Molecular Evaluation of Resistance to Rifampicin and Isoniazid of Tuberculosis Patients by test “Genotype® MTBDR Plus” in Senegal

Babacar Faye1,2,3, Issa Jessika1, Mame Cheikh Seck1,4, Cheikh Tidiane Ndour5, Papa Amadou Lamine Gueye6, Fatoumata Ba3, Marie Sarr6, Michael Grillo7, Sharon Reed2 and Alioune Dieye3

1Laboratory of Molecular Biology, Military hospital of Ouakam (HMO), Dakar-Senegal
2AIDS Program of the Senegalese Armed Forces, Senegal
3Service of Immunology, Department of Pharmacy, Faculty of Medicine, Pharmacy and Odonto-Stomatology of Cheikh Anta Diop University of Dakar, Senegal
4Parasitology Unit, Department of Pharmacy, Faculty of Medicine, Pharmacy and Odonto-Stomatology of Cheikh Anta Diop University of Dakar, Senegal
5Service des maladies infectieuses centre national hospitalier de FANN Dakar Senegal
6National Program against Tuberculosis (NTP) in Senegal, Senegal
7Department of Defense HIV/AIDS Prevention Program (DHAPP), San Diego, California, United States

Corresponding author: Dr. Babacar Faye, Laboratory of Molecular Biology, Military hospital of Ouakam (HMO), Dakar-Senegal, Tel: +221 77 6341070/+221 33 8601989; E-mail: bab_faye@yahoo.fr

Received date: 30 May 2018; Accepted date: 25 July 2018; Published date: 01 August 2018

Copyright: © 2018 Faye B. This is an open-access article distributed under the terms of the creative Commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Multi Drug Resistant-Tuberculosis (MDR-TB) is a major public health problem in Senegal with less successful treatment of tuberculosis. There were 208 cases of MDR-TB in 2015 surveyed at 31 treatment sites by the National Program against Tuberculosis.

Objectives: The aim of our study is to evaluate the prevalence of Mycobacterium tuberculosis isolates resistant to Rifampicin and Isoniazid and their associated resistance mutations in Senegalese patients.

Materials and methods: MDR-TB was diagnosed by molecular testing (Genotype MTBDRplus Hain Life Science) among sputum samples obtained from 185 Senegalese TB patients and was defined as resistance to both Isoniazid (INH) and Rifampicin (RIF).

Results: The sex-ratio M/W was of 2.2. The median age was 33.5 (8-72 years). Out of 185 positives samples for TB by microscopy, 95% (174/185) were Mycobacterium tuberculosis complex by Genotype MTBDRplus. The rate of TB-MDR+ in the total study population, treatment naïve, and previously treated was 64.94%, 46.25%, and 80.85%, RIF mono-resistance was 10.92%, 7.50%, and 13.83% and INH mono-resistance was 6.32%, 8.75%, and 4.26%.

Our strains of Mycobacterium tuberculosis have mutations conferring resistance in the rpoB, katG and inhA genes among 75.86%, 68.69%, and 13.21% respectively.

Conclusion: Our results demonstrate a high degree of resistance to Rifampicin and/or Isoniazid among Mycobacterium tuberculosis isolates from patients with a treatment history or contact with a patient with MDR TB which were rapidly detected with the use of Genotype MTBDRplus.

Keywords: Tuberculosis; Mycobacterium; Resistance; Rifampicin; Isoniazid; Genotype®MTBDRplus

Introduction

Tuberculosis (TB) is a major public health throughout the world. According to WHO 2017 report, there were 10.4 million people who had TB in 2016: 90% were adults, 10% were children, 65% were male and10% were people living with HIV in 2016. There were 1.7 million TB deaths among which 0.4 million HIV-positive [1].

In Senegal, there are 20,000 recorded TB cases with a mortality rate of 21 deaths for 100,000 inhabitants. The prevalence and the incidence were estimated at 205 cases per 100,000 inhabitants and 138 new cases per 100,000 inhabitants, respectively [2].

The WHO estimates that early diagnosis and effective therapy of tuberculosis saved 53 million lives between 2000 and 2016, but there are still large gaps in detection and treatment. However, increasing resistance to anti-TB drugs constitutes a major challenge for many TB treatment programs [3]. The prevalence of multi-drug resistant tuberculosis (MDR-TB) among new cases and previously treated cases has increased everywhere in the world [3,4].

In 2016, there were 600 000 new cases with resistance to rifampicin (RIF-TB), of which 490 000 had multidrug-resistant TB (MDR-TB) [1]. In Senegal, the external review performed in 2016 by the National Program to Fight against Tuberculosis (PNT) detected 208 MDR-TB
cases in 2015 at 31 treatment sites. A TB patient is resistant if he is positive at Ziehl Neelsen staining microscope after two months of anti-TB treatment.

It is therefore both urgent and important for early diagnosis to identify resistant TB cases in order to provide appropriate therapy and limit the spread of mycobacterial strains resistant to anti-TB drugs [5]. During the past few years, several molecular techniques have been developed including sequencing, pyrosequencing, real-time PCR, and hybridization to detect the frequency and profile of the mutations associated with drug resistance [6,7]. It is now recommended to use molecular line probes assays (LPA), which include the Genotype™MTBDR Plus [8]. Such assays detect MDR-TB, allowing prompt diagnosis of Mycobacterium tuberculosis and detection of the mutations responsible for the resistance to Rifampicin (RIF) and Isoniazid (INH), the first line TB-drugs in Senegal. The LPA uses multiplex PCR for the identification of M. tuberculosis complex (MTBC) and detection of the rpoB, katG and inhA genes mutations that confer a resistance to Rif and INH [6,9].

In Senegal, investigation of the frequency and specific mutation profiles associated with anti-TB drug resistance has not previously been done. The aim of our study is to determine the prevalence of anti-TB drug resistance (RIF and INH) among 1) treatment naïve patients and 2) patients with a history of treatment for TB and to assess the frequency and profile of the mutations associated with TB drug resistance.

Materials and Methods

Study design

This retrospective study was carried out at the Laboratory of Molecular Biology of the Military Hospital of Ouakam (HMO) of Dakar, Senegal. The study did not require ethics approval because the test has already been approved by the WHO for sensitivity testing. The samples used in this study were collected from 2012 to 2015 from TB-positive patients enrolled following microscopic TB diagnosis using the Ziehl Neelsen staining.

The study participants belong to the cohort of the National Program against Tuberculosis (NTP) for the assessment of first line anti-TB drugs resistance to Rifampicin and Isoniazid. Medical history of the patients included report of previous treatment as well as other chronic diseases. The patients were divided into two groups including those naïve to TB treatment (n=83) and those having history of (n=102).

Collection and processing of the sputum samples

Two sputum samples of 2-10 ml sputum were collected for each patient for Ziehl Neelsen staining. Collected sputum samples were kept in an icebox and preserved at -4°C. 3ml of each sample were then decontaminated using an equal volume of BBL NALC N-acetyl-L-cysteine (NaOH 4% 2.9% Citrate) in a plastic Falcon tube according to the manufacturer’s instruction (BBLTM MycoPrepTM Becton Dickson). From the sputum, smears were made on glass slide, fixed and stained using the Ziehl Neelsen method and read at the microscope at X100. Only patients with a positive Ziehl Neelsen test were enrolled in the study.

Line probe assay (LPA)

LPA are nitro-cellulose strips which have specific probes attached to them which are complementary to the DNA that is targeted in the specimen. Genotype MTBDRplus® is a LPA available for detection of Mycobacterium tuberculosis resistance to rifampicin and Isoniazid. LPA was carried out only on the smear-positive samples.

DNA Extraction

The extraction of the mycobacterial DNA was carried out using the Genolyse kit (Hain). A volume of 500µL of the decontaminated specimen was centrifuged at 10,000 g for 15 minutes, and the supernatant was discarded. Then, 100 µL of lysis buffer (A-LYS) solution was added to pellet. The suspension was homogenized by vortexing and inactivated by incubation in block heating at 95°C for 5 minutes. The neutralizing buffer solution (A-NB) was then added and vortexed. The mixture was centrifuged for 5 min, and the supernatant containing the DNA was recovered and transferred into a conical tube.

DNA amplification

DNA amplification was performed using Genotype MTBDRplus® kit version 2.0 according to the manufacturer’s instructions (Life Hain science, Nehren, GMBH, Germany). The reactional medium for the PCR was composed by a mix of nucleotidic primer and amplification probes in MgCl₂, sterile water, and Taq polymerase. The amplification consisted of 15 mn of denaturation to 95°C, followed by 10 cycles of denaturation at 95°C during 30 s and primers annealing at 65°C during 2 mn. This was followed by 20 cycles of denaturation at 95°C during 25 s, at annealing at 50 °C during 40 s, at 70°C during 40 seconds, and a final extension at 70°C during 8 mn.

Revelation

The PCR results were displayed by hybridization and revelation using a twincubator. The revelation method used the MTBDRplus version2 (Hain) with covered strips of highly specific probes, which are selected complementary to the amplified nucleic acid sequences. After hybridization and washing, the bands were fixed on paper, and the results were interpreted. The Genotype™ MTBDRplus kit band contains 27 probes, including 6 six controls (combined, amplification, complexes, rpoB, katG and inhA of Mycobacterium tuberculosis, for PCR validation. For the detection of resistance to RIF, 8 eight wild-type (WT) probes (probes WT1 to with WT8) included the regions area of the amino acids corresponding to 500 to 531 of the coding rpoB gene. Four probes (probes rpoB D516V, rpoB H526Y, rpoB H526D, and rpoB S313L) targeted the mutations specifically conferring a resistance to RIF. For the detection of resistance to INH, a probe covered the area of the codon 315 of wild-type of katG gene (WT), while two other probes (katG MUT1 and MUT2) were designed to detect S315T1 and S315T2 mutations on the katG gene. Finally, for the detection of resistance to INH related to the translation of the inhA genes, two probes were used to detect the wild type WT1 and WT2 bands and 4 other probes detected the band of MUT1, MUT2, MUT3A and MUT3B mutations.

The absence of one or more wild-type probe(s) and/or the presence of one or more mutant probe (s) were indicative of a resistant strain (Figure 1).

Figure 1: MTBDRplus interpretation Interprétation de Genotype MTBDRplus results (cf revelation in material and method): Patient 1 is multi-resistant because of the presence of the MUT3 band in the rpoB gene, the absence of WT band, and the presence of MUT1 band in the katG gene showing a resistance to both RIF and INH. Patients 2 and 3 are not Mycobacterium tuberculosis because the control TUB band does not appear. Patient 4 is RIF mono-resistant because of the presence MUT3 band in rpoB gene.

Statistical analysis

The data were obtained from the computerized records and file review. Descriptive analysis was done for demographic, clinical and mutation profiles features. Data analysis was performed using Statistical Package for the Social Sciences version 20 (SPSS Inc, Chicago, IL, USA). Frequencies were obtained for categorical clinical variables. Median and interquartile range were determined for continuous variables. Categorical variables were compared using Fisher’s exact test, and continuous variables were compared using the Mann-Whitney U-test. P< 0.05 was considered statistically significant.

Results

The study population

Among the patients, male gender represented 68.63% compared to 31.35% females, resulting in a sex-ratio (man/women) of 2.19. According to the history of the treatment, the tuberculosis patients were classified as treatment naïve (44.86%) and previously treated (55.13%) (Table 1; P< 0.001) A certain number of treated naïve patients were sent to the laboratory because they have been initially in contact with MDR-TB patients. Previously treated patients are in priority composed of: treatment failures, relapses and retreatment after abandonment of the first treatment.

Table 1: Distribution according to sex and treatment status of patients.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Naive</th>
<th>Previously Treated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>52</td>
<td>28.11</td>
<td>75</td>
</tr>
<tr>
<td>Women</td>
<td>31</td>
<td>16.76</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>44.87</td>
<td>102</td>
</tr>
</tbody>
</table>

The median age of the patients was 33.5 (range between 8-72 years). In the study population, the highest frequency was found in the age group of 21-30 years with 43.78% (81/185) followed by age group 31-40 years 24.32% (45/185). When compared by gender, the same age groups were the most impacted, with 39.66% (23/185) and 22.41% (13/185) among women, and 45.67% (58/185) and 25.20% (32/185) among men. (Table 2; P=0.26) Out of 185 TBM+ samples, 94% (174/185) were confirmed as being Mycobacterium tuberculosis Complex by Genotype MTBDRplus test (absence of the TUB band, Figure 1, patient 2 and 3 for example).

Table 2: Infection rate relative to age and gender

<table>
<thead>
<tr>
<th>Group of age</th>
<th>Women</th>
<th>Men</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>year</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>0-10</td>
<td>1</td>
<td>1.72</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>8</td>
<td>13.72</td>
<td>6</td>
</tr>
<tr>
<td>21-30</td>
<td>23</td>
<td>39.66</td>
<td>58</td>
</tr>
<tr>
<td>31-40</td>
<td>13</td>
<td>22.41</td>
<td>32</td>
</tr>
<tr>
<td>41-50</td>
<td>6</td>
<td>10.34</td>
<td>15</td>
</tr>
<tr>
<td>51-60</td>
<td>7</td>
<td>12.06</td>
<td>11</td>
</tr>
<tr>
<td>61-70</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>71-80</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sub total</td>
<td>58</td>
<td>31.35</td>
<td>127</td>
</tr>
</tbody>
</table>

Table 3: Distribution of resistance.

<table>
<thead>
<tr>
<th>Types of patients</th>
<th>Sensitive</th>
<th>Mono-resistant</th>
<th>MDR (RIF+INH)</th>
<th>Global</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>NRI</td>
<td>%</td>
</tr>
<tr>
<td>Treatment naïve</td>
<td>30</td>
<td>37.5</td>
<td>6</td>
<td>7.5</td>
</tr>
<tr>
<td>Previously Treated</td>
<td>1</td>
<td>1.06</td>
<td>13</td>
<td>13.83</td>
</tr>
</tbody>
</table>

The median age of the patients was 33.5 (range between 8-72 years). In the study population, the highest frequency was found in the age group of 21-30 years with 43.78% (81/185) followed by age group 31-40 years 24.32% (45/185). When compared by gender, the same age groups were the most impacted, with 39.66% (23/185) and 22.41% (13/185) among women, and 45.67% (58/185) and 25.20% (32/185) among men. (Table 2; P=0.26) Out of 185 TBM+ samples, 94% (174/185) were confirmed as being Mycobacterium tuberculosis Complex by Genotype MTBDRplus test (absence of the TUB band, Figure 1, patient 2 and 3 for example).

The median age of the patients was 33.5 (range between 8-72 years). In the study population, the highest frequency was found in the age group of 21-30 years with 43.78% (81/185) followed by age group 31-40 years 24.32% (45/185). When compared by gender, the same age groups were the most impacted, with 39.66% (23/185) and 22.41% (13/185) among women, and 45.67% (58/185) and 25.20% (32/185) among men. (Table 2; P=0.26) Out of 185 TBM+ samples, 94% (174/185) were confirmed as being Mycobacterium tuberculosis Complex by Genotype MTBDRplus test (absence of the TUB band, Figure 1, patient 2 and 3 for example).

Resistance to RIF and INH of the 174 isolates belonging to Mycobacterium tuberculosis complex, 17.82% (31/174) were sensitive to both RIF and INH. Specifically, 37.59% (30/80) of the strains of treatment naïve patients and 1.06% (1/94) of the previously treated patients were sensitive to both RIF and INH (Table 3; P<0.001).
RIF mono-resistance was 10.92% (19/174) among the overall study population, 7.5% (6/80) in the treatment naïve patients, and 13.83% (13/94) in previously treated patients (Table 3; P<0.001).

INH mono-resistance was 6.32% (11/174) among the whole study population, 4.26% (4/94) in the treatment naïve patients, and 8.75% (7/80) in previously treated patients. (Table 3; P<0.001)

Multi-resistance to both RIF and INH (MDR-TB) was observed among 64.94% (113/174) of all the samples tested and belonging to *Mycobacterium tuberculosis* complex (MTC). MDR-TB was observed among 46.25% (37/80) of treatment naïve patients and 80.85% (76/94) of and previously treated patients (Table 3; P<0.001).

Mutation profile (Table 4)

According to the Genotype MTBDRplus (Figure 1), a *Mycobacterium tuberculosis* strain is resistant when there is absence of the Wild Type bands (WT) or presence of the mutation bands (MUT).
had limited therapeutic failures and the propagation of drug resistant tuberculosis. Hence, the efforts to control tuberculosis are focused on the detection of the new cases to initiate early treatment. Initial tuberculosis therapy uses mainly the first line anti-TB drugs RIF and INH along with Pyrazinamide and Ethambutol. Incomplete treatment because of poor adherence can lead to resistance to anti-TB drugs and requires the use of second line drugs. The increase of MDR-TB worldwide, particularly in low-income countries requires the use of second line anti-TB drugs RIF and INH. The GenotypeMTDRplus test has a specificity of 95.8% for the detection of Rif resistance and sensitivity 96% for the detection of INH resistance [6,10-17].

Our results show that the infection rate of tuberculosis was higher in men compared to women (P=0.15). This result is similar to the ratios found in India [18] and South India [19]. This ratio (2.19) is however higher than that found in certain countries of Africa which is equal to 1 [20], and to the worldwide ratio of 1.7.

Of 185 TB patients, 68.11% were young with an age ranging between 20 and 40 years in both sexes. Similar results were noted in India with 63.3% [18]. These observations on the gender/age of the TB-infected populations allow a better targeting in the tuberculosis prevention activities of the national program [21].

The bacteriological diagnosis of tuberculosis is first done by the identification of acid fast bacilli (AFB) by microscopic examination, which does not allow differentiation of Mycobacterium tuberculosis complex (MCTB) from other mycobacteria atypical [22]. Among the 185 positive strains by Ziehl-Nielsen staining, 94% belonged to the complex tuberculosis (MTBC) according to the criteria of the GenotypeMTDRplus test. This result is similar to those previously recorded in Zambia (93.3%) [23] and India (90.76%) [18].

Our study showed that the RIF mono-resistance was 10.92%, 13.83% and 7.5% respectively for the general population, treatment naïve, and previously treated patients. Our results for the treatment naïve (13.83%) is higher than those found by several studies in India with figures going from 0.5% to 6.8% [24-29], 6.6% in China, 1.5% in Uganda, 1.6% in Germany, and 2.6% in Australia [30]. On the other hand, our results are lower than those found in other studies performed in India 19.42%, [18], 23.5% in Saudi Arabia and 50% in Bangladesh [31,32]. RIF mono-resistance in the previously treated patients is lower than that reported in India, 28.2% with Jaipur [27] 74.4% with Mumbai [33] and 33.7% with New Delhi [25], 46.1% in Ethiopia [34], 29.7% in China [30], 62.5% with Uzbekistan [35], and 80% in Bangladesh [32]. However RIF mono-resistance at these patients was described similar to Uganda 13.4%, and low in Germany 7.7% and in Sri Lanka 2.6% [30].

Our results show an INH mono-resistance of 6.32%, 8.75% and 4.26% respectively on the level of the general population of study, treatment naïve and previously treated patients. A study in Ethiopia [35] found a similar result of 6.3% to that of the general study population. Contrary to the other types of resistance, the INH mono-resistance is lower among previously treated patients. Among treatment naïve patients, our results were higher compared to those found in Uganda (5.8%) and Germany (7.1%), but similar to that of Australia (8.9%) [30], while lower than those found in Bangladesh 54.5% [32], Saudi Arabia 33.8% [31], and India 21.35% [18]. For the previously treated patients, our results reveal an INH mono-resistance lower than those found in India 39.7% [27], Ethiopia 56.1%, Bangladesh 82.6%, China 38%, Germany 15.4%, Uganda 20%, Australia 29.2%, and Sri Lanka 5.3% [30].

Resistance to both RIF and INH (MDR-TB) was 64.94%, 46.25% and 80.85% respectively in the overall study population, treatment naïve, and previously treated patients.
Among naive patients, the level of MDR was higher than that described in India 24% [36], Bangladesh 40.9% [32], and Saudi Arabia 20.6% [31]. This high rate of MDR was likely related to the recruitment of the treatment naive patients who were in contact with MDR patients.

Our study shows a very high MDR resistance rate among previously treated patients of 80.5%. A similar rate was found in studies from Bangladesh for RIF and INH resistance with 80% [32], 82.6% respectively [30]. Our results were higher than described in New Delhi 33.7% [25], in Mumbai 41% [36], Ethiopia 36.3% [37], and Burkina Faso 55.5% [38].

Our high MDR-TB rate is likely explained by testing patients who were strongly suspected of MDR-TB, had treatment failures, relapses and retreatment after abandonment of the first treatment. According to the WHO-2015 report on tuberculosis, Senegal recorded 887 relapses of pulmonary tuberculosis of which 73.6% were confirmed bacteriologically and 16.4% clinically diagnosed (MDR) [2]. These findings justify the importance of the early detection of resistance to anti-TB drugs.

For the first time in Senegal, the profiles of resistance mutations genes of Mycobacterium tuberculosis are documented. RIF inhibits the transcription of mRNA by blocking the beta unit encoded by polymerase rpoB genes of Mycobacterium tuberculosis [39]. RMP resistance is related to mutations in a restricted area of this gene rpoB. In the Genotype MTDR plus test, RIF resistance is related to the absence of one or more bands WT (WT1 to WT8) and/or the presence of the mutation band (MUT1, MUT2A, MUT2B and MUT3) in area 500-531 of the rpoB gene (Figure 2).

![Figure 2: Area of the gene rpoB and its mutations of resistance to Rif](Image)

In our study, 75.86% of the strains of the MTC have mutation of rpoB genes. Our results show that the most frequent mutations are the absence of bands WT8 (55.30%), WT3 (15.15%), WT4 (12.12%), WT7 (11.36%), and the presence of mutation MUT3 in 9.09% (Table 4). The other types of mutations of this gene are negligible. Studies of the RIF resistance on Mycobacterium tuberculosis strains showed that the frequent mutation is MUT3 with 88.6% in South Africa, [10] and in India 62.2% [7,18].

INH is activated by the catalase-peroxidase encoded by KatG gene. The INH resistance of Mycobacterium tuberculosis is associated with mutations in the katG and inhA genes [10,40]. Our results show that 71.26% (124/174) of isolates of Mycobacterium tuberculosis are INH resistant (MDR+ INH- monoresistant) with 68.96% with mutations of katG and 13.21% of mutations of inhA genes (Table 4). Other studies showed mutations of katG of 81.8% [41] and from 50 to 100% [9] with mutations of inhA genes.

The most frequent mutations were the absence of WT (88.71%) and the presence of MUT1 (S315T1) with 7.26% at the katG gene. For the inhA gene, the absence of WT1 and WT2 were noted at 7.26% and 8.06% respectively.

Other studies show a higher frequency of MUT1 mutation 74.32% [18] and 93.3% [7] in India. Most often, we found that our Mycobacterium tuberculosis (MTC) strains with mutations of RIF and INH had absent WT bands rather than the presence of MUT bands. Because the patients included in the study had a therapeutic failure, relapse, or contact with MDR-TB patients, our population was enriched for MDR strains.

Since Genotype MTDR plus test has a reported sensitivity of 95.8% for the detection of RIF resistance and of 96% for the detection of INH resistance [6,10-17], our high prevalence is likely to be accurate and additional mutations that may not hybridize with the MUT bands may be present. The clinical exams of the patients are in favor of the liability of our results. To answer this question in a rigorous way, it will be necessary to conduct a phenotypic study of sensitivity to Rifampicin and Isoniazid of the Mycobacterium tuberculosis strains from cultures followed by sequencing of the rpoB, katG and inhA genes.

**Conclusion**

Our data have shown that MDR-TB is higher among patients with antecedents of treatment compared to new cases. The mono-resistance in Rifampicin and Isoniazid is much less common than MDR-TB.

The MDR-TB presented more mutations on the rpoB gene than mutations on the katG gene level. The resistance mutations of Mycobacterium tuberculosis strains were more related to the absence of WT bands than with the presence of the MUT bands. Further studies of the phenotypic sensitivity of cultures and sequence analysis of the rpoB, katG and inhA genes in Senegal and other countries will better define the profiles of mutation resistances to anti-TB drugs and help evaluate the reliability of the molecular testing.

**Acknowledgments**

The authors thank the National Program against Tuberculosis in Senegal (NTP) to have placed at our disposal necessary reagents, and the Department of Defense HIV/AIDS Prevention Program (DHAPP) for the support in material/equipment of molecular biology.

**Author’s contribution**

Conceived and designed the experiments: Babacar Faye, Alioune Dieye.

Performed the experiments: Babacar Faye, Issa Jessika.

Analyzed the data: Cheikh Tidiane Ndour, Babacar Faye, Issa Jessika.

Contributed for reagents/materials/analysis tools: Papa Amadou Lamine Gueye, Fatoumata Ba, Marie Sarr.

Wrote the paper: Babacar Faye, Alioune Dieye, Cheikh Tidiane Ndour.

Reviewed critically the manuscript for important intellectual content: Michael Grillo, Sharon Reed.

Approved the final version to be published: Alioune Dieye, Cheikh Tidiane Ndour.

Conflicts of Interest: There are no conflicts of interest.
References
