Detection of Drug-Resistant Mycobacterium tuberculosis Strains by Genotype MtbdRplus Assay

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Abstract

Rapid identification of drug-resistance in Mycobacterium tuberculosis infections is crucial for early treatment and control of transmission. The MTBDR plus assay is potentially a practical and rapid alternative to the slower phenotypic drug susceptibility testing (DST) for detection of drug resistant tuberculosis (TB). The purpose of this study was to determine the diagnostic accuracy of the MTBDRplus assay.

Sixty strains of Mycobacterium tuberculosis isolated from TB patients diagnosed in different parts of Poland in the two year period 2015-2016 were used. All strains were analyzed for drug susceptibility using both a conventional indirect proportion method and the MTBDRplus assay.

The MTBDRplus assay identified 72% (43) of isolates as multidrug-resistant TB (MDR-TB), 7% (4) as INH-monoresistant and 5% (3) as RIF-monoresistant. The sensitivity and specificity of the MTBDRplus assay were 96% and 100% with respect to both RIF- and INH-resistance, while sensitivity of the assay was lower (91%) for MDR-TB. In our study, codon 531 of rpoB gene and codon 315 of katG gene were found to have highest mutational frequency for RIF resistance (74% – 34 strains) and INH resistance (89% – 42 strains) respectively.

The use of MTBDRplus assay as a supplement to the gold standard DST in obtaining a rapid preliminary DST result may contribute to early optimization of treatment.

ABBREVIATIONS

DST: Drug Susceptibility Testing; TB: Tuberculosis; MDR-TB: Multi-Drug Resistant Tuberculosis; RIF: Rifampicin; INH: Isoniazid; HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; RR-TB: Rifampicin-Resistant Tuberculosis; WHO: World Health Organization; L–J: Löwenstein-Jensen; MTBC: Mycobacterium Tuberculosis Complex; ATTC: American Type Culture Collection; DNA: Deoxyribonucleic Acid; PCR: Polymerase Chain Reaction; AM-A: Amplification Mix A; AM-B: Amplification Mix B; MDR: Multi-Drug Resistant; RNA: Ribonucleic Acid; ACP: Acyl Carrier Protein; WT: Wild Type; MUT: Mutant; UK: Unknown Mutation Characterized by no Hybridization to wild-type Probes nor to any of Mutation Probes; R: Resistant; D: Aspartic Acid; V: Valine; H: Histidine; S: Serine; L: Leucine; T: Threonine; C: Cysteine

INTRODUCTION

Tuberculosis (TB) is a significant global health problem. It is currently one of the top 10 causes of death worldwide, ranking above HIV/AIDS as a leading cause of mortality from an infectious disease [1].

In 2015 there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) caused by strains resistant to at least rifampicin and isoniazid, the two most powerful anti-TB drugs. An additional 100 000 new cases presented with rifampicin-resistant TB (RR-TB) [1]. Overall, an estimated 3.9% of new cases and 21% of previously treated cases had MDR/RR-TB [1].

According to the data from the Central Register of Tuberculosis, in Poland in 2015 there were an estimated 140 TB cases resistant to at least one anti-TB drug and 38 patients with MDR-TB, representing 2.1% and 0.6% of all of the registered cases of tuberculosis respectively [2].

Drug resistance in tuberculosis is a major global public health challenge globally. Early and accurate detection of drug resistance is one of the priorities of TB control programs [1]. Phenotypic drug susceptibility testing (DST) of M. tuberculosis is considered the gold standard [3]. However, this method is technically demanding, and is time consuming: DST on solid media by the indirect proportion method takes at least 4 weeks, while the use of liquid media in automatic systems can take up to 14 days [4]. The slow diagnosis of drug resistance can result in delay of proper treatment, increasing the risk of treatment failure and onward...
transmission of drug-resistant TB [5]. Molecular methods are an attractive alternative to reduce the time of detection of drug-resistant strains, which may contribute to the effective control of drug-resistant TB [6]. Since 2008, the World Health Organization (WHO) recommends use of the GenoType MTBDR plus line-probe assay for rapid detection of rifampicin and isoniazid resistance [7]. This assay provides information on the most common mutations and levels of resistance within 24 hours [8].

The aim of this study was to evaluate the ability of the GenoType MTBDR plus assay to detect rifampicin and isoniazid resistance among M. tuberculosis strains isolated in Poland.

MATERIALS AND METHODS

Clinical strains

Sixty clinical isolates of M. tuberculosis, collected by the National Tuberculosis Reference Laboratory in Warsaw, were used in this study.

All samples were cultured according to standard mycobacteriological procedures. Species identification was performed with a niacin test and the GenoType MTBC assays (Hain Lifescience, Germany).

Drug susceptibility

The drug susceptibility testing (DST) to rifampicin (RIF) and isoniazid (INH) was performed using a conventional indirect proportion method on Löwenstein-Jensen (L–J) medium. The following drug concentrations were used: 40µg/ml (RIF) and 0.2µg/ml (INH). Resistance was defined by the growth of ≥ 1% of a bacillary inoculum on drug-containing medium compared to growth on the drug free control medium.

The reference strain M. tuberculosis H$_{37}$R$_{v}$ ATCC 25618, susceptible to all the drugs tested, was used as a quality control strain.

GenoType MTBDR plus assay

The GenoType MTBDR plus (v2.0) assay (Hain Lifescience, Nehren, Germany) was carried out according to the manufacturer’s instructions [9]. The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization. Genomic DNA was extracted from bacterial culture using the GenoLyse kit according to the manufacturer’s instructions [10]. Following PCR amplification, the reverse hybridization step and the interpretation of the hybridization results were done as previously described [11].

Data analysis

Data were entered and analyzed in MS Excel 2007. The sensitivity and specificity were calculated for each drug compared to the gold standard of culture-based DST.

RESULTS AND DISCUSSION

Drug resistance appears in Mycobacterium tuberculosis through the selection of spontaneously preexisting drug-resistant mutants [12]. In a wild-type bacillary population, these mutants are present at a predictable frequency of between 1/10$^{-6}$ and 1/10$^{-8}$ and are selectively amplified by monotherapy or inadequate combination therapy [13]. Delay in the confirmation of drug resistance results in a delay in initiation of treatment or ineffective chemotherapy, which is the major factor that contributes to drug-resistant TB outbreaks. At present drug resistance can be assessed rapidly within 1 day by molecular assays such as MTBDR plus, Xpert MTB/RIF, INNO-LiPA Rif. TB, and MTBDRsl [14].

The MTBDR plus assay is a rapid and reliable method for the detection of the most common and frequent mutations leading to rifampicin and isoniazid resistance [6]. This assay is now used routinely in many countries due to its shorter turnaround time, making it a more effective procedure [5].

In this study, out of 60 M. tuberculosis isolates tested for drug resistance by the GenoType MTBDR plus assay and the Löwenstein-Jensen medium-based indirect proportion method, 50 were obtained from patients with drug-resistant TB, and 10 from patients with drug-sensitive TB. Patients were diagnosed in different parts of Poland in a 2 year period (2015-2016). Among the patients were 18 women between the ages of 16-66, and 42 men between the ages of 12-72 years. There were 47 newly detected and 13 previously treated patients.

The results of the GenoType MTBDR plus assay showed that 72% (43) of M. tuberculosis strains tested in this study were resistant to RIF and INH (MDR-TB). The GenoType MTBDR plus assay also detected 4 (7%) INH-monoresistant, 3 (5%) RIF-monoresistant. L–J proportion DST method identified 78% (47), 3% (2), and 2% (1) of analyzed strains as MDR-TB, RIF-monoresistant and INH-monoresistant strains, respectively. Ten strains were identified as susceptible by both methods (Table 1).

We observed that the GenoType MTBDR plus assay results showed good concordance with the conventional DST, with the additional advantage of a shorter turnaround time.

The results of the evaluation of the performance of the GenoType MTBDR plus assay in detecting drug resistance are summarized in Table (2).

The concordances of the GenoType MTBDR plus assay and the conventional DST for the detection of RIF and INH resistance were 96%. There were 4% discrepant isolates for both RIF and INH resistance, which were resistant in L–J-DST but sensitive in the GenoType MTBDR plus assay.

The concordance of the two tests in detecting MDR M. tuberculosis strains was 91%. Divergent results were obtained for 4 strains, which were identified as MDR-TB by L–J proportion method but in the GenoType MTBDR plus assay as INH-monoresistant (2 isolates) and RIF-monoresistant (2 isolates). Discordance of the results obtained by genotypic and phenotypic DST may be due to mutations in other gene regions which are not targeted by the assay [15].

The sensitivity and specificity of the GenoType MTBDR plus assay for detection of RIF- and INH-resistant M. tuberculosis isolates in this study were very high. The sensitivity and specificity of the GenoType MTBDR plus assay for the detection of RIF-resistant M. tuberculosis isolates were 96% and 100%,
The molecular mechanisms of isoniazid resistance are not fully understood, although numerous studies have linked them to distinct mutations in various genetic loci of the *M. tuberculosis* genome. Two loci most commonly affected are the katG gene, encoding catalase-peroxidase, which transforms isoniazid to its pharmacologically active form and the inhA gene (with the mabK-inhA promoter region), coding for enoyl-acyl carrier protein (ACP) reductase, a mycolic acid biosynthetic pathway enzyme [39,40]. In our current study, mutations in the katG gene were observed in 89% of INH resistant isolates (42) (Table 3): all of them possessed the S315T1 mutation in katG region that results in amino acid serine substitution to threonine. Huyen et al. [41], and Van Rie et al. [42], reported similar results, whereas Barnard et al. [11], reported that this mutation occurred less frequently (37.6%). In a study reported by Vijdia et al. [19], in Lithuania, 100% of the isolated strains of *Mycobacterium tuberculosis* complex had the S315T mutation. In previously published results, the incidence of this mutation is found 65% to 93.3% of the time [11,29,43,44].

In the MTBDR-plus assay in our study, an unusual pattern was observed in three isolates in which WT katG deletion band was not accompanied by a concomitant presence of a MUT band (Table 3). The same observation was reported in other study from Central India [8]. This may be due to mutation of one or more genes other than katG and inhA, notably atbpC, cpyk, kasA, and ndh [15].

Detection of the mutation in the inhA gene was less frequent than in katG. Eleven (23%) of the INH resistant strains showed a mutation in inhA gene. Combined mutation in inhA and katG gene were found in 19% of INH-resistant strains [9]. This observation was also reported in other studies [16,43,46]. The prevalence of mutations in the katG and inhA genes seems to vary widely in different geographic locations [47]. 97% of katG mutations and 24% of inhA mutations were found in the INH-resistant isolates from KwaZulu-Natal [48], whereas Van Rie et al., reported 72% of katG mutations and 2% mutations in the inhA gene of INH-resistant isolates in the Western Cape province of South Africa [42]. Extensive studies from other countries have confirmed this variability in the contribution of different mutations to INH resistance [44,49].

**CONCLUSION**

The high sensitivity and specificity of the MTBDR-plus assay for RIF and INH resistance suggests that this assay should be used as a supplement to the gold standard DST to obtain a rapid preliminary DST result to early optimization of treatment.
Table 3: Mutation patterns of the GenoType MTBDRplus assay.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Failing wild type band(s)</th>
<th>Gene regions</th>
<th>Developing mutation band</th>
<th>Mutation</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB</td>
<td>WT2/WT3</td>
<td>510-517</td>
<td>-</td>
<td>-</td>
<td>3 (7%)</td>
</tr>
<tr>
<td></td>
<td>WT3/WT4</td>
<td>513-519</td>
<td>MUT1</td>
<td>DS16V</td>
<td>7 (15%)</td>
</tr>
<tr>
<td></td>
<td>WT7</td>
<td>526-529</td>
<td>MUT2B</td>
<td>HS26D</td>
<td>2 (4%)</td>
</tr>
<tr>
<td></td>
<td>WT8</td>
<td>530-533</td>
<td>MUT3</td>
<td>SS31L</td>
<td>34 (74%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All RIF(^{+}) isolates: 46</td>
</tr>
<tr>
<td>katG</td>
<td>WT</td>
<td>315</td>
<td>MUT1</td>
<td>S315T1</td>
<td>42’ (89%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All INH(^{+}) isolates: 47</td>
</tr>
<tr>
<td>inhA</td>
<td>WT1</td>
<td>-15</td>
<td>MUT1</td>
<td>C-15T</td>
<td>11’ (23%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All INH(^{+}) isolates: 47</td>
</tr>
</tbody>
</table>

*Nine isolates had mutations in katG and inhA genes.
C: Cysteine; D: Aspartic acid; H: Histidine; INH: Isoniazid; L: Leucine; RIF: Rifampicin; S: Serine; T: Threonine; UK: Unknown Mutation characterized by no hybridization to wild-type probes nor to any of mutation probes; V: Valine; WT: Wild Type

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REFERENCES


