Molecular Evaluation of Primary and Secondary Resistance of M. Tuberculosis in Senegalese Patients by Seegene Anyplex™II MTB/MDR/XDR

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Abstract

Background: Drug-resistant TB has become a public health problem. In 2017 WHO estimates that 3.6% of new TB cases and 17% of previously treated cases were multidrug-resistant (MDR-TB). However, 8.5% of people with MDR-TB had extensively drug-resistant TB (XDR-TB). Object: To evaluate the primary and secondary resistance of M.tuberculosis in TB patients and the resistance mutations. Materials and methods: 137TB-specimens are examined by the Seegene Anyplex™II MTB/MDR/XDR test. These patients are 45 new cases and 92 TB patients with treatment history. The Chi-square and Fisher tests allowed us to calculate p-values. When the p-value was less than 0.05 (significance), we estimated the odds ratio and their interval reliability at 95% to measure the risks of association.

Results: The median age was 30 years. 95.6% of the strains were tuberculosis complex (MTBC) and 61.06% were M. tuberculosis. Strains of Mycobacterium tuberculosis harbored mutations conferring resistance to rifampicin, to isoniazid and fluoroquinolones: 70.2%, 63.3% 12.2% and 4.5% respectively at the rpoB, KatG, inhA and gyrA. We did not observe mutations in the rrs gene and its promoter region eis conferring resistance to injectable drugs (amikacin, kanamycin and capreomycin). Mono-resistance (RIF, INH), multi-resistance (MDR, XDR) and resistances mutations rpoB, KatG, InhA were significantly higher in patients with treatment history than in new cases. Conclusion: Our study shows that MDR-TB is high in patients with treatment or in contact with MDR patients and that XDR-TB is rare in Senegal.

Keywords: M. tuberculosis, mutations-resistance, PCR-Multiplex, AnyplexII.

INTRODUCTION

In 2017, WHO estimated about 10 million new cases of tuberculosis (TB), with 133 cases per 100 000 inhabitants of which 5.8 million are men, 3.2 million are women and 1 million are children, 90% were adults, 64% were men and 9% were people living with HIV [1].

In Senegal, according to the National Tuberculosis Program (NTP), 3,800 died from TB in 2016 and the incidence was of 140 cases/100 000 inhabitants in 2017 [2]. WHO estimates that between 2000 and 2017, 54 million lives were saved by early diagnosis and effective treatment of tuberculosis [3]. However, antituberculosis-resistant germs have become a public health problem and represent a threat to the global fight against TB [4]. In 2017, 558 000 cases rifampicin resistance (RR, the most effective first-line drug) have been detected worldwide of which 82% were multidrug-resistant tuberculosis (MDR). 3.5% of new cases and 18% of previously treated TB cases were MDR-TB [1]. At the African level, the incidence of MDR-TB is about 9% [1].

In Senegal, the incidence of multidrug-resistant tuberculosis is estimated at 440 cases that is 3 cases per 100 000 inhabitants in 2017 [2]. In fact, multi-resistant...
infections are difficult to cure and are more likely to remain sources of infection longer than in patients with drug susceptible TB. Thus, it is essential to develop rapid diagnostic tests and appropriate chemotherapy to prevent the spread of resistant \textit{M. tuberculosis} [5]. Microscopic examination is the known-standard method for diagnosing TB because of its speed, its simplicity and its low cost. However, its low sensitivity limits the usefulness of this technique. The culture is the reference method for the diagnosis of \textit{M. tuberculosis} and evaluation of the antibiotic resistance thanks to its high sensitivity, but it is time consuming, labor intensive and represents a high infectious risk for the technicians, which limits its clinical interest. Therefore, molecular tests provide a sensitive, specific and faster diagnosis of \textit{M. tuberculosis}, as well as the identification of mutations conferring resistance to the most important first-line antibiotics Isoniazid (INH) and Rifampicin (RIF) [6].

INH is a prodrug activated by catalase peroxidase, an enzyme encoded by the \textit{katG} gene. Once activated, INH inhibits mycolic acid synthesis via NADH reductase dependent enoyl-acyl encoded by the \textit{inhA} gene [7]. The mutation of the \textit{katG} gene is the main mechanism of resistance to INH and secondarily mutation in the \textit{inhA} gene [4].

Rifampicin binds to the β-subunit of RNA polymerase encoded by the \textit{rpoB} gene of \textit{M. tuberculosis}, resulting in the inhibition of the elongation of the mRNA [7]. Most of the resistance to RIF is associated with point mutations occurring in a target region of 81 base pairs (bp) or region RRDR (RIF Resistance Determining) of the \textit{rpoB} gene coding for the β subunit of RNA polymerase [8].

Fluoroquinolones exert potent antibacterial activity by trapping bacterial gyrase as ternary complexes of DNA, thus blocking replication and transcription. \textit{M. tuberculosis} contains the genes \textit{gyrA} and \textit{gyrB} coding respectively for the subunits A and B of DNA gyrase [9]. A mutation in the conserved QRDR region of \textit{gyrA} (320 bp) and \textit{gyrB} (375 bp) has been shown to be the region most implicated in FQ resistance in \textit{M. tuberculosis} [4].

Kanamycin (KM) and its amikacin derivative (AMK) are also inhibitors of protein synthesis by modifying the ribosomal structure by binding to the 30 S unit of bacterial ribosomes. Mutations in the \textit{rrs} gene (16S rRNA) are associated with high-level resistance to KM, AMK and CPM (capreomycin) [4].

The Seegene Anyplex™ II MTB/MDR/XDR Detection test is a real-time multiplex PCR for the identification of \textit{M. tuberculosis} and the simultaneous detection of MDR and XDR resistance alleles. In the analysis by Anyplex™ II MTB/MDR/XDR, the target sequences of \textit{M. tuberculosis} and each resistance mutation is amplified and specifically detected by the double-priming oligonucleotide (DPO) method and tagging oligonucleotide cleavage extension (TOCE) technology without additional downstream processing. This double PCR procedure increases the blocking specificity of the extension of non-specific primed sequences and the multiplexing capability by simultaneous real-time detection of multiple dot mutations with high specificity. The Anyplex™ II MTB/MDR/XDR real-time PCR assay has been recommended by WHO in reference laboratories [10]. Its use is very limited or non-existent in developing countries. With the exception of our study on the resistance of \textit{M. tuberculosis} in Senegalese TB patients by the GenoType MTBDRplus technique [11], there has been no molecular study on the frequency and patterns of specific mutations in antituberculosis drug resistance in Senegal.

The main objective of this study is to evaluate by molecular Anyplex™ II MTB/MDR/XDR, the frequency of resistance to first-line drugs (RIF and INH) and second-line drugs (fluoroquinolones and injectables, amikacin, kanamicin and capreomycin) of \textit{M. tuberculosis} in Senegalese TB patients. The mutation profiles related to these resistances are also evaluated.

**MATERIAL AND METHOD**

**Type and population of study**

This is a retrospective study conducted at the molecular biology laboratory of the Ouakam Military Hospital (HMO) in Dakar (Senegal) on tuberculosis patients with suspected resistance in the period of 2013 to 2016. In this study no ethical approval was required because the data was collected during patient’s therapeutic follow-up. The patients included in the study had positive sputum on light microscopy after Zielh Neelsen staining. The information regarding the patient, his family and his TB treatment were collected by a questionnaire and recorded. Patients were divided into two groups: a group consisting of anti-tuberculosis treatment-naive patients (new cases n=45) and a group of patients with antituberculosis treatment history (known cases n= 92) referred to the laboratory for TB drug resistance testing. The new case consisted mainly of new cases (N=34) and new cases in contact with MDR-TB patients (N=11). Known cases consisted of patients with relapse (N= 34), positive microscopic control after three months of treatment (N=18), treatment failure (N=34), and with previous treatment history (N=06).

**Collection and decontamination of samples**

Two samples of 3 to 5 ml of sputum per patient were collected in a sterile jar. They were treated with the Microprep BBL NALC N-Acetyl-L-Cysteine Kit (4% NaOH 2.9% Citrate). 3 ml of sputum were transferred into a 15ml Falcon tube and then treated according to the kit method (BBLTM MycoPrep™ Becton Dickson).
Samples were decontaminated and stored in the refrigerator at -20 °C pending DNA extraction.

**Multiplex PCR for detection of resistance mutations DNA extraction**

Decontaminated sputum was manually extracted with the DNA extraction solution included in the Seegene kit. The extraction is carried out according to the instructions of the manufacturer Anyplex ™ II MTB/MDR/XDR Detection assay kit manufacturer (TB7500Y).

![Image](image-url)

Altenhofstrasse 80D-66386 St.ingbert, Germany/Taewon Bldg., 91, ogeum-ro, Songpa-gu, Seoul, Republic of Korea). A DNA extract of 50 μl is obtained of which 10 μl are used for the multiplex PCR.

**Amplification**

According to the manufacturer's protocol, the DNA extracts were tested by the Anyplex ™ II MTB/MDR/XDR Detection assay Kit (TB7500Y / Altenhofstrasse 80 D-66386 St. ingbert, Germany/Taewon Bldg., 91, ogeum-ro, Songpa-gu, Seoul, Republic of Korea). Which is a qualitative test for the identification of *M. tuberculosis* and the detection of mutations leading to resistance to first-line (INH and RIF) and second-line (FLQ) antituberculosis drugs by multiplex PCR on a real-time biopharmaceutical Bio-Rad CFX96. Multiplex PCR uses seven pairs of primers that detect 7 mutations of the *katG* gene and the promoter region of the *inhA* gene conferring resistance to INH; 18 pairs of primers detecting 18 mutations of the *rpoB* gene responsible for resistance to RIF; 7 pairs of primers detecting 7 mutations of the *gyrA* gene causing resistance to FLQ and 6 pairs detecting 6 mutations of the *rrs* gene and its promoter region *eis* [10] causing resistance to injectable anti-TB drugs (amikacin, kanamycin and capreomycin) (Figure 1).

Amplification is performed according to the manufacturer's instructions. Each sample was tested in two separate reactions (MTB / MDR and MTB / XDR) according to the kit procedure. These two reaction solutions contain Master Mix (EM1), RNase-free Water and TOM MTB / MDR or TOM MTB / XDR. EM1 contains DNA polymerase, Uracil-DNA glycosylase (UDG) Buffer containing dNTPs. TOM MTB / MDR and TOM MTB / XDR are TOCE oligo mix consisting of reagents for the amplification and detection of MTB and MDR-TB or XDR-TB on the CFX96 ™ Real-time PCR System (Bio-Rad version 1.6). The amplification protocol is defined in the Bio-Rad CFX Manager software.

**Revelation:** The interpretation of the melting curves was performed automatically with a computer connected to the CFX 96 Bio-Rad with Seegene view visualization software, Version 2.0 (Seegene Technologies).

Data entry and analysis: Seegene visualization software results were imported in Excel 2013. The questionnaires were entered into an EPI info template to form a database that was analyzed by the R software v3.3.3. For the qualitative variables we have calculated the absolute frequencies and the relative frequencies. A comparison of proportions allowed us to compare the types of resistance compared to cases with treatment history and new cases. The Chi-square and Fisher tests allowed us to calculate p-values. The degree of significance was set at 5% (P < 0.05).

When the p-value was less than 0.05, we estimated the odds ratio and their 95% confidence interval to measure the risks of association.

**RESULTS**

The median age of the 137 patient’s cohort was 30 years with extremes ranging from 8 to 72 years? The patients were composed of 96 men and 41 women with a M/F ratio of 2.34. Of the study patients 45 were new cases and 92 known cases with treatment history.

**Resistance of first line and second line of mycobacteria**

Of 137 samples tested by PCR, 131 (95.6%) were tuberculosis complex strains and 6 (4.5%) non-tuberculosis strains. Non-tuberculosis strains were excluded from the study. 24.42% of patients (32/131) were sensitive to first-line drugs (RIF, INH) and second-line drugs (fluoroquinolones, Amikacin, Kanamicin and capreomycin). This sensitivity was higher in new cases, 56.1% (23/41) than in cases with treatment history 10% (9/90).

The Monoresistance (INH) multiresistance (MDR, XDR) and mutations of resistance mono-resistance to the RIF was 9.6% (12/131), 9.75%, and 8.88% for the whole study group, for new cases, and in cases with treatment history respectively. This mono-resistance to rifampicin is higher in new cases than in cases with treatment history (P=0.02 and OR=5.11) (Table II).

The mono-resistance-INH: was 4.58% (6/131) and 6.66% respectively for the whole group and for the cases with treatment history. This mono-resistance was not observed in the group of new cases (Table II).

The Mono-Resistance-FQ: Only one patient in the new case group was mono-resistant to Fluoroquinolones. Multi-resistance (MDR-TB): 61.1% (80/131) of our patients had MDR-TB in the general population. This multi-resistance was higher in the group of cases with treatment history, 74.4% than in the group of new cases, 31.7% (P=0.0000001; OR=13.17; Table II).
Extensively drug-resistant TB (XDR-TB): XDR-TB is the multi-resistance (RIF + INH) associated with resistance to at least one second-line anti-TB drug. The prevalence was 3.8% (5/131) in the study group. This resistance although low, was higher in cases with treatment history, 4.4% than in new cases 2.4% (P=0.04; OR=10.22; Table II).

**Frequency and type of resistance mutation**

The resistances noted above are related to the presence of mutations at the target genes of Mycobacterium tuberculosis (Table I).

Mutation on the rpoB gene: found in mono-resistance-RIF, Multi-resistance MDR-TB and XDR-TB. 70.2% (92/131) of MTBC strains showed a mutation on the rpoB gene in the study group. The frequency was higher in the cases with treatment history with 83.3% than in the new case group with 41.5% (p=0.0000001; OR=11.27; Table III).

Kat G mutation: found in INH mono-resistance of the MDR-TB and XDR-TB. 63.3% (83/131) of strains of Mycobacterium tuberculosis showed mutations in the katG gene in the cohort. These katG mutations were higher in cases with treatment history, 77.7% than in new cases, 31.7% (p=0.000001; OR=13.76; Table III).

**In inhA mutation:** They were present in 12.2% (16/131) of strains in the cohort. These inhA mutations were significantly more common in cases with treatment history with 13.3% of prevalence in new cases the prevalence was 9.7% (p=0.001; OR=7.66; Table III). Gyr A mutation: mutations in the gyrA gene confer mycobacterial resistance to fluoroquinolones (Table I). These mutations of the gyrA gene were present at 4.5% (6/131) 4.8% and 4.4% respectively for the general population new and cases with treatment history. There was no difference between cases with treatment history and new cases (P=0.09 Table III). Mutations of the rrs gene and its promoter region eis conferring resistance to injectable drugs (amikacin, kanamicin and capreomycin) were not observed.

**Tables**

**Table-I: Target mutations of the Anyplex™ II MTB/MDR/XDR Detection (Seegene)**

<table>
<thead>
<tr>
<th>Drug resistance</th>
<th>Related gene</th>
<th>Target mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isoniazid resistance (INH-R)</strong></td>
<td>KatG</td>
<td>S315I (AGC→ATC)</td>
</tr>
<tr>
<td></td>
<td>inhA promoter</td>
<td>-15 (C→T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L511P (CTG→CCG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 a.a. deletion in 513~516</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q513K (CAA→AAA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q513L (CAA→CTA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q513P (CAA→CCA)</td>
</tr>
<tr>
<td></td>
<td>rpoB</td>
<td>S315N (AGC→ACC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8 (T→A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D516V (GAC→GTC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H526C (CAC→TGC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H526R (CAC→CCG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S531W (TCG→TTG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S531P (TCG→CCG)</td>
</tr>
<tr>
<td></td>
<td>gyrA</td>
<td>S315T (AGC→ACA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8 (T→C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D516Y (GAC→TAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H526D (CAC→GAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H526L (CAC→CTC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S531L (TCG→TTG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S531L (TCG→CCG)</td>
</tr>
</tbody>
</table>

**Table-II: Type of resistance of M. Tuberculosis to antituberculosis drugs**

<table>
<thead>
<tr>
<th>Type of resistance</th>
<th>Known cases N=90</th>
<th>New cases N=41</th>
<th>P-value</th>
<th>Odd-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-resistance</td>
<td>Mono-RIF</td>
<td>8 (8.88)</td>
<td>4 (9.75)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Mono-INH</td>
<td>6 (6.66)</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mono-FQ</td>
<td>0</td>
<td>1 (2.43)</td>
<td>0.53</td>
</tr>
<tr>
<td>Multi-resistance</td>
<td>MDR</td>
<td>67 (74.44)</td>
<td>13 (31.70)</td>
<td>0.0000001</td>
</tr>
<tr>
<td></td>
<td>XDR</td>
<td>4 (4.4)</td>
<td>1 (2.4)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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**DISCUSSION**

The low sensitivity of microscopy in the diagnosis of tuberculosis explains a significant percentage of bacilli transmission due to negative smears. The culture, although being a diagnostic reference technique has a long delay between 3 and 8 weeks to be confirmed. Effective TB control in the world requires accurate diagnosis and early treatment of the TB patient. In addition, the diagnosis of mycobacteria resistance to antituberculous drugs became a major issue in the limitation of therapeutic failures and the non-expansion of resistant strains. The use of molecular techniques has thus enabled early diagnosis and adequate antituberculosis treatment by the detection of resistance mutations.

In this study, we used an Anyplex ™ II MTB/MDR/XDR Detection assay, a multiplex PCR to assess the frequency of first- and second-generation of anti-TB drug resistance and the associated mutations at rpoB, KatG, inhA, gyrA, rrs and its promoter eis in a population of tuberculosis patients in Senegal. Studies have shown a performance of this test with a sensitivity of 83.3% and a specificity of 100% in the detection of INH resistance [10], 100% and 90.3% for resistance to RIF [12], 50% and 100% for resistance to FLQ and 100% and 94.4% for resistance for KAN and CAP [13].

Our results showed that the population with tuberculosis is predominantly male let be a sex-ratio M/F of 2.35. This male predominance is similar to the 2.6 and 2.1 described in India [14,15] and Burkina Faso (2.12) [16]. Our ratio is higher than that found in Kenya (1.8) [17] and below the 3 described in Ivory Coast [18]. Men in different sectors of activity are more exposed than women hence facilitating the transmission of TB [16-18].

Our results showed that 95.6% (131/137) Ziehl-Neelsen-positive sputum in light microscopy was a tuberculosis complex (TCM) infection and 4.3% was a non-tuberculosis mycobacteria infection. This result is similar to that of Igarashi (92.6%) [19] and 93.3% in Zambia [20]. This molecular test has the advantage of identifying specifically *Mycobacterium tuberculosis* unlike optical microscopy.

<table>
<thead>
<tr>
<th>Type of mutations</th>
<th>Known cases N=90</th>
<th>New cases N=41</th>
<th>P-value</th>
<th>Odd-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rpoB</td>
<td>75 (83.3)</td>
<td>17 (41.5)</td>
<td>0.00000001</td>
<td>11.27 [4.43-28.67]</td>
</tr>
<tr>
<td>katG</td>
<td>70 (77.7)</td>
<td>13 (31.7)</td>
<td>0.00000001</td>
<td>13.76 [5.20-36.36]</td>
</tr>
<tr>
<td>inhA</td>
<td>12 (13.3)</td>
<td>4 (9.7)</td>
<td>0.001</td>
<td>7.66 [1.95-30.14]</td>
</tr>
<tr>
<td>gyrA</td>
<td>4 (4.4)</td>
<td>2 (4.8)</td>
<td>0.09</td>
<td>5.11 [0.79-32.96]</td>
</tr>
</tbody>
</table>

In our study, rifampicin mono-resistance was 9.75% in the new-case group and 8.88% in the group with a history of treatment. Our rate of rifampicin mono-resistance in treatment-naive patients is higher when comparing with 3.5% in Somalia [21] and 2.2% in India [22] and under the ones observed with 19.42% in India [14] and in 23.5% in Saudi Arabia [23]. In the group of patients with treatment history, our level of mono-RIF is similar to 7.7% in Germany, higher than 2.6% in Sri Lanka [24], at 1.2% in Bangladesh [25] and is below 13.4% in Uganda [24]. This mono-resistance rate was relatively low in favor of multi-resistance.

The mono-resistance to INH in our study was 4.58% (6/131) in our cohort, 6.66% in patients with a history of treatment and not detected in new cases. This mono-resistance in cases with treatment history was higher than the 4.26% described in Senegal by Faye et al. [11] to 4.5% Bangladesh [25] and 4.5% in Zambia [20] and below 10.6% in Somalia [21], to 8.61% in India [14]. A similar rate has been reported in Uganda [24].

MDR-TB resistant to RIF and INH was 61.06% (80/131) in our cohort, with 31.70% for new cases and 74.44% for cases with treatment history. This multi-resistance was greater in patients with a history of treatment than in new cases. This is explained by the fact that cases with treatment history were composed mainly of therapeutic failure of relapse and positive microscopic control after three months of treatment. The MDR-TB level in our general population (61.1%) was higher at 12.8% in Morocco [26]. However, it is similar to the 65.5% described in India [27]. MDR-TB for new cases was greater than 6.3% in Iran [28], to 12.6% in India [14]. MDR-TB among the new cases results from the direct transmission of resistant M. tuberculosis. In this group, some of the new patients were referred by contact with MDR-TB.

The MDR-TB in the case group with treatment history (74.4%) was very high and similar to that found in Bangladesh (77.8%) [25] but higher than that found in New Delhi in India (47.1%) [29]. The high level of multi-resistance of new cases and patients with treatment justifies the establishment of a system for monitoring resistance mutations by reliable and rapid techniques to limit the therapeutic failures and the circulation of
resistant strains. In Senegal XDR-TB is rare. Extensively drug-resistant TB (XDR-TB) is MDR-TB associated with resistance to at least one second-line anti-TB drug. In this paper, we found 3.8% (5/131) of XDR-TB in our cohort. Only resistance to fluoroquinolones was noted in the second line. Our rate of XDR-TB was higher than that described in Egypt 6.5% [10]. The lack of resistance to injection drugs (amikacin, kanamicin and capreomycin) in our study was also described in Egypt.

This is the first time that the detection of primary and secondary resistance mutation patterns is documented in Senegal. Rifampicin blocks bacterial transcription by inhibiting the β-unit of the polymerase encoded by the rpoB gene of Mycobacterium tuberculosis (TCM) [30]. RIF resistance is related to 18 mutations in this gene (Table I).

Our results showed that 70.2% (92/131), 41.5% and 83.3% of Mycobacterium tuberculosis strains in our cohort had rpoB gene mutations respectively in the general population, naïve patients and patients with treatment history. The frequency of mutations in our cohort was higher at 46.1% described in Ethiopia [31] and below 97% in South Africa [32].

INH is a catalase-peroxidase activated prodrug encoded by the Mycobacterium tuberculosis katG gene. The resistance of Mycobacterium tuberculosis to isoniazid is related to mutations in the katG and inhA genes [32; 33] (Table I). Our results showed that 63.3% (83/131), 31.7% and 77.7% of the MTC strains had mutations in the katG gene respectively in the study group, new cases and cases with treatment history. This level of mutation in the katG gene was similar to 68.96% in Senegal [11] in the general population and below 86% in South Vietnam [34] and 81.8% described in the CDC (US) by Campbell and al, in 2011 [35]. Mutations in the inhA gene were found in 12.2% (16/131), 9.7% and 13.3% respectively of the general population new cases and cases with treatment history. The frequency found in the general population was low compared to 18% described by Huyen [34] and similar to 13.21% in Senegal [11]. Our results show that isoniazid resistance is mainly related to mutations in the katG gene than those in the inhA gene. In addition to giving the results of rapid resistance, the test used is sensitive in that it detects 38 mutations and specific by a selection of Mycobacterium tuberculosis sequences. These performances allow a fast management of TB patients but also have an interest for the molecular epidemiology. Our methodological approach allowed us to evaluate the resistance of Mycobacterium tuberculosis to second-line anti-TB drugs such as fluoroquinolones and injectable drugs (aminoglycosides and capreomycin). Only mutations on the gyrA gene were detected in proportions of 4.5% (6/131), 4.8% and 4.4% respectively our cohort, new cases and cases with treatment history. ElFeky et al. [10] described in Egypt a 6.5% resistance to fluoroquinolones relatively higher than our result. Our study showed no resistance mutation on the rrs gene and its promoter eis, that means no resistance to amikacin, kanamicin and capreomycin as described in Egypt [10]. Our results bring a certain interest to the clinical diagnosis and treatment of mycobacteria. They contribute to the effective control of tuberculosis public health problem, in particular the resistance management of mycobacteria. Our method is sensitive and highlights the gene mutated among rpoB, katG, inhA, gyrA, rrs and eis. We have information on the susceptibility or resistance of Mycobacterium tuberculosis strains for each anti-TB drug. However, information on the precise location of the resistance mutation among the 38 detected (Table I) on the gene targets are not obtained in the disclosure of the technique. The limit of our study could be corrected by sequencing resistant strains; this would allow seeing the different types of mutation, their location in the bacterial genome and their frequency in antituberculous drug resistance. To fully understand the impact of the identified mutations culture technique to evaluate level of antibiotic resistance would be needed. It is not expected that every mutation will have the same impact on antibiotic resistance. Identification by sequencing non-tuberculosis strains would complement the interest of molecular epidemiology.

CONCLUSION

Our results showed that mono-resistances to rifampicin, with isoniazid and fluoroquinolones and extensively drug-resistant XDR-TB are rare whereas MDR multi-resistance is high in our study population. The rifampicin mono-resistance, MDR and XDR multi-resistances are significantly higher in patients with a history of treatment than in naïve patients. The resistance mutations on the rpoB and katG genes are very high and those on the inhA, gyrA, rrs and its promoter eis are low but all these mutations are significantly higher in the former patients than in the treatment-naive patients.

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REFERENCES


