**ORIGINAL ARTICLE**

# Detection of Mutations Associated with Resistance to Second-line Drugs in Isolates of *Mycobacterium tuberculosis*

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## ABSTRACT

***Background:*** *Mycobacterium tuberculosis (M. tuberculosis) develops resistance toward second-line anti-tuberculous drugs, mostly through mutations of the chromosome. The mechanism of resistance is complicated and accompanies several genes as rrs and eis promoter mutations.* ***Objective:*** *To assess the validity of the Real-time PCR (Rt-PCR) in recognizing the mutations of the resistance to second-line injectable agents (capreomycin, amikacin and kanamycin) in multidrug resistant tuberculosis (MDR-TB) clinical isolates in comparison with the gold standard proportion method (PM) using Lowenstein–Jensen (LJ) media.* ***Methodology:*** *This study was conducted on 48 MDR-TB clinical isolates (from sputum and bronchioalveolar lavage samples) obtained from the Egyptian National Central Laboratory of the Ministry of Health, Egypt. The isolates obtained were resistant to a minimum one of the second-line anti-tuberculous drugs (ofloxacin, capreomycin, amikacin, and kanamycin) identified by PM using LJ media. Isolates were tested by Rt-PCR to track mutations in rrs and the eis promoter.* ***Results:*** *Thirty-eight (38) isolates were positive for rrs, and 32 isolates for the promoter of eis using Rt-PCR. Comparing the results to the gold standard PM, an agreement of 100%, and 69% were found to rrs, and the eis promoter, respectively.* ***Conclusion:*** *Using the Real-time PCR for recognizing mutations related to second-line anti-tuberculous drugs highly agrees with the PM. This could help in MDR-TB early detection and screening for extensively drug-resistant TB (XDR-TB) strains.*

## INTRODUCTION

Before the emergence of the coronavirus (COVID-19) pandemic, Tuberculosis (TB) was the most prominent cause for death from a single infectious agent, with a rate violating those of HIV/AIDS. According to the World Health Organization (WHO), 1.3 million HIV-negative persons died from TB in 2021. These increased TB deaths is due to the significant decrease in TB case detection and documentation throughout 2020, which possibly reveals reduced access to TB diagnosis and treatment services. In geographic context, most 2020 TB cases were reported from the WHO regions of South-East Asia (43%), Africa (25%) and the Western Pacific (18%). Additionally, fewer cases were documented from Eastern Mediterranean (8.3%), Americas (3.0%) and Europe (2.3%) [1].

It was believed that TB could be eliminated by the culmination of the 20th century [2]. Nevertheless, by the mid-1990s, MDR-TB (resistant to both rifampicin and isoniazid) appeared in most countries. In 2006, extensively drug-resistant TB (XDR-TB) emerged (resistant to fluoroquinolone, and to one, at minimum, of the three second-line drugs (SLDs) (capreomycin (CM), kanamycin (KM), and amikacin (AMK) besides the MDR) [3].

The injectable SLDs comprise a cyclic-peptide antibiotic, CM, and two aminoglycoside antibiotics, KM and AMK. The three drugs influence protein translation, share a molecular target, and bind at comparable positions. Consequently, cross-resistance has often been recognized [4]. The SLDs resistance occur mainly through chromosome mutations, which and is more complex and accompanied with numerous genes compared to those of the first-line anti-tuberculous drugs. The mutation of *rrs* is accompanied with CM, KM, and AMK cross-resistance. Mutations in the *eis* promoter are linked to less KM resistance level [5].

The proportion method (PM) is the gold standard classical conventional methods for drug susceptibility testing (DST) of *M. tuberculosis* but has its limitations, mainly the long turnaround time with duration of six to eight weeks required before obtaining the results [6]. Many methods were introduced to provide quick detect of the anti-tuberculous drugs resistance [7]. One of the rapid DST that are applicable in developing countries is Real-time PCR (Rt-PCR) [8]. Rt-PCR is simple, easy to perform, and reduces the analysis time to three or four days, helping in early detection of MDR-TB and screening for XDR-TB strains [9]. Recognizing drug resistance rabidly make early treatment in the course of

the disease possible and consequently help to eliminate morbidity, infectiousness, and mortality [1].

The aim of the present research is to assess the Rt-PCR validity for detecting mutations related to the resistance against injectable SLDs (CM, KM, and AMK) in *M. tuberculosis* clinical isolates in comparison to PM using LJ media.

## METHODOLOGY

The study was conducted on 48 MDR-TB clinical isolates (from sputum and bronchioalveolar lavage samples) identified in the Egyptian Ministry of Health's National Central Laboratory, by PM on L-J medium and showed resistance to one of the SLDs (ofloxacin (OFX), CM, KM, and AMK) at least. The drugs and their concentrations in the L-J medium were as follow: isoniazid (0.2 $\mu$g/ml), rifampicin (40 $\mu$g/ml), streptomycin (4 $\mu$g/ml), ethambutol (2 $\mu$g/ml), OFX (2 $\mu$g/ml), capreomycin (40 $\mu$g/ml), kanamycin (30 $\mu$g/ml), and amikacin (20 $\mu$g/ml). Results were read after 28 days of incubation and the reference *M. tuberculosis* H37Rv strain was employed as a control. A strain considered resistant toward the antimicrobial agent once the rate of growing surpassed 1% relative to the control [10]. The results were further confirmed in Supranational TB laboratory; The Tropical Institute of Medicine, Antwerp, Belgium. The isolates were obtained during the period from July 2015 to December 2016. The present work has been accomplished after the approval of Research Ethics Committee, Ain Shams University, Faculty of Medicine (FWA 000017585) (FMASU MD 90/2015) and agrees with The Ethics Code of the World Medical Association (Declaration of Helsinki) for experiments in humans.

**Real -Time PCR:**

The 48 resistant isolates were tested by Rt- PCR to identify mutations exhibited by *rrs* and *eis* promoter. Contextually, two double-labeled probes as well as two pairs of primers were developed (Table 1) [11]. Asymmetrical PCR was accomplished for each target. This was achieved by changing the concentrations of one of the primers to be 20 fold higher in concentration than the other primer. The aTaq DNA polymerase (Promega, USA) without 5′ to 3′ exonuclease activity was adopted to prevent the probes hydrolysis during amplification. The DNA was acquired by QIAMP DNA Mini Kit with modification (Qiagen, Germany Cat. No.51504). Reaction mixture was assembled for a final volume of 25 µL as follow: 12.5 µL of aTaq DNA polymerase Master Mix, 2.5 µL forward Primer, 2.5 µL reverse Primer, 1 ul of corresponding probe and 1.5 DNase free water and 5 ul of extracted DNA. Amplification was done by Step One™ Real-Time PCR Systems (Applied Biosystems, USA) with preliminary denaturation step at 95°C for 1 minute. This was then followed by 40 denaturation cycles for 20 seconds at 95°C, annealing for 30 s at 55°C, and extension for 30 s at 72°C.

**Table 1:** Sequence of Primers and Probes [11].

| Primers & probes | Description | Target | Nucleotide Sequence | Gene location | Length (bp) |
|---|---|---|---|---|---|
| ***eis*-F** | Forward primer | *eis* promotor | 5′ CGT GAT CCT TTG CCA GAC ACT 3′ | -55 to -75 | 21 |
| ***eis*-R** | Reverse primer | *eis* promotor | 5′ TCGG TCG GGC TAC ACA GGG TCA 3′ | 8-29 | 22 |
| ***eis*P** | Probe | *eis* promotor | FAM-5′ ATC CGA CTG TGG CAT ACG TGC ACG TG-BHQ1-3′ | -4 to-18 & -30 to -40 | 26 |
| ***rrs*-F** | Forward primer | *rrs* | 5′ CGC GAG GTT AAG CGA ATC C 3′ | 1257-1275 | 19 |
| ***rrs*-R** | Reverse primer | *rrs* | 5′ GGT ACG GCT ACC TTG TTA CGA CTT 3′ | 1485-1508 | 24 |
| ***rrs*P** | Probe | *rrs* | FAM-5′ TGT TAC CGA CTT TCA TGA CGT GAC GGG-BHQ1-3′ | 1396-1421 | 26 |

A negative control with double-distilled water and a standard wild-type control with DNA of H37Rv without mutations and susceptible to the entire drugs had been accomplished for each Rt-PCR experiment. Reference strain *M. tuberculosis* H37Rv was offered by the Egyptian Ministry of Health's National Central Laboratory.

**Melting curve analysis:**

Once PCR amplification has been finished, melting curves were achieved through the preliminary holding step at 95°C for 30 s. Afterwards, persistent detection of fluorescence from 45°C to 85°C, with temperature increasing level of 0.5°C per step, was done. The data derived from the melting curve were analyzed and standardized, where the temperature shifts were further analyzed by Step One™ Real-Time PCR Systems. The software distinguishes the variance in the melting curve pattern of the investigated sample from the melting curve pattern of the control strain H37Rv to identify sequence variants and produces a difference plot curve.

This aid to allocate clustered samples with alike melting curves in groups so sequence polymorphisms can be distinguished.

**Statistical analysis:**

Diagnostic and agreement statistics (DAG) program was utilized in data analysis [12]. Data were presented in two formats, number and percentage of categorized data. Diagnostic validity test involves; the diagnostic sensitivity {proportion of truly diagnosed resistant cases (True positive; TP) amongst the entire resistant cases (TP + False negative; FN)}, the diagnostic specificity {proportion of susceptible cases truly omitted by the test (True negative; TN) amongst the entire susceptible cases (TN + False positive; FP)}, positive predictive value (proportion of TP within the whole TP and FP), and negative predictive value (proportion of TN amongst TN and FN).

## RESULTS

Results of Rt-PCR for the 48 MDR-TB isolates displayed that 38 isolates (79.2%) were resistant to AMK and CM, and 32 isolates (66.6%) were resistant to KM. Validity of Rt-PCR to detect the resistance toward the tested four SLDs is presented in *table 2*. *Table 3* shows perfect agreement of Rt-PCR test with the PM for AMK and CM (100%), and substantial agreement for KM (69%).

**Table 2:** Validity of real-time PCR for detection of resistance to the tested second line anti-tuberculous

| | | | **Susceptibility by PM** | | |
|---|---|---|---|---|---|
| | | | Resistant | Sensitive | Total |
| **Susceptibility by Rt-PCR** | **Amikacin (AMK)** | Resistant | 38 | 0 | 38 |
| | | Sensitive | 0 | 10 | 10 |
| | | **Total** | **38** | **10** | **48** |
| | **Capreomycin (CM)** | Resistant | 38 | 0 | 38 |
| | | Sensitive | 0 | 10 | 10 |
| | | **Total** | **38** | **10** | **48** |
| | **Kanamycin (KM)** | Resistant | 32 | 0 | 32 |
| | | Sensitive | 6 | 10 | 16 |
| | | **Total** | **38** | **10** | **48** |

**Table 3:** Performance characteristics of Rt-PCR assay for the tested second line anti-tuberculous

| Drug | Agreement | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|
| **AMK** | 100% | 100% | 100% | 100% | 100% |
| **CM** | 100% | 100% | 100% | 100% | 100% |
| **KM** | 69% | 84.2% | 100% | 100% | 62.5% |

PPV: positive predictive value, NPV: negative predictive value

Melting curve analysis showed that in the resistant strains the melting temperature shifted from 1 to 7.5 degrees less than the sensitive control strain. Melting temperature for sensitive control strain H37RV was 66.5°C for *rrs* (Figure 1), and 63.5°C for the promoter of *eis* (Figure 2). Shift in the melting temperature between the wild and the mutated *rrs* gene was from 66.5°C to 61°C (Figure 1). Shift in the melting temperature between the wild and the mutated DNA in the promoter of *eis* was from 63.5°C to 57°C (Figure 2).

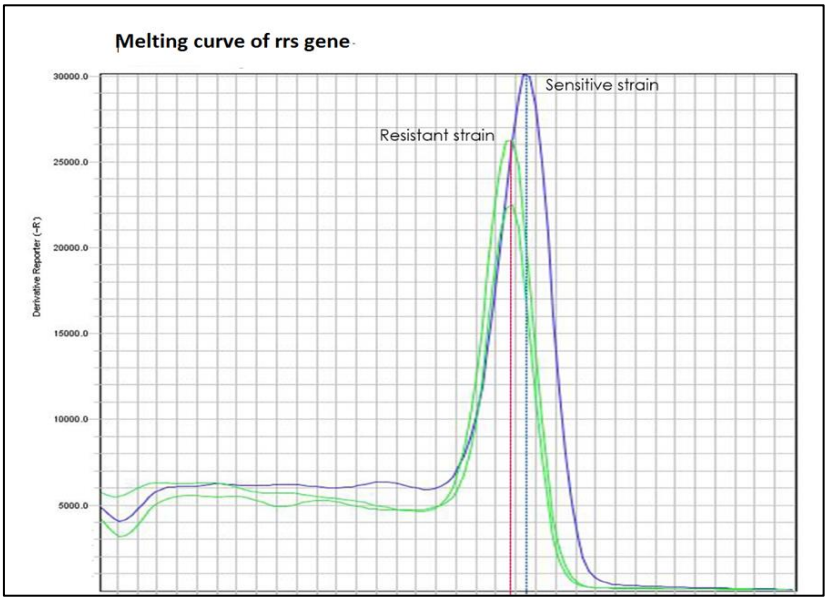


**Figure 1:** Detection of mutation in *rrs* gene through melting curve analysis.
Shift in the melting temperature between the wild and the mutated DNA from 66.5°C to 61°C.

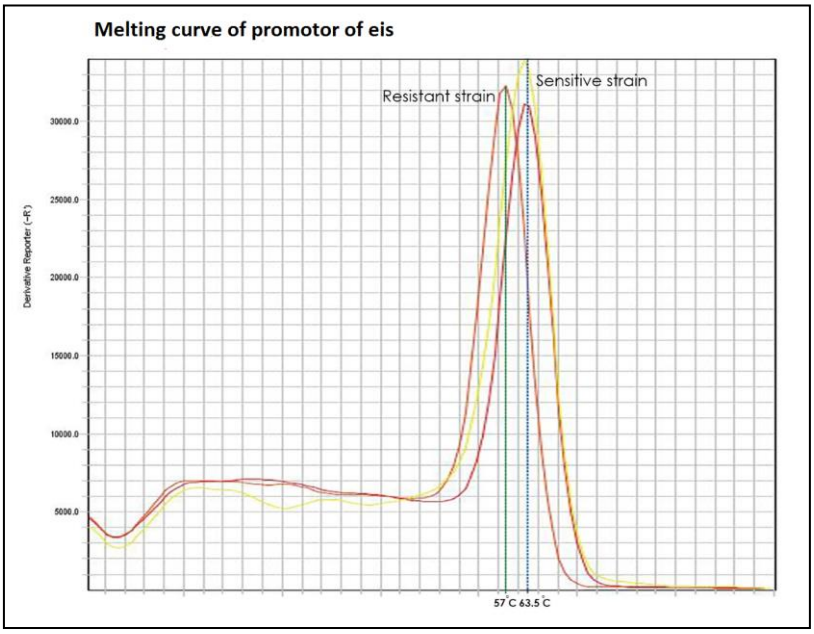


**Figure 2:** Detection of mutation in the promoter of *eis* through melting curve analysis.
Shift in the melting temperature between the wild and the mutated DNA from 63.5°C to 57°C.

## DISCUSSION

Since 1998 up to 2015, the idea of a high burden country (HBC) was well-known and extensively adopted within the TB context. In 2015, WHO declared three HBC categories (TB, MDR-TB, and HIV-associated TB), and fortunately Egypt was not on these categories. There was an intersection within these three categories, in which 14 countries were represented in all of them and accounting for about 63% of the appraised worldwide number of incident cases of TB in 2019. These countries are China, Angola, the Democratic Republic of the Congo, India, Ethiopia, Kenya, Indonesia, Mozambique, Nigeria, Myanmar, Papua New Guinea, Thailand, South Africa, and Zimbabwe [13].

In our study, the MDR-TB isolates showed high resistance capacity toward the tested SLDs by PM, as 38 out of 48 isolates (79.2%) were resistant to CM, KM, and AMK. Results of a study conducted in Viet Nam on 1312 cases showed similar patterns of resistance, as all

TB cases that resist KM were resistant to CM as well [14]. *Yon and his colleagues* found lower rates of resistance among the 217 isolates tested in Shanghai, as 17.1% (37) of the cases showed resistance to AMK / KM, and 20.3% (44) resist the CM [15]. Considering the study done in the European Economic Area and European Union on 1310 cases, a comparable resistance to KM (846; 64.6%) were reported, while 536 (40.9%) were resistant to AMK, 491(37.5%) were resistant to CM of MDR-TB cases [16]. High rates of resistance might prove the weakness of the administration of these drugs in health services, where some SLDs, are broadly prescribed for treating respiratory infections and other bacterial infections. Furthermore, these drugs are, often accessible with no prescription in the local drug stores[17].

One of the important anti-tuberculous SLDs is KM inhibiting protein synthesis by 16S rRNA, and its resistance can be diagnosed by *rrs* gene mutation detection and/or the *eis* promoter which detects lowered resistance to KM [18, 19]. In the same context, *Liu and his colleagues* [11] found that the prominent *rrs* mutation was identified in 78.6% (11 out of 14) of the isolates that exhibited cross resistance to CM, AMK and KM, while 7 out of 17 KM mono-resistant isolates displayed mutations in the *eis* promoter. In another study, amongst all 156 MDR-TB isolates investigated, 26 (16.6%) isolates exhibited resistant to KM, and 19 (73.1%) of their mutations occurred within the *rrs* and only 4 (15.4%) occurred within the *eis* region [20]. Our study supports these results, where 32 out of 48 isolates (66.7%) were mono-resistant to KM and carried mutations in the *eis* promoter suggesting that the remaining 6 isolates of this study diagnosed by PM, which didn't show mutations in the *eis* promoter, may attribute resistance through mutations in the *rrs.* This postulated that the sequencing of *rrs* and *eis* region might contribute as a screening process for KM resistance before the DST results. Combining recognition of the two regions could enhance the sensitivity and precision of the drug resistance to KM.

An exciting output of this study is that validity of Rt-PCR compared to PM showed a perfect agreement (100%) for AMK and CM between and substantial agreement (69%) for KM. These results were relatively concordant to another study conducted in India on 90 *M. tuberculosis* clinical isolates to correlate *rrs* and *eis* promoter mutations with phenotypic susceptibility levels toward the injectables of the second-line using GenoType MTBDR*sl* assay (Hain LifeScience GmbH, Germany), the agreement of results was 97% for AMK, 96% for KM and 86% for CM [21]. Our results exceeded the results of another Line Probe Assay (LPA) currently proposed by WHO for the early drug resistance screening of sputum smear-positive samples; GenoType MTBDR*plus* VER2.0, which displays inconstant sensitivity and specificity for the screening of resistance to injectable SLDs [22, 23]. The overall specificity ranging from 59 to 100% and sensitivity from 83 to 87% for identifying XDR isolates [24, 25]. The performance characteristics of Rt-PCR assay for the tested SLDs of this study showed 100% specificity for CM, KM, and AMK, 100% sensitivity for CM and AMK, and 84.2% sensitivity for KM.

Our results were also in agreement with tests' results of DNA microarray-based techniques, which exhibit the maximum capability of various target detection with thousands of sequences involved in one reaction. As these tests demonstrate high sensitivity and specificity for detecting mutations related to the anti-TB drug resistance within clinical isolates (95.7% and 90.2% respectively) [26, 27].

Our outcomes depend upon a pair of strengths. In the first place, isolates were obtained from the National Central Laboratory, Ministry of Health, Egypt with high competency and skilled personnel and whose results are confirmed by the Supranational TB laboratory; The Tropical Institute of Medicine, Antwerp, Belgium, which guaranteed the validity of the presented drug resistance results. Our study results depend upon Rt-PCR with lowered contamination risk, where all steps following the DNA extraction are accomplished in a single tube.

However, one pitfall of this study is the shortage in studying all types of mutations related to SLDs resistance (such as *gidB* and *tlyA*) due to restricted resources. Another limitation was the inability of application of duplex Rt-PCR for detection of the two mutations simultaneously due to limited access to high technology device for performing this technique at the time.

## CONCLUSIONS

In conclusion, this study showed that the use of Real-time PCR for detecting mutations related to SLDs consistently agrees with the gold standard PM. Besides providing quick DST beyond first-line anti-TB drugs, this SLDs resistance molecular testing additionally affords data that can improve drug resistance investigation with the lack of drug resistance surveys. This would act as a guide for the TB surveillance, and control efforts from the disease control courses.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

**Author contributions**

All authors significantly contributed in the conceptualization and design of the study. **FYF:** collected the samples. **FYF,** and **NNS**: did the experiments. **FYF, MFA & NNS:** performed the statistical analysis. **NNS:** wrote the first draft of the manuscript. **MFA** and **AMK:** provided critical suggestions on study design and manuscript writing. All authors have made contributions in revising the manuscript and approved the final version. All authors provided final approval of the version to be published.

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## REFERENCES

1. World Health Organization. Global tuberculosis report 2021. Geneva, Switzerland: World Health Organization, 2021.
2. Centers for Disease Control and Prevention. Treatment of tuberculosis. American Thoracic Society, CDC, and Infectious Diseases Society of America. Morbidity and Mortality Weekly Report 2003; 52 (No RR-11).
3. World Health Organization. Anti-tuberculosis drug resistance in the world. Fourth global report. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. WHO/HTM/TB/2008.394. Geneva, Switzerland: World Health Organization, 2008.
4. Centers for Disease Control and Prevention. Model performance evaluation program, *Mycobacterium tuberculosis* complex drug susceptibility program report, November survey, 2014.
5. Gikalo MB, Nosova EY, Krylova LY and Moroz AM. The role of *eis* mutations in the development of kanamycin resistance in *Mycobacterium tuberculosis* isolates from the Moscow region. J Antimicrob Chemother 2012; 67: 2107–9.
6. Dubrous P, Alaoui H, N'Dounga Mikolo B and Koeck JL. Diagnosis of tuberculosis in developing countries: new perspectives. Med. Trop 2009; 69: 618-628.
7. Zhao Y, Xu S, Wang L, Zhang H, Wang S and Zhao B. National survey of drug-resistant tuberculosis in China. N Engl J J Med Dis 2012; 366: 2161–70.
8. Hershkovitz I, Donoghue HD, Minnikin DE, May H, Lee OY, Feldman M, et al. Tuberculosis origin: the Neolithic scenario. Tuberculosis 2015; 95 Suppl 1:S122–6.
9. Zhong QL, Mingxin Z, Hui Z and Xinxin L. Utility of Real-Time Quantitative Polymerase Chain Reaction in Detecting Mycobacterium tuberculosis. BioMed Research International 2017; 366: 2161–70.
10. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull. World Health Organ 1969; 41: 21–43.
11. Liu Q, Luo T, Li J, Mei J, Li J and Gao Q. Triplex real-time PCR melting curve analysis for detecting Mycobacterium tuberculosis mutations associated with resistance to second-line drugs in a single reaction. J Antimicrob Chemother 2013; 68: 1097–1103.
12. Diagnostic and agreement statistics (DAG). Available at: www.mhri.edu.au/biostats/DAG-Stat
13. World Health Organization. Global tuberculosis report 2020. Geneva, Switzerland: World Health Organization, 2020.
14. Hoa BN, Nhung V, Nguyen AC, Huong TG, Hai VN and Quyen TTB. Prevalence of resistance to second-line tuberculosis drug among multidrugresistant tuberculosis patients in Viet Nam. Antimicrob Agents Chemother 2016; 383: 1572–1579.
15. Yon GC, Zhengan Y, Xin S, Jie W, Zheyuan W and Biao X. Resistance to Second-Line Antituberculosis Drugs and Delay in Drug Susceptibility Testing among Multidrug-Resistant Tuberculosis Patients in Shanghai. BioMed Research International 2016; 3(3):220–34.
16. Van der Wer MJ, Ködmön C, Hollo V, Sandgren A, Zucs. Drug resistance among Tuberculosis cases in European union and European Economic Area. Eur Respir J 2007;16(2):364-71.
17. Centers for Disease Control and Prevention. Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. MMRW 2009; 58(01): 7-10.
18. Maus CE, Plikaytis BB, and Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy 2005; 49(8): 3192–3197.
19. Zaunbrecher MA, Sikes Jr. RD, Metchock B, Shinnick TM, and PoseyJE. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase *eis* confers kanamycin resistance in Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America 2009; 106(47): 20004–20009.

20. Hu Y, Xu L, He YL, Pang Y, Lu N, Liu J, Shen J, Zhu DM, Feng X, Wang YW, and Yang C. Prevalence and Molecular Characterization of Second-Line Drugs Resistance among Multidrug-Resistant *Mycobacterium tuberculosis* Isolates in Southwest of China. BioMed Research International; 2017: Article ID 4563826.
21. Kambli P, Ajbani K, Nikam C, Sadani M, Shetty A, Udwadia Z, et al. Correlating *rrs* and *eis* promoter mutations in clinical isolates of Mycobacterium tuberculosis with phenotypic susceptibility levels to the second-line injectables. Int J Mycobacteriol 2016; 5:1–6.
22. Brossier F, Guindo D, Pham A, Reibel F, Sougakoff W, Veziris N, et al. Performance of the new version (v2.0) of the GenoType MTBDR sl test for detection of resistance to second-line drugs in multidrug-resistant Mycobacterium tuberculosis complex strains. J. Clin. Microbiol.2016; 54: 1573–1580.
23. Mujuni D, Kasemire DL, Ibanda I, et al. Molecular characterisation of second-line drug resistance among drug resistant tuberculosis patients tested in Uganda: a two and a half-year's review. BMC Infect. Dis.2022; 22: 363.
24. Tomasicchio M, Theron G, Pietersen E, Streicher E, Stanley-Josephs D, van Helden P, et al. The diagnostic accuracy of the MTBDRplus and MTBDRsl assays for drug-resistant TB detection when performed on sputum and culture isolates. Sci. Rep.2016; 6:17850.
25. Meaza A, Kebede A, Yaregal Z, Dagne Z, Moga S, Yenew B, et al. Evaluation of genotype MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in smear positive and negative sputum samples. BMC Infect. Dis. 2017; 17:280.
26. Yao C, Zhu T, Li Y, Zhang L, Zhang B, Huang J, et al. Detection of *rpoB, katG* and *inhA* gene mutations in Mycobacterium tuberculosis clinical isolates from Chongqing as determined by microarray. Clin. Microbiol. Infect.2010; 16: 1639–1643.
27. Zimenkov DV, Kulagina EV, Antonova OV, Zhuravlev VY, Gryadunov DA. Simultaneous drug resistance detection and genotyping of Mycobacterium tuberculosis using a low-density hydrogel microarray. J. Antimicrob. Chemother. 2016; 71:1520–1531.