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Xpert MTB/RIF detection of rifampin resistance and time to treatment initiation in Harare, Zimbabwe

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Abstract

Background—Patients at elevated risk of drug-resistant tuberculosis are prioritized for testing with Xpert MTB/RIF® (“Xpert”), though clinical utility in this population is understudied.

Design—From November 2011 to June 2014, consecutive outpatients with history of prior tuberculosis in high-density suburbs of Harare, Zimbabwe were tested with Xpert, solid and liquid culture, and the microscopically-observed drug susceptibility assay. Diagnostic accuracy for rifampin-resistance and time to second-line regimens were ascertained. The *rpoB* gene was sequenced in cases of culture-confirmed rifampin resistance and genotypic sensitivity.

Results—Among 352 retreatment patients, 71 (20%) had rifampin-resistant, 98 (28%) rifampin-susceptible, 64 (18%) culture-negative/Xpert-positive, and 119 (34%) culture-negative/Xpert-negative TB. Xpert was 86% (95% CI 75-93%) sensitive and 98% (95% CI 92-100%) specific for rifampin-resistant TB. The positive predictive value of Xpert-determined rifampin resistance for MDR-TB was 82% (95% CI 70-91%). Fifty-nine of 71 (83%) participants initiated SLDs, with a median time to regimen initiation of 18 days (IQR, 10-44 days).

Conclusion—The diagnostic accuracy of Xpert for rifampin-resistance is high, though predictive value for MDR-TB is lower than anticipated. Xpert allows for faster SLD initiation under programmatic conditions, relative to culture-based drug susceptibility testing.

Keywords

Xpert MTB/RIF; rifampin resistance; HIV; retreatment

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Introduction

Prior anti-tuberculosis (TB) drug exposure is the strongest predictor of drug resistant TB, [1-3] and retreatment active TB is a common clinical problem, affecting over 700,000 persons in 2013. [4] The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) is strongly recommended by the World Health Organization (WHO) as an initial diagnostic test for patients with history of prior treatment in any retreatment category.[5] Yet, individuals with retreatment TB have been infrequently included in studies of Xpert.[6]

Concerns regarding suboptimal positive predictive value for rifampin resistance with previous versions of Xpert,[7] case reports of decreased specificity of rifampin-resistance detection due to non-viable mycobacteria,[8] and health system challenges related to implementation of second-line anti-TB drugs (SLDs) may impact the utility of PCR-based molecular TB drug susceptibility tests (DST) in resource limited settings. Further, in high HIV burden areas, patients presenting with presumptive retreatment TB have a broad differential diagnosis and often lack microbiologic evidence of *M. tuberculosis*.[9]

We prospectively enrolled individuals with retreatment TB in Harare, Zimbabwe over a two-year period to determine the diagnostic accuracy of Xpert for rifampin (RIF) resistant- and multidrug resistant (MDR)-TB, utility of Xpert among persons failing to respond to first-line drugs (FLD), and factors associated with delayed initiation of SLDs following Xpert-determined RIF-resistance. Information on false-positivity for *M. tuberculosis* detection among persons with recurrent TB[10] and preliminary data on empiric TB treatment within this cohort[9] have been reported elsewhere.

Study Population and Methods

Study Population

From November 2011 through June 2014, we prospectively enrolled consecutive individuals notified as retreatment TB cases within two central infectious diseases referral clinics and eight polyclinics within the southern high-density suburbs of Harare, Zimbabwe (metropolitan population 2.8 million, 2009). Studies reporting the burden of drug resistant TB and detection of *M. tuberculosis* in this setting have been previously published.[9-11] Eligible participants had at least one month of prior TB treatment and were symptomatic at time of enrolment with cough (any duration), fever, night sweats, or weight loss.[12] Clinical data including HIV status and CD4+ T-lymphocyte (CD4) counts were collected by participant interview and abstracted during medical record review. All participants provided written informed consent, and ethical approval was obtained from the Medical Research Council of Zimbabwe and the UCSF Human Research Protection Program.

Definitions

Notified cases were categorized according to the outcome of their most recent course of treatment as either (1) “recurrent TB” (incident relapse or reinfection following prior completion of TB treatment), or (2) “prevalent retreatment TB” (lost to follow-up during prior treatment course, “late smear conversion,” and treatment failure). Late smear

conversion and treatment failure were defined as AFB sputum smear-positivity at month three and at month five or later, respectively, of treatment with a standard FLDs. Infectious periods were estimated as patient-reported symptom onset through 2 weeks following initiation of appropriate (according to final drug-susceptibility) antimicrobial therapy.[13]

Laboratory Methods

The Biomedical Research and Training Institute (BRTI) Tuberculosis Laboratory within the National Microbiology Reference Laboratory is a center for Trials of Excellence in Southern Africa and accredited for International Organization for Standardization (ISO) 151589. BRTI collaborates with the Ministry of Health and Child Welfare (MOHCW) in laboratory capacity building and regularly undergoes External Quality Assurance (EQA) of DST for FLDs. The most recent Centre for American Pathologists (CAP) assessment in 2014 demonstrated 100% agreement for isoniazid (INH), RIF, ethambutol (EMB), and streptomycin (STR) resistance testing.

All participants provided two spontaneously expectorated sputum specimens on the day of enrolment ("spot-spot" specimens, separated by at least one hour). Both specimens were mechanically homogenized, combined, and divided into aliquots; individual patient specimens less than 1mL were not accepted. From the combined specimens, one aliquot (≥ 0.5 mL) was submitted for Xpert MTB/RIF testing, and one aliquot (≥ 2 mLs) was submitted for microscopically observed drug-susceptibility (MODS) testing; remaining aliquots (≥ 3 mLs) were submitted for both solid (Löwenstein-Jensen (LJ)) and liquid (BBL™ MGIT™ Mycobacterial Growth Indicator Tubes (Becton Dickinson, Sparks, MD)) culture. Therefore, one Xpert and three culture results were available for each patient. Ziehl-Neelsen staining was used to confirm growth of Mycobacteria in all test-positive tubes. All positive cultures underwent a rapid MPT64 antigen detection-based (Beckton Dickinson TBc rapid immunochromatographic assay) to determine presence of *M. tuberculosis* complex, or growth at 25 °C, 45 °C temperatures and on Paranitrobenzoic acid Löwenstein–Jensen (LJ) slope if rapid kit assay was negative. Culture-based drug-susceptibility testing was performed on all *M. tuberculosis*-confirmed isolates using the absolute concentration measurement on LJ media.[14] Culture for mycobacteria and direct DST were also performed using the MODS assay (TB MODS Kit™, Hardy Diagnostics, Santa Maria, CA USA) in accordance with standard operating procedures. [15] The definition of *M. tuberculosis* drug resistance in our study was a positive DST result by either culture-based method.

Xpert MTB/RIF (Cepheid, Generation 3 November 2011 through March 2012; Generation 4 (n=293/352 (83%)), thereafter) was conducted and interpreted according to the manufacturer's recommendations by a trained operator masked to clinical information. Briefly, the sample reagent was mixed in a 2:1 ratio with 1 ml of raw sputum, incubated at room temperature for 15 minutes, and 2 ml of this mixture was added to an Xpert MTB/RIF cartridge and processed in a 4-module GeneXpert (Cepheid) instrument. Laboratory turnaround time for Xpert was defined as the time from receipt of patient specimen to reporting of results, and was two days or less in all cases. In cases of genotypic-phenotypic discordance for RIF resistance, Xpert was repeated and the *rpoB* gene from the sample sediment or culture isolate was amplified and sequenced using Sanger sequencing.

Statistical Analysis

For assessment of Xpert rifampin drug-susceptibility, we included all patients with a positive *M. tuberculosis* culture result. We excluded patients if: 1) culture-based DST results were not available; 2) Xpert MTB/RIF was not performed; or 3) culture-based DST indicated RIF susceptibility but RIF resistance could not be assessed by Xpert (because Xpert did not detect M.tb). The latter criteria avoided inflating estimates of Xpert specificity for RIF resistance. We calculated proportions with exact binomial 95% confidence intervals (CI) for the primary analyses of sensitivity, specificity, positive predictive value, and negative predictive value.

Because of censored data and lack of a specific time point of interest, we used survival analysis to describe determinants of time to effective treatment of RIF-resistant TB. We generated a multivariate Cox proportional hazard model including HIV status, gender, and referral from rural areas outside of Harare. The number of cases in participating clinics during the study period determined the sample size. All analyses were performed using Stata 12.1 (Stata Corporation, College Station, TX).

Results

Study population

From November 2011 through June 2014, 393 ambulatory patients with retreatment TB were enrolled. Of 352 analyzed patients (Figure 1), 152 (43.2%) had recurrent TB, and 200 (56.8%) had prevalent retreatment TB. Most (n=238/352, 67.6%) had HIV co-morbidity with a median CD4 count of 198 (IQR, 90-350). Patients had a median of 1 (IQR, 1-2) prior treatment courses, and none had prior experience with second-line drugs.

Ultimately, 71 (20%) had Xpert- or culture-positive RIF-resistant TB, 98 (28%) had culture-positive RIF-susceptible TB, 64 (18%) had culture-negative/Xpert-positive TB, and 119 (35%) had culture-negative/Xpert-negative TB. Relative to other groups, those with RIF-resistant TB were more often younger (p=0.13), female (p=0.02), to have not responded to FLDs (p<0.001), and to have had early relapse (p<0.001) (Table 1 and Figure 2). Individuals with RIF-resistant TB also had a significantly longer estimated infectious period relative to other groups.

Diagnostic accuracy for RIF-resistant TB

Overall, of 71 RIF-resistant cases, 54 (76%) were Xpert- and culture-confirmed, 8 (11%) were Xpert-determined only, and 9 (13%) were culture-confirmed only. Xpert detected RIF resistance in 54 of 63 culture-confirmed RIF resistant cases (sensitivity 85.7%, 95% CI 74.6-93.3%) and RIF susceptibility in 90 of 92 cases (specificity 97.8%, 95% CI 92.4-99.7%); both false-positive tests were generation 4 assays. RIF resistance was detected in 14/86 (16.3%, 95% CI 9.2-25.8%) of those with late smear conversion (smear-positive at 3 months) vs. 19/66 (28.8%, 95% CI 18.3-41.3%) of those with treatment failure (smear-positive at 5 months or later) (p=0.06 for difference). Xpert was indeterminate for RIF resistance in four (1.1%) subjects with mean cycle thresholds near the upper assay limit (C_T=40); upon re-testing, three of four tests provided valid results.

Of the 62 Xpert-determined RIF-resistant cases, 43 (69%) had probe results. The *rpoB* mutations associated with RIF resistance were located within probe E (60.5%, 95% CI 44.4-75.0%; n=26/43), probe B (14.0%, 95% CI 5.3-27.9%; n=6/43), probe C (11.6%, 95% CI 3.9-25.1%; n=5/43), probe D (9.3%, 95% CI 2.6-22.1%; n=4/43) and probe A and B (4.7%, 95% CI 0.57-15.8%; n=2/43). Sequencing of *rpoB* was performed for four specimens demonstrating phenotypic resistance to RIF but genotypic susceptibility by Xpert (Table 3). Two isolates had wild-type *rpoB* sequences, one had two high-confidence drug resistance conferring mutations (DRM) (*rpoB* L511P and *rpoB* D516P), and the final isolate showed an unusual deletion at codon 519, previously described in a single case from Russia (RIF MIC 100) and the Netherlands (RIF MIC of 10). [16]

Other drug resistance

Eight of 54 (14.8%) individuals with culture-confirmed, Xpert-determined RIF-resistant TB were sensitive to INH. Most (n=10/11, 91%) rifampin-monoresistant TB occurred among persons with HIV-coinfection. STR and EMB resistance occurred among 42.3% (n=22/52) and 57.7% (n=30/52) of MDR-TB cases, respectively. Among MDR isolates that underwent second-line DST, nine percent (n=3/33) were resistant to ethionamide; no fluoroquinolone or aminoglycoside resistance was detected.

Initiation of second-line anti-tuberculosis regimens

Fifty-four of the 62 individuals (87.1%) with Xpert-determined RIF-resistance initiated SLDs. Of these 54, five (9%) were initiated based on test results other than Xpert. Culture-based DST results were available a median of 79 days (IQR, 64-96 days) following sputum collection. The median time to SLD initiation for those with RIF-resistant TB was 18 days (IQR, 10-44 days) (Figure 3). Neither HIV status (hazard ratio (HR) 0.7 95% CI 0.4-1.3), male sex (HR 0.8 95% CI 0.5-1.5), nor referral from outside Harare (HR 0.8 95% CI 0.4-1.5) were associated with treatment initiation time.

Discussion

We used a stringent definition based on multiple reference standards to detect *M. tuberculosis* drug resistance in a high HIV burden, resource limited setting. Our primary findings were that (1) the sensitivity and negative predictive value of Xpert was lower than anticipated from prior literature; (2) although much improved relative to a median turnaround time for culture-based DST of 79 days, median time to initiation of second-line regimens following Xpert diagnosis of rifampin resistance was still prolonged at 18 days (IQR, 10-44 days); (3) approximately one in seven persons with an Xpert RIF-resistant result had rifampin mono-resistant TB rather than MDR-TB; and (4) sixteen percent of individuals who remained sputum smear-positive three months into treatment were found to harbor RIF-resistant *M. tuberculosis*, supporting WHO recommendations to perform phenotypic DST at this stage.[4]

The majority (>80%) of newly diagnosed TB patients treated with standard short course therapy will be sputum smear-negative by month three of treatment,[17,18] though limited data exist for HIV-infected individuals. Accordingly, the WHO recommends phenotypic DST to

assess for drug-resistance in patients who remain sputum smear-positive at month three rather than month five, when programmatic notification as treatment failure would occur according to current international standards.[4] In our study, nearly fifteen percent of individuals with sputum smear-positivity at month three had MDR-TB, providing support for this approach. Overall, we found a sensitivity for detection of RIF-resistant TB lower than that reported in demonstration studies[7,19] and pooled estimates.[6] This decreased sensitivity is accounted for by inclusion of Xpert-negative specimens in our DST diagnostic accuracy assessment, since failure to report RIF resistance in these cases is problematic, regardless of cause.

Although improvements in treatment outcome have yet to be demonstrated in randomized trials,[20] Xpert accelerates treatment initiation for both drug-susceptible [7,21] and drug-resistant TB.[22] In our study, the median delay between sputum collection for Xpert and initiation of effective treatment was 18 days, with some patients not initiated on SLDs due to clinical response to FLDs. Although far longer than initial demonstration studies of molecular diagnostics would suggest,[7,23] similar delay following Xpert detection of RIF resistance has been noted in routine settings in South Africa,[22,24] and compares favorably with turnaround time for culture-based DST in multiple settings,[25-27] as well as historic data from Harare. Of note, health system delays in Zimbabwe have accounted for only a small proportion of total treatment delay in drug-sensitive TB,[28] and this may be similar in proportion for persons with MDR-TB.

Rifampin-monoresistant TB was common in our study, leading to a positive predictive value of Xpert for MDR-TB substantially lower than expected. Although rare in high-income settings,[29] less is known about rifampin-monoresistant TB in low- and middle-income settings; however, prevalence may be increasing in the Southern Africa region.[30,31] Rifampin-monoresistant TB was likely enriched in our target population of individuals with presumptive drug-resistant TB relative to isoniazid-monoresistant strains, with those with persistently sputum smear-positive AFB more likely to harbor rifampin-monoresistant strains. In addition, advanced HIV/AIDS is the most consistent risk factor for rifampin-monoresistant TB in high-income settings,[32-35] and was common in our cohort. The suboptimal surrogacy of Xpert-detected RIF resistance for true MDR-TB is concerning if generalizable to similar settings. First, given the lack of evidence-based rifampin-monoresistant TB treatment regimens (e.g., 12–18 months of INH, EMB, fluoroqiunolone, with at least 2 months of PZA)[36] and the lag in scale-up of phenotypic DST relative to Xpert globally, most individuals with rifampin-monoresistant TB in resource limited settings (as in ours) will be treated with second-line regimens including injectables. Second, the increasing reliance of MDR-TB clinical trial enrollment on the “rule-in” value of Xpert could be less efficient than hoped.

Our study has limitations. Although the study was not a population-based sample, the 10 enrollment sites accounted for approximately 50% of all retreatment TB cases diagnosed in Harare during the study period. In addition, despite use of a rigorous reference standard, some proportion of false-positive Xpert results may have been true-positive, as has been noted in previous studies from high HIV-burden settings.[37,38]

Conclusion

In conclusion, patients with retreatment TB in a high HIV burden setting have high probabilities of both RIF-resistant TB and empiric treatment without microbiologic confirmation. Within this group, we found that Xpert MTB/RIF had a sensitivity for detection of RIF-resistance that was lower than pooled estimates derived from demonstration studies, and that RIF-resistance was common among individuals remaining smear-positive at three months, supporting international recommendations to perform DST at this stage.

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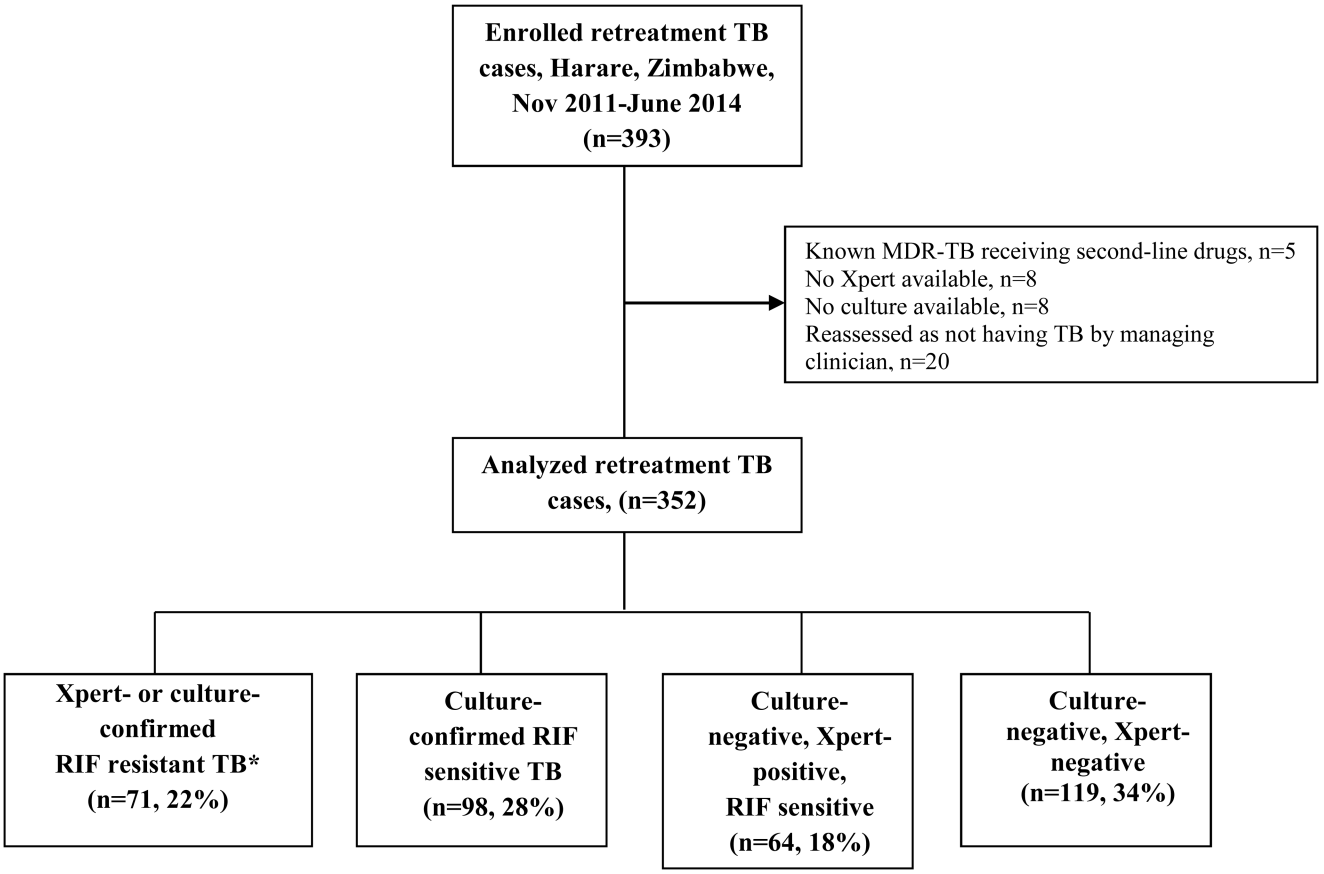

Figure 1. Screening and Analysis of the Study Population

*Includes patients diagnosed with both Xpert and phenotypic DST (n=54, 76%), DST only (n=9, 13%), and Xpert only (n=8, 11%).

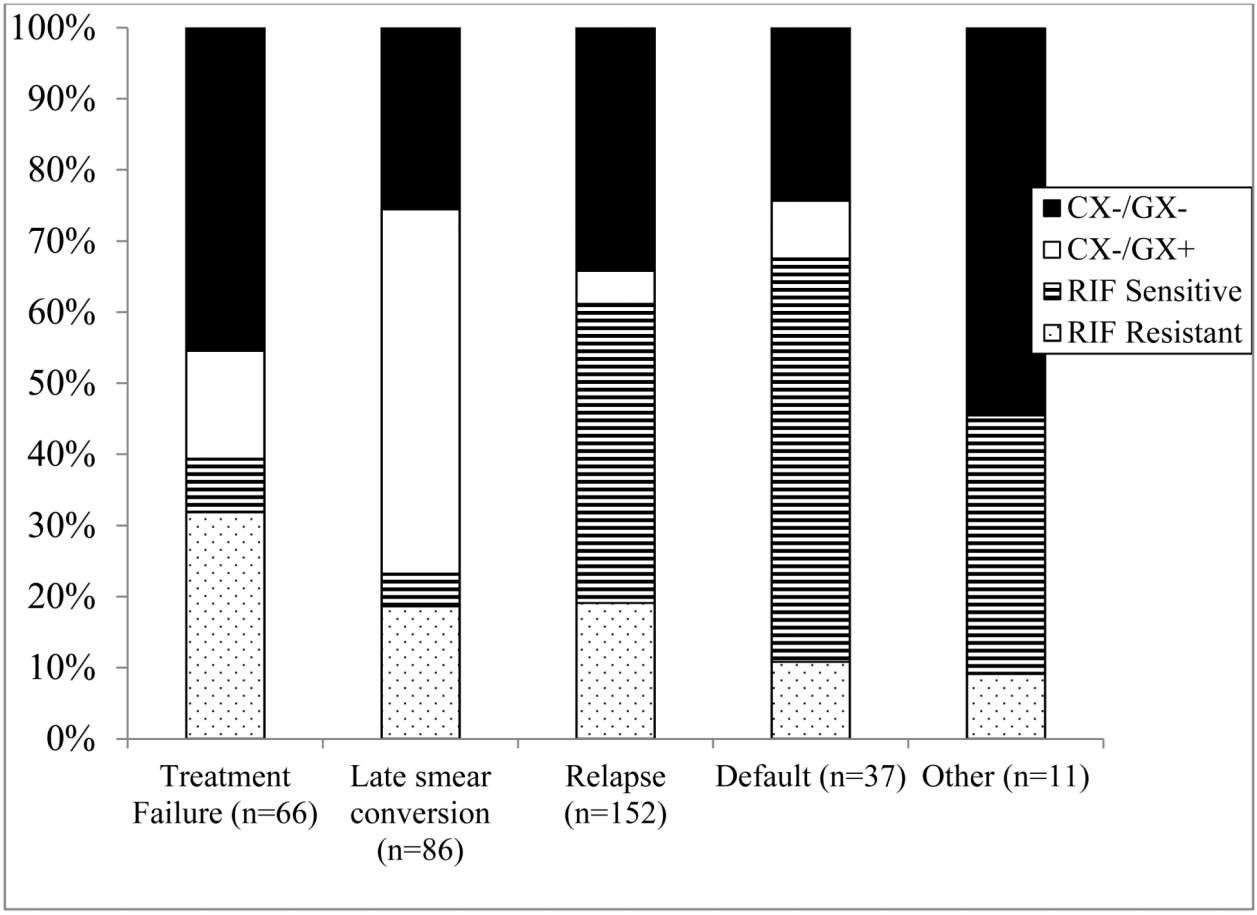

Figure 2. Diagnostic Classification According to Retreatment Category

Cx-/GX-: culture-negative/Xpert-negative; Cx-/GX+: culture-negative/Xpert-positive; RIF: rifampin

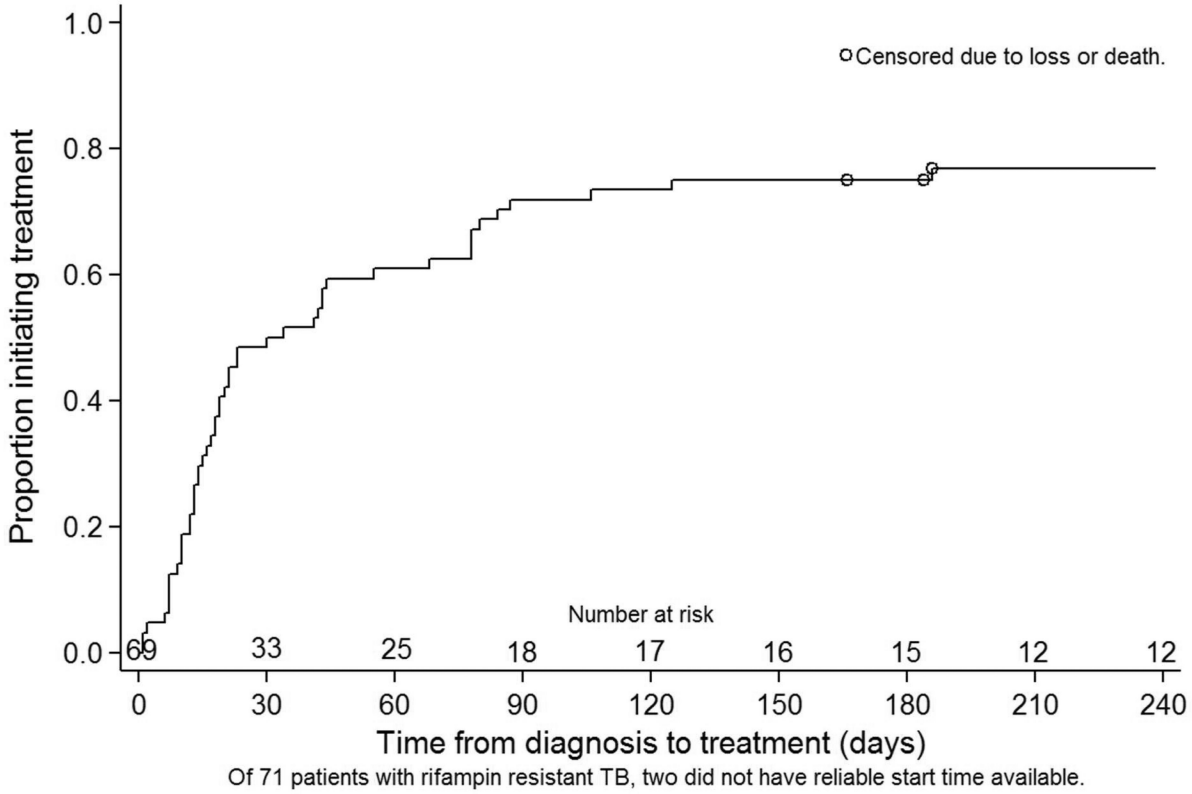

Figure 3. Time to treatment initiation for patients with rifampin-resistant TB

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Table 1

Characteristics of the study population

	Xpert- or culture-confirmed RIF resistant TB (n=71)	Culture-confirmed RIF sensitive TB (n=98)	Culture-negative Xpert positive (n=64)	Culture negative Xpert negative (n=119)	P value
Age, median years (IQR)	33 (27-43)	35 (28-43)	37 (29-45)	39 (31-46)	0.04
Female (%)	34 (47.9)	30 (30.6)	22 (34.4)	50 (42.0)	0.03
HIV Infection	47 (69.1)	61 (62.2)	41 (64.1)	89 (74.8)	0.21
CD4+ T-cell count/mm^3, median (IQR)	189 (100-374)	195 (105-390)	211 (90-253)	249 (89-372)	0.79
On ART (%)	41 (89.1)	36 (60.0)	38 (92.7)	68 (76.4)	<0.001
Retreatment TB Type (%)					<0.001
Treatment failure (%)	21 (29.6)	5 (5.1)	10 (15.6)	30 (25.2)	
"Category I" (%) *	10 (47.6)	1 (20.0)	6 (60.0)	19 (63.3)	
"Category II" (%) †	11 (52.4)	4 (80.0)	4 (40.0)	11 (36.7)	
Late smear conversion (%)	16 (22.5)	4 (4.1)	44 (68.8)	22 (18.5)	
Relapse (%)	29 (40.9)	64 (65.3)	7 (10.9)	52 (43.7)	
Early (1-12 months) (%)	14 (48.3)	8 (12.5)	0 (0.0)	12 (23.1)	<0.001
Late (>12 months) (%)	15 (51.7)	56 (87.5)	7 (100)	40 (76.9)	
Default (%)	4 (5.6)	21 (21.4)	3 (4.7)	9 (7.6)	
Other (%)	1 (1.4)	4 (4.1)	0 (0.0)	6 (5.0)	
Xpert cycle threshold, median (IQR)	19.7 (16.9-24.1)	21.3 (18.3-25.2)	27.1 (24.5-30.3)	N/A	<0.001
Number of Prior Rx Courses					<0.001
None (%)	20 (28.2)	4 (4.1)	45 (70.3)	29 (24.4)	
One (%)	35 (49.3)	67 (68.4)	13 (20.3)	62 (52.1)	
Two or more (%)	16 (22.5)	27 (27.6)	6 (9.4)	28 (25.3)	
Infectious Period,§ median days (IQR), p<0.001	269 (161-458)	86 (44-147)	72 (45-143)	100 (45-326)	<0.001
Number of symptoms at presentation //					0.04
1	0 (0.0)	7 (7.1)	4 (6.3)	15 (0.8)	
2	14 (19.7)	19 (19.4)	13 (20.3)	15 (12.6)	
3	24 (33.8)	41 (41.8)	23 (35.9)	30 (25.2)	

	Xpert- or culture-confirmed RIF resistant TB (n=71)	Culture-confirmed RIF sensitive TB (n=98)	Culture-negative Xpert positive (n=64)	Culture negative Xpert negative (n=119)	P value
4	30 (42.3)	27 (27.6)	19 (29.7)	31 (26.1)	
5	1 (1.4)	4 (4.1)	5 (7.8)	5 (4.2)	
Body Mass Index (%)					0.06
<18	25 (38.5)	25 (26.0)	11 (18.3)	25 (22.1)	
18-25	40 (61.5)	97 (69.8)	47 (78.3)	80 (70.8)	
>25	0 (0.0)	4 (4.2)	2 (3.3)	8 (7.1)	
Contact with known TB patient	31 (43.7)	29 (30.0)	13 (20.6)	26 (21.9)	<0.01
Travelled to another country	22 (31.0)	33 (33.7)	21 (32.8)	43 (36.1)	0.90
Travelled to South Africa	15 (21.1)	19 (19.4)	13 (20.3)	25 (21.0)	0.99
Hospitalized in the past 5 years	16 (22.5)	27 (27.6)	19 (29.7)	28 (23.5)	0.71
Prison in the past 5 years	2 (2.8)	2 (2.0)	2 (3.1)	5 (4.2)	0.84
Household income (quartiles)					0.88
Lowest ($0-100/month)	21 (29.6)	27 (27.6)	15 (23.4)	34 (29.1)	
Second ($106-200/month)	23 (32.4)	25 (25.5)	19 (29.7)	37 (31.6)	
Third ($220-300/month)	11 (15.5)	22 (22.5)	13 (20.3)	16 (13.7)	
Highest ($328-5000/month)	16 (22.5)	324 (24.5)	17 (26.6)	30 (25.6)	

The denominator for each characteristic excludes missing or unknown values. Categorical data was analysed using Chi-square and Fisher's exact tests; continuous data was analysed using Kruskal–Wallis equality-of-populations rank test.

[*] Category I refers to standard first-line TB treatment (2HRZE/4HR)

[†] Category II refers to the retreatment regimen with first-line drugs (2HRZES/1HRZE/5HRE)

[‡] Late smear conversion was defined as AFB sputum smear-positivity at month three during treatment with first-line drugs

[§] Days symptomatic prior to initiation of appropriate anti-TB medications.

[//] Assessed symptoms included cough, fever, night sweats, and weight loss.

Table 2a

Recurrent Tuberculosis (N=87)

	Sensitivity	Specificity	PPV	NPV
Any rifampin resistance	23/28 (82.1) (63.1-93.9)	59/59 (100) (93.9-100)	100 (85.2-100)	92.2 (82.7-97.4)
Multidrug resistance	16/18 (88.9) (65.3-98.6)	62/69 (89.9) (80.2-95.8)	69.6 (47.1-86.8)	96.9 (89.2-99.6)

PPV: positive predictive value; NPV: negative predictive value; Multidrug resistance includes resistance to at least INH and RIF.

Table 2b
Prevalent Retreatment Tuberculosis (N=68)

	Sensitivity	Specificity	PPV	NPV
Any rifampin resistance	31/35 (88.6) (73.3-96.8)	31/33 (93.9) (79.8-99.3)	93.9 (79.8-99.3)	88.6 (73.3-96.8)
Multidrug resistance	30/34 (88.2) (72.5-96.7)	31/34 (91.2) (76.3-98.1)	90.9 (75.7-98.1)	88.6 (73.3-96.8)

PPV: positive predictive value; NPV: negative predictive value; Multidrug resistance includes resistance to at least INH and RIF.

Table 3

Sequencing of discordant drug susceptibility results

Patient	Sputum smear	Xpert MTB/RIF	Mean Ct	LJ DST	MODS INH/RIF	*rpoB* sequencing	Retreatment TB Type	Patient outcome (12-mo)
A	NEG	S	15.0	POS SENS	S/R	WT	Relapse (late)	Failed Cat II [*] treatment/Died
B	++	S	13.8	POS MDR	C/C	WT	Late smear conversion [≠]	Continuing MDR-TB Rx
C	+	S	31.8	POS MDR	NEG	codon 519 deletion	Late smear conversion	Improved on Cat I [†] treatment
D	NEG	S	30.3	POS MDR	NEG	L511P and D516P	Treatment failure (Cat I)	Improved on Cat II treatment

S: sensitive; R: resistant; C: contaminated; MDR: multidrug resistant; MODS: microscopically observed drug-susceptibility; WT: wild-type; ASx: asymptomatic

[*] Category II refers to the retreatment regimen with first-line drugs (2HRZES/1HRZE/5HRE)

[†] Category I refers to standard first-line TB treatment (2HRZE/4HR)

[≠] Late smear conversion was defined as AFB sputum smear-positivity at month three during treatment with first-line drugs