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MAJOR ARTICLE

Discordance of the Repeat GeneXpert MTB/RIF Test for Rifampicin Resistance Detection Among Patients Initiating MDR-TB Treatment in Uganda

Willy Ssengooba,[1,2] Jean de Dieu Iragena,[3] Kevin Komakech,[2] Iginitius Okello,[1] Joanitah Nalunjogi,[1] Winceslaus Katagira,[1] Ivan Kimuli,[1] Susan Adakun,[1,4] Moses L. Joloba,[1,2] Gabriela Torrea,[5] and Bruce J. Kirenga[1]

[1]College of Health Sciences, Makerere University Lung Institute, Kampala, Uganda, [2]Mycobacteriology (BSL-3) Laboratory, Department of Medical Microbiology, Makerere University, Kampala, Uganda, [3]Communicable Diseases Cluster, HIV/TB and Hepatitis Programme, World Health Organization Regional Office for Africa, Brazzaville, Congo, [4]National Tuberculosis Treatment Unit, Mulago Hospital, Kampala, Uganda, and [5]Unit of Mycobacteriology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Background. The Global Laboratory Initiative (GLI) guidelines recommend repeat for GeneXpertMTB/RIF (XpertMTB/RIF) in patients with a low pretest probability of rifampicin resistance (RR).

Methods. This was a cross-sectional study using results of sputum specimens collected from participants screened for the STREAM 2 trial. We recruited all patients with XpertMTB/RIF RR-TB detected who were referred for RR/multidrug-resistant (MDR) TB treatment initiation at Mulago National Referral Hospital, Kampala, between September 2017 and October 2019. At baseline, smear microscopy, repeat XpertMTB/RIF, Xpert Ultra, and MTBDR*plus* assays were done on sputum specimens. Culture-based drug susceptibility testing (DST) was performed on discordant specimens. We analyzed the prevalence and factors associated with discordance between initial and repeat XpertMTB/RIF RR and false XpertMTB/RIF RR. False XpertMTB/RIF RR was defined as no RR detected by any of Xpert Ultra, LPA, or culture DST (reference comparator).

Results. A total of 126/130 patients had repeat XpertMTB/RIF results, of whom 97 (77.0%) had *M. tuberculosis* detected, 81 (83.5%) had RR detected, and 1 (1.0%) had RR indeterminate. The prevalence of discordant XpertMTB/RIF RR was 15/96 (15.6%), whereas false XpertMTB/RIF RR prevalence was 10/96 (10.4%).

Low–bacillary load sputum specimens were more likely to have discordant XpertMTB/RIF RR and false XpertMTB/RIF RR results (adjusted odds ratio [aOR], 0.04; 95% CI, 0.00–0.37; $P = .01$; aOR, 0.02; 95% CI, 0.01–0.35; $P = .01$, respectively).

Conclusions. Our findings show a high false-positive rifampicin resistance rate in low–TB burden patients, which calls for repeat testing in order to prevent unnecessary prescription of anti-MDR-TB therapy.

Keywords. detection; Repeat; RR/MDR-TB; Xpert.

Efforts toward tuberculosis (TB) control are challenged by the emergence of multidrug-resistant TB (MDR-TB). The World Health Organization (WHO) reported in 2019 approximately half a million (range, 417 000–556 000) new cases of rifampicin-resistant TB (of whom 78% had multidrug-resistant TB) [1]. Treatment for RR/MDR-TB is not only longer, but also more expensive (≥US$1000 per person), with only a 55% success rate globally [1]. There are still huge gaps between diagnosis and treatment initiation. As part of the effort to reduce the diagnostic gap, the WHO endorsed the use of the GeneXpert MTB/RIF test (XpertMTB/RIF; Cepheid, Sunnyvale, CA, USA) in 2011 as the initial diagnostic test in individuals presumed to have RR/MDR-TB or HIV-associated TB [2]. This was followed by the WHO End TB strategy, aimed at reducing the RR/MDR-TB burden. The strategy recommends key actions including universal screening for drug resistance, TB treatment informed by drug resistance patterns, and use of shorter regimens with drugs that are more effective [3]. Like susceptible TB, early diagnosis and treatment of RR/MDR-TB is crucial for TB control and elimination efforts; however, this remains a challenge in most low- and middle-income countries (LMICs) [4]. The Xpert MTB/RIF test has played a leading role in enabling early diagnosis of RR-TB, which is used by most LMICs to inform RR/MDR-TB treatment initiation [5]. In 2017, the WHO endorsed the use of the GeneXpert Ultra (Xpert Ultra; Cepheid, Sunnyvale, CA, USA) assay [6, 7], which is a second-generation Xpert test with improved sensitivity for diagnosis of TB as well as detection of RR-TB.

The other rapid but less accessible molecular diagnostic for RR/MDR-TB is the line probe assay (LPA): the Genotype

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Correspondence: Willy Ssengooba, Mycobacteriology (BSL-3) Laboratory, Department of Medical Microbiology, Makerere University, P.O.BOX 7072, Kampala, Uganda (willyssengooba@gmail.com).

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MTBDR*plus* (Hain Life Sciences, Nehren, Germany). Although LPA is rapid and offers drug susceptibility test results for rifampicin and isoniazid, it requires more technical skills and infrastructure to perform. Studies have documented XpertMTB/RIF discordance/false RR results compared with other rifampicin resistance–determining methods [8–13]. However, none has compared this discordance with the Xpert Ultra cartridge, which is being rolled out in most high–TB burden countries. Uganda implemented the "Xpert for all" TB diagnostic strategy and has transitioned to Xpert Ultra at all health facilities.

The guidelines from the Global Laboratory Initiative (GLI) recommend repeat Xpert MTB/RIF testing for RR among patients at low risk of having RR/MDR-TB (ie, new TB patients). Due to limited resources in most of the high–TB burden countries including Uganda, Xpert repeat testing to confirm RR is not usually done. Uganda is categorized as a low-RR/MDR-TB-prevalent setting [1], and with the use of Xpert as a frontline test for TB diagnosis, a significant number of TB cases may be classified as low-risk patients for RR and may require a repeat test before RR/MDR-TB treatment initiation.

We set out to investigate the prevalence of discordance between the initial test and repeat XpertMTB/RIF testing for RR-TB determination and factors associated with discordant and false XpertMTB/RIF RR among patients referred from peripheral health care facilities to Mulago National Referral Hospital TB unit, Kampala, Uganda, for RR/MDR-TB treatment initiation.

METHODS

Study Setting and Population

This was a cross-sectional study using results of sputum specimens collected from participants screened for the STREAM 2 trial. Patients were diagnosed as having RR using XpertMTB/RIF G4 cartridge within 24–48 hours of sample collection at the health care facilities in Uganda. Patients diagnosed with RR-TB were referred to Mulago National Referral Hospital in Kampala for RR/MDR-TB treatment initiation, where they were invited to participate in the STREAM 2 trial. During screening, patients provided 3 sputum specimens.

Laboratory Procedures

All laboratory procedures were performed at the College of American Pathologist (CAP) ISO15189 Accredited Mycobacteriology (BSL-3) Laboratory at the Department of Medical Microbiology, Makerere University, Kampala, Uganda.

Smear microscopy was done to select any smear-positive sputum specimen for repeat XpertMTB/RIF and Genotype MTBDRplus assay (LPA) as key screening tests for the STREAM2 trial. Repeat XpertMTB/RIF was done to verify their RR status and determine the bacterial load based on the cycle threshold (Ct) value, whereas LPA was done to confirm the patient's MDR-TB status. Both XpertMTB/RIF and LPA were done within 24 hours of sample collection and according to the manufacturer's protocols.

All sputum specimens with discordant XpertMTB/RIF (ie, RR not detected on repeat testing) were retested with Xpert Ultra. The 3 sputum specimens were processed by decontamination and concentration according to standard procedures [14, 15]. The pellets of the 3 sputum specimens were inoculated in mycobacterial growth indicator tubes (MGITs) for *M. tuberculosis* isolation. Xpert Ultra was also performed on culture isolates from the sputum specimens that had scanty or smear-negative results. Drug susceptibility testing (DST) for rifampicin was done on the same sample as that used for repeat XpertMTB/RIF, Xpert Ultra, and LPA using MGIT at a critical concentration of 1 μg/mL according to the manufacturer's instructions [15].

Data Analysis

We compared the results of the initial XpertMTB/RIF test with the repeat XpertMTB/RIF test for determination of discordance. The results of the additional DST methods (ie, Xpert Ultra, first line LPA, and culture DST) were used to determine false RR results. False RR was defined as no RR detected by any of the additional DST methods done (reference comparator). Factors associated with discordance for rifampicin susceptibility as well as false resistance were determined using logistic regression analysis. Factors included gender, HIV status, CD4 cell/mm^3 at enrollment, smear microscopy grade at enrollment (high [1+, 2+, and 3+], scanty, and negative), initial Xpert bacillary load (semiquantitatively; high [Ct <16], medium [Ct 16–<22], low [Ct 22–28], and very low [Ct >28]), and TB treatment history. Factors with a P value <.2 at the bivariate level were included in the multivariate analysis, and those with adjusted odds ratios (aORs) having a P value <.05 were considered statistically significant.

Patient Consent Statement

This study used the results of samples collected from participants in the STREAM 2 trial. The patient's written consent was obtained. The STREAM 2 trial was approved by the Mulago Hospital Research and Ethics Committee (MREC) and the Uganda National Council of Science and Technology (UNCST). No additional approval was needed for secondary data analysis.

RESULTS

A total of 130 participants were screened at the TB clinic between September 2017 and October 2019. Participants were 73 (56.0%) male, 53 (41.0%) female, and 4 (3.0%) with unknown gender status. The median age (interquartile range [IQR]) was 33 (30–35) years, and 67 (52.0%) were HIV positive. A total of 65 participants had CD4 results with a median (IQR) of 233

(149–356) cells/mm^3, and 43 (66.2%) had a CD4 cell count of >100 cell/mm^3. A total of 78 (60.0%) were new TB patients (Table 1).

Results of Repeat GeneXpert Testing for Newly Diagnosed RR-TB Patients

Of the 97 (77.0%) patients with *M. tuberculosis* detected, RR-TB was detected in 81 (83.5%), indeterminate in 1 (1.0%), RR not detected among 15 (15.5%), and *M. tuberculosis* not detected/RR not confirmed in 29 (29.8%) participants (Figure 1). Repeat XpertMTB/RIF semiquantitative results were as follows: 5 (5.1%) very low, 19 (19.6%) low, 24 (24.7%) medium, and 49 (50.5%) high. Among patients with RR-TB not detected on repeat, the median number of days since initial XpertMTB/RIF to repeat testing (IQR) was 12 (5–20) days. Of the patients with repeat XpertMTB/RIF RR-TB not detected, 9 (60.0%) were smear positive and 6 (40.0%) were smear negative. The smear microscopy grades were as follows: 2 (22.2%) scanty, 1 (11.1%) 1+, 4 (44.4%) 2+, and 2 (22.2%) 3+.

Comparison of Repeat XpertMTB/RIF Results With Other Drug Susceptibility Testing Methods

Of the 15/96 (15.6%) patients with RR not detected on repeat/discordant XpertMTB/RIF, MTBDR*plus* assay was RR not detected in 8 (53.3%) and RR detected among 4 (26.7%) and indeterminate among 3 (20.0%) participants. These results were further confirmed by the Xpert Ultra test; only 1 out of 15 (6.7%) patients with RR not detected status was found to be RR positive. Of the 8 patients with RR not detected on both XpertMTB/RIF and MTBDR*plus* assay, 2 were negative and 6 were positive by smear microscopy with high smear grades (1+, 2+, 3+). Using MGIT960 DST, only 2 patients had RR, 11 were rifampicin susceptible, and 2 had no culture growth and therefore DST was not possible (Table 2). A repeat Xpert Ultra on isolates from sputum culture of patients who had scanty and smear-negative results (n = 6) found no rifampicin resistance in all of them (Table 2). A total of 10/96 (10.4%) patients had false RR detected (ie, no RR confirmed by any of Xpert Ultra, LPA, or MGIT-DST).

Table 1. Characteristics of RR/MDR-TB Patients Referred to the TB Clinic for Treatment Initiation

Parameter	Frequency	Percentage
Gender		
Female	53	41.0
Male	73	56.0
Unknown	4	3.0
Median age (IQR), y	33 (30–35)	
HIV status		
Negative	59	45.0
Positive	67	52.0
Unknown	4	3.0
CD4 cell count at screening (n = 65)		
Median cells/mm^3 (IQR)	233 (149–356)	
<100 cells/mm^3	22	33.8
>100 cells/mm^3	43	66.2
Smear microscopy status		
Negative	44	34.0
Positive	82	63.0
Unknown	4	3.0
Smear positive grade at screening		
Scanty	9	11.0
1+	16	19.5
2+	21	25.6
3+	36	43.9
History of TB treatment		
New	78	60.0
Previously treated	50	38.0
Unknown	2	2.0

Abbreviations: IQR, interquartile range; MDR-TB, multidrug-resistant tuberculosis; RR, rifampicin resistance; TB, tuberculosis.

Factors Associated With Discordant and False Rifampicin Resistance of the Initial XpertMTB/RIF Test

Patients with very low *M. tuberculosis* detected on the initial XpertMTB/RIF test were 4 times more likely to have discordant RR-TB detected on repeat XpertMTB/RIF (aOR, 0.04; 95% CI, 0.004–0.37; $P = .01$) (Table 3). Having false-positive RR was associated with low bacillary load of the initial Xpert test (aOR, 0.02; 95% CI, 0.01–0.35; $P = .01$) (Table 4). Additionally, of the patients with MTB not detected on repeat XpertMTB/RIF (n = 29) and LPA (n = 19), 8 were culture positive; of these, RR-TB was detected in 4 and not detected in the remaining 4.

DISCUSSION

Our study has shown that repeat XpertMTB/RIF testing has the potential to correctly exclude a significant number of TB patients from unnecessary RR/MDR-TB treatment. Having a very low bacillary load on the initial Xpert was significantly associated with false XpertMTB/RIF RR results. These findings are in agreement with several studies that documented high levels of XpertMTB/RIF discordant RR results mostly attributed to technical challenges, resistance mechanisms, or sputum specimens with low bacillary load [8–13].

The Xpert assay has revolutionized the diagnosis of TB and resistance to rifampicin in the last decade [16]. The XpertMTB/RIF test has been used in Uganda since 2011 and is increasingly deployed at 244 testing sites across the country. In Uganda, the current testing strategy is to use Xpert Ultra as the frontline test for TB diagnosis. The GLI guidelines recommend repeat XpertMTB/RIF testing for patients with a low pretest probability of RR such as new TB cases (with no history of RR-TB contact) [17]. However, in agreement with the previous study [13], in our study the high pretest probability of RR did not lower the rates of discordant resistance. Specifically, almost half of the participants with discordant XpertMTB/RIF RR results had been previously treated for TB.

In 2017, a novel Xpert Ultra cartridge was endorsed by the WHO to further improve the limit of detection (LOD)

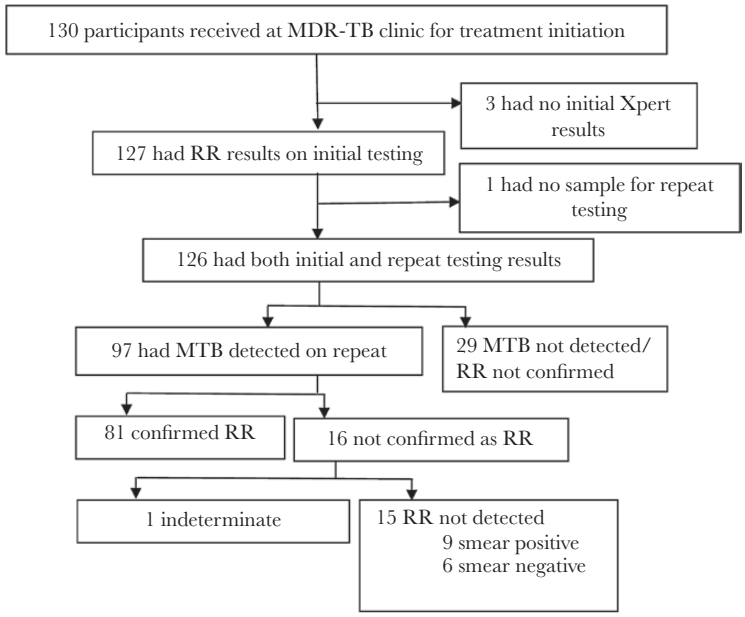

Figure 1. Flow diagram of Xpert MTB/RIF repeat testing for RR/MDR-TB patients included in the study. Abbreviations: MDR-TB, multidrug-resistant tuberculosis; RR, rifampicin resistance; XpertMTB/RIF, GeneXpert MTB/RIF assay.

for TB diagnosis and to increase the specificity for RR detection [18]. In addition to the *rpoB* target included in the classic XpertMTB/RIF, Xpert Ultra includes multicopy insertion sequences (IS6110 and IS1081) specific to the MTB complex, thus increasing its sensitivity to detect TB for paucibacillary disease. Xpert Ultra is expected to yield fewer false RR results, as it uses melting curve analysis for the *rpoB* gene, while the classical XpertMTB/RIF relied on the absence of probe binding

Table 2. Comparative Results for Rifampicin Susceptibility Among Patients With Discordant Repeat Xpert Results

Peripheral Lab XpertMTB/RIF			Repeat XpertMTB/RIF			Other Parameters				MTBDR*plus*		MGIT 960 Culture/DST	
SNO	MTB	RIF	MTB	RIF	Mean *Ct* value	Xpert Ultra	Treatment Category	Smear Microscopy Grade	Days Since Previous XpertMTB/RIF	MTB	RIF	MTB	RIF
1	DVL	R	DVL	S	29.9	S	New	8/length	14	POS	Inconclusive[d]	POS[e]	S
2	DVL	R	DVL	S	32.7	S	New	Smear negative	2	POS	Inconclusive[d]	POS[e]	S
3	DL	R	DVL	S	30.0	S	New	Smear negative	10	POS	**S**	NG	N/A
4	DVL	R	DL	S	30.5	S	New	Smear negative	10	POS	**S**	POS[e]	S
5	DVL	R	DL	S	30.8	S	Previously treated	Smear negative	14	POS	R	NG	N/A
6[a]	DVL	R	DL	S	27.5	S	Previously treated	Smear negative	16	POS	Inconclusive[d]	POS[e]	S
7	DVL	R	DL	S	27.0	S	New	Smear negative	21	POS	R	POS[e]	S
8	DL	R	DL	S	23.7	S	New	2+	0	POS	**S**	POS	S
9	DL	R	DL	S	23.9	S	Previously treated	15/length	11	POS	R	POS[e]	R
10[b]	DVL	R	DM	S	19.0	S	Previously treated	2+	25	POS	**S**	POS	S
11	DL	R	DM	S	22.5	S	Previously treated	2+	27	POS	**S**	POS	S
12	DL	R	DM	S	22.2	S	New	1+	27	POS	**S**	POS	S
13	DH	R	DM	S	17.7	R[e]	New	3+	4	POS	**S**	POS	S
14	DH	R	DH	S	16.2	S	New	3+	0	POS	**S**	POS	S
15	DH	R	DH	S	14.3	S	Previously treated	2+	12	POS	R[c]	POS	R

Abbreviations: Ct, cycle threshold; DH, detected high; DL, detected low; DST, drug susceptibility testing; DVL, detected very low; MGIT, mycobacterial growth indicator tube; MTB, *M. tuberculosis*; N/A, not applicable; NG, no growth; POS, positives; R, resistant; RIF, rifampicin; S, sensitive.

[a]On treatment for 8 days.

[b]On treatment for 14 days.

[c]Hetero resistance detected to rifampicin.

[d]Absence of or uninterpretable TUB control band.

[e]Xpert Ultra done on isolates were rifampicin sensitive. Only this sample among the discordant was found to have mixed strain in MIRU-VNR 24 loci (results not included).

Table 3. Factors Associated with Discordant Repeat XpertMTB/RIF Results Among Patients Initiating RR/MDR-TB Treatment (n = 96)

Variable	RR Detected on Repeat	RR Not Detected on Repeat	OR (*P* Value; 95% CI)	aOR (*P* Value; 95% CI)
Gender (n = 95)[a]				
Female	33	7	*Ref*	
Male	47	8	1.24 (.69; 0.41–3.77)	
HIV- status (n = 95)[b]				
Negative	46	3	Ref	Ref
Positive	34	12	0.18 (.01; 0.05–0.71)	0.40 (.27; 0.08–2.04)
CD4 cell count category				
<100 cell/mm^3	11	4	*Ref*	
>100 cells/mm^3	12	8	1.00 (1.00; 0.25–4.06)	
Smear microscopy grade (enrollment)				
High (1+–3+)	66	7	*Ref*	*Ref*
Scanty	6	2	0.32 (.21; 0.53–1.88)	1.11 (.92; 0.13–9.79)
Negative	9	6	0.16 (.01; 0.04–0.58)	1.28 (.80; 0.18–9.22)
Initial XpertMTB/RIF bacterial burden				
High (Ct <16)	36	3	*Ref*	
Medium (Ct 16–22)	25	0	*(empty)*	*(empty)*
Low (Ct 22–28)	13	5	0.21 (.06; 0.45–1.04)	0.25 (.11; 0.05–1.37)
Very low (Ct >28)	3	7	0.36 (.00; 0.01–0.21)	0.04 (.01; 0.00–0.37)*
Previous TB treatment				
Previously treated	34	6	*Ref*	
New	47	9	0.92 (.89; 0.29–2.83)	

Abbreviations: AFB, acid fast bacilli; aOR, adjusted odds ratio; Ct, cycle threshold; MDR-TB, multidrug-resistant tuberculosis; OR, odds ratio; RR, rifampicin resistance; TB, tuberculosis; XpertMTB/RIF, GeneXpert MTB/RIF assay.

*Statistically significant.

[a]One patient had unknown gender.

[b]One patient had unknown HIV status.

Table 4. Factors Associated With Rifampicin Resistance Not Confirmed by Any of the Additional DST Methods (n = 96)

Variable	RR Detected by any DST Method	RR Not Confirmed by Any DST Method (n = 10)	OR (*P* Value; 95% CI)	aOR (*P* Value; 95% CI)
Gender (n = 95)[a]				
Female	35	5	*Ref*	
Male	50	5	1.42 (.59; 0.38–5.30)	
HIV- status (n = 95)[b]				
Negative	48	1	*Ref*	*Ref*
Positive	37	9	0.08 (.02; 0.10–0.71)	0.15 (.13; 0.01–1.73)
CD4 cell count category				
<100 cell/mm^3	12	3	Ref	
>100 cells/mm^3	24	6	0.79 (1.00; 0.21–4.71)	
Smear microscopy grade (enrollment)				
High (1+ to 3+)	68	5	*Ref*	*Ref*
Scanty	7	1	0.51 (.57; 0.52–5.05)	3.35 (.39; 0.21–52.56)
Negative	11	4	0.20 (.03; 0.05–0.87)	2.74 (.39; 0.27–27.24)
Initial XpertMTB/RIF bacterial burden				
High (Ct <16)	38	1	*Ref*	*Ref*
Medium (Ct 16–22)	25	0	*(empty)*	*(empty)*
Low (Ct 22–28)	14	4	0.92 (.04; 0.01–0.89)	0.09 (.05; 0.01–1.08)
Very load (Ct >28)	5	5	0.03 (.02; 0.01–0.27)	0.02 (.01; 0.01–0.35)*
Previous TB treatment				
Previously treated	31	3	*Ref*	
New	49	7	0.57 (.43; 0.14–2.34)	

Abbreviations: AFB, acid fast bacilli; aOR, adjusted odds ratio; Ct, cycle threshold; DST, drug susceptibility testing; OR, odds ratio; RR, rifampicin resistance; TB, tuberculosis; XpertMTB/RIF, GeneXpert MTB/RIF assay.

*Statistically significant.

[a]One patient had unknown gender.

[b]One patient had unknown HIV status.

to detect RR. Xpert Ultra can also detect resistance better in the presence of mixed-strain or heteroresistant and ambiguous mutations [19], unlike the classic XpertMTB/RIF assay.

In our study, we performed Xpert Ultra on all discordant raw sputum specimens and culture isolates of the patients with discordant results whose sputum specimens were scanty or negative on smear microscopy. Only 1 patient had RR-TB detected with Ultra and was found to have mixed strains (Table 2). Apart from 1 patient in our study, Xpert Ultra did not provide further clarity on rifampicin susceptibility in patients who had rifampicin indeterminate results in XpertMTB/RIF testing.

In line with previous studies [20–22], our findings further confirm that when the level of *M. tuberculosis* is low in the sample, the DNA needed for the XpertMTB/RIF assay may be very low to reliably rule out RR (absence of probe binding). This did not improve with Xpert Ultra despite the documented improvement in detection of RR-TB. From these findings, it is evident that discordant RR-TB is still very challenging to resolve, yet XpertMTB/RIF and Xpert Ultra are rapidly being deployed for better detection of TB among patients expected to have a low bacillary load, such as those who are HIV positive who usually have paucibacillary TB disease. All patients in this study were treated as RR/MDR-TB according to national guidelines.

There is a need for urgent review of the available findings and development of guidelines that will protect the patients from inappropriate second-line RR/MDR-TB treatment. Evidence from such a review may facilitate better RR-TB estimates for countries in light of increasing Xpert deployment. Given the low prevalence of RR in most of the LMICs, the diagnostic gain from repeating XpertMTB/RIF or Xpert Ultra for those few individuals may outweigh the burden of falsely treating a susceptible TB patient as having RR/MDR-TB, given the long treatment duration, associated adverse events, and treatment costs. On the other hand, if repeat XpertMTB/RIF were used as a confirmatory test, true rifampicin resistance would be missed in 5/127 (4%) of the cohort who were RR positive by either LPA, Xpert Ultra, or phenotypic DST. This suggests that risk of overtreatment of false RR vs harm of continued transmission and suffering due to missed true RR detection should be balanced while interpreting discordant results.

The strength of our findings includes the fact that patients were recruited at the largest RR/MDR-TB treatment center in Uganda coming from all parts of the country, and this makes our findings generalizable. Second, participants were those screened for the possibility of being included in a large clinical trial, STREAM 2 trial, with all evaluations done in accordance with standards acceptable for a clinical trial, hence ensuring high-quality data. Third, we compared the initial XpertMTB/RIF results with 3 other tests (ie, Xpert Ultra, LPA, and MGIT-DST) including repeat Xpert Ultra on culture isolates to conclude false RR-TB.

Some of the limitations of our study findings include that the repeat XpertMTB/RIF was not done on the same day or on the same sample as the initial XpertMTB/RIF test, which may modify the results in terms of the yield. However, the days from the initial test to repeat testing were minimal (median, 12 days) and unlikely to have significantly affected the results; moreover, a significant number of the repeat XpertMTB/RIF results were medium and smear positive. Discordance has been previously attributed to probe delay [12]; however, we were unable to retrieve initial XpertMTB/RIF probe data from the peripheral health facilities' GeneXpert machines as patients came from all over Uganda, and this would have required extra effort.

Furthermore, all sputum specimens that were low and/or smear negative but culture positive had their culture *M. tuberculosis* isolates repeat-tested using Xpert Ultra, and results remained rifampicin susceptible. In our study, the prevalence of false XpertMTB/RIF RR-TB may be underestimated as among the 29 patients not detected by repeat XpertMTB/RIF testing, 8 were culture positive, of whom 4 (50%) were negative for RR-TB (results not shown). Due to the facts that neither LPA nor phenotypic DST is a suitable gold standard test for rifampicin resistance determination [8–12] and that false Xpert TB/RR can be detected at a high bacillary load [23], additional considerations are needed when interpreting such discordance. Sample splitting for XpertMTB/RIF, Xpert Ultra, LPA, and culture could have resulted in a lower bacillary burden for each test and could have had an impact on the result; however, of the 6 smear-negative sputum specimens, 4 were culture positive and RR not detected using Xpert Ultra on the isolates. Whole-genome or targeted sequencing for *rpoB* would have supported our conclusions better; however, resources were not available, and these findings have been confirmed in a more recent study that used sequencing, which reported 47% false RR [13].

In conclusion, our findings show a high false-positive rifampicin resistance rate in low–TB burden patients, which calls for repeat testing in order to prevent unnecessary prescription of anti-MDR-TB therapy. We recommend that patients with *M. tuberculosis* detected very low but with rifampicin resistance detected on initial testing have their Xpert test repeated. If on repeat Xpert testing the patient has RR-TB detected, she/he should be initiated on second-line treatment otherwise, managed as a susceptible TB patient. However, the risks and benefits should be weighed by the clinician while making such treatment decisions. If managed as susceptible patients based on results of repeat XpertMTB/RIF, sputum specimens should be sent for culture and phenotypic DST and rapid molecular testing such as LPA or Xpert may be repeated during treatment if they do not respond well to treatment. MGIT-DST is known to miss most rifampicin resistance–conferring mutations with borderline MIC distribution [24–26]. A repeat DST with MGIT using a lower critical concentration of 0.5 μg/mL, as recently

recommended by the WHO, would help to rule out rifampicin resistance.

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