Evaluation of TBMDR® and XDRA® for the detection of multidrug resistant and pre-extensively drug resistant tuberculosis

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1. Introduction

Drug-resistant tuberculosis (TB) is still remaining a worrisome public health problem even though the overall TB incidence has been decreased. In 2019, an estimated 10 million people developed TB and 1.4 million people died. Among the newly developed TB cases, almost half million were rifampicin-resistant TB (RR-TB), of which 78% were multidrug-resistant TB (MDR-TB). Considering the large gap between notified 7.1 million and estimated 10 million new cases in 2019, drug resistance can be more serious than reported. Another gap (38%) between estimated MDR/RR-TB and enrolled in treatment might support such assumption [1].

To minimize such gaps, it is essential to expend TB diagnosis and drug resistance detection in various extents. Recently, the World Health Organization (WHO) recommended to use oral drugs more extensively [2,3] and revised the definition of extensively drug-resistant TB (XDR-TB). The updated XDR-TB is defined as infection with an MDR-TB strain that is also resistant to any fluoroquinolone (FQ) and at least one additional Group A drug and pre-XDR-TB is MDR/RR-TB that is also resistant to any FQ [4]. Thus second-line injectable drugs (SLID) were no longer the part of XDR-TB nor widely recommended. However, it will take times to change all previous injectable drug containing regimens to all oral drug regimens including newly introduced TB drugs. Thus, the use of currently available drug resistant detection methods must be continued and development of new improved methods is still needed. Culture-based phenotypic drug susceptibility testing (DST) remains as a gold standard for drug resistance determination to detect MDR-TB and XDR-TB although it is labor-intensive and time-consuming [5]. Currently used molecular-based DSTs such as Xpert MTB/RIF assay and line probe genotypic assays (LPAs) which are designed for rapid detection of specific drug-resistance conferring mutations in Mycobacterium tuberculosis (MTB), have some limitations in each test. Xpert MTB/RIF assay is a cartridge based nucleic acid amplification test which detects TB and RR-TB rapidly but it has limitation for ruling out rifampicin (RIF) sensitive polyresistant TB. The World Health Organization (WHO) endorsed LPAs for rapid molecular detection of MDR-TB, FQ and SLID resistance but there is requirement of laboratory infrastructure and trained persons to perform the tests [6–9].

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https://doi.org/10.1016/j.jctube.2022.100303

Available online 9 February 2022
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Recently two new assays (TBMDR® and XDRA®), which are rapid and affordable in-vitro diagnostics solutions using Real-Time PCR platform, were designed for rapid simultaneous detection of Rif/isoniazid (INH) resistance and FQ/SLID resistance. These assays are based on fully automated system which minimize all manual handling processes such as pipetting to reduce cross-contamination for better results. Total running time of the assays is short as it takes about three hours. In this study, these two new assays were evaluated to access their diagnostic performance to detect MDR-TB and pre-XDR-TB.

2. Methods

2.1. Clinical isolates

Clinical Mycobacterium tuberculosis (MTB) isolates cultured from sputum samples of active pulmonary TB patients who enrolled in a prospective observational cohort study (ClinicalTrials.gov identification number, NCT00341601) at International Tuberculosis Research Center (ITRC) in South Korea during the study period 2005–2018 were used for the present study. The study was reviewed and approved by the NMMH ethics review board. A total of 234 phenotypically well-characterized MTB isolates comprising XDR (as previous definition), FQ/SLID resistant MDR, MDR, mono-resistant to any drugs, and pan-susceptible based on previous phenotypic DST were selected. These isolates were subcultured on the Lowenstein-Jensen (LJ) egg slants and incubated at 37 °C for 4 weeks [10].

2.2. Phenotypic drug susceptibility test (DST)

The standard protocol for DST in MGIT 960 was strictly followed as recommended for isoniazid (INH) (0.1ug/ml), rifampicin (RIF) (1ug/ml), ofloxacin (OFX) (2.0ug/ml), moxifloxacin (MOX) (0.25ug/ml), kanamycin (KM) (2.5ug/ml), and capreomycin (CAP) (2.5ug/ml). To each 7 ml MGIT tube, 0.8 ml of MGIT 960 Growth Supplement and 0.1 ml of the drug stock solutions were aseptically added, and finally 0.5 ml of the test inoculum was added. For each isolate, a growth control (GC) tube with Growth Supplement and without drug was included. For this GC, the inoculum was prepared by pipetting 0.1 ml of the test inoculum with 10 ml of sterile water to make a 1:100 dilution; 0.5 ml of GC inoculum was added to a drug-free MGIT. All of the inoculated tubes were placed into the BACTEC MGIT 960 instrument on the same day of inoculation. The relative growth ratio between the drug-containing tube and drug-free GC tube was determined by the system’s software algorithm. If the relative growth in the drug-containing tube was equal to or exceeded that of the GC tube, the isolate was considered drug resistant; if the relative growth was less than in the GC tube, the isolate was considered drug susceptible. The instrument did the final interpretation and reported the susceptibility results automatically [11].

2.3. AccuPower® TB & MDR Real-Time PCR (TBMDR) and AccuPower® XDR-TB Real-Time PCR Kit-A (XDRA) assays

Preparation of kit materials and specimens, assay protocol, and data analysis were carried out according to the ExiStation™ system User Guide (Bioneer, Daejeon, South Korea). The process included two steps: i) DNA extraction by Exiprep™ 16Dx ii) real-time PCR reaction, and data analysis by Exicycler™. After preparing samples, DNA is extracted using Exiprep™ 16Dx (A-5050) instrument using the Exiprep™ Dx Mycobacteria genomic DNA Kit (K-4418). After the kit is installed in the instrument, DNA extraction proceeds automatically, and DNA is dispensed into the PCR reaction tube. After that, the PCR tube is taken out from the instrument, goes through the vortexing, spins down, and then installed in the Exicycler™ Real-Time Quantitative Thermal Block (A-2060-1) perform Real-Time PCR. After PCR is finished, Existation S/W automatically analyzes the results. The running time is approximately 1 h and 15 min for DNA extraction and 1 h and 40 min for Real-Time PCR, totaling 3 h. This workflow includes all processes from sample preparation through the actual extraction process using the Exiprep™ 16Dx, which automatically deposit the extracted genomic DNA into the Elution buffer cartridge.

Target gene region of AccuPower® TB & MDR Real-Time PCR (TBMDR) and AccuPower® XDR-TB Real-Time PCR Kit-A (XDRA) assays were shown in Table 1.

2.4. Limit of detection (LoDs) of the assays

The experiment followed the CLSI guideline EP17-A2. Six or more dilutions were used, including the concentration values before and after the estimated minimum detection limit. The experiment was carried by diluting step by step from a high concentration. More than 24 repetitions per concentration were tested, and Probit Analysis was performed. Through the analysis, the minimum concentration showing the 95% detection rate was set as the LoD value, and the value calculated as the 95% confidence interval (CI) was set as the confidence interval.

2.5. DNA sequencing

Nucleotide sequence alterations in each target gene from test samples were characterized by sequencing. Genes or genetic loci that were known as involved in drug resistances according to the updated WHO recommendation were characterized [12]. Primers to amplify targets for sequencing and amplification conditions are described in Supplementary Table 1. Direct sequencing was carried out on the ABI3730 in Bioneer (Daejeon, South Korea).

3. Results

3.1. Phenotypic DST profile

Of 234 M. tuberculosis isolates included in the study, 37 (15.8 %) were susceptible, 6 (2.6%) were mono-resistant, 20 (8.5%) were poly-resistant other than MDR-TB, 41 (17.5%) were MDR-TB, 33 (14.1%) were MDR-TB plus FQ resistant, 13 (5.6%) were MDR-TB plus SLID resistant and 84 (35.9%) were XDR-TB. Total resistance to individual drug/drug group were 186 (79.5%), 188 (80.3%), 126 (53.8%) and 92 (39.3%) for INH, RIF, FQ and SLID respectively (Table 2).

3.2. Limit of detection (LoDs) of TBMDR and XDRA

LoDs of each gene conferring drug resistance were in an average range of 97.7 to 380.2 copies/test (Table 3).

3.3. Diagnostic performance of MDRTB and XDRA

Sensitivity and specificity of TBMDR and XDRA assays for INH, RIF, FQ and SLID were calculated compared to phenotypic DST results. Diagnostic accuracy of TBMDR was 84.2 % and 95.7% for INH and RIF respectively and that of XDRA was 91.0% and 87.2% for FQ and SLID respectively. Discordant samples (37 isolates for INH, 10 for RIF, 21 for...
Table 2
Phenotypic DST profiles of 234 clinical M. tuberculosis isolates.

| Drug resistance profile                       | No. of isolates (%) |
|-----------------------------------------------|---------------------|
| Susceptible to all drugs                      | 37 (15.8)           |
| MDR-TB                                        | 41 (17.5)           |
| MDR-TB plus FQ resistant                      | 33 (14.1)           |
| MDR-TB plus SLID                              | 13 (5.6)            |
| XDR-TB                                        | 84 (35.9)           |
| Mono-resistant                                | 6 (2.6)             |
| Poly-resistant other than MDR-TB              | 20 (8.5)            |
| Individual drug/drug group resistance         |                     |
| INH                                           | 186 (79.5)          |
| RIF                                           | 188 (80.3)          |
| FQ                                            | 126 (53.8)          |
| SLID                                          | 92 (39.3)           |

DST = Drug susceptibility testing, MDR-TB = Multi-drug-resistant TB, XDR-TB = Extensively drug-resistant TB, INH = Isoniazid, RIF = Rifampicin, FQ = Fluoroquinolones, SLID = Second-line injectable drugs.

Table 3
Limit of detection (LoDs) of TBMDR and XDRA.

| Name of assay | Target | Limit of detection (Average (Range) copies/number/test) |
|---------------|--------|---------------------------------------------------------|
| Accupower TBMDR | Mycobacterium tuberculosis | 63.10 (40.74 – 95.50). |
|                | RIF    | 144.54 (95.50 – 213.80) |
|                | INH    | 112.20 (69.18 – 181.97) |
| Accupower XDRA | FQ gyrA1W | 309 (186.2 – 512) |
|                | FQ gyrA1M | 104.7 (64.6 – 166.0) |
|                | FQ gyrA2W | 380.2 (213.8 – 660.7) |
|                | FQ gyrA2M | 92.72 (60.26 – 158.5) |
|                | SLID r1W | 281.8 (154.88 – 524.8) |
|                | SLID r1M | 199.5 (123.03 – 323.6) |
|                | SLID r2W | 288.4 (186.2 – 446.7) |
|                | SLID r2M | 158.5 (97.72 – 251.2) |

INH = Isoniazid, RIF = Rifampicin, FQ = Fluoroquinolones, SLID = Second-line injectable drugs.

FQ and 30 for SLID) between phenotypic DST and TBMDR/XDRA assays were detected. High concordance rates (greater than 95%) were observed when compared to direct-sequencing for each drug-resistant conferring genes. Diagnostic performance of TBMDR and XDRA compared to phenotypic DST, drug-resistant gene mutations pattern and concordance rates between sequencing and TDMDR and XDRA assays were described in Table 4. Discordant results for each drug resistance and target gene sequence variation were shown in Supplementary Table 2.

4. Discussion

Bioneer’s TBMDR and XDRA are in vitro diagnostic Real-Time PCR-based assays, designed for rapid detection of MDR-TB and XDR-TB. These assays can be used in human samples such as sputum and bronchoalveolar lavage and culture isolates. The present study evaluated the diagnostic performance of these assays on culture isolates compared to phenotypic DST. LoDs of each gene conferring drug resistance in TBMDR and XDRA assays showed an average range of 97.7 to 380.2 copies/test. Overall diagnostic accuracy was satisfactory as it ranged from 84.2% to 95.7%. This result is comparable to that of WHO recommended molecular-based GenoType MTBDRplus and MTBDRsl assays reported by other studies [7,8,13].

For INH resistance detection, TBMDR showed a sensitivity of 81.2% (95% CI 74.81 – 86.53%) and specificity of 95.8% (95% CI 85.75 – 99.49%). Relatively low sensitivity might be explained from the fact that, i) several genes or genetic loci have been known to involve INH resistance (three major targets; katG, inhA, aphC), ii) there are additional genes or genetic loci which suspected to involve in INH resistance (suspects around 10–15% of INH resistance) [14]. Two phenotypic DST susceptible/TBMDR resistant isolates showed katG mutation (S315N) which is associated with low level resistance [15]. Most of the INH resistance discrepancies, phenotypic DST resistant/TBMDR sensitive samples showed katG and inhA genes mutation points that TBMDR does not target and one sample was due to kit error as it failed to detect C-15 T inhA mutation.

Table 4
Discordant results for each drug resistance and target gene sequence variation were shown in Supplementary Table 2.

| Individual drug/drug group resistance | Discordant result |
|--------------------------------------|-------------------|
| INH                                  |                    |
| RIF                                  |                    |
| FQ                                   |                    |
| SLID                                 |                    |

DISCLOSURE

There were some limitations in our study. There was lack of sputum smear data and we can not perform the testing directly on the sputum samples. The present study mainly focused on diagnostic performance of the kits and we did not correlate the genotypic resistance profiles of the assays to the clinical treatment data. We can not use WHO endorsed LPAs in our study for direct comparison with the tested assays. Further study was suggested to evaluate the performance of these assays for Multidrug-resistant TB, XDR-TB = extensively drug-resistant TB, INH = Isoniazid, RIF = Rifampicin, FQ = Fluoroquinolones, SLID = Second-line injectable drugs.

Although XDRA targets only gyrA and rrs, sensitivities for FQs and SLIDs were comparable to other reports on XDR detection or even higher [8,12,17]. Sequencing results of gyrA and gyrB for FQ and rrs for SLID. One phenotypic DST susceptible/XDRA resistant discordant result was found to be caused by isolate with A90V mutation which is associated with FQ low level resistance and it was missed by phenotypic DST. However most of FQ and SLID resistance discordant results were due to presence of gyrB and eis promoter mutations that XDRA do not target. Regarding phenotypic DST error for FQ, some MOX resistant isolates were suggested involved in low level resistance. In such a case, current drug concentrations might not good enough to detect FQ resistance [19-21].

Although XDRA targets only gyrA and rrs, sensitivities for FQs and SLIDs were comparable to other reports on XDR detection or even higher [8,12,17]. Sequencing results of gyrA and gyrB for FQs and rrs/eis promoter region for SLIDs showed significant concordance with XDRA assay; 95.3% for FQs and 96.6% for SLIDs, respectively.

In the present study, we found that TBMDR and XDRA assays can detect INH, RIF and FQ resistance in isolates with low level resistance which were missed by phenotypic DST. The sensitivities, specificities and diagnostic accuracies of TBMDR and XDRA assays can also be improved if we take consider phenotypic DST error and low level resistance.

We also observed two TBMDR kit error cases which failed to detect RIF and INH common resistance mutations. This can be due to mutation ratio of these isolates were below the limit of detection. “ExiStation™ is an automated molecular diagnostic system consisting of an automatic nucleic acid extraction instrument (ExiPrep™/16 Dx) and a nucleic acid amplification instrument (Exicycler™/96). We minimized user errors by automating the contamination prevention system and PCR reaction set up in the instrument. In addition, errors were minimized by reducing the hands-on step using a pre-filled nucleic acid extraction cartridge, and vacuum dried premix type PCR reagent. Minimizing TB-MDR kit error is important because this could lead to wrong selection of the treatment regimen for patients.

There were some limitations in our study. There was lack of sputum smear data and we can not perform the testing directly on the sputum samples. The present study mainly focused on diagnostic performance of the kits and we did not correlate the genotypic resistance profiles of the assays to the clinical treatment data. We can not use WHO endorsed LPAs in our study for direct comparison with the tested assays. Further study was suggested to evaluate the performance of these assays for...
XDRA assays could be useful tools for detection of MDRTB and XDRTB.

Appendix A. Supplementary data

Table 4 Diagnostic performance of TBMDR and XDRA assays compared to phenotypic DST and gene sequencing.

| Test kit          | Ant-TB drugs | pDST (MGIT) | Susceptible | % Sensitivity (95% CI) | % Specificity (95% CI) | Diagnostic accuracy (%) | Concordancesequencing vs AccuPower® kits (%) |
|-------------------|--------------|-------------|-------------|------------------------|------------------------|-------------------------|---------------------------------------------|
|                   |              |             | No. of isolates | Mutation types (sequencing) | No. of isolates | Mutation types (sequencing) |                                             |
|                   |              |             | Susceptible   | No mutation            | 35          | katG 312GCG-GGG<sup>®</sup> | 81.2 (74.81-86.53) | 95.8 (85.75-99.49) | 84.2 | 95.3 |
|                   |              |             |              |                        |             | katG 390TAT-TGT*            |                            |                                             |
|                   |              |             |              |                        |             | inhA -34C>T<sup>®</sup>     |                            |                                             |
|                   |              |             |              |                        |             | inhA -15T*                  |                            |                                             |
|                   |              |             | Resistant     | 2                      | 151        | katG 315AGC-AAC<sup>®</sup> |                            |                                             |
|                   |              |             |              |                        |             | Not shown                  |                            |                                             |
|                   |              |             | Susceptible   | 44                     | 8          | 522TCG-TGG<sup>®</sup>      | 95.7 (91.79-96.15) | 95.7 (85.15-99.47) | 95.7 | 98.7 |
|                   |              |             |              |                        |             | 176GTC-TTC<sup>®</sup>      |                            |                                             |
|                   |              |             |              |                        |             | 531TGC-TTG<sup>®</sup>      |                            |                                             |
|                   |              |             | Resistant     | 2                      | 180        | 511CTG-CCG<sup>®</sup>      |                            |                                             |
|                   |              |             |              |                        |             | 516GAC-TAC<sup>®</sup>      |                            |                                             |
|                   |              |             | Susceptible   | 107                    | 20         | gyrB 540GAA-GAC<sup>®</sup> | 84.1 (76.56-90.03) | 99.1 (94.95-99.98) | 91.0 | 95.3 |
|                   |              |             |              |                        |             | gyrB 538AAC-GAC<sup>®</sup> |                            |                                             |
|                   |              |             |              |                        |             | gyrB 539ACC-GCC<sup>®</sup> |                            |                                             |
|                   |              |             |              |                        |             | gyrB 551GGG-AGG<sup>®</sup> |                            |                                             |
|                   |              |             |              |                        |             | gyrB 486TCC-TTC<sup>®</sup> |                            |                                             |
|                   |              |             | Resistant     | 1                      | 106        | gyrA 90GCC-GTG<sup>®</sup>  |                            |                                             |
|                   |              |             |              |                        |             | Not shown                  |                            |                                             |
|                   |              |             | Susceptible   | 142                    | 30         | rrs A1401G<sup>®</sup>      | 67.4 (56.82-76.80) | 100.0 (97.44-100.0) | 87.2 | 96.6 |
|                   |              |             |              |                        |             | rrs CI-402T<sup>®</sup>     |                            |                                             |
|                   |              |             |              |                        |             | eis -8C-A<sup>®</sup>       |                            |                                             |
|                   |              |             |              |                        |             | eis -10G-A<sup>®</sup>      |                            |                                             |
|                   |              |             |              |                        |             | eis -12C-T<sup>®</sup>      |                            |                                             |
|                   |              |             |              |                        |             | eis -14C-T<sup>®</sup>      |                            |                                             |
|                   |              |             | Resistant     | 0                      | 62         | No mutation                |                            |                                             |

pDST: Phenotypic drug susceptibility test.
Ψ: Low level resistant.
#: Kit does not target.
*: Phenotypic DST error.
§: Kit error.

rapid detection of MDR and XDR-TB directly from clinical samples.

In conclusion, the sensitivities of TBMDR and XDRA TB drug resistant detection kits for each drug were equivalent to other molecular drug susceptibility testing methods. Our study showed Bioneer’s TBMDR and XDRA assays could be useful tools for detection of MDRTB and XDR-TB.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank Bioneer, Daejeon, South Korea for donating equipment and kits for evaluation study.

Funding

This work was supported (in part) through continuing support from the Ministry for Health and Welfare of the Republic of Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2022.100303.

References

[1] World Health Organization. Global tuberculosis report 2020. Geneva, Switzerland: WHO; 2020.
[2] World Health Organization. WHO operational handbook on tuberculosis. Module 4: treatment- drug-resistant tuberculosis treatment. Geneva, Switzerland: WHO; 2020.
[3] World Health Organization. Rapid communication: key changes to treatment of drug-resistant tuberculosis 2019. Geneva, Switzerland: (WHO/CDS/TB/2019.26). 2020.
[4] World Health Organization. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27–29 October 2020. Geneva, Switzerland: World Health Organization; 2021.
[5] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva, Switzerland: WHO; 2018.
[6] World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. Geneva, Switzerland: WHO; 2013.
[7] Bai Y, Wang Y, Shao C, Hao Y, Jin Y, Chatterji D. GenoType MTBDRplus assay for rapid detection of multidrug resistance in Mycobacterium tuberculosis: a meta-analysis. PLoS One 2016;11(3):e0150321. https://doi.org/10.1371/journal.pone.0150321.
[8] Jian J, Yang X, Yang J, Chen L. Evaluation of the Genotype MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, China. Infect Drug Resist 2018;11:627–34.
[9] Hopmeier D, Lampio T, Rycroft J, Tiberi S, Melzer M. The limitations of the Cepheid GeneXpert® Mtb/Rif assay for the diagnosis and management of polypotent resistant pulmonary tuberculosis. Clin Infect Pract 2020;7–8. https://doi.org/10.1016/j.jcinpr.2020.100038.
[10] Cho E, Shamputa IC, Kwak HK, Lee J, Lee M, Hwang S, et al. Utility of the REBA MTB- Rif® assay for rapid detection of rifampin resistant Mycobacterium tuberculosis. BMC Infect Dis 2013;13:478.

[11] Siddiqi SH, Rusch-Gerdes S. MGIT Procedure Manual. Geneva, Switzerland: Foundation for Innovative New Diagnostics; 2006. p. 41-51.

[12] World Health Organization. WHO operational handbook on tuberculosis. Module 3: diagnosis- rapid diagnostics for tuberculosis detection, 2021 update. Geneva, Switzerland: WHO; 2021.

[13] Gardee Y, Dreyer AW, Koornhof HJ, Omar SV, da Silva P, Bhyat Z, et al. Evaluation of the Genotype MTBDRsl version 2.0 assay for second line drug resistance detection of Mycobacterium tuberculosis isolates in South Africa. J Clin Microbiol 2017;55(3):791-800.

[14] Seifert M, Catanzaro D, Catanzaro A, Rodwell TC, Mokrousov I. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One 2015;10(3):e0119628. https://doi.org/10.1371/journal.pone.0119628.

[15] Kandler JL, Mercante AD, Dalton TL, Ezewudo MN, Cowan LS, Burns SP, et al. Validation of novel Mycobacterium tuberculosis isoniazid resistance mutations not detected by common molecular test. Antimicrob Agents Chemother 2018;62(10):e00974-1018.

[16] Mohajeri P, Sadri H, Farahani A, Noroozi B, Atashi S. Frequency of mutations associated with rifampicin resistance to Mycobacterium tuberculosis strains isolated from patients in west of Iran. Microb Drug Resist 2015;21(3):315-9.

[17] Rahmo A, Hamdar Z, Kasaa I, DABBousi F, Hamze M. Genotypic detection of rifampicin-resistant M. tuberculosis strain in Syrian and Lebanese patients. J Infect Public Health 2012;5:381-7.

[18] Li J, Gao X, Luo T, Wu J, Sun G, Liu Q, et al. Association of gyrA/B mutations and resistance levels to fluoroquinolones in clinical isolates of Mycobacterium tuberculosis. Emerg Microbes Infect 2014;3(3):e9.

[19] Bakuła Z, Napiórkowska A, Kamiński M, Augustynowska-Kopeć E, Zwalda Z, Bielecki J, et al. Second-line anti-tuberculosis drug resistance and its genetic determinants in multidrug-resistant Mycobacterium tuberculosis clinical isolates. J Microbiol Immunol Infect 2016;49(3):439-44.

[20] Feng Y, Liu S, Wang Q, Wang L, Tang S, Wang J, et al. Rapid diagnosis of drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol using genotype MTBDRsl assay: a meta-analysis. PLoS One 2013;8(2):e55292. https://doi.org/10.1371/journal.pone.0055292.

[21] Iszeva Y, Bukatina A, Krylova L, Nosova E, Makarova M, Moroz A. Determination of critical concentrations of moxifloxacin and gatifloxacin for drug susceptibility testing of Mycobacterium tuberculosis in the BACTEC MGIT 960 system. J Antimicrob Chemother 2013;68(10):2274-81.