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

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Abstract

We recruited all patients with Xpert Rifampicin-Resistant (RR)-TB detected, referred to the MDR-TB ward at Mulago National Referral Hospital, Kampala, Uganda for MDR-TB treatment initiation between September 2017 and October 2019. Using baseline samples collected for patients screened for STREAM 2 trial, smear microscopy, repeat Xpert test and first-line MTBDR*plus* assay were done. Culture-based drug-susceptibility testing was done on discordant samples. We analysed for factors associated with discordant results and false RR as determined as no RR detected by at least two of the methods used (reference standard).

Of 126/130 patients who had results of repeat Xpert, 97 (77.0%) had *M. tuberculosis* detected of which 81 (83.5%) had RR-detected, 1 (1.0%) indeterminate. A total of 10/96 (10.4%) patients were rifampicin susceptible by at least two of the methods. Having false Xpert RR was associated with low bacillary load measured by high cycle threshold (Ct) value i.e. low (Ct 22–28) and very low (Ct > 28) of the initial Xpert test 0.09 (0.05; 0.01–1.08) and 0.02 (0.01; 0.01–0.35) respectively. Our results show that a repeat Xpert test on another sputum sample for patients with initial low *M. tuberculosis* detected RR-detected, would exclude 10% of the TB patients from unnecessary MDR-TB treatment initiation.

Background

Efforts towards tuberculosis (TB) control are challenged by the emergency of Multi Drug 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 which 78% had multidrug-resistant TB) in 2018. [1] . Treatment for MDR-TB is no only longer, but also more expensive, ≥US$ 1000 per person, with only 55% success rate globally [1] . There are still huge gaps between diagnosis and treatment initiation. As part of the efforts to reduce the diagnostic gap for MDR-TB, the WHO endorsed the use of GeneXpert MTB/RIF test (Xpert; Cepheid, Sunnyvale, CA) in 2011 as the initial diagnostic test in individuals suspected of having MDR-TB or HIV associated TB [2] .

This was followed by the recommendation of WHO End TB strategy towards the reduction of the MDR-TB burden. The WHO End TB 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]. As it is for susceptible TB, early diagnosis of MDR-TB is paramount towards TB control and elimination efforts, however, this remains a challenge in most of the low and middle income countries (LMICs) such as Uganda[4,5]. Xpert test has played a big part towards early diagnosis of MDR-TB in which most of the LMICs are basing MDR-TB treatment initiation on the Xpert results only i.e. patients with Xpert rifampicin resistance (RR) detected are initiated on MDR-TB treatment. In 2017, WHO endorsed the use of Xpert Ultra (Ultra; Cepheid, Sunnyvale, CA, USA) assay[6,7] which is a second generation Xpert with improved sensitivity and specificity for diagnosis of TB as well as detection of rifampicin resistance.

The other rapid, but less accessible molecular MDR-TB diagnostic is the line probe assay (LPA) the Genotype® MTBDR*plus* (Hain Life Sciences, Nehren, Germany). Although LPA is rapid and offers drug susceptibility test results to rifampicin and isoniazid, it requires extra technical skills as well as infrastructure requirements. Studies have documented discordant results between LPA and Xpert for RR determination, although at a lower frequency, mostly attributed to differences in the regions they cover[8].

The guidelines from the Global Laboratory initiative (GLI) recommend repeat Xpert testing for RR among patients with low risk of having MDRTB (i.e. new TB patients). Due to limited resources in most of the high TB burden countries including Uganda, repeat testing for RR is usually not done. Uganda is categorized as low 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 requiring a repeat test before MDR-TB treatment initiation.

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

Methods

Study setting and population

This was a cross-sectional study using results of samples collected from participants screened for the STREAM 2 trial. Patients were diagnosed as having RR from Xpert testing health facilities in Uganda and then referred to Mulago National Referral Hospital, Kampala,

Uganda for MDR-TB treatment initiation from where they were requested to participate in the STREAM 2 trial. During screening, patients were requested to provide three sputum samples for repeat Xpert to categorize the bacterial load based on the cycle threshold value according to the protocol. Smear microscopy was also performed followed by line probe assay (LPA) Genotype MTBDRplus and baseline culture. Xpert and LPA were done on a sample with highest bacillary load as measured by smear microscopy, and if all were smear negative, on any of the submitted samples.

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.

Two sputum samples were collected and screened for positivity using fluorescent smear microscopy. The highest grading smear positive sample, and any sample, if all were smear negative, was processed by concentration according to standards procedures[9,10]. The pellet was reconstituted in 2 ml of which a portion was used in LPA testing and the other in Xpert testing according to manufacturer's instructions i.e. Hain Life Science GmbH, Nehren, Germany and Cepheid, Sunnyvale, CA respectively. The remaining volume was inoculated in mycobacterial growth indicator tube (MGIT) for isolation of *M. tuberculosis*.

For MGIT-DST, tests were performed according to manufacturer's instructions for rifampicin with a standard critical concentration of 1 μg/ml[10]. All discordant samples were re-tested with Xpert Ultra to investigate the potential gain from the newer generation of Xpert. We also performed Ultra on culture isolates for the MTB not detected on Xpert and/or LPA including those with inconclusive LPA results.

Data analysis

We compared results for the first Xpert testing with the repeat Xpert. The LPA results were used as confirmatory test for genotypic method. In light of the fact that studies have also documented incomplete agreement between LPA and Xpert for some resistances, we performed MGIT-DST on the discordant isolates and agreement by at least any two methods was taken as a valid result for drug susceptibility testing. Factors associated with rifampicin susceptibility discordance as well as false resistance as determined by the reference standard were determined by odds ratios using logistic regression at bivariate and multivariate analysis. Factors included; gender, HIV-status, CD4 cell/mm3 at enrolment, smear microscopy grade at enrolment (high (1 + to 3+), scanty (actual number of bacilli), and negative, initial Xpert bacillary load (semi-quantitatively; high (Ct = cycle threshold; <16), Medium (Ct 16-to -<22), low (Ct 22–28) and very low (Ct > 28), and TB treatment history. Factors with p-value less than 0.2 at bivariate level were included in the multivariate analysis and those with p-value less than 0.05 at 95% confidence interval (CI) were considered statistically significant.

Ethical statement

This study used results of samples collected from participants in the STREAM 2 trial. 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). All participants gave a written informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Results

A total of 130 participants were screened at the TB clinic between September 2017 and October 2019. Participants were 73 (56.0%) male, median Age (years; Interquartile range (IQR)) of 33 (30–35) of whom, 67 (52.0%) were HIV-positive. A total of 65 participants had CD4 results with a median (cells/mm^3; (IQR)) of 233 (149–356) and 43 (66.2%) had CD4 cell count of > 100 cells/mm^3. A total of 78 (60.0%) were new TB patients, Table 1.

Results of repeat GeneXpert testing for newly diagnosed MDR-TB patients

A total of 126 had repeat Xpert results of which, 29 (23.0%) had *M. tuberculosis* not detected and 97 (77.0%) had *M. tuberculosis* detected. Repeat Xpert semi quantitative results were; 5 (5.1%) very low, 19 (19.6%) low, 24 (24.7%) medium and 49 (50.5%) high,. Rifampicin resistance (RR) among the repeat Xpert testing was detected in 81 (83.5%), indeterminate in 1 (1.0%) and RR not detected among 15 (15.5%) participants, Fig. 1. The median days since initiation Xpert testing to repeat was 9 days (IQR 7–11) overall, and 12 days (IQR 5–20) among participants with RR not detected on repeat. Smear microscopy was positive among 9/15 (60.0%) and negative among 6/15 (40.0%). Among the smear positive patients with RR not detected on repeat, smear microscopy grades were; 2 (22.2%) scanty, 1 (11.1%) 1+, 4 (44.4%) 2 + and 2 (22.2%) 3+.

Comparison repeat Xpert results with other drug susceptibility methods

The LPA (MTBDR*plus* assay) was done among 124 participants of whom, *M. tuberculosis* was detected among 105 (84.7%) and not detected among 19 (15.3%). Analysis for RR using MTBDR*plus* assay was detected among 74 (70.5%), not detected among 22 (20.9%) and indeterminate among 9 (8.6%) of the participants.

Of the 15 patients with RR not detected on repeat Xpert, MTBDR*plus* assay was; RR not detected in 8 (53.3%) and RR detected among 3 (20.0%) and indeterminate among 4 (26.7%) participants. Xpert Ultra test was also done on the 15 patients with RR not detected of whom only one had RR detected. Of the eight patients with RR not detected on both Xpert 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 two patients had RR, eleven were R susceptible and 2 had no culture growth and therefore DST was not possible, Table 2. A repeat Xpert Ultra was done on isolates from patients who had scanty and smear negative results and smear with actual numbers using culture isolates and all had rifampicin resistance not detected (data not shown).

False rifampicin resistance on initial Xpert test is associated with low bacillary load

For factors associated with false RR detected on initial Xpert test as determined by the repeat and the reference standard as defined by no RR detected by any of the DST methods. Only low bacillary load on the initial test remained significant at multivariate level of analysis after adjusting for other factors. False resistance to rifampicin as determined by the repeat Xpert was associated with low bacillary load of the initial sputum sample as measured by Xpert semi-quantitative grade. Patients with low and very low *M. tuberculosis* detected were 2.1 and 3.6 times likely to have a false RR detected on repeat Xpert i.e. OR (p-value; 95%CI); 0.21 (0.06; 0.45–1.04) and 0.36 (0.001; 0.01–0.21) respectively, Table 3. A total of 10/15 (66.7%) patients with RR not detected on repeat had false RR as determined by the reference standard. Having false RR by a reference standard was associated with low bacillary load of the initial Xpert test i.e. 0.92 (0.04; 0.01–0.89) and 0.26 (0.002; 0.02–0.27) respectively, Table 4. Of the patients with MTB not detected on Xpert (n = 29) and LPA (n = 19), eight were culture positive of which 4 had RR-TB detected and 4 had RR-TB not detected (results not shown).

Discussion

In a study among rifampicin resistant patients referred for MDR-TB treatment initiation, a repeat Xpert testing would correctly exclude a significant number of the TB patients from MDR-TB treatment. Specifically, in our study, 10/96 (10.4%) TB patients with MTB detected could not have RR detected by at least two of the DST methods done. Having low bacillary load on the initial Xpert was significantly associated with discordant repeat Xpert for RR and false RR results as determined by the reference standard. The burden may be underestimated since among the 29 repeat Xpert MTB not detected patients, eight were culture positive of which 4 (50%) had RR-TB not detected. A recent study in Rwanda found high levels of false RR, after repeat, attributed to low bacillary load samples[11]. In our study, 7/15 (46.7%) patients with false Xpert RR had very low bacillary load on the initial Xpert. Studies have also found the low positive predictive value of Xpert assay to be as a result of low specificity in a low MDR-TB prevalent setting such as Uganda[11]. Furthermore, increased testing capabilities has also been found to reduce test specificity as patients are detected in the early stages of the disease[11].

The GeneXpert® MTB/RIF assay, a rapid qPCR-based assay, has revolutionized the diagnosis of TB and its resistance to rifampicin in the last decade [12]. The Xpert test has been used in Uganda since 2011 and increasingly deployed in 244 testing sites across the country. In Uganda the testing strategy is to use Xpert as the frontline test for TB diagnosis and detection of RR. The high discordance of repeat Xpert for RR has been documented in previous studies[11,13]. The GLI guidelines recommends repeat Xpert testing for patients with a low pretest probability of RR such as the new TB cases[14]. However, in agreement with the previous study[11], in our study the high pretest probability of RR did not lower the rates of false resistance as there were almost half of patients previously treated who had no RR detected on repeat Xpert. Some studies have associated the discordance of Xpert for RR due to low bacterial load clinical specimens[11,15]. In our study, 7/15 (46.7%)of the TB patients with discordant RR results had very low bacterial load on the initial Xpert test (Table 2). An earlier study found false-positive RR on Xpert MTB/RIF to be caused by a silent mutation in the *rpoB* gene[16]. However, the issues with probe and silent mutations have been reduced with improved versions of the Xpert cartridge. Moreover, the Xpert results discordance has been associated with poor clinical outcome among the affected patients[17,18]. It remains to investigate the potential strategy towards reducing false RR among patients with low bacillary load.

A novel Xpert cartridge, GeneXpert® MTB/RIF (Ultra) was developed to further improve the limit of detection (LOD) for TB diagnosis and to increase the specificity for rifampicin resistance detection[19]. This was endorsed by the WHO in 2017 as a replacement for the Xpert cartridge. In addition to the *rpoB* target included in the classic Xpert, Xpert Ultra includes multi-copy insertion sequences (IS6110 and IS1081) specific for the MTBc, thus increasing its sensitivity to detect TB for paucibacillary disease. Xpert Ultra is expected to yield fewer false-RR results, as melting curve analysis for the *rpoB* gene is used, while the classical Xpert relied on absence of probe binding to detect RR. In our study, we performed Xpert Ultra on raw sputum and culture isolates of the patients with RR not detected on repeat Xpert. All apart from one had RR not detected with Ultra. The one patient with RR detected using Ultra was later found to have hero-resistance (Table 2. Like Ultra, LPA has capabilities of detecting hetero-resistance for rifampicin. Given the expected high specificity for RR detection on Ultra, our findings which shows no difference with Xpert among low bacillary load samples require more studies on a larger scale to further guide which semi-quantitative levels of Ultra may require repeat testing to ascertain true rifampicin resistance.

In line with the previous studies, our findings further confirm that when the *M. tuberculosis* is low in the sample, the DNA needed for Xpert assay may be very low to reliably rule-out RR [20–22]. This did not improve with Ultra despite the documented improvement in the detection of RR. From these findings, it is evident that false RR challenge is increasing yet Xpert is rapidly being deployed for better detection of TB among patients expected to have low bacillary load such as those who are HIV-positive. Although not statistically significant, in our study, HIV-positive patients had more false RR than HIV-negative TB patients. This was expected as HIV-positive individuals usually have paucibacillary TB disease. In Uganda, as it may be in other LMICs, once a TB patient test RR on Xpert, the practice is that the patient is initiated on MDR-TB treatment and a baseline sample is sent to the National TB Reference laboratory (NTRL) for second line DST. Indeed, all the false resistant TB patients identified in this study became screen failure for STREAM 2 trial and were handed over to the National TB program (NTP) and continued with MDR-TB treatment under NTP. There is a need for urgent review of the available findings by the WHO and develop guidelines which will protect the patients from inappropriate second line MDR-TB treatment. Evidence from such review may facilitate better RR-TB estimates for country with increasing Xpert deployment. After a thorough review of the available information, countries may revise their diagnostic algorithm for Xpert including Ultra to repeat Xpert testing RR basing on the semi-quantitative results. Such has already been adopted by the Rwanda national TB program[11]. In such scenarios, patients with *M. tuberculosis* detected from Low Ct values and below with RR detected could be repeated regardless of treatment history. Patients with *M. tuberculosis* detected Medium and above may be initiated on MDR-TB treatment without repeat, if they have a high pretest probability for RR-TB. Given the low prevalence of RR in most of the LMICs, the again from repeating Xpert for those few individuals may outweigh the burden of falsely treating a susceptible TB patient as having MDR-TB, given the long treatment duration and the associated adverse events and treatment costs.

The strength of our study findings include the fact that patients were recruited at the largest MDR-TB treatment centre in Uganda without sampling and this makes our findings generalizable. Secondly, participants were those screened for possibility of being included in one of the largest 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 Xpert results with five other tests including repeat Xpert Ultra on culture isolates to conclude false RR-TB.

Some of the limitations of our study findings include; the repeat Xpert was not done on the same day and on the same sample which may modify the results in terms of the yield. However, the days from the initial testing to repeat testing were minimal (median, 9-days) to significantly vary the results, moreover, a significant number of the repeat Xpert results were medium and smear positive proving that the discordance is driven by the difference in bacillary load. Furthermore, all samples having low and/or smear negative but culture positive had their *M. tuberculosis* isolates repeated on Xpert Ultra and results remained rifampicin susceptible.

In conclusion, in our setting, a repeat Xpert test on another sputum sample for patients with initial low *M. tuberculosis* detected, would exclude 10% of the TB patients from unnecessary MDR-TB treatment initiation. We recommend that patients with *M. tuberculosis* detected low and below but with rifampicin resistance detected on initial testing should 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 susceptible TB patient. The susceptible patients on repeat Xpert should have their samples sent for culture and phenotypic DST and Xpert testing may be repeated during treatment in case they do not respond well to treatment. Patients with MTB detected medium or high with RR-TB detected should be initiated on second line TB treatment without repeat testing.

Declarations

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Author Contributions

WS and BJK designed the study, WS, JDI, KK, IO, JN, WK, IK, SA, MLJ, GT and BJK performed the experiments, WS, GT and BJK analysed the data, WS, wrote draft manuscript, and all authors, read and approved the final version of the manuscript.

Competing interests

The author(s) declare no competing interests.

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Tables

Table 1
Characteristics of 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 (years; IQR)	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)	
Less than 100 cells/mm^3	22	33.8
Greater than 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

TB= Tuberculosis, MDR–TB = Multi–drug Resistant Tuberculosis, IQR = Inter quartile Range,

Table 2
Comparative results for rifampicin susceptibility among patients with discordant results

Peripheral lab Xpert			Repeat MTB/RIFXpert			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 Xpert	MTB	RIF	MTB	RIF
1	DVL	R	DVL	S	29.9	S	New	8/length	14	POS	Inconclusive	POS*	S
2	DVL	R	DVL	S	32.7	S	New	smear negative	2	POS	Inconclusive	POS*	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*	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	POS*	S
7	DVL	R	DL	S	27.0	S	New	Smear negative	21	POS	R	POS*	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*	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[c]	DH	R	DM	S	17.7	R	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	Inconclusive	POS	R

a = on treatment for 8 days and b = on treatment for 14 days, c = patient found to have hetero–resistance, DVL = detected very low, DL = Detected low, DH = Detected High, S= sensitive, R = Resistant, Ct = cycle threshold, POS = Positives, NG = No Growth, RIF = Rifampicin, MTB = *M. tuberculosis*, DST = Drug Susceptibility Testing, MGIT = Mycobacterial Growth Indicator Tube, N/A = Not applicable, * XpertMTB/RIF Ultra done on isolates were rifampicin sensitive

Table 3
; Factors associated with Xpert rifampicin resistance not detected on repeat Xpert testing (n = 96)

Variable	Rifampicin resistance detected on repeat	Rifampicin resistance Not detected on repeat	Unadjusted OR (P-value; 95%CI)	Adjusted OR (P-value; 95%CI)
Gender				
Female	33	7	*Ref*	..
Male	47	8	1.24 (0.69; 0.41–3.77)	..
HIV- Status				
Negative	46	3	Ref	Ref
Positive	34	12	**0.18 (0.01; 0.05–0.71)**	0.40 (0.27; 0.08–2.04)
CD4 Cell count category				
< 100 cell/mm3	11	4	*Ref*	..
> 100 cells/mm3	12	8	1 (1.00; 0.25–4.06)	..
Smear microscopy grade (enrollment)				
High (1^+ to 3^+)	66	7	*Ref*	*Ref*
Scanty (Actual AFB number)	6	2	0.32 (0.21; 0.53–1.88)	1.11 (0.92; 0.13–9.79)
Negative	9	6	**0.16 (0.01; 0.04–0.58)**	1.28 (0.80; 0.18–9.22)
Initial Xpert bacillary grade				
High (Ct < 16)	36	3	*Ref*	
Medium (Ct 16 to 22)	25	0	*(empty)*	*(empty)*
Low (Ct 22 to 28)	13	5	**0.21 (0.06; 0.45–1.04)**	**0.25 (0.11; 0.05–1.37)**
Very low (Ct > 28)	3	7	**0.36 (0.00; 0.01–0.21)**	**0.04 (0.01; 0.004–0.37)**
Previous TB treatment				
Pretreatment	34	6	*Ref*	..
New	47	9	0.92 (0.89; 0.29–2.83)	..

Table 4
; Factors associated with rifampicin resistance not detected by any DST method on repeat (n = 96)

Variable	Rifampicin resistance detected by any DST method	Rifampicin resistance Not detected by any DST method	Unadjusted OR (P-value; 95%CI)	Adjusted OR (P-value; 95%CI)
Gender				
Female	35	5	*Ref*	..
Male	50	5	1.42 (0.59; 0.38–5.30)	..
HIV- Status				
Negative	48	1	*Ref*	*Ref*
Positive	37	9	**0.08 (0.02; 0.10–0.71)**	0.15 (0.13; 0.01–1.73)
CD4 Cell count category				
< 100 cell/mm3	12	3	Ref	..
> 100 cells/mm3	24	6	0.79 (1.00; 0.21–4.71)	..
Smear microscopy grade (enrollment)				
High (1^+ to 3^+)	68	5	*Ref*	*Ref*
Scanty (Actual AFB number)	7	1	0.514 (0.57; 0.52–5.05)	3.35 (0.39; 0.21–52.56)
Negative	11	4	**0.20 (0.03; 0.05–0.87)**	2.74 (0.39; 0.27–27.24)
Initial Xpert bacillary grade				
High (Ct < 16)	38	1	*Ref*	*Ref*
Medium (Ct 16 to 22)	25	0	*(empty)*	*(empty)*
Low (Ct 22 to 28)	14	4	**0.92 (0.04; 0.01–0.89)**	**0.09 (0.05; 0.01–1.08)**
Very load (Ct > 28)	5	5	**0.03 (0.02; 0.01–0.27)**	**0.02 (0.01; 0.01–0.35)**
Previous TB treatment				
Pretreatment	31	3	*Ref*	..
New	49	7	0.567 (0.43; 0.14–2.34)	..

Figures

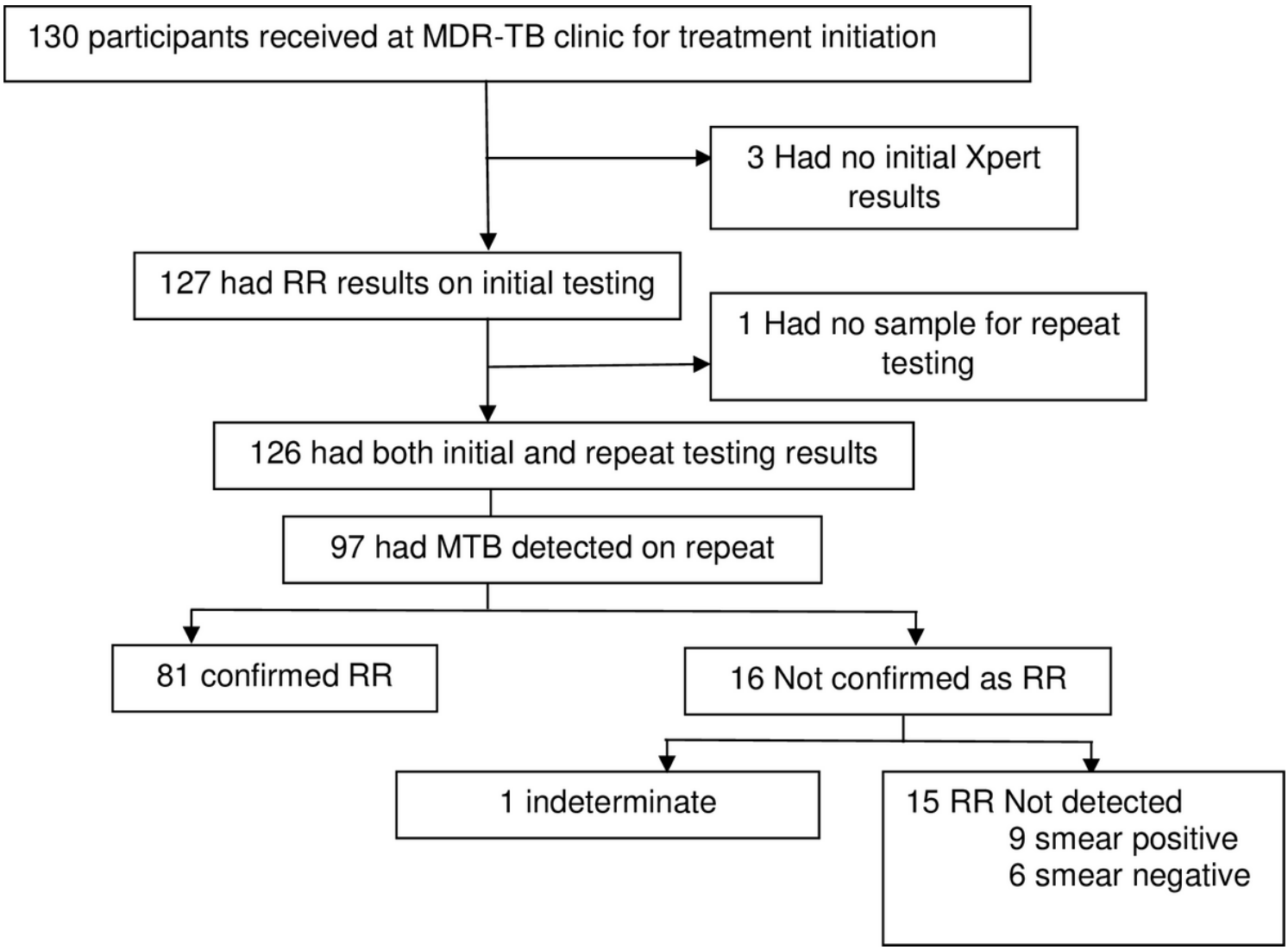

Figure 1

Flow diagram of Xpert MTB/RIF repeat testing for MDR-TB patients before treatment initiation. RR= rifampicin resistance