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Comparison of Three Molecular Assays for the Detection of Rifampin Resistance in *Mycobacterium tuberculosis*

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Background: Rifampin (RIF) is the most important first-line antituberculosis drug, and resistance to this drug may result in treatment failures. We evaluated the diagnostic performances of recently introduced, molecular assays for the detection of RIF resistance. *Methods:* A total of 100 isolates (50 RIF resistant and 50 RIF susceptible) were studied. Their RIF resistances were determined by conventional drug-susceptibility test. These results were compared with those of three molecular assays: Xpert MTB/RIF assay (MTB is *Mycobacterium tuberculosis*), Sacace MTB Real-TM resistance, and AdvanSure MDR-TB GenoBlot assay (MDR is multidrug resisitant). *Results:* Sensitivities for RIF resistance detection of Xpert MTB/RIF assay, Sacace MTB Real-TM resistance, and Advansure GenoBlot assay were 94.0%, 91.8%, and 84.0%, respectively. Their specificities for RIF resistance detection were all 100%. *Conclusion:* Three molecular assays for the detection of RIF resistance have various performances. Xpert MTB/RIF assay shows the highest sensitivity among the three molecular assays and can be an effective choice in clinical laboratories. J. Clin. Lab. Anal. 29:142–145, 2015. © 2014 Wiley Periodicals, Inc.

Key words: tuberculosis; resistance; rifampin; molecular assay

INTRODUCTION

The burden of tuberculosis (TB) remains enormous worldwide. The mortality rate of TB is still high, and there were 1.7 million human deaths due to TB in 2009 (1). Rifampin (RIF) is the most important first-line anti-TB drug, and resistance to this drug may result in treatment failures. Globally, the emergence and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB are increasing concerns and cause significant challenges to control this disease (2). A recent report revealed that 20% of the isolates met the MDR criteria and 2% of them were classified as XDR (3).

Although culture-based, conventional drug-sensitivity testing (DST) has been considered gold standard to detect drug resistance of *Mycobacterium tuberculosis* (MTB), it is time consuming and labor intensive, causing a diagnostic delay. To prevent and control the spread of MDR and XDR TB, a simple and convenient DST is essential.

Recently, the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) was introduced and was endorsed by World Health Organization (WHO) (4). This assay simultaneously detects the presence of MTB and its susceptibility to RIF in a single reaction, which integrates sample processing and polymerase chain reaction in a disposable plastic cartridge (5–7). Because most RIF-resistant isolates also exhibit resistance to isoniazid (INH), the detection of RIF resistance serves as a surrogate marker for MDR MTB (8). Also, several molecular assays for the detection of RIF resistance have been recently developed. In this study, we wanted to evaluate the diagnostic performances of recently introduced, molecular assays for the detection of RIF resistance.

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MATERIALS AND METHODS

Clinical Isolates

A total of 100, nonduplicated isolates (50 RIF resistant and 50 RIF susceptible) from respiratory specimens of the patients with TB were studied. Their RIF resistance was determined by conventional DST (resistance ratio method). The results of DST were considered gold standard and were compared with those of three molecular assays: Xpert MTB/RIF assay, Sacace MTB Real-TM resistance (Sacace Biotechnologies, Como, Italy), and AdvanSure MDR-TB GenoBlot assay (LG Life Sciences, Seoul, Korea).

Xpert MTB/RIF Assay

The Xpert MTB/RIF assay is an automated assay in sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR. The system requires the use of single-use disposable Xpert cartridges that hold the PCR reagents and host the PCR process. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81-bp "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

Sacace MTB Real-TM Resistance

MTB Real-TM resistance is an in vitro allele-specific real-time PCR test for specific mutations in *rpoB*, *katG*, and *inhA* genes associated with INH or RIF resistance: *rpoB* codon 531 (two mutations: Ser-Leu and Ser-Trp), *rpoB* codon 526–1 (three mutations: His-Tyr, His-Asp, and His-Arg), *rpoB* codon 526–2 (three mutations: His-Leu, His-Asn, and His-Pro), *rpoB* codon 516 (one mutation: Asp-Val), *rpoB* codon 533 (one mutation: Leu-Pro), and *katG* codon 315 (three mutations: Ser-Thr, Ser-Asn, and Ser-Thr).

AdvanSure MDR-TB GenoBlot Assay

AdvanSure MDR-TB GenoBlot assay is a reverse-hybridization line blot assay using one-tube nested multiplex asymmetric PCR, targeting *rpoB*, *katG*, *inhA*, and *ahpC* genes. This assay has 21 strip lines, which include 14 for RIF resistance, 2 for INH high-level resistance, and 5 for INH low-level resistance.

Statistical Analysis

Sensitivities and specificities for each assay were calculated with 95% confidence interval (CI). The McNemar's test was used to decide the statistical difference between assays. Statistical analysis was performed using SPSS software, (version 12.0, SPSS Inc., Chicago, IL), MedCalc Statistical Software (version 11.2.1, MedCalc Software, Mariakerke, Belgium), and Analyse-it Method Evaluation Edition (Analyse-it Software, Ltd., Leeds, UK). *P*-values less than 0.05 were considered statistically significant.

RESULTS

Among the 50 RIF-resistant isolates, Xpert MTB/RIF assay detected the RIF resistance in 47 isolates, Sacace MTB Real-TM resistance in 45 isolates, and Advansure GenoBlot assay in 42 isolates (Table 1). False resistance was not detected in three assays with 50 RIF-susceptible isolates.

Sensitivities for RIF resistance detection of Xpert MTB/RIF assay, Sacace MTB Real-TM resistance, and Advansure GenoBlot assay were 94.0%, 91.8%, and 84.0%, respectively. Their specificities for RIF resistance detection were all 100%. Compared to culture-based DST, the results of Xpert MTB/RIF assay and MTB Real-TM resistance were not different, but Advansure GenoBlot assay showed a significant difference ($P = 0.0078$).

DISCUSSION

The Xpert MTB/RIF assay is a WHO-endorsed diagnostic tool and has many advantages, including simultaneous detection of MTB and RIF resistance, automated processing, reaction in disposable plastic cartridge, and rapid turnaround time (4–6). Recent studies have evaluated the performance of Xpert MTB/RIF assay for MTB detection, and the reported sensitivities were about 98.0–100.0% in smear-positive, culture-positive patients and 57.0–72.5% in smear-negative, culture-positive patients (5, 9–11). Recent reports also evaluated the performances of Xpert MTB/RIF assay for MTB detection in nonrespiratory specimens or in HIV-positive patients, and the results of these studies were variable and need to be confirmed through further studies (10, 12–14).

To the best of our knowledge, the studies on the performance of Xpert MTB/RIF assay for detection of RIF resistance are sparse (13), and most studies included only limited number of resistant isolates (14, 15). Moreover, the performances of MTB Real-TM resistance and AdvanSure MDR-TB GenoBlot assay have not been evaluated so far. This study has focused on the performance of detection of RIF resistance and compared the results of three molecular assays for resistance of RIF.

The Xpert MTB/RIF assay showed excellent sensitivity (94.0%) and specificity (100.0%) for RIF resistance. A

TABLE 1. Performances of Three Molecular Assays for Detection of RIF Resistance in Comparison to Culture-Based Method

Test method	No. of samples	True positive	False positive	True negative	False negative	Sensitivity (95% CI)	Specificity (95% CI)	P^a
Xpert MTB/RIF	100	47	0	50	3	94.0% (83.5–98.8)	100.0% (92.9–100.0)	NS
Sacace MTB Real-TM resistance	100	45	0	50	5	91.8% (80.4–97.7)	100.0% (92.9–100.0)	NS
AdvanSure MDR-TB GenoBlot	100	42	0	50	8	84.0% (70.9–92.8)	100.00% (92.9–100.0)	0.0078

[a]There was no statistical difference between the results for any two molecular assays ($P > 0.05$, McNemar's test).

recent study also showed similar performance of Xpert MTB/RIF assay (sensitivity 94.4% and specificity 98.3% for RIF resistance) (13). On the other hand, Salvo et al. (16) concerned the discrepant results between results of the Xpert MTB/RIF test for RIF resistance and those of conventional DST. They suggested that the results of Xpert MTB/RIF test need to be confirmed with a second test or conventional DST, before treatment for MDR TB.

MTB Real-TM resistance also showed excellent sensitivity and specificity (sensitivity 91.8% and specificity 100.0%) for RIF resistance, but its sensitivity was lower compared with that of Xpert MTB/RIF assay. AdvanSure MDR-TB GenoBlot assay showed lower sensitivity (84.0%) for RIF resistance compared with the other assays. Principle of AdvanSure MDR-TB GenoBlot assay is different from the other two assays. Blot hybridization does not require real-time PCR device, therefore it could be more optimal to the small-setting laboratory. Since we did not perform DNA sequencing and the results were compared with those of conventional DST, the reasons of false-negative results could be related to low sensitivity of assay to detect mutations or the resistance due to mechanisms other than covered mutations.

Overall, Xpert MTB/RIF assay and MTB Real-TM resistance showed excellent performance for detection of RIF resistance. The Xpert MTB/RIF assay is fast, technically simple and can be performed as a point-of-care test, while MTB Real-TM resistance and AdvanSure MDR-TB GenoBlot assay require the more complex processing step and separate DNA extraction step, which take more hands-on time and manual workload. Moreover, the result of AdvanSure MDR-TB GenoBlot assay needs to be further evaluated using more specimens with various susceptibilities. In conclusion, our data show that molecular assays for the detection of RIF resistance have various performances. The Xpert MTB/RIF assay shows the highest sensitivity among the three molecular assays and can be an effective choice in routine clinical laboratories.

ABBREVIATIONS

CI = confidence interval
DST = drug sensitivity testing
MDR = multidrug resistant
MTB = *Mycobacterium tuberculosis*
NS = not significant
RIF = Rifampin
TB = tuberculosis
XDR = extensively drug-resistant

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