First evaluation in Argentina of the GenoType® MTBDRplus assay for multidrug-resistant Mycobacterium tuberculosis detection from clinical isolates and specimens

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ABSTRACT

Tuberculosis (TB) and multidrug and extensively drug-resistant (DR) TB are important public health problems that are spreading worldwide. The aims of this study were to determine the sensitivity and specificity of the GenoType® MTBDRplus assay from smear-positive clinical specimens and isolates and to explore its possible application in routine work. Clinical samples were previously decontaminated using NaOH-n-acetyl-l-cystein or NaOH-CiNa hypertonic solution for Ziehl-Neelsen staining and cultures. The leftover sediments of smear-positive samples were stored at –20 ºC, 70 of which were selected to be included in this study according to their DR profile. Thirty DR Mycobacterium tuberculosis isolates were also assessed. Sequencing was used as gold standard to detect mutations conferring isoniazid (INH) and rifampicin (RIF) resistance. Valid results were obtained in 94.0 % of the samples and 85.5 % (53/62) of the INH-R samples were properly identified. Mutations in the katG S315T gene and inhA C-15T gene promoter region were present in 59.7 % (37/62) and 25.8 % (16/62) of the INH-R samples, respectively. The system could also identify 97.7 % (41/42) of the RIF-R samples; the mutations found were rpoB S531L (66.7 %, 28/42), D516V (19.0 %, 8/42), H526Y and S531P/W (4.8 %, 2/42 each one), and S522L/Q (2.4 %, 1/42). A 98.8 % concordance between the genotype assay and sequencing was obtained. GenoType® MTBDRplus has demonstrated to be easy to implement and to perform in clinical laboratories and useful for a rapid detection of DR M. tuberculosis from decontaminated sputa and clinical isolates. Therefore, this assay could be applied as a rapid tool to predict INH-R and/or RIF-R in DR risk cases.

Key words: molecular detection, multidrug-resistant tuberculosis, GenoType® MTBDRplus

RESUMEN

Primera evaluación en Argentina de GenoType® MTBDRplus para la detección de Mycobacterium tuberculosis multidrogo-resistente desde aislamientos y especímenes clínicos. La tuberculosis (tBC), y la tBC multi y extensivamente drogo-resistentes (dR) tBC son importantes problemas de salud pública mundial. El objetivo de este estudio fue determinar la sensibilidad y especificidad del sistema GenoType® MTBDRplus a partir de esputos (baciloscopía positiva) y aislamientos clínicos de Mycobacterium tuberculosis, explorando su aplicación clínica. Previo a la tinción de Ziehl-Neelsen y al cultivo, las muestras fueron descontaminadas mediante NaOH-N-acetil-L-cisteina o la solución hipertónica NaOH-NaCl. Los sedimentos remanentes se conservaron a –20 ºC, y 70 de ellos fueron incluidos en este estudio según su perfil de DR. Treinta cepas de M. tuberculosis DR fueron también evaluadas. La secuenciación fue utilizada como método de referencia para la detección de mutaciones que confieren resistencia a isoniazida (INH) y rifampicina (RIF). Se obtuvieron resultados válidos en el 94,0 % de las muestras, identificándose al 85.5 % (53/62) de las INH-R. La mutación katG S315T estuvo presente en 59,7 % (37/62), y la mutación C-15T del promotor del gen inhA en 25,8 % (16/62) de las mismas. El sistema identificó el 97,7 % (41/42) de las muestras RIF-R. Las mutaciones encontradas fueron rpoB S531L (66,7 %, 28/42), D516V (19,0 %, 8/42), H526Y y S531P/W (4,8 %, 2/42 cada una ellas) y S522L/Q (2,4 %, 1/42). La concordancia entre el GenoType y la secuenciación fue del 98,8 %. El sistema GenoType® MTBDRplus resultó ser útil, fácil de realizar e implementar para la detección rápida de M. tuberculosis DR. Por lo tanto, este ensayo podría ser aplicado como una herramienta rápida para el diagnostico de TBC DR, principalmente en aquellos casos asociados a factores de riesgo.

Palabras clave: detección molecular, tuberculosis multidrogo-resistente, GenoType® MTBDRplus
INTRODUCTION

Tuberculosis (TB) and especially multidrug-resistant TB (MDR-TB), TB resistant to isoniazid (INH) and rifampicin (RIF), and the most recently described extensively drug-resistant TB (XDR-TB) caused by MDR Mycobacterium tuberculosis, which is also resistant to kanamycin, amikacin or capreomycin and one fluoroquinolone, are important public health problems that are spreading worldwide (16, 17). Currently, the World Health Organization (WHO) estimates that approximately 500,000 new cases of MDR-TB occur each year (32), 5 to 7 % of which might evolve to XDR (29, 31). These alarming figures have highlighted the urgency for rapid screening methods to detect DR in a short turnaround time, especially in those patients with high risk of having MDR/XDR-TB, such as potential institutional transmission and cases with HIV co-infection (30).

Argentina is a country with middle incidence TB rate (23.2 per 100,000 inhabitants in 2010), (14) being Buenos Aires the province having the highest number of cases, 4,298 and an incidence of 28.1 per 100,000 inhabitants. Moreover, 80 % of these cases are concentrated in the overcrowded suburban areas of Buenos Aires City (23).

According to the last national drug-resistance (DR) surveillance, it was observed that 15.5 % of patients with a previous anti-TB treatment history and 2.2 % of the new cases were caused by MDR strains and that the prevalence of INH resistance is about 17 % (26).

INH and RIF are two of the main anti-tuberculosis first line drugs used in the standard treatment regime for TB. Resistance to INH is generally the first step for developing MDR-TB and resistance to RIF is commonly a marker of MDR-TB, since more than 90 % of the M. tuberculosis RIF-R strains are also resistant to INH. Furthermore, INH is currently used for chemoprophylaxis in children with a proven fully drug-susceptible TB contact.

When resistance to INH and RIF occurs with or without associated resistance to any other drug, the case is defined as a MDR-TB case (16, 17).

As it was previously described, DR in M. tuberculosis is mainly caused by point mutations in certain genes (3). A base-pair change in codon 315 of the katG gene is the most common mutation associated to INH-R, followed by mutations in position -15 of the inhA gene promoter region (4, 10, 13). Mutations within the hot spot region of the rpoB gene of M. tuberculosis, mainly in codons 516, 526 and 531 are responsible for about 95 to 97.0 % of RIF-R (5, 18, 21).

In our setting, detection of DR-TB is mainly based on phenotypic drug susceptibility testing (DST). Faster methods for rapid detection of DR-TB to avoid incorrect anti-TB treatment and to prevent transmission of resistant forms of the disease in the community are urgently needed.

The GenoType® MTBDRplus assay is a rapid molecular method that can be used from M. tuberculosis clinical isolates or directly from pulmonary smear-positive clinical specimens. This method has been designed to identify RIF-R by the detection of the most common mutations within the hot spot region of the rpoB gene. For the INH-R detection, the katG gene including codon 315 and the promoter region of the inhA gene, position –15 are examined.

The GenoType® MTBDRplus assay is a rapid molecular method that can be used from M. tuberculosis clinical isolates or directly from pulmonary smear-positive clinical specimens and isolates, in comparison to conventional drug susceptibility testing (DST) methods and sequencing of katG, inhA (promoter region) and rpoB genes of M. tuberculosis; b) to explore its possible application in routine work and under clinical conditions.

MATERIALS AND METHODS

It was a retrospective study that included smear-positive clinical specimens and M. tuberculosis isolates obtained from patients diagnosed in the Reference Laboratory of Tuberculosis Control Program at Dr. Cetrangolo Hospital of Buenos Aires Province.

Clinical isolates. A total of 30 previously characterized DR strains of M. tuberculosis were assessed.

Clinical specimens. In order to obtain isolates, clinical samples were decontaminated by the NaOH-N-acetyl-L-cystein or by the hypertonic solution of NaCl following previously described protocols (20). The concentrated sediment was used for preparing smears for Ziehl-Neelsen staining (15) and for cultures in solid [Löwenstein-Jensen (LJ), and Stonebrink (Sk)], and liquid media BACTEC MGIT 960 system (BD, Buenos Aires, Argentina) (8).

The leftover sediments of smear-positive samples were stored at –20°C to be used with the GenoType assay.

Drug-susceptibility testing to first anti-TB drugs were
performed—from cultures and in all specimens—by the indirect proportion method on LJ, the BACTEC MGIT 960 SIRE kit (BD, Argentina) and in some cases by the microplate colorimetric method (2, 9, 19).

According to the drug resistance pattern obtained by the DST, 70 frozen leftover sediments of smear-positive samples were selected to be tested by the GenoType system.

Table 1 shows the total number of clinical specimens and isolates included in the study and their drug-resistant profiles.

### Table 1. Samples included in the study and their drug-resistant profile

<table>
<thead>
<tr>
<th>Drug resistance</th>
<th>Material</th>
<th>INH</th>
<th>RIF</th>
<th>MDR</th>
<th>XDR</th>
<th>DS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>dS+</td>
<td>15</td>
<td>1</td>
<td>12</td>
<td>2</td>
<td>16</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>dS++</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>dS+++</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Isolates</td>
<td>7</td>
<td>4</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>27 (27)</td>
<td>6 (6)</td>
<td>38 (38)</td>
<td>2 (2)</td>
<td>27 (27)</td>
<td>100 (100)</td>
<td></td>
</tr>
</tbody>
</table>

The pluses indicate bacillary load within the clinical specimen (+: about 1x10^4 bacilli per ml of sputum; ++: ≥ 1x10^5 bacilli/ml; +++: ≥ 1x10^6 bacilli/ml); dS: decontaminated sputa; INH: isoniazid; RIF: rifampicin; MDR: multidrug-resistant; XDR: extensively drug-resistant; DS: drug-susceptible.

The fully drug-susceptible (DS) reference strain *M. tuberculosis* H37Rv ATCC 29274 was used as control for DST.

### M. tuberculosis identification

The molecular technique spoligotyping (27), which is specific for the differentiation of most of the members of the *M. tuberculosis* complex, was performed on clinical isolates (30 prior clinical isolates plus the 70 cultures grown from the decontaminated sputa tested) to confirm that they were all *M. tuberculosis*.

### DNA sequencing

This technique was used as a gold standard molecular method to detect mutations conferring INH and RIF resistance. The *M. tuberculosis* clinical isolates and the cultures obtained from the decontaminated sputa included in the study were sequenced.

To characterize the mutations conferring INH and RIF-R in the samples included in the study, *M. tuberculosis* clinical isolates and cultures obtained from the decontaminated sputa were sequenced. To accomplish the above mentioned aim, a 435 bp fragment containing codon 315 of the katG gene, 648 bp of the inhA promoter (including position −15) and 250 bp of the flanking region of the “hot spot” rpoB gene were amplified and sequenced.

Both DNA strands were sequenced in all cases. H37Rv ATCC 27294 was used as wild type (WT) control for sequencing.

Table 2 shows the primers used for PCR and sequencing of the studied genes. These primers and the PCR protocols used for sequencing were adopted from previous studies (13).

### Table 2. Primers used for PCR and DNA sequencing of *M. tuberculosis* drug-resistant genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers (5´-3´)</th>
</tr>
</thead>
</table>
| katG | F: GCAGATGGGGGTGTAGTCTACG  
R: AACGGTTCCGGGATGGTG |
| inhAP| R: CTCGCCGATGCCCGTGGAGC |
| rpoB | F: GTCGGCCGAGCATCAAGGA  
R: TGACCGCGCGTACAC |

F: primer forward; R: primer reverse

The MyCycler thermal cycler (BioRad Richmond, CA, USA) was used to perform the PCR reactions. PCR products were purified by the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) and quantified on a 0.8 % agarose gel (BioRAD). The purified PCR products were later sequenced using a DNA sequencer 3130xl Genetic Analyzer (Applied Biosystems, Buenos Aires, Argentina). Double- stranded DNA was sequenced for each isolate in order to avoid discrepancies and the results were analyzed by the Basic Local Alignment Search Tool (National Center for Biotechnology Information, Bethesda, MD, USA).

### GenoType® MTBDRplus assay

The previously mentioned 100 samples were tested by the GenoType assay according to the manufacturer’s instructions (Hain Lifescience). In order to obtain DNA from the clinical specimens, the decontaminated sputa stored at -20°C were thawed and 500 µl of each were centrifuged during 15 min at 10,000 x g, then the pellet was resuspended in 100 µl of molecular biology-
grade water, heated at 95 °C for 20 min, sonicated (Lysort™ LCx Probe System, Abbott Laboratories) for 15 min and centrifuged for 5 min at 13,000 x g.

To obtain DNA from clinical isolates, 2 colonies from solid media were resuspended in 300 µl of molecular biology-grade water and heated at 95 °C during 20 min, then the samples were centrifuged for 5 min at 13,000 x g.

The PCR mixture contained 5 µl of crude extracted DNA, 35 µl of a primer nucleotide mixture (provided by the kit), 5 µl of 10X PCR buffer containing 15 mM MgCl₂, 2 µl of 25 mM MgCl₂, 1 U hot start Taq polymerase (QIAGEN, GmbH, Hilden, Germany) and molecular-grade water to a final volume of 50 µl.

The thermal cycler parameters consisted of 15 min of initial hot start Taq polymerase activation and DNA denaturation at 95 °C, followed by 10 cycles of 30 s at 95 °C and 2 min at 58 °C; then 20/30 cycles (isolates/clinical samples) of 25 s at 95 °C, 40 s at 53 °C and 40 s at 70 °C; finally, 8 min at 70 °C for elongation.

RESULTS

Molecular identification of the samples

All 100 specimens, both the 30 previously obtained isolates and the 70 sputa cultured as part of the routine laboratory procedures, were identified as M. tuberculosis by spoligotyping.

GenoType® MTBDRplus assay results

Valid results were obtained in 94 out of 100 (94 %) samples included in the study.

Six decontaminated smear-positive sputa classified as one plus (+, about 10,000 bacilli per ml of sputum) from 2 INH-R specimens, 1 RIF-R, 2 MDR and 1 XDR did not show any signals on the GenoType strip for any one of the three different genes.

Table 3 shows the mutations found by the GenoType assay for the samples that yielded valid results.

By using the GenoType assay, we could properly identify 85.5 % (53/62) of the INH-R samples detected by DST.

The mutation S315T in the katG gene was present in 59.7 % (37/62) of the INH-R samples, whereas the mutation C-15T of the inhA gene promoter region was in 25.8 % (16/62) of the INH-R specimens.

In 2 INH-R samples, the GenoType assay detected an unknown mutation in katG315 but AGC315AGA mutation was later confirmed by sequencing.

Regarding the rpoB gene mutations, the GenoType system identified 97.7 % (41/42) of the RIF-R samples. The S531L was the main mutation found in the rpoB gene (66.7 %, 28/42) of the RIF-R samples; 19 % (8/42) presented the D516V mutation, mutations H526Y and S531P/W were both found in 4.8 % (2/42) each one, and the S522L/Q mutation was present only in one sample (2.4 %, 1/42).

All DS clinical specimens and H37Rv strains assessed in the study showed the WT sequence for all the fragments of the three genes included in the strips.

Being a retrospective study, the specificity was evaluated by taking into account the DS isolates

<table>
<thead>
<tr>
<th>DR(1)</th>
<th>N</th>
<th>KatG</th>
<th>InhAP</th>
<th>Mutated genes</th>
<th>rpoB</th>
<th>WT profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S315T</td>
<td>C-15T</td>
<td>D516V</td>
<td>S522L/</td>
<td>H526Y</td>
</tr>
<tr>
<td>INH(2)</td>
<td>25</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RIF</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MDR</td>
<td>36</td>
<td>23</td>
<td>11</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>XDR</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTS</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>94</td>
<td>37</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Overall detection (%)</td>
<td>(100.0)</td>
<td>(59.7)</td>
<td>(25.8)</td>
<td>(19.0)</td>
<td>(2.4)</td>
<td>(4.8)</td>
</tr>
</tbody>
</table>

N: number; WT: wild type; INH: isoniazid; RIF: rifampicin; MTS: fully drug-susceptible; DR: drug-resistance; MDR: multidrug-resistant; XDR: extensively drug-resistant; 1: DR based on phenotypic drug-susceptibility testing (indirect proportion method on Löwenstein Jensen and the BACTEC MGIT 960 SIRE kit); 2: two DS carried another mutation in katG315; 3: one sample showing M. tuberculosis XDR profile by DST had the GAC516GTC mutation of the rpoB by sequencing, which was detected as WT by the GenoType assay.

<p>| Table 3. Mutations found in clinical specimens and strains with GenoType®MTBDRplus assay |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>DR(1)</th>
<th>N</th>
<th>KatG</th>
<th>InhAP</th>
<th>Mutated genes</th>
<th>rpoB</th>
<th>WT profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S315T</td>
<td>C-15T</td>
<td>D516V</td>
<td>S522L/</td>
<td>H526Y</td>
</tr>
<tr>
<td>INH(2)</td>
<td>25</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RIF</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MDR</td>
<td>36</td>
<td>23</td>
<td>11</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>XDR</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTS</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>94</td>
<td>37</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

N: number; WT: wild type; INH: isoniazid; RIF: rifampicin; MTS: fully drug-susceptible; DR: drug-resistance; MDR: multidrug-resistant; XDR: extensively drug-resistant; 1: DR based on phenotypic drug-susceptibility testing (indirect proportion method on Löwenstein Jensen and the BACTEC MGIT 960 SIRE kit); 2: two DS carried another mutation in katG315; 3: one sample showing M. tuberculosis XDR profile by DST had the GAC516GTC mutation of the rpoB by sequencing, which was detected as WT by the GenoType assay.
from clinical specimens, the reference strain H37Rv and considering the DR patterns of the isolates. For instance: one resistant strain to RIF only should have wild type (WT) \textit{katG} and \textit{inhA} promoter genes, while a strain only resistant to INH should show a WT \textit{rpoB} gene. No false positive signals were observed in any case, therefore the specificity of the GenoType assay in this study was considered to be 100 %.

**Concordance between GenoType® MTBDRplus assay and DNA sequencing**

DNA sequencing results were obtained in 84 out of the 100 samples studied by the GenoType assay.

A 98.8 % global concordance between the GenoType assay and DNA sequencing was obtained. When the mutation was present in C-15T of \textit{inhA} or in \textit{katG}315 the concordance between both methods was 100 %, but a 97.2 % concordance was obtained for detection of \textit{rpoB} mutations: one sample showing \textit{M. tuberculosis} XDR profile by DST carried the GAC516GTC \textit{rpoB} mutation by sequencing, which was detected as WT by the GenoType assay.

The kappa (\( \kappa \)) coefficient for the concordance between both methods was calculated using the MedCalc® Software v 9.5.2.0 (Mariakerke, Belgium) and it was 0.996, indicating an excellent agreement between both methods (\( \kappa > 0.8 \)) and a very good concordance (24).

This misdetected sample by GenoType was confirmed as carrying the GAC516GTC mutation by sequencing it twice. The RIF-R detected by phenotypic DST methods was also confirmed by therapeutic evidence, based on patient response to anti-TB specific treatment.

**DISCUSSION**

In this study, the evaluation of the clinical performance of GenoType® MTBDRplus was carried out with the main objective of comparing its sensitivity and specificity to conventional DST and to explore its possible application in routine work under clinical conditions. The overall sensitivity of the GenoType assay obtained in this study was 94 % because 6 decontaminated sputum samples did not show any signals in the GenoType strips. The presence of inhibitors in the 6 clinical samples was discarded by \( \beta \)-actin gene amplification (25) and no GenoType PCR products from any of the samples were evidenced on an agarose gel. Therefore, a possible explanation for the negative GenoType results could be an insufficient amount of mycobacteria in those six samples. Since the global sensitivity of this kit is around 10,000 mycobacteria in 500 \( \mu l \) of decontaminated sputa used for DNA extraction, a loss of DNA during the extraction process or its relative inefficiency would explain, at least partially, the decreased sensitivity. Valid sequencing results for the different genes were obtained from all these samples. Nevertheless, global specificity and concordance were very good, accounting for 100 % and 98.8 %, respectively.

As previously reported by other authors, in this study the \textit{katG} gene mutation S315T and the \textit{rpoB} gene mutation S531L were the most prevalent mutations found to be responsible for INH-R and RIF-R, respectively (17, 18, 20, 21).

The GenoType® MTBDRplus assay was demonstrated to be easy to implement and perform in clinical laboratories and useful for a rapid detection of \textit{M. tuberculosis} resistant to INH and RIF from both decontaminated sputa and clinical isolates. Therefore, this assay could be applied as a rapid screening tool to predict INH-R and RIF-R, especially in those cases at high risk of becoming infected by a DR or a MDR strain. The screening would also be useful in other situations such as HIV co-infection, immunosuppression or malignancies and both adult and children household contacts of MDR-TB cases.

This assay has been used worldwide (1, 6, 11, 12, 22, 24, 28) and it has been recently endorsed by WHO to be used as a molecular screening tool for rapid detection of MDR-TB cases (30).

However, it is worth noting that this test cannot totally replace the conventional phenotypic DST method in clinical practice (30) because DST is still necessary to confirm XDR-TB. As it was shown in this study, a few DR cases could not be detected by the Genotype assay. The most plausible explanation may reside in the fact that only the most common mutations conferring drug-resistance are detected by the strip. Moreover, different drug-resistance mechanisms such as those implying efflux pumps might be involved in the occurrence of this phenomenon. Based on the sensitivity and specificity values found in this study, the method could be reliable with a result showing “resistance” to any given drug. Only those cases with invalid results (band pattern showing heteroresistance) or those designated as “susceptible” to a drug but being highly suspicious for drug-resistance should be confirmed by a phenotypic DST method. Consequently, only few cases should require confirmation after obtaining either drug-resistant or drug-susceptible results by the Genotype assay. The importance of adding a molecular test for drug-resistance detection at a clinical level is also evident at the moment of considering rapidness and optimal assignment of economic and personnel resources. When the GenoType assay is applied as screening tool, there is proven time-saving in technicians’ work and also a reduction in the costs of commercial DST methods, which together compensate for the cost of molecular techniques used as their replacement.
The whole healthcare system could benefit from the implementation of rapid diagnostic tools, leading to a shorter hospitalization time, with all the implications brought about to the affected families by this particular socio-economic setting.

Acknowledgements: we especially thank Hain Lifescience for kindly providing us the GenoType® MTBDRplus kit assay. AC is a career member and BI is a fellow from CONICET, Argentina.

REFERENCES


