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Original article

Performance of REBA MTB-XDR to detect extensively drug-resistant tuberculosis in an intermediate-burden country

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ABSTRACT

Extensively drug-resistant tuberculosis (XDR-TB) is a serious worldwide problem. The REBA MTB-XDR (REBA-XDR) was recently developed in Korea to detect resistance to ofloxacin, kanamycin, and streptomycin. The aim of this study is to evaluate the diagnostic accuracy of the REBA-XDR. We prospectively enrolled 104 patients with acid-fast bacilli smear-positive specimens between July 2010 and January 2013. Performance characteristics were compared between REBA-XDR and conventional drug-susceptibility testing. The sensitivity values of REBA-XDR for detecting resistance to ofloxacin, kanamycin, and streptomycin were 66.7%, 90.9%, and 60.0%, and the specificity values were 93.3%, 93.5%, and 85.4%, respectively. The positive predictive values were 62.5%, 62.5%, and 40.9%, and the negative predictive values were 94.3%, 98.9%, and 92.7%, respectively. Accuracy was 89.4%, 93.3%, and 81.7%, respectively. REBA-XDR seems to be a useful kit for "ruling in" XDR-TB in intermediate-burden countries, and especially useful for detecting kanamycin resistance.

1. Introduction

Despite global efforts to control tuberculosis (TB), multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) are serious worldwide problems because of the high rates of treatment failure, morbidity, and mortality [1–5]. By the end of 2012, 3.7% of new cases and 20% of previously treated cases were estimated to be MDR-TB [1]. The burden of XDR-TB-defined as MDR-TB with additional resistance to a fluoroquinolone and ≥1 of 3 injectable drugs (capreomycin, kanamycin, or amikacin)-has increased annually [1,2,6]. The prognosis of XDR-TB is poorer than that of MDR-TB because the treatment options are very limited [3,7–10]. There is concern that the true burden of drug-resistant TB cannot be accurately estimated by global studies because of the absence of appropriate laboratory infrastructure in countries with a high-TB-burden [1].

The availability of rapid and accurate methods for detecting drug-resistant TB is very important because delayed diagnosis and treatment leads to further transmission, thereby increasing mortality and morbidity [1–5,11,12]. However, conventional phenotypic drug-susceptibility testing (DST) delays diagnosis by weeks to months. For this reason, polymerase chain reaction (PCR)-based techniques have provided new opportunities for the rapid diagnosis of drug resistance. In 2008, the World Health Organization (WHO) recommended that rapid DST with PCR-based techniques should be considered when diagnosing TB [11,13]. The GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) has already been endorsed by WHO for detecting rifampicin and isoniazid resistance [11,14,15]. Although the GenoType MTBDR*sl* assay (Hain Lifescience GmbH) was developed for detecting fluoroquinolone, amikacin/capreomycin, and ethambutol resistance, the usefulness of this assay in detecting XDR-TB has not been

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clarified in clinical practice [16,17]. WHO recently recommended that the MTBDR*sl* assay should not be used as a replacement test for conventional DST [18].

Given this situation, the REBA MTB-XDR line probe assay (REBA-XDR; YD Diagnostics, Gyeonggi-do, Korea) was developed to detect ofloxacin, kanamycin, and streptomycin resistance in Korea. The reason for including streptomycin-resistant probes is that streptomycin resistance may affect the treatment outcomes of MDR-/XDR-TB patients [3,19–22]. The aim of our current study was to evaluate the performance of REBA-XDR for detecting XDR-TB by detecting the key mutations associated with resistance to fluoroquinolone and kanamycin in acid-fast bacilli (AFB) smear-positive clinical specimens.

2. Materials and methods

2.1. Study oversight

This study was conducted at four tertiary hospitals in South Korea. South Korea is an intermediate TB-burden country with reported prevalence rates of 2.9% and 9.3% MDR-TB in new and previously treated TB cases in 2012, respectively; the prevalence of MDR-TB is also increasing in new cases [1,2,23]. This trial was approved by the institutional review boards of all affiliated hospitals. Informed consent was waived because REBA-XDR was performed on the residual portions of sputum samples that were submitted for routine culture, and no personal information on the study subjects except for age and sex was collected. Between July 2010 and January 2013, 119 AFB smear-positive sputum specimens were obtained from patients with a previous history of TB or patients with previously diagnosed MDR-TB.

2.2. REBA (reverse blot hybridization assay) MTB-XDR

REBA-XDR is a commercial kit for detecting ofloxacin, kanamycin, and streptomycin resistance in AFB smear-positive sputum specimens and uses molecular line probe assay technology. REBA-XDR consists of 25 probes (9 wild-type-specific probes and 16 mutant specific probes) that detect mutations in 3 codons (88, 91, and 94) of the *gyrA* gene, which is related to ofloxacin resistance; in 4 nucleotides (A1401G, C1402A, C1402T, and G1484T) of the *rrs* gene, which is related to kanamycin resistance; in 2 nucleotides (A514C and C517T) of the *rrs* gene; and in 2 codons (43, 88) of the *rpsL* gene, which is related to streptomycin resistance (Fig. 1). The wild-type probes cover codons 79–98, nucleotide 1400–1484, nucleotide 507–553, and codons 32–98 of the *gyr*A, *rrs* 1400, *rrs* 500, and *rps*L genes, respectively.

DNA was extracted by boiling with resin. Briefly, at least 1–5 mL sputum was treated with 4% NaOH-N-acetyl-L-cysteine at room temperature for 15 min. After adding 300–500 μL distilled water to the pellets, the specimen was vortexed and transferred to a 1.5 mL sterile tube. After completely removing the supernatant, 50–100 μL of DNA extraction solution (MolecuTech REBA MTB-XDR, YD diagnostics, GyeongGi-Do, Korea) was added to the pellets, which was further incubated for 10 min at 100 °C. After centrifugation, the supernatant was used in PCR analysis, and the amplified products were used in the REBA-XDR assay. In the REBA-XDR, multiplex PCR amplifications are performed, with the same condition, for several resistance-inducing genes. The multiplex PCR condition includes an initial denaturation step of 5 min at 95 °C, followed by 50 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, and a final extension step of 7 min at 72 °C. The 5′ end of all reverse primers was labeled with biotin for chromogenic detection. The amplified PCR products were used in a reverse blot hybridization assay for detection of drug resistance-inducing gene mutations.

Five to ten microliters of clinical genomic DNA was used for amplification, which was performed in an automated thermocycler. The biotin-labeled PCR products were denatured, hybridized to the membrane-bound probes, and washed thoroughly, and streptavidin-alkaline phosphatase conjugate was added to the strips. For the chromogenic reaction, an alkaline phosphatase-mediated staining solution was added, and the strips were incubated until color appeared. As a final step, further development of color was stopped by adding distilled water to the strip and pasted dried strip and stored protected from light. The results were interpreted using a respective line assignment of provided data sheet (Fig. 1).

The visible band of the mutant probe was used to interpret "resistance" to certain drugs. The absence of a visible band for a wild-type probe without a visible band for a mutant probe was interpreted as "resistance" to a certain drug.

2.3. Study procedures

All sputum specimens were examined using Ziehl–Neelsen staining with positive results and cultured in both solid Ogawa

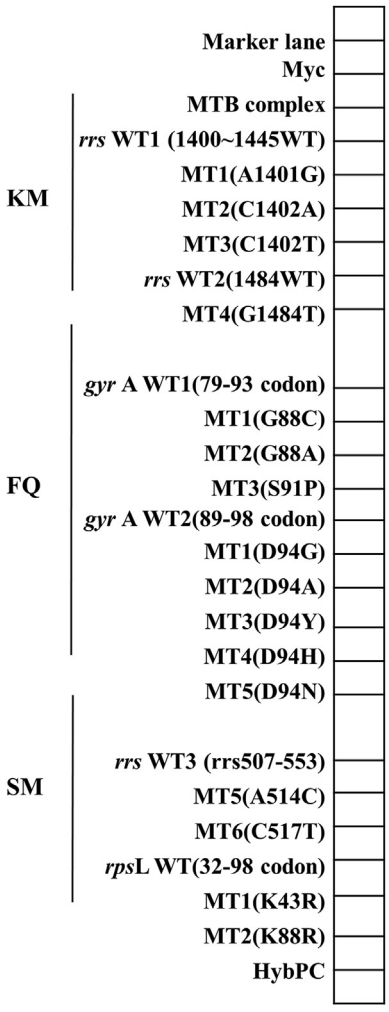

Fig. 1. REBA MTB-XDR kit REBA-XDR consists of 25 probes (9 wild-type-specific probes and 16 mutant specific probes) that detect mutations in 3 codons (88, 91, and 94) of the *gyrA* gene, which is related to ofloxacin resistance; in 4 nucleotides (A1401G, C1402A, C1402T, and G1484T) of the *rrs* gene, which is related to kanamycin resistance; in 2 nucleotides (A514C and C517T) of the *rrs* gene; and in 2 codons (43, 88) of the *rpsL* gene, which is related to streptomycin resistance.