A	HRS	Tyr526(TAC)	Start Category IV
MDR_242	54	F	Retreatment	–	+	MTB+/RMP+	No mutation	HRS	Pro533 (CCG), Ala 562 (GCA)	Start Category IV
MDR_260	47	M	Retreatment	–	+	MTB+/RMP+	*rpo*B	H(0.2)RE	Leu531(TTG)	Start Category IV
MDR_265	33	F	Retreatment	–	–	MTB+/RMP+	Uninterpretable	Culture-negative	Not done	None
MDR_274	73	F	Retreatment	not done	+	MTB+/RMP+	Uninterpretable	S	Wild type	Start Category IV
MDR_281	18	F	New	+	–	MTB+/RMP+	*rpo*B, *inh*A	H(0.2)S	Pro511 (CCG), Ser508 (TCC)	Start Category IV
MDR_284	62	F	New	–	+	MTB+/RMP+	*rpo*B	Culture-negative	Not done	Start Category IV
MDR_292	56	F	Retreatment	–	+	MTB+/RMP+	*rpo*B	Culture-negative	Not done	Start Category IV
MDR_296	33	F	New	+	+	MTB+/RMP+	*kat*G	HRS	Leu526 (CTG)	Start Category IV
MDR_308	50	F	New	–	–	MTB+/RMP+	Uninterpretable	No resistance	Wild type	Start Category IV
MDR_312	33	F	New	+	–	MTB+/RMP+	Uninterpretable	HRSE	Leu531 (TTG)	Start Category IV
MDR_316	38	F	New	+	+	MTB+/RMP+	*rpo*B	R	No result	Start Category IV
MDR_317	38	M	Retreatment	–	+	MTB+/RMP+	*rpo*B, *kat*G	HR	Leu531(TTG)	Start Category IV
MDR_320	30	F	New	–	+	MTB+/RMP+	*rpo*B, *kat*G	Not done	Not done	Start Category IV
MDR_321	50	M	New	–	–	MTB+/RMP+	Uninterpretable	No resistance	No amplification	Start Category IV
MDR_322	30	F	New	–	–	MTB+/RMP+	Uninterpretable	Culture-negative	Not done	Start Category IV
MDR_325	75	F	New	Not done	+	MTB+/RMP+	No mutation	Culture-negative	Not done	Refused
MDR_334	32	F	Retreatment	–	–	MTB+/RMP+	No mutation	Culture-negative	Not done	Start Category IV
MDR_336	44	M	Retreatment	+	+	MTB+/RMP+	Not done	HRS	Tyr526(TAC)	Start Category IV
MDR_340	58	F	New	–	–	MTB+/RMP+	Not done	S	No amplification	Start Category I
MDR_341	33	M	Retreatment	+	–	MTB+/RMP+	Not done	Culture-negative	Not done	Start Category IV
MDR_348	53	M	Retreatment	–	+	MTB+/RMP+	*rpo*B, *inh*A	R	No result	Start Category IV
MDR_351	60	F	Retreatment	–	+	MTB+/RMP+	*rpo*B, *inh*A	H(0.2)R	No result	Start Category IV
MDR_352	48	M	Retreatment	+	+	MTB+/RMP+	*rpo*B, *inh*A	H(0.2)R	Gln531(CAG)	Start Category IV
MDR_353	71	M	Retreatment	–	+	MTB+/RMP+	*rpo*B	Culture contaminated	Not done	Start Category IV
MDR_354	23	M	Retreatment	–	+	MTB+/RMP+	*rpo*B	RSOfx	Tyr516(TAC)	Start Category IV
MDR_355	34	F	Retreatment	–	+	MTB+/RMP+	*rpo*B, *kat*G	Culture-negative	Not done	Start Category IV

M = male; F = female; + = positive; – = negative; HIV = human immunodeficiency virus; MTB+/RMP+ = *M. tuberculosis* DNA detected/RMP resistance mutation detected; H, INH = isoniazid; R, RMP = rifampicin; S = streptomycin; E = ethambutol; Km = kanamycin; Ofx = ofloxacin; H(0;2) = INH resistance at a (low) inhibitory concentration of 0;2 μg/ml; Category IV = standardised second-line anti-tuberculosis treatment; Category I = first-line anti-tuberculosis treatment (4RHEZ/2HR).

RESUME

CADRE : L'accès aux tests de sensibilité (DST) aux hôpitaux de référence est limité, ce qui contribue à un délai dans le diagnostic de la tuberculose multirésistante (TB-MDR).

OBJECTIF : Documenter l'impact sur le délai diagnostique et thérapeutique de l'identification de résistance à la rifampicine (RMP) par Xpert, et évaluer sa performance sous conditions programmatiques.

METHODES : Etude prospective observationnelle. Nous avons dépisté des individus à présomption de TB-MDR par Xpert et culture de medium solide/DST conventionnel. La résistance à la RMP était confirmée par test de sondes en ligne (LPA). Nous avons enregistré le délai diagnostique et thérapeutique. Le séquençage post hoc du gène *rpo*B nous a permis de résoudre les sensibilités à la RMP discordants.

RESULTATS : Nous avons dépisté 299/345 individus à présomption de TB-MDR. Entre eux, 44 étaient résistants à la RMP à l'Xpert : 16/165 (10%) de nouveaux cas et 28/136 (20%) de retraitements. Le délai médian était de 2 jours (Xpert) (vs. 6 jours additionnels pour le LPA) ; le médian délai thérapeutique était de 14 jours. Le LPA confirmatrice fait sur 39/44 cas était concordant en 27, discordant en 6 et invalide en 6. La résistance à la RMP détecté par Xpert était confirmé en 24/30 (80%) et 21/23 (91%) cas par le DST phénotypique et le séquençage *rpo*B, respectivement.

CONCLUSION : Dépister par Xpert des malades à présomption TB-MDR a permis un diagnostic et initiation au traitement rapide. La performance de Xpert était bonne pourvu qu'une évaluation du risque soit faite. Des tests confirmatrices rapides ne contribuaient que peu à la décision clinique. Nos résultats appuient les dernières directives de l'Organisation Mondiale de la Santé en terme de renoncer au LPA confirmatrice en faveur de répéter l'Xpert en cas de doute clinique, tout en attendant le DST final phénotypique.

RESUMEN

MARCO DE REFERENCIA: La limitación del acceso a las pruebas de sensibilidad a los medicamentos (DST) en los hospitales de referencia contribuye al retraso en la detección de la tuberculosis multidrogorresistente (TB-MDR).

OBJETIVO: Documentar la repercusión de la detección de la resistencia a rifampicina (RMP) mediante la prueba Xpert sobre el lapso hasta obtener un diagnóstico y el lapso hasta comenzar el tratamiento y evaluar su desempeño en condiciones programáticas.

METODOS: Fue este un estudio prospectivo. Se investigaron los casos con presunción de TB-MDR mediante la prueba Xpert, el cultivo en medio sólido y las DST corrientes. Se confirmó la resistencia a RMP con la prueba de hibridación con sonda en tiras (LPA) con el fin de confirmar la resistencia a RMP. Se registraron los retrasos en el diagnóstico y el comienzo del tratamiento. En un análisis a posteriori, se resolvieron las discordancias sobre la sensibilidad a RMP mediante la secuenciación del gen *rpo*B.

RESULTADOS: Se investigaron 299 de las 345 personas con presunción diagnóstica de TB-MDR y la prueba Xpert reveló 44 casos de resistencia a RMP, a saber, 16 casos nuevos en 165 (10%) y 28 casos de retratamiento en 136 (20%). La mediana del lapso hasta el diagnóstico fue 2 días con Xpert, contra 6 días más al practicar la LPA; la mediana del lapso hasta iniciar el tratamiento fue 14 días. La prueba confirmatoria LPA en 39/44 casos reveló 27 concordancias, 6 discordancias y 6 resultados inválidos. La resistencia a RMP diagnosticada por la prueba Xpert se confirmó en 24/30 casos (80%) mediante pruebas fenotípicas de DST y en 21/23 casos (91%) mediante la secuenciación del gen *rpo*B.

CONCLUSION: La investigación de los pacientes con presunción diagnóstica de TB-MDR con la prueba Xpert facilitó el diagnóstico rápido y el tratamiento precoz de la TB-MDR. El desempeño de esta prueba es adecuado, siempre y cuando se realice una evaluación clínica adecuada de los riesgos. La contribución de la prueba confirmatoria rápida a la toma de decisiones clínicas fue escasa. Los presentes resultados respaldan las directrices de la Organización Mundial de la Salud con respecto al abandono de la prueba confirmatoria LPA en favor de la repetición de la prueba Xpert cuando existen dudas clínicas, a la espera del resultado de las pruebas fenotípicas DST.