Evaluation of Genotype® MTBDRplus Assay for Rapid Detection of Resistance to Isoniazid and Rifampin in Mycobacterium tuberculosis Isolates Collected in Tunisia

Manel Marzouk*, Imen Ben Kahla, Mohamed Dhaou, Asma Ferjani, Naila Hannachi and Jalel Boukadida

Abstract

TB diagnosis and resistance detection remain to suffer from complex techniques and long time taking. The rapid identification of drug-resistant strains of Mycobacterium tuberculosis is crucial for the timely initiation of appropriate antituberculosis therapy. Over the years, several new technologies have been proposed to accelerate and simplify the detection of resistant M. tuberculosis. In this context, we evaluated the Genotype MTBDRplus to detect resistance to isoniazid and rifampin in positive M. tuberculosis cultures. The performance of the Genotype MTBDRplus assay was compared with that of the phenotypic drug susceptibility tests, a gold standard culture based method. The Genotype MTBDRplus assay was quicker and more cost-effective for the detection of rifampin and isoniazid resistance, with a slightly lower detection of rifampin-resistant strains in our setting.

Keywords: Antituberculosis therapy; Genotype; Mycobacterium tuberculosis

Introduction

Tuberculosis (TB) remains a global health problem worldwide and the prevalence of TB is high among the developing world [1]. Multidrug-resistant (MDR) Mycobacterium tuberculosis organisms are defined as organisms resistant to both isoniazid (INH) and rifampicin (RIF). INH resistance is based on complex genetic alterations, most commonly in the katG and inhA genes [2,3]. RIF resistance is due to mutations within the 81 base pair (bp) hotspot region of the rpoB gene [4,5]. World Health Organization (WHO) estimates current MDR-TB rates in new and previously treated cases globally at 2.9% and 15.3% respectively [6]. In Tunisia, specifically 2.6% and 36% of newly and previously treated tuberculosis (TB) patients are estimated to be infected by MDR-TB isolates [7]. The diagnosis of MDR-TB is based on the mycobacterial culture and drug susceptibility testing (DST) on liquid or solid media and the results are too long (4-8 weeks at least) [8]. So, prompt but correct detection of MDR strains is essential for appropriate management. Since 2008, WHO recommended the use of molecular line probe assay for the diagnosis of MDR-TB [7].

The aim of this study was to evaluate the GenoType® MTBDRplus assay for rapid detection of resistant TB cases to INH and RIF in the central region of Tunisia.

Materials and Methods

GenoType® MTBDRplus assay (Hain Lifescience, Nehren, Germany)

The GenoType® MTBDRplus assay (Hain Lifescience, Nehren, Germany) was used according to the protocol recommended by the manufacturer. This kit is a commercially available assay that combines detection of Mycobacterium tuberculosis complex (MTBC) with prediction of resistance to RIF and INH. The GenoType® MTBDRplus strip contains 17 probes, including amplification and hybridization controls to verify the test procedures. For the detection of rifampicin resistance, eight rpoB wild-type probes (probes WT1 to WT8) encompass the region of the rpoB gene encoding amino acids 509-533. Four probes (probes rpoB MUT D516V, rpoB MUT H526Y, rpoB MUT H526D and rpoB MUT S531L) specifically target the most common mutations conferring resistance to RIF. For the detection of INH resistance, one probe cover the wild-type S315 region of katG, while two others (probes katG MUTT1 and MUTT2) are designed to assess the AGC-to-ACC (S315T) and the AGC-to-ACA (S315T) mutations. Furthermore, the promoter region of the inhA gene is included on the new strip and encompasses the regions from positions -15 to -16 for the inhA WT1 probe and positions -8 for the inhA WT2 probe. Four mutations (-15C/T, -16A/G, -8T/C and -8T/A) can be targeted with the inhA MUT1, MUT2, MUT3A and MUT3B probes. Again, either the absence of one or more wild-type probe(s) or the presence/staining of mutant probes were indicative of the resistant strain.

Population study

This study was conducted in the coastal region of east-central Tunisia, about 500 000 people.

It included TB positive cultures diagnosed during six months on the basis of bacteriological criteria in the Microbiology and Immunology Laboratory of Farhat Hached Hospital in Sousse. This hospital serves 557 000 people, recruiting all TB patients from Central Tunisia. TB incidence in Tunisia has been estimated to be 25 per 100 000 population.

Patients included in the study were both new or previously treated suspected DR-TB cases from all age groups, in whom TB was confirmed by culture and in whom DST against MTBC strains had been performed. Those infected with mycobacteria other than TB (MOTT) were not included in this study.

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This study was conducted after approval by the local research ethics committee.

**Mycobacterial strains**

The samples were inoculated on Löwenstein Jensen (LJ) and Coletos culture media and incubated at 37°C in an inclined position up to 3 months. All the positive vials were subjected to smear microscopy for the presence of acid fast bacilli (AFB). Positive cultures for mycobacterium were typed by conventional biochemical techniques and confirmed by the GenoType molecular kits (Hain Lifescience GmbH, Nehren, Germany). Susceptibility to the first line drugs was testing by the phenotypic DSTs by 1% proportional method (Biorad). This technique was used as the reference standard.

**Statistical analysis**

The EPI6.EXE epidemiology software was used for data analysis.

**Results**

Among a total of 1895 specimens collected from 1543 (81.4%) pulmonary TB (PTB) and 352 (18.6%) extra-pulmonary TB (EPTB) patients of highly suspected cases of treatment defaulters, re-treatment and relapse cases, 168 (8.9%) cultures were positive for mycobacteria which were typed by conventional biochemical techniques and confirmed by the GenoType MTBC, GenoType CM, and GenoType AS molecular kits (Hain Lifescience, Nehren, Germany): 160 (95.2%) strains confirmed as MTBC and 8 (4.8%) were confirmed as one of the species of MOTT.

Only one specimen per patient was used in the present analysis. Thus, we included 56 positive cultures: PTB (n=35) and EPTB (n=21), newly diagnosed cases (n=43) and re-treatment cases (n=13).

A total of 20 strains were identified as resistant to one or more than one anti-tubercular drug. Out of 20 resistance cases, only one strain was identified as MDR-TB and 19 strains were mono-resistant to either one anti-tubercular drug. Out of 20 resistance cases, only one strain newly diagnosed cases (n=43) and re-treatment cases (n=13). Thus, we included 56 positive cultures: PTB (n=35) and EPTB (n=21), as MTBC and 8 (4.8%) were confirmed as one of the species of MOTT.

Comparison of MTBDRplus results and phenotypic DSTs.

<table>
<thead>
<tr>
<th>MTBDRplus</th>
<th>Phenotypic DST</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>VPP</th>
<th>VPN</th>
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<tbody>
<tr>
<td>INH</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>S</td>
<td>43 0</td>
<td>100% 100% 100% 100%</td>
<td></td>
<td></td>
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<tr>
<td>R</td>
<td>0 13</td>
<td>94.7-100% 94.7-100% 94.7-100% 98.2-100%</td>
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<tr>
<td>RIF</td>
<td>S (n=49) R (n=7)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>49 1</td>
<td>85.7% 100% 100% 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0 6</td>
<td>42-99.2% 91-100% 52-100% 88-99.9%</td>
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</tbody>
</table>

Table 1: Comparison of MTBDRplus results and phenotypic DSTs.

There were no strains showing negative signals for the wild-type probes in drug-sensitive strains.

**Discussion**

TB diagnosis and resistance detection remain to suffer from complex techniques and long-time taking. These facts are highly affecting and compromising the management of TB infections. In this context, we evaluated the MTBDRplus assay in an area with high TB incidence.

The results of the present study have shown that the MTBDRplus assay is easy to perform and has the capability for the rapid detection of RMP- and INH-resistant *M. tuberculosis*. With respect to culture isolates, the sensitivities of the MTBDR assay for the detection of RIF resistance were recently reported to be in the range of 95% to 99% [9-12]. Both the rarity of RMP-resistance-associated mutations in codons other than the *rpoB* 81-bp hot spot region and the rarity of silent mutations in the hot spot region are responsible for the high rate of detection of RMP resistance by investigation of this region [12-14]. However, in our study sensitivity of the MTBDRplus assay measured for resistance to RIF was lower than those reported. Our results confirmed that the distribution can differ in some settings [15]. Although the prevalence of false-resistant and false-susceptible results seems to be infrequent, some authors have discussed the relevance of mutations in codon 533 to RMP resistance [16]. *M. tuberculosis* isolates with mutations in codon 533 showed RMP susceptibility [16,17] or low-level or high-level resistance [18,19]. The recommendation in these cases is either to control the result by DNA sequencing or confirm the result by the conventional DST (article).

Some authors discussed a main limitation of the MTBDR test system, which is the low sensitivity for the detection of INH resistance [11,20]. In our study we could show that sensitivity and specificity of detection of INH resistance were excellent. Although the number of cases used in the present study is too small for a statistical analysis, this trend can be approved.

Although the MTBDRplus assay has limitations, as, the assay detects resistance only when the origin of resistance is in the *M. tuberculosis rpoB*, *katG*, and *inhA* regions, it saves several weeks of time which is required for primary isolation and conventional DST.

In conclusion, the high sensitivities of RIF and INH resistance detection is a merit point for the MTBDRplus test, as it can be used for the detection of RIF and INH resistances in our area with high TB incidence.

Acknowledgments

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**References**


