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 Iragena3, Kevin Komakech2, Iginitius Okello1, Joanitah Nalunjogi1, Winceslaus Katagira1, Ivan Kimuli1, Susan Adakun1,4, Moses L Joloba1,2, Gabriela Torrea5, Bruce J Kirenga1

1Makerere University Lung Institute, College of Health Sciences, Kampala, Uganda.
2Makerere University, Department of Medical Microbiology, Mycobacteriology (BSL-3) Laboratory, P.O.BOX 7072, Kampala, Uganda
3Communicable Diseases Cluster, HIV/TB and Hepatitis Programme, World Health Organization Regional Office for Africa, Brazzaville, Congo
4National Tuberculosis treatment unit, Mulago Hospital, Kampala, Uganda
5Unit of Mycobacteriology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Correspondences: Dr. Ssengooba Willy; willyssengooba@gmail.com

Summary of the article’s main point

Among patients with low risk of resistance, a repeat Xpert is recommended

We report high false-positive rifampicin resistance rate in low TB burden patients

Repeat Xpert test will prevent unnecessary prescription of anti MDR-TB therapy.
ABSTRACT

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

Design/Methods: In 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/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 MTBDRplus assays were done on sputum specimens. Culture-based drug-susceptibility testing (DST) were done on discordant specimens. We analysed 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 which, 97 (77.0%) had M. tuberculosis detected of whom, 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, aOR (p-value: 95%CI), 0.04 (0.01; 0.00-0.37) and 0.02 (0.01; 0.01-0.35) respectively.
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.

Key words: Repeat; Xpert; RR/MDR-TB; detection
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 417000–556000) new cases of rifampicin-resistant TB (of which 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 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, the WHO endorsed the use of GeneXpert MTB/RIF test (XpertMTB/RIF; Cepheid, Sunnyvale, CA) 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 recommendation of WHO End TB strategy towards the reduction of 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 \(^3\). In 2017, WHO endorsed the use of 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® MTBDRplus (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 to other rifampicin resistance determining methods\(^8\).
However, none has compared this discordance with the Xpert Ultra cartridge which is being roll-out in most of high TB burden countries. Uganda implemented the “Xpert for all” TB diagnostic strategy and currently 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 with low risk of having RR/MDR-TB (i.e. 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 low RR/MDR-TB prevalent setting 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 RR/MDR-TB treatment initiation.

We set out to investigate the prevalence of discordance between the initial and repeat XpertMTB/RIF test for RR-TB determination and factors associated with discordant and false XpertMTB/RIF RR among patients referred from peripheral healthcare 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 healthcare facilities in Uganda. Patients diagnosed with RR-TB were referred to Mulago National Referral Hospital in Kampala for RR/MDR-TB treatment initiation from where they were requested to participate in the STREAM 2 trial. During screening, patients provided three 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) whereas LPA was done to confirm patient’s MDR-TB status. Both XpertMTB/RIF and LPA were done within 24 hrs of sample collection and according to the manufacturer’s protocols.

All sputum specimens with discordant XpertMTB/RIF i.e. RR not detected on repeat testing, were re-tested with Xpert Ultra. The three sputum specimens were processed by decontamination and concentration according to standards procedures. The pellets of the three sputum specimens were inoculated in mycobacterial growth indicator tubes (MGIT) for *M. tuberculosis* isolation. Xpert Ultra was also performed on culture isolates from the sputum specimens which 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 manufacturer’s instructions.

**Data analysis**

We compared results of the initial XpertMTB/RIF testing with the repeat XpertMTB/RIF for determination of discordance. Results of the additional DST methods i.e. 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/mm³ at enrolment, smear microscopy grade at enrolment (high (1+ 2+ and 3+), scanty, and negative, initial Xpert bacillary load (semi-quantitatively; high (Ct=cycle threshold; <16), medium (Ct 16 to <22), low (Ct 22 to 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 adjusted odds ratio (aOR) having a p-value less than 0.05 at 95% confidence interval (CI) were considered statistically significant.

Patient Consent Statement
This study used 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 (years; Interquartile range (IQR)) was 33 (30-35) of whom, 67 (52.0%) were HIV-positive. A total of 65 participants had CD4 results with a median (cells/mm³; (IQR)) of 233 (149-356) and 43 (66.2%) had CD4 cell count of >100 cells/mm³. 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%) of the participants, (Figure 1). Repeat XpertMTB/RIF semi-quantitative results were; 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 days since initial XpertMTB/RIF to repeat testing was 12 days (IQR 5-20). 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: 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 methods

Of the 15/96 (15.6%) patients with RR not detected on repeat/discordant, XpertMTB/RIF, MTBDR<sub>plus</sub> 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 Xpert Ultra test of which only one out of 15 (6.7%) patients with RR not detected status was found RR positive. Of the eight patients with RR not detected on both XpertMTB/RIF and MTBDR<sub>plus</sub> 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 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 i.e. no RR confirmed by any of Xpert Ultra, LPA or MGIT-DST.
Factors associated with discordant and false rifampicin resistance of the initial XpertMTB/RIF test

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

Discussion

Our study has shown that a repeat XpertMTB/RIF testing has potential to correctly exclude a significant number of the TB patients from unnecessary RR/MDR-TB treatment. Having 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 which documented high levels of XpertMTB/RIF discordant RR 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 increasingly deployed in 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 recommends repeat XpertMTB/RIF testing for patients with a low pretest probability of RR such as the 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 were previously treated for TB.
In 2017, a novel Xpert Ultra cartridge was endorsed by WHO to further improve the limit of detection (LOD) for TB diagnosis and to increase the specificity for RR detection. In addition to the rpoB target included in the classic XpertMTB/RIF, Xpert Ultra includes multi-copy insertion sequences (IS6110 and IS1081) specific for 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 absence of probe binding to detect RR. Xpert Ultra can also detect resistance better in presence of mixed strain or heteroresistance and ambiguous mutations, 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 one patients had RR-TB detected with Ultra and was found to have mixed strains (Table 2). Apart from one 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 the previous studies, our findings further confirm that when the M. tuberculosis is low in the sample, the DNA needed for 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 the detection for 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 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 develop guidelines which will protect the patients from inappropriate second-line RR/MDR-TB treatment. Evidence from such 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 risks of overtreatment of false RR versus harm of continued transmission and suffering due to missed true RR detection should be balanced while interpreting discordant results.

The strength of our findings include the fact that patients were recruited at the largest RR/MDR-TB treatment centre in Uganda coming from all parts of the country 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 XpertMTB/RIF results with three other tests i.e. 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 the repeat XpertMTB/RIF was not done on the same day and 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 testing to repeat testing were minimal (median, 12-days) to significantly vary 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\textsuperscript{12}, however, we were unable to retrieve initial XpertMTB/RIF probe data from the peripheral health facilities GeneXpert machines since patients came from almost all over Uganda and this would require extra effort.

Furthermore, all sputum specimens having low and/or smear negative but culture positive had their culture \textit{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 since among the 29 patients not detected by repeat XpertMTB/RIF test, eight were culture positive of which 4 (50\%) were negative for RR-TB (results not shown). Due to the fact that neither LPA nor phenotypic DST are suitable gold standard tests for rifampicin resistance determination\textsuperscript{8-12}, and that false Xpert TB/RR can be detected at high bacillary load\textsuperscript{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 can have an impact on the result, however of the six smear negative sputum specimens, four were culture positive and RR not detected using Xpert Ultra on the isolates. Whole genome or targeted sequencing for \textit{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\textsuperscript{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 \textit{M. tuberculosis} detected very low 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 a susceptible TB patient. However, the risks and benefits should be weighed in by the clinician while making such treatment decision. 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 in case they do not respond well to treatment. MGIT-DST is known to miss most rifampicin resistance conferring mutations with borderline MIC distribution\textsuperscript{24-26}. A repeat DST with MGIT using a lower critical concentration of 0.5\mu g/ml as recently recommended by WHO, would help to rule-out rifampicin resistance.
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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 (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 repeat Xpert results

| SNO | MTB | RIF | Repeat XpertMTB/RIF MTB | Repeat XpertMTB/RIF RIF | Mean Ct value | Xpert Ultr a | Treatment category | Smear microscopy grade | Days since previous XpertMTB/R IF | MTBDR plus | MTB | RIF | MGIT 960 Culture/DS T |
|-----|-----|-----|--------------------------|-------------------------|----------------------|------------|-------------------|------------------------|----------------------------------|-------------|-----|-----|----------------------|
| 1   | DVL | R   | DVL                      | S                       | 29.9                 | S          | New               | 8/length               | 14                               | POS         | Inconclusi ve d      | POS S | S   |
| 2   | DVL | R   | DVL                      | S                       | 32.7                 | S          | New               | smear negative         | 2                                | POS         | Inconclusi ve d      | POS S | S   |
| 3   | DL  | R   | DL                       | S                       | 30.0                 | S          | New               | Smear negative         | 10                               | POS         | S               | NG     | N/ A |
| 4   | DVL | R   | DL                       | S                       | 30.5                 | S          | New               | Smear negative         | 2                                | POS         | S               | POS S | S   |
| 5   | DVL | R   | DL                       | S                       | 30.8                 | S          | Previously treated | Smear negative         | 14                               | POS         | R               | NG     | N/ A |
| 6a  | DVL | R   | DL                       | S                       | 27.5                 | S          | Previously treated | Smear negative         | 16                               | POS         | Inconclusi ve d      | POS S | S   |
| 7   | DVL | R   | DL                       | S                       | 27.0                 | S          | New               | Smear negative         | 21                               | POS         | R               | POS S | S   |
| 8   | DL  | R   | DL                       | S                       | 23.7                 | S          | New               | 2+                     | 0                                | POS         | S               | POS S | S   |
| 9   | DL  | R   | DL                       | S                       | 23.9                 | S          | Previously treated | 15/length              | 11                               | POS         | R               | POS S | S   |
| 10b | DVL | R   | DM                       | S                       | 19.0                 | S          | Previously treated | 2+                     | 25                               | POS         | S               | POS S | S   |
| 11  | DL  | R   | DM                       | S                       | 22.5                 | S          | Previously treated | 2+                     | 27                               | POS         | S               | POS S | S   |
| 12  | DL  | R   | DM                       | S                       | 22.2                 | S          | New               | 1+                     | 27                               | POS         | S               | POS S | S   |
|    | DH | R | DM | S |     |    | POS | S | POS | S |
|----|----|---|----|---|-----|----|-----|---|-----|---|
| 13 | DH | R | DM | S | 17.7 | New | 3+ | 4 | POS | S |
| 14 | DH | R | DH | S | 16.2 | New | 3+ | 0 | POS | S |
| 15 | DH | R | DH | S | 14.3 | Previously treated | 2+ | 12 | POS | R |

a = on treatment for 8 days and b = on treatment for 14 days, c= hetero resistance detected to rifampicin, d= absence of or uninterpretable TUB control band, 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. *Xpert Ultra done on isolates were rifampicin sensitive, e = 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)**              |                       |                           |                     |                     |
| Female                         | 33                    | 7                         | Ref                 | ..                  |
| Male                           | 47                    | 8                         | 1.24 (0.69; 0.41-3.77) | ..                  |
| **HIV- Status (n=95)**         |                       |                           |                     |                     |
| 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                         | 1.00 (1.00; 0.25-4.06) | ..                  |
| **Smear microscopy grade (enrollment)** |               |                           |                     |                     |
| High (1+ to 3+)                | 66                    | 7                         | Ref                 | Ref                 |
| Scanty                         | 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 XpertMTB/RIF bacterial burden** |           |                           |                     |                     |
| High (Ct <16)                  | 36                    | 3                         | Ref                 | (empty)             |
| 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; 0.00-0.37) |
| **Previous TB treatment**      |                       |                           |                     |                     |
| Previously treated             | 34                    | 6                         | Ref                 | ..                  |
| New                            | 47                    | 9                         | 0.92 (0.89; 0.29-2.83) | ..                  |

**Key:** TB = Tuberculosis, Ct = Cycle threshold, AFB = Acid Fast Bacilli, XpertMTB/RIF= GeneXpert MTB/RIF Assay, OR = Odds Ratio, aOR = adjusted OR, CI = Confidence Interval, ⃰ = statistically significant, a = one patient had unknown gender, b = one patient had unknown HIV status, RR= rifampicin resistance
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)**                      |                               |                                          |                     |                      |
| Female                                 | 35                            | 5                                        | Ref                 | .                    |
| Male                                   | 50                            | 5                                        | 1.42 (0.59; 0.38-5.30) | ..                  |
| **HIV- Status (n=95)**                 |                               |                                          |                     |                      |
| 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                                 | 7                             | 1                                        | 0.51 (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 XpertMTB/RIF bacterial burden**|                               |                                          |                     |                      |
| 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**              |                               |                                          |                     |                      |
| Previously treated                     | 31                            | 3                                        | Ref                 | ..                  |
| New                                    | 49                            | 7                                        | 0.57 (0.43; 0.14-2.34) | ..                  |

Key: TB = Tuberculosis, Ct = Cycle threshold, AFB = Acid Fast Bacilli, XpertMTB/RIF = GeneXpert MTB/RIF Assay, OR = Odds Ratio, aOR= adjusted OR, CI = Confidence Interval, DST = Drug Susceptibility Testing, RR= rifampicin resistance, * = statistically significant, a = one patient had unknown gender, b= one patient had unknown HIV status
Figures and Figure Legends

Figure 1. Flow diagram of Xpert MTB/RIF repeat testing for RR/MDR-TB patients included in the study

RR= rifampicin resistance
130 participants received at MDR-TB clinic for treatment initiation

127 had RR results on initial testing

3 Had no initial Xpert results

1 Had no sample for repeat testing

126 had both initial and repeat testing results

97 had MTB detected on repeat

29 MTB not detected/RR not confirmed

81 confirmed RR

16 Not confirmed as RR

1 indeterminate

15 RR Not detected
   9 smear positive
   6 smear negative