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Original Article

Performance of the GenoType MTBDR*sl* assay for the detection second-line anti-tuberculosis drug resistance

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ABSTRACT

The rapid detection of drug-resistant tuberculosis (TB) is important to improve treatment outcomes and prevent disease transmission. The GenoType MTBDR*sl* assay (MTBDR*sl* assay) was developed to detect fluoroquinolone (FQ) and second-line injectable drug (SLID) resistance. The aim of this study was to evaluate the performance and clinical utility of MTBDR*sl* assay. We retrospectively reviewed patient medical records with MTBDR*sl* assay data between December 2011 and February 2017. MTBDR*sl* assay results were compared with that of phenotypic drug susceptibility testing. In addition, treatment outcomes were analyzed to evaluate the clinical utility of the MTBDR*sl* assay. Among 107 clinical isolates (84 cultured isolates and 23 sputum specimens), 85 (79.4%) were multidrug-resistant TB and 9 (8.4%) were extensively drug-resistant TB (XDR-TB). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of MTBDR*sl* assay for detecting FQ resistance was 87.5%, 94.7%, 87.5%, 94.7%, and 92.5%, respectively. The sensitivity, specificity, PPV, NPV, and accuracy of MTBDR*sl* assay for detecting SLID resistance was 88.9%, 98.9%, 94.1%, 97.8%, and 97.2%, respectively. Novel drugs such as bedaquiline and linezolid were more commonly used in patients with FQ or SLID resistance detected by the MTBDR*sl* assay and, probably therefore, the treatment outcome was favorable irrespective of FQ or SLID resistance. The MTBDR*sl* assay could be used as a rule-in test to detect FQ and SLID resistance. By detecting FQ- and SLID-drug resistance rapidly, novel or repurposed drugs could be initiated earlier, suggesting that better treatment outcomes would be expected in patients with pre-XDR- and XDR-TB.

1. Introduction

Although global efforts to control tuberculosis (TB) have reduced the overall incidence of TB, these have failed to reduce the global burden of drug resistance TB [1–6]. The incidence of multidrug-resistant TB (MDR-TB; defined as resistance to isoniazid and rifampin [RMP]) continues to increase, and the mortality rate of MDR-TB has not decreased. Especially, extensively drug-resistant TB (XDR-TB; defined as MDR-TB with additional resistance to a fluoroquinolone [FQ] and at least one of three injectable drugs [capreomycin, kanamycin, or amikacin]) raises global concern because the prognosis of XDR-TB is poorer than that of MDR-TB, due to very limited treatment options [3–7]. Delayed diagnosis and treatment of MDR-TB, including XDR-TB, leads to increased ongoing transmission and TB-related mortality in communities [8–10]. Thus, the rapid detection of drug-resistant TB is very important. However, the diagnosis of MDR-TB using conventional phenotypic drug susceptibility testing (DST) can be delayed for several months [11]. For this reason, the application of genotypic DST continues to increase, which uses polymerase chain reaction-based techniques [7,8,12,13]. Actually, the World Health Organization (WHO) recommended that the use of GenoType MTBDR*plus* assay (MTBDR*plus* assay; Hain Lifescience GmbH, Nehren, Germany) and GeneXpert MTB/RIF assay (Cepheid Inc. Sunnyvale, California, USA) should be considered when diagnosing drug-resistant TB [7,8,12].

In 2009, the GenoType MTBDR*sl* assay (MTBDR*sl* assay; Hain Lifescience GmbH, Nehren, Germany) was developed as a

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commercial kit for detecting FQ, amikacin/capreomycin, and ethambutol resistance. Recently, the importance of early detection of FQ-resistant and second-line injectable drug (SLID)-resistant TB has been highlighted because a short-course treatment of 9–12 months is recommended in patients with FQ- and SLID-susceptible MDR-TB [7]. Thus, WHO recommended that the MTBDR*sl* assay should be considered for the early detection of resistance to FQ and SLID when initiating treatment for MDR-TB [7,13].

The aim of our current study was to evaluate the performance and clinical utility of MTBDR*sl* assay for detecting FQ- and SLID-resistant TB in routine clinical practice.

2. Materials and methods

2.1. Study design

This retrospective-design study was conducted at Asan Medical Center, a tertiary referral hospital in Seoul, South Korea. South Korea reported prevalence rates of MDR-TB of 2.9% and 9.3% in 2012 in new and previously treated TB cases, respectively [1,14]. From December 2011, we investigated TB resistance to FQ and SLID using the MTBDR*sl* assay to test isolates from patients with RMP-resistant TB detected by phenotypic DST, MTBDR*plus* assay, or GeneXpert MTB/RIF assay. The results of the assay were applied to the treatment of patients. In this study, we retrospectively reviewed patient medical records with MTBDR*sl* assay results between December 2011 and February 2017. All clinical specimens were investigated by the MTBDR*sl* assay from the same DNA extract that was used for the phenotypic DST and MTBDR*plus* assay. The result of the MTBDR*sl* assay was compared with that of the phenotypic DST. Treatment outcomes and new or repurposed drug (bedaquiline and linezolid) use were also analyzed.

This study was approved by the Institutional Review Boards at the Asan Medical Center (No. 2017-0638). Informed consent was waived because of the retrospective nature of the study.

2.2. MTBDRsl assay

MTBDR*sl* assay (version 1.0) was performed according to the instructions provided by the manufacturer [15,16]. All specimens showing resistance to RMP and isoniazid or to RMP-alone by the phenotypic DST and MTBDR*plus* assay were concurrently tested by the MTBDR*sl* assay using the same DNA extract. For each gene, the test evaluates the presence of wild-type (WT) and/or mutant sequences, thus covering all high-confidence resistance mutations. The absence of a WT band or appearance of a mutant (MUT) band at least as strong as the amplification control band must be interpreted as resistance to the respective drug.

2.3. Drug susceptibility testing

All sputum specimens were examined using Ziehl-Neelsen staining with positive results and cultured in both solid Ogawa medium and the liquid MGIT system (Becton Dickinson Diagnostics, Sparks, MD). Conventional DST was performed using the absolute concentration method with Lőwenstein-Jensen media at the Korean Institute of Tuberculosis (Osong, South Korea), which is a Supranational TB Reference Laboratory. Isolates were tested for resistance to critical concentrations of isoniazid (0.2 μg/mL), RMP (40.0 μg/mL), kanamycin (30.0 μg/mL), capreomycin (40.0 μg/mL), amikacin (30.0 μg/mL), ofloxacin (4.0 μg/mL), levofloxacin (2.0 μg/mL), and moxifloxacin (2.0 μg/mL). The critical concentration used for DST of kanamycin changed from 40.0 μg/mL to 30.0 μg/mL at January 2014. In addition, the critical concentration used for DST of ofloxacin changed from 2.0 μg/mL to 4.0 μg/mL at December 2015 [16–18].

2.4. Definitions of treatment outcomes

The seven categories of outcome definitions included in the 2014 WHO recommendation were as follows: cured, treatment completed, treatment failed, died, lost to follow-up, and not evaluated. Treatment success was defined as the sum of cured and treatment completed [19]. All outcomes were the initial treatment outcomes during the study period and were analyzed based on AFB culture results.

2.5. Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the accuracy of the MTBDR*sl* assay in detecting resistance to ofloxacin, kanamycin, and ethambutol were calculated using the results of conventional DST as the reference standard. Concordance between the MTBDR*sl* assay and conventional DST results was assessed using kappa (κ) coefficients, with $\kappa \geq 0.81$ defined as almost perfect agreement, κ between 0.61 and 0.80 defined as substantial agreement, κ between 0.41 and 0.60 defined as moderate agreement, κ between 0.21 and 0.40 defined as fair agreement, κ between 0.01 and 0.20 defined as none to slight agreement, and $\kappa \leq 0$ defined as no agreement [20]. SPSS software (version 21.0; IBM Corp., Armonk, NY) was used for all analyses.

3. Results

3.1. Clinical specimens

Among the 107 clinical specimens, 23 (21.5%) were sputum specimens and 84 (78.5%) were cultured isolates. Of those, 96 (89.7%) were RMP-resistant by conventional phenotypic DST (85 MDR-TB and 11 RMP-mono-resistance) and another 11 were RMP-resistant by MTBDR*plus* assay or GenoXpert MTB-RIF assay (Fig. 1). Eighty-five (79.4%) were MDR-TB and nine (8.4%) were XDR-TB. Drug-resistant patterns of other drugs are presented in Table 1.

3.2. Performance of MTBDRsl assay according to clinical specimens

In a total of 107 specimens tested, the MTBDR*sl* assay showed a sensitivity of 87.5%, specificity of 94.7%, PPV of 87.5%, NPV of 94.7%, and accuracy of 92.5% for detecting FQs-resistant TB. The sensitivity, specificity, PPV, NPV, and accuracy of MTBDR*sl* assay for detecting SLID-resistant TB were 88.9%, 98.9%, 94.1%, 97.8%, and 97.2%, respectively. There was an excellent agreement between the MTBDR*sl* assay and conventional DST in the detection of FQ resistance ($\kappa = 0.822$; 95% CI, 0.703–0.940) and SLID resistance ($\kappa = 0.898$; 95% CI, 0.784–1.012).

The performance of the MTBDR*sl* assay in clinical specimens is presented in Table 2. In 84 cultured isolates, the MTBDR*sl* assay showed a sensitivity of 88%, specificity of 98.3%, PPV of 95.7%, NPV of 95.1%, and accuracy of 95.2% for detecting FQ resistance. The sensitivity, specificity, PPV, NPV, and accuracy of the MTBDR*sl* assay for detecting SLID resistance was 86.7%, 100%, 100%, 97.2%, and 97.6%, respectively. The κ value was higher for detecting SLID resistance than for detecting FQ resistance in both sputum specimens and cultured isolates.

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