Effectiveness of Genotype MTBDRsl to exclude drug-resistance of *Mycobacterium tuberculosis* in a clinical trial

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**Running title**: Effectiveness of the LPAs/ in STREAM Trial

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Abstract (199 words)

Objectives: In this study, we assessed the ability of the Genotype MTBDRsl line probe assay Version 1 (V1.0) (LPAsl V1.0) to exclude baseline fluoroquinolones (FQ) and second-line injectable drugs (SLI) resistance in the STREAM 1 trial.

Methods: Direct sputum LPAs result in the site laboratories were compared to indirect phenotypic drug-susceptibility testing (pDST) results in the central lab, with DNA sequencing as a reference standard.

Results: Of 413 multidrug-resistant tuberculosis (MDR-TB) patients tested with LPAsl and pDST, 389 (94.2%) were FQ susceptible, and 7 (1.7%) FQ resistant, while 17 (4.1%) had an inconclusive LPAsl result. For SLI, 372 (90.1%) were susceptible, 5 (1.2%) resistant and 36 (8.7%) inconclusive. There were 9 (2.3%) FQ discordant pDST/LPAsl results, of which 3 revealed a mutation, and 5 (1.3%) SLI discordant pDST/LPAsl results, none of which were mutants by sequencing. Among the 17 FQ and SLI LPAsl inconclusive samples, sequencing showed 1 FQ- and zero SLI-resistant results, similar to frequencies among the conclusive LPAsl. The majority of inconclusive LPAsl were associated with low bacillary load samples (AFB smear-negative or scantily positive) compared to conclusive results (p<0.001).

Conclusion: LPAsl can facilitate the rapid exclusion of FQ and SLI resistances for enrolment in clinical trials.

Keywords: Clinical trial; fluoroquinolones; line probe assay; M. tuberculosis; resistant; second-line injectables
Background

Despite the availability of curative anti-TB therapy for nearly half a century, the emergence and spread of MDR strains is a major public health concern and threatens global control of the disease. In 2019, according to World Health Organization (WHO), an estimated 465,000 people developed rifampicin-resistant TB (RR-TB).

The evaluation of new drugs and new regimens requires randomized clinical trials. STREAM 1 was a phase III, multicentre, open-label, randomized controlled trial that enrolled patients between 2012 to 2015 to evaluate safety, efficacy, and cost-effectiveness of a standardized shorter regimen for MDR-TB similar to a regimen described by Van Deun et al. 2010 compared to long regimen recommended in 2011 by WHO. Enrolment criteria and trial procedures have been previously reported elsewhere (STREAM 1, ISRCTN78372190 and clinicaltrials.gov NCT02409290). The trial enrolled adults with rifampicin-resistant (RR)/MDR-TB and no evidence of resistance to fluoroquinolones (FQ) or second-line injectable drugs (SLI) by line probe assays (LPAs) in four weeks prior to randomization.

Second-line drug LPAs are the fastest and most commonly used genotypic drug susceptibility testing (gDST) method available although still requiring an adequate level of molecular technical expertise. The MTBDRsI LPA Version 1 (LPAsI) detects the most frequent
mutations conferring resistance to FQ in \textit{gyrA} and \textit{gyrB} gene, and SLI in 16S rRNA (\textit{rrs}) and \textit{eis} promoter gene \textsuperscript{14}. A systematic review from Cochrane database showed that LPAsl had sensitivities and specificities of 85.1\% and 98.2\% and 76.9\% and 99.5\% for detection of FQ and SLI resistance from clinical samples, respectively \textsuperscript{14}. At the time STREAM 1 was planned, it was not clear whether this would lead to sufficiently high negative predictive values (NPV) to reliably exclude FQ- and/or SLI-resistant cases from enrolment \textsuperscript{15}. Moreover, indeterminate results have been reported in 7.1\% of FQ bands and 13.5\% for SLI by LPAsl \textsuperscript{15,16}.

Using data and \textit{M. tuberculosis} isolates from the STREAM 1 trial, we assess this LPAsl effectiveness and investigate the most appropriate interpretation of inconclusive LPAsl results directly from sputum, including their association with sputum bacillary load.

**Ethical statement**

This study used microbiological data and isolates from STREAM 1 clinical trial. The isolates tested to resolve discordant LPAsl/DST results were identified using the central laboratory accession number, without any patient identifiers. The Institutional Review Board of Institute of Tropical Medicine (ITM), Antwerp, was notified of these analyses. Full protocol review was not requested, in line with the low-risk nature of microbiological analyses.

**Materials and methods**
Study design and population

This study was a retrospective comparison of LPAs/ results obtained from baseline clinical samples (defined as participant’s sputum specimens collected before initiation of MDR-TB treatment) in seven STREAM 1 sites; Ethiopia, Mongolia, South Africa, and Vietnam from patients randomized between July 2012 and June 2015 with phenotypic DST (pDST) on baseline isolates.8,11

Microscopy

Sputum smear microscopy was conducted at each STREAM trial site following WHO standard protocol.17 The site TB laboratories currently participated in External Quality Assessment (EQA) programs ensuring the quality of AFB-smear microscopy results. Additionally, the reference laboratory at ITM, Antwerp, warranted the quality of the site results through periodical monitoring visits.

Drug susceptibility testing (DST)

Phenotypic drug susceptibility testing (pDST) of M. tuberculosis isolates received from three countries (Ethiopia, South Africa, and Vietnam) was performed at ITM, Antwerp, using proportion method on Middlebrook 7H11 agar at critical concentrations recommended by WHO, whereas pDST of M. tuberculosis isolates from Mongolian site was conducted in...
National Tuberculosis Reference Laboratory (NRL), Ulaanbaatar following WHO standard protocol for indirect proportion method on Löwenstein-Jensen for first and second-line drugs following [18].

**GenoType line probe assay (LPAs/I)**

The GenoType LPAs/I V1.0 was performed and interpreted according to the manufacturer instructions (Hain Lifescience, Nehren, Germany) for clinical samples [19]. The results were recorded inconclusive based on presence of overall weak bands or absence of WT and MUT bands, together with the absence of the loci control bands for one or more of the genes tested. The DNA extracted from the reference *M. tuberculosis* H37Rv strain and molecular-grade water were used as positive and negative controls for each run.

**DNA sequencing**

To resolve discordant results of FQ LPAs/I and pDST, extracted DNA was amplified using gyrA/B specific primers and sequenced by Sanger method as previously described [20]. Sequences were compared to that of H37Rv reference strain (NCBI, GenBank accession number NC_000962) using CLC Sequence Viewer software. For SLIs, next-generation sequencing (NGS) was used to resolve discordance between pDST and LPAs/I methods. Illumina NGS short sequencing was performed by TGen/C-Path platform in which the whole genome is analyzed for variants that
are known to SLI resistance-conferring genes. TB-profiler (version 2.6.0) was used to analyze raw fastq data.

Statistical analysis

Statistical analysis was performed using STATA version 15.0 (STATA Inc., USA). LPA results discordant with those of pDST were resolved by sequencing as the reference standard. As evidence of FQ or SLI resistance excluded patients from the trial, only a small fraction of the strains obtained at the screening visit that had LPA resistant results also had pDST done at the reference laboratories, only the predictive value of a susceptible, but not that of a resistant result in the population screened could be determined. Logistic regression was used for analysis of the association between inconclusive results and a low bacillary load. The Chi-square test was used to compare proportions.

Results

A total of 689 patients were screened from the seven sites, of whom 579 patients with both LPA and smear microscopy results were used to investigate the association between inconclusive LPA results and bacillary load. Only 413 patients with both LPA and pDST results available were also included for the assessment of LPA performance (Figure 1).
Of 413 patients, LPA\textsubscript{sl} identified 396 (95.9%) patients with FQ conclusive, and 17 (4.1%) inconclusive results (Table 1). LPAs\textsubscript{sl} also identified 377 (91.3%) SLI conclusive, and 36 (8.7%) with inconclusive results (Table 1).

Among all 579 patients, 503 (86.9%) were reported as FQ-susceptible (FQ-S), 26 (4.5%) FQ-resistant (FQ-R), and 50 (8.6%) as having inconclusive results. For SLI LPAs\textsubscript{sl} results, 478 (82.6%) were susceptible, 25 (4.3%) resistant and inconclusive for 76 (13.1%) patients. The inconclusive LPAs\textsubscript{sl} results were significantly more common in South Africa compared to other trial sites (p<0.001 and p<0.001 respectively) (Supplement Table 1).

**Phenotypic drug susceptibility testing (pDST) versus LPAs\textsubscript{sl} results and sequencing**

Of the total 413 pDST results (Figure 1 and Table 1), 403 (97.6%) and 406 (98.3%) \textit{M. tuberculosis} isolates were FQ-S and SLI-S, respectively.

For 396 patients with conclusive LPAs\textsubscript{sl} and pDST results for FQ, nine patients showed discordant results: 5 LPA-S/pDST-R and 4 LPA-R/pDST-S (Table 1). For 377 patients with conclusive results for SLI, 5 showed discordant results: 2 LPA-S/pDST-R and 3 LPA-R/pDST-S (Table 1). gyrA/B sequencing of the discordant results showed wildtype gyrA/B for all 4 LPA-R, whereas 2 wildtypes and 3 resistance-conferring mutations (2 Ala90Val, 1 Asp94Gly) were observed for the LPA-S. Together with the 3 concordant resistant cases, this brings the total FQ-
R to 6 (1.5%) (Supplement Table 2). At this very low prevalence, the negative predictive value (NPV) of LPAs/ for FQ resistance was very high, 99.2% (95%CI, 0.98-1.00; Table 2). For the strains with SLI discordance between LPAs/ and pDST, sequencing did not show SLI-R-conferring mutations. Hence, only two strains were finally classified as SLI resistant (0.5%; Supplement Table 2). The NPV for the exclusion of SLI-R by LPAs/ was thus 100% (95%CI: 0.99-1.00; Table 2).

As patients identified as FQ or SLI resistant by LPAs/ screening were excluded from the trial no pDST is available from strains for most of these patients. Hence, we could not evaluate false resistance in the screened population. Sensitivity, specificity, and PPV of the FQ or SLI resistant results could thus not be determined in this study.

Resistance missed by inconclusive MTBDRs/ LPA results

pDST identified 15 FQ-S and 2 FQ-R samples among the 17 with FQ-inconclusive LPAs/ results (Table 1). Sequence analysis could confirm only 1 gyrA Ala90Val resistance-conferring mutation among the 2 phenotypic FQ-R, all other samples being wildtype (Supplement Table 2). Thus, among the 17 inconclusive LPAs/, 1 FQ-R had not been identified by LPA. Considering the 26 (4.9%) FQ-R cases identified at the initial screening of 529 patients with conclusive results, the 1/17 (5.9%) confirmed by the reference standard from FQ inconclusive LPAs/ results is very similar.

The pDST identified 3 (8.3%) SLI-R patients among 36 with SLI-inconclusive LPAs/ results (Table 1). Only half (17) of those - and among them, only one pDST SLI-R - were available for sequencing. No SLI resistance-conferring mutations were detected for these 17 (Supplement
Due to insufficient confirmatory testing, it was not possible to compare the proportions of resistance for inconclusive versus conclusive SLI LPAs/ results.

**Association of inconclusive MTBDRs/ LPA results with bacillary load**

Additionally, we analyzed the association between the 579 LPAs/ results and sputum bacillary load as determined by smear microscopy (Table 3). Among 140 (24.2%) with low-bacillary load, 94 (16.3%) were smear-negative and 46 (7.9%) had scanty-positive smear microscopy results, while the 439 (75.8%) high-bacillary load comprised of 146 (25.2%) 1+, 118 (20.4%) 2+, and 175 (30.2%) 3+ smear-positive results. Higher proportion of patients with Inconclusive FQ LPAs/ and SLI LPAs/ had a lower bacillary load compared to those with conclusive results (p<0.001), (Table 3).
Rapid and accurate diagnostic tools to exclude pre-XDR and XDR-TB patients are essential to decide on the eligibility of patients for enrolment in some MDR clinical trials, and also for timely management of MDR-TB. In this study, we evaluate the performance of LPAs/ in such a context, the STREAM 1 trial. The agreement between LPAs/ and pDST to detect true susceptibility was excellent for both FQ and SLI. Although DNA sequencing showed most of the discordant results for LPAs/ FQ and/or SLI resistance were false, as a clinical trial screening tool, LPAs/ still did very well. Similar to results achieved in other settings,14,22, in STREAM, LPAs/ performance for the exclusion of resistance was very good, with a NPV over 99% for both FQ and SLI. In addition to minimizing testing delay, LPAs/ were almost 100% effective in identifying FQ- and/or SLI-resistant TB patients in trial settings with a low prevalence of resistance and thus a low pre-test probability such as Ethiopia and Vietnam (Supplementary Table 1). Others have also evaluated LPAs/ V2.0 and reported the specificity of the LPAs/ to be close to 99%23. Therefore provided that DNA sequencing is used to resolve discordances with pDST, in high-prevalence settings, the NPV should still be good enough in a trial setting; only five FQ-resistant cases would be missed when screening 1000 patients at 50% prevalence. Although the proportion of patients with SLI resistance missed among the inconclusive could not be determined in this study, these test failures may constitute a serious problem where SLI resistance prevalence is high, since they were not rare, and not as strongly associated with the low bacillary load.

In addition to overall prevalence, the distribution of specific FQ-R mutations might vary geographically24–26 partly explaining LPAs/ performance variation. In the study by Pantel et al.27
compensatory mutations that restore FQ susceptibility in *M. tuberculosis* strains have been also described in the screening of MDR-TB.

The assay's good performance in all STREAM 1 sites for exclusion of resistance to FQ and SLI is similar to that reported in a feasibility study describing the molecular assay for screening of patients in TB clinical trials except for some false LPAs/ results, particularly in South Africa. The WHO expert Group also noted that given high assay NPV for detecting resistance to FQ and SLI, the results of the LPAs/ could be used for screening, pending the results of pDST results while avoiding placing patients who have resistance to FQ and SLI on the regimen and start eligible patients on treatment.

Our analysis showed an overall rate of inconclusive LPA results of 8.6% for FQ and 13.1% for SLI, which is within the reported range from other studies. Although not fully understood, poor test execution or suboptimal amounts of DNA in low bacillary burden samples are obvious possible reasons. However, in principle, inconclusive results could also be associated with mutations or deletions in the locus control region, as well as the complete or partial deletion of a target gene. A significantly higher proportion of inconclusive LPAs/ was seen in patients from South Africa, where the majority had no or only scanty bacilli on sputum microscopy, possibly due to a higher proportion of patients enrolled with HIV co-infection. Previous studies have also documented more inconclusive results for smear-negative and lower bacillary load specimens and in those who are HIV positive. WHO recommends that direct use of sputum for the LPAs/ test is not suitable for smear-negative clinical specimens. Ongoing DR-TB trials increasingly enroll larger proportions of patients with low bacillary burdens, e.g. those
who test Xpert very low, AFB negative. As shown in our study, such samples are more likely to yield inconclusive LPA results, lowering LPA utility for rapid exclusion of baseline resistance.

In our study, the proportion of FQ-resistant organisms among LPAs/ inconclusive results did not appear to differ from that observed among conclusive results, but we could not arrive at any conclusion on this point for SLI because of insufficient confirmatory testing. At least for FQ, enrolment of patients with an inconclusive LPAs/ result can be fully justified at low FQ-resistance prevalence, and it would seem reasonable to believe this to be true for SLI.

This study has limitations. We could not review the strips for each LPAs/ performed in the sites, so we were not able to investigate banding profiles suggestive of heteroresistance or errors of reading at these laboratories as possible causes of the false results observed. Exclusion of FQ and SLI resistant LPAs/ samples from a trial further limits our ability to estimate the level of false resistance in the screened population and causes incapable of determining sensitivity and PPV of resistant results. Besides, we could only apply the reference standard, sequencing, to half the samples with inconclusive results for SLI, leaving some uncertainty regarding their importance and most suitable interpretation for clinical trial patient screening.

Conclusion

In this study, LPAs/ proved a good screening tool for rapid exclusion of resistance to FQ and SLI using a composite reference standard of pDST plus DNA sequencing to resolve discordant
results. Although the prevalence of both FQ and SLI resistance was low to moderate in our study populations, the high specificity suggests that also in high prevalence settings LPAs can facilitate screening for FQ and SLI resistance. Relatively frequent inconclusive results, particularly for SLI, may constitute a larger problem, especially when enrolling patients with AFB smear-negative disease, such as those diagnosed by Xpert. Inconclusive results may conceal a proportion of resistance proportional to the prevalence of FQ or SLI resistance in the test setting.

Competing interests

The authors stated that they have no conflict of interest.

Author Contributions

Conception and design of the study: Gabriela Torrea, Armand Van Deun, Andrew Nunn, Sarah Meredith, Saim Ahmed, Doljinsuren Dalai, Daniel Kokebu, Tesfamariam Mebrahtu, Ermias Diro, Nosipho Ngubane, Ronelle Moodliar, Francesca Conradie, Phan Thuong Dat, Bouke C. de Jong; Experiments/research work: Oyuntuya Tumenbayar, Bazarragchaa Tsogt, Mekonnen Teferi, Dang Thi Minh Ha, Pham Thu Hang, Lynette Duckworth, Elie Nduwamahoro, Jelle Keysers, Pim De Rijk, Wim Mulders; Sequence analysis: Mebrat Ejo, Jelle Keysers, Pim De Rijk, Wim Mulders; Analysis of the data: Mebrat Ejo, Gabriela Torrea, Armand Van Deun, Andrew Nunn, Saim Ahmed, Ermias Diro, Leen Rigouts, Bouke C. de Jong; Preparation of the
Acknowledgments

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References


Global Laboratory Initiative (GLI). Line probe assays for drug-resistant tuberculosis detection: Interpretation and reporting guide for laboratory staff and clinicians. Stop TB Partnership; 1–44.


Table 1. Cross-tabulation of LPAs/ versus pDST results

<table>
<thead>
<tr>
<th></th>
<th>Phenotypic DST</th>
<th>Phenotypic DST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FQ Susceptible (n=403)</td>
<td>FQ Resistant (n=10)</td>
</tr>
<tr>
<td>Conclusive</td>
<td>388 (98.0%)</td>
<td>8 (2.0%)</td>
</tr>
<tr>
<td></td>
<td>396</td>
<td></td>
</tr>
<tr>
<td>FQ LPAs/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>384 (98.7 %)</td>
<td>5 (1.3 %)</td>
</tr>
<tr>
<td></td>
<td>389</td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>4 (57.1%)</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td>15 (88.2%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>SLI Susceptible</td>
<td>373 (98.9%)</td>
<td>4 (1.1%)</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td></td>
</tr>
<tr>
<td>SLI LPAs/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>370 (99.5%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td></td>
<td>372</td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>3 (60.0%)</td>
<td>2 (40.0%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td>33 (91.7%)</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

FQ= fluoroquinolone; SLI = second-line injectable; LPAs/ = second-line drugs Line Probe Assay; pDST = phenotypic drug-susceptibility testing
Table 2. LPA sl effectiveness for exclusion of resistance

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Resistant</th>
<th>Susceptible</th>
<th>Totals</th>
<th>Negative Predictive Value % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolone</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>386</td>
<td>389</td>
<td>99.2 (0.98-1.00)</td>
</tr>
<tr>
<td>Second-line injectable</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>372</td>
<td>372</td>
<td>100 (0.99-1.00)</td>
</tr>
</tbody>
</table>

CI = Confidence Interval; LPA sl = second-line drugs Line Probe Assay; pDST = phenotypic drug-susceptibility testing; #DNA sequencing used as a reference standard to resolve discordant between pDST and LPA sl results.
<table>
<thead>
<tr>
<th>LPAs/ (N)</th>
<th>Direct smear-microscopy</th>
<th><strong>p-value</strong></th>
<th>[95%CI]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Low-bacillary load*</td>
<td>High-bacillary load#</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td>50</td>
<td>37 (74.0%)</td>
<td>13 (26.0%)</td>
</tr>
<tr>
<td>Conclusive</td>
<td>529</td>
<td>103 (19.5%)</td>
<td>426 (80.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
<td>140 (24.2%)</td>
<td>439 (75.8%)</td>
</tr>
<tr>
<td>Second-line injectable</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td>76</td>
<td>46 (60.5%)</td>
<td>30 (39.5%)</td>
</tr>
<tr>
<td>Conclusive</td>
<td>503</td>
<td>94 (18.7%)</td>
<td>409 (81.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
<td>140 (24.2%)</td>
<td>439 (75.8%)</td>
</tr>
</tbody>
</table>

FQ= fluoroquinolone; SLI= second-line injectable; LPAsl= second-line drug Line Probe Assay

*Low-bacillary load included negative and scanty smear microscopy results

#High-bacillary load included 1+, 2+, and 3+ smear microscopy results

**Univariable logistic regression analysis (LPAsl result versus Smear-microscopy result)
Supplement Table 1. Analysis of FQ- and SLI-LPA sl results by country (STREAM stage 1 sites).

<table>
<thead>
<tr>
<th>STREAM sites</th>
<th>FQ LPAs/</th>
<th><strong>p-value</strong></th>
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<tbody>
<tr>
<td></td>
<td>Conclusive</td>
<td><strong>p-value</strong></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>159</td>
<td>147 (92.5%)</td>
</tr>
<tr>
<td>Mongolia</td>
<td>40</td>
<td>38 (95%)</td>
</tr>
<tr>
<td>South Africa</td>
<td>278</td>
<td>216 (77.7%)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>102</td>
<td>102 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
<td>503 (86.9%)</td>
</tr>
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<table>
<thead>
<tr>
<th>SLI LPAs/</th>
<th><strong>p-value</strong></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Conclusive</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
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<td>Ethiopia</td>
<td>159</td>
</tr>
<tr>
<td>Mongolia</td>
<td>40</td>
</tr>
<tr>
<td>South Africa</td>
<td>278</td>
</tr>
<tr>
<td>Vietnam</td>
<td>102</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
</tr>
</tbody>
</table>

FQ = Fluoroquinolones; SLI = second-line injectable; LPAsl = second-line drug Line Probe Assay
**Univariable logistic regression analysis (LPAsl result versus STREAM site (Country, factor variable))**
Supplement Table 2. Final results applying the reference standard comparing conclusive and inconclusive LPAs/l results

<table>
<thead>
<tr>
<th>LPAs/l</th>
<th>Fluoroquinolones</th>
<th>Reference standard (Sequencing)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Conclusive</td>
<td>6 (1.5%)</td>
<td>390 (98.5%)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>1 (5.9%)</td>
<td>16 (94.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (1.7%)</td>
<td>406 (98.3%)</td>
</tr>
<tr>
<td></td>
<td>Second-line injectables¹</td>
<td>1.000</td>
</tr>
<tr>
<td>Conclusive</td>
<td>2 (0.5%)</td>
<td>375 (99.5%)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>0 (0%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (0.5%)</td>
<td>392 (99.5%)</td>
</tr>
</tbody>
</table>

LPAs/l = second-line drugs Line Probe Assay; pDST = phenotypic drug-susceptibility testing; 19/36 SLI inconclusive were not sequenced of which 18-S and 1-R by pDST

**Chi-squared test (Comparison between the reference standard (sequencing) and LPAs/l results)**
Figure 1. Flow diagram displaying patients and samples included in the study
689 MDR-TB patients screened
(Ethiopia (n=191), Mongolia (n=47),
South Africa (n=349), Vietnam (n=102))

579 patients with LPAs/ and smear microscopy results among 689 screened
- 503 FQ-S, 26 FQ-R, and 50 inconclusive
- 478 SLI-S, 25 SLI-R, and 76 inconclusive
- 485 smear-positive (46 scanty, 146 1+, 118 2+, 175 3+), and 94 smear-negative

166 without pDST result were excluded
- 155 not enrolled
  - 19 resistant to FQ and/or SLI
- 11 enrolled but no pDST result
  - 1 resistant to SLI

413 patients with LPAs/ and pDST results

Fluoroquinolone (FQ) LPAs/
- 396 conclusive (7 FQ-R)
  - 387 pDST concordant
  - 9 pDST discordant
- 17 inconclusive

Second-line injectable (SLI) LPAs/
- 377 conclusive (5 SLI-R)
  - 372 pDST concordant
  - 5 pDST discordant
- 36 inconclusive