Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 27 (2022) 100303

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

journal homepage: www.elsevier.com/locate/jctube

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

Eunjin Cho [a], Su jin Lee [b], Jiyoung Lim [b], Dong Sik Kim [b], Namil Kim [b], Han Oh Park [b], Ji-im Lee [a], Eunsoon Son [a], Sang Nae Cho [a], Wah Wah Aung [c], Jong Seok Lee [a,*]

[a] ITRC (International Tuberculosis Research center): 236 Gaposunhwan-ro, Masanhappo-gu, changwon-si, Gyeongsangnam-do, Republic of Korea
[b] BIONEER: 8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon 34302, Republic of Korea
[c] Advanced Molecular Research Centre, Department of Medical Research, Yangon, Republic of the Union of Myanmar

ARTICLE INFO

Keywords:
TBMDR®, XDRA®
Diagnosis
MDR-TB
pre-XDR-TB

ABSTRACT

This study evaluated the diagnostic performance of the AccuPower® TB&MDR Real-Time PCR (TBMDR®) and AccuPower® XDR-TB Real-Time PCR Kit-A (XDRA®) to detect multidrug-resistant (MDR-TB) and pre-extensively drug-resistant tuberculosis (pre-XDR-TB) in comparison with phenotypic drug susceptibility testing (DST) using MGIT 960 on 234 clinical *Mycobacterium tuberculosis* isolates. Discrepant results were confirmed by direct-sequencing. Sensitivity and specificity of TBMDR and XDRA for cultured isolates were 81.2% and 95.8% for isoniazid (INH) resistance, 95.7% and 95.7% for rifampicin (RIF) resistance, 84.1% and 99.1% for fluoroquinolone (FQ) resistance, and 67.4% and 100% for second-line injectables resistance. The sensitivities of each drug were equivalent to other molecular DST methods. High concordance was observed when compared to direct-sequencing. We also found that TBMDR and XDRA assays can detect INH, RIF and FQ resistance in isolates with low level resistance-associated mutations which were missed by phenotypic DST. Our study showed TBMDR and XDRA assays could be the useful tools to detect MDR-TB and pre-XDR-TB.

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 a 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].

* Corresponding author.
E-mail address: cosmosljs@gmail.com (J. Seok Lee).

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

Available online 9 February 2022

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 human 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 NMH 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*™ 96. 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*™ 96 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 (LoD)s 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 1
Target regions of *Accupower*® TBMDR and XDRA Real-Time PCR Kits.

Kit	Drug	Gene target	Codon region	Nucleotide
Accupower® TBMDR Real-Time PCR Kit	Rifampicin	*rpoB*	501–531	–
	Isoniazid	*inhA* promoter	–	−15
		katG	315	–
Accupower® XDRA Real-Time PCR Kit	Fluoroquinolones injectable second-line drugs	*gyrA*	90,91,94	–
		rrs	–	1401,1402,1484

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 = Multidrug-resistant TB, XDR-TB = Extensively drug-resistant TB, INH = Isoniazid, RIF = Rifampicin, FQ = Fluoroquinolones, SLID = Second-line injectable drugs.

Table 3
Limit of detection (LoD)s of TBMDR and XDRA.

Name of assay	Target	Limit of detection Average (Range) copies numbers/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 assay	FQ gyrA1W	309 (186.2 ~ 512)
	FQ *gyrA*1M	104.7 (64.6 ~ 166.0)
	FQ *gyrA*2W	380.2 (213.8 ~ 660.7)
	FQ *gyrA*2M	92.72 (60.26–158.5)
	SLID *rrs*1W	281.8 (154.88 ~ 524.8)
	SLID *rrs*1M	199.5 (123.03 ~ 323.6)
	SLID *rrs*2W	288.4 (186.2 ~ 446.7)
	SLID *rrs*2M	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 than95%) 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

Bioneers'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 MTBDR*plus* and MTBDR*sl* 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 has been known to involve INH resistance (three major targets; *katG*, *inhA*, *ahpC*), 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.

Much higher sensitivity and specificity were shown for RIF. Sensitivity was 95.7% (95% CI 91.79–96.15%) and specificity was 95.7% (95% CI 85.16–99.47%) which were similar to the 95% pooled sensitivity and 98% pooled specificity values of Xpert MTB/RIF assay [6] and 96–98.7% sensitivity and 88.9 to 99% specificity values of MTBDRplus assays reported by the previous studies [7,8,9]. In comparison with sequencing results of RRDR it showed 98.7% concordance. Of eight phenotypic DST resistant/TB-MDR kit RIF susceptible samples, five were confirmed as susceptible by sequencing, thus those five samples can be phenotypic DST error or due to other resistance determinants. Two samples had V176F and S522W mutations which kit does not target. Kit error was found in one sample which had S531L mutation. The results also showed that TB-MDR kit can detect two RIF low level resistant isolate with L511P and D516T mutations which were missed by phenotypic DST [16,17].

gyrA and *gyrB* are two major genes that explain about 90% of FQ resistances. Similarly, *rrs* gene and *eis* promoter region are known to be major resistance determinants which can explain about 75–90% of SLIDs resistance [18]. However, current Bioneer XDRA targets only *gyrA* 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* promotor 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/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

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

Test kit	Ant-TB drugs		pDST (MGIT)				% Sensitivity (95% CI)	% Specificity (95% CI)	Diagnostic accuracy (%)	Concordance sequencing vs AccuPower® kits (%)
			Susceptible		Resistant					
			No. of Isolates	Mutation types (sequencing)	No. of Isolates	Mutation types (sequencing)				
AccuPower® TBMDR	INH	Susceptible	46	No mutation	35	*katG* 312GCG-GGG[#] *katG* 390TAT->TGT[#] *inhA* -34C-T[#] *inhA* -15T[§]	81.2 (74.81–86.53)	95.8 (85.75–99.49)	84.2	95.3
		Resistant	2	*katG* 315AGC-AAC[Ψ]	151	Not shown				
	RIF	Susceptible	44	No mutation	8	522TCG-TGG[#] 176GTC-TTC[#] 531TCG-TTG [§]	95.7 (91.79–96.15)	95.7 (85.16–99.47)	95.7	98.7
		Resistant	2	511CTG-CCG[Ψ] 516GAC-TAC[Ψ]	180	Not shown				
AccuPower® XDRA	FQs	Susceptible	107	No mutation	20	*gyrB* 540GAA-GAC[#] *gyrB* 538AAC-GAC[#] *gyrB* 539ACC-GCC[#] *gyrB* 551GGG-AGG[#] *gyrB* 486TCC-TTC[#]	84.1 (76.56–90.03)	99.1 (94.95–99.98)	91.0	95.3
		Resistant	1	*gyrA* 90GCG-GTG[Ψ*]	106	Not shown				
	SLIDs	Susceptible	142	No mutation	30	*rrs* A1401G[§] *rrs* C1402T[§] *eis* -8C-A[#] *eis* -10G-A[#] *eis* -12C-T[#] *eis* -14C-T[#]	67.4 (56.82–76.80)	100.0 (97.44–100.0)	87.2	96.6
		Resistant	0	No mutation	62					

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 XDRTB.

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).
[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 MTBDR*plus* 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 MTBDR*plus* and MTBDR*sl* for the detection of drug-resistant *Mycobacterium tuberculosis* on isolates from Beijing, China. Infect Drug Resist 2018;11:1627–34.
[9] Hopmeier D, Lampejo T, Rycroft J, Tiberi S, Melzer M. The limitations of the Cepheid GeneXpert® Mtb/Rif assay for the diagnosis and management of polyresistant pulmonary tuberculosis. Clin Infect Pract 2020:7–8. https://doi.org/10.1016/j.clinpr.2020.100038.

[10] Cho E, Shamputa IC, Kwak HK, Lee J, Lee M, Hwang S, et al. Utility of the REBA MTB- Rifa® 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, Norozi B, Atashi S. Frequency of mutations associated with rifampicin resistance in *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, Dabboussi 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 fluoquinolones in clinical isolates of *Mycobacterium tuberculosis*. Emerg Microbes Infect 2014;3(3):el9.
[19] Bakuła Z, Napiórkowska A, Kamiński M, Augustynowicz-Kopeć E, Zwolska 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 MTBDR*sl* assay: a meta-analysis. PLoS One 2013;8(2):e55292. https://doi.org/10.1371/journal.pone.0055292.
[21] Isaeva 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.