

Research Article

Molecular Diagnosis of Resistant Tuberculosis Against Rifampicin to the Patients of the Centre Hospitalier Universitaire de Référence Nationale (CHU-RN): Preliminary Study During the COVID-19 Pandemic Period in N'Djamena, Chad

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Abstract

Background: The fight against Tuberculosis (TB) remains a challenging public health problem worldwide. Nowadays, resistance forms increase sharply in developed countries. With a rifampicin resistance rate of 7.2% higher than the World Health Organization (WHO) level, the Tuberculosis Control Program in Chad has many challenges to overcome in the fight against resistant and multiresistant forms of TB. This present study aims to evaluate the proportion of resistant tuberculosis against rifampicin at the Centre Hospitalier Universitaire de Référence Nationale (CHU-RN) of N'Djamena in Chad.

Material and Methods: From August 1st to December 31st, 2020, sputum samples collected in the laboratory of CHU-RN were automatically included in our study. We directly analyzed sputum samples using the GeneXpert MTB/RIF, which is a molecular diagnosis method, to identify the sensitivity of the Mycobacterium tuberculosis complex to rifampicin.

Results: The study population consisted of 680 sputum samples collected. It was mainly represented by men (66.62%), with a ratio sex of about 1.99. The age range varies from 2 to 85 years old. Most of the patients in the study lived in N'Djamena (95.68%), and 4.32% were from other localities. Mycobacterium Tuberculosis Complex (MTBC) was detected in 51.63% (N = 696), including 244 men and 104 women (a sex ratio of 2.35). Many tuberculosis patients are young people aged 15–34 (49.14%). Children whose samples tested positive for TB accounted for 2% of the 348 MTBC-positive samples, 7.18% (20 males and 5 females) were resistant to rifampicin, confirming a rifampicin sensitivity of 92.53%. Among these 25 cases of rifampicin resistance, 15 patients (4.31%) were already treated, and the other 10 patients represented new cases of TB (2.87%).

Conclusion: This study showed 7.18% resistance to rifampicin. These results highlighted the question linked to the post-COVID-19 period. The DOTS strategy should include the use of Xpert MTB/RIF® technology in all provinces of Chad. This will lead to the early detection of new cases of TB and MDR-TB. Overall, it will provide rapid treatment, as recommended by the WHO for developing countries like Chad.

Keywords: Molecular Diagnosis; Resistance; Rifampicin; Tuberculosis Disease; GeneXpert

Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. The transmission occurs primarily through the air when a person with TB coughs or sneezes. The disease continues to cause preventable suffering and death in many countries around the world, including Chad [1]. Tuberculosis is a curable disease, but it still kills millions of people around the world every year and forms of resistance to this disease are increasing sharply today in developed countries. The incidence rate of pulmonary tuberculosis in Chad is approximately 142 cases per 100,000 inhabitants which is considered high. The country ranks among the countries most affected by the disease in sub-Saharan Africa according to the World Health Organization (WHO) [1]. In 2018, there were approximately half a million new cases of rifampin-resistant tuberculosis worldwide [1]. Globally, 3.4% of new TB cases and 18% of previously treated cases have Multidrug- or Rifampicin-Resistant TB (MDR/RR-TB). In Chad, resistant and Multidrug-Resistant forms of Tuberculosis (MDR-TB) constitute a major public health problem that must be addressed because the diagnosis of resistant tuberculosis is based solely on resistance to rifampicin. With a rifampicin resistance rate of 7.2% higher than the WHO level, the Tuberculosis Control Program has many challenges to overcome in the fight against resistant and multiresistant forms of tuberculosis. This present study aims to evaluate the proportion of resistant tuberculosis against rifampicin at the centre hospitalier universitaire de référence nationale of N'Djamena in Chad.

Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

Material and Methods

Study Design

This was a descriptive cross-sectional study that took place over a period of five months, more precisely from August 1st to December 31st, 2020. Throughout this period, the Centre Hospitalier de Référence Nationale (CHU-RN) hosted the study, during which samples received at the laboratory were systematically included in the study. These samples, collected mainly from routine activities, were sputum collected for Acid Fast Bacilli (AFB) research. These sputa were directly analyzed using the GeneXpert MTB/RIF molecular diagnostic method. This method was carried out in the mycobacteria unit of the CHU-RN laboratory in N'Djamena (Chad). Patients included in this study were individuals who had a documented history of cough episodes or who were actively experiencing cough symptoms at the time of data collection. The sampling method used in this study was a non-probability sampling technique, meaning that the selection of participants was not based on chance. Rather, individuals included in the study were purposefully selected based on their availability and willingness to participate in the research.

Laboratory Analysis

GeneXpert molecular analysis allows the detection of the *Mycobacterium tuberculosis* complex and mutations associated with rifampicin resistance. This technique uses the GeneXpert MTB/RIF® kit (Cepheid, Maurens-Scopont, France). It is an automated semi-quantitative test based on semi-nested real-time amplification of the core region (RDRR) of the *rpoB* locus for the diagnosis of TB and for the detection of genetic mutations associated with resistance to RIF. The amplification is carried out in a closed environment (cartridge) to minimize contamination (Fig. 1) between samples [2]. It uses 5 fluorescent wild-type probes that cover the RDRR (Fig. 1). During the amplification process, the hybridization of each of these probes reflects the wild nature of the RDRR sequence and therefore the sensitivity of the strain to RIF, however, the absence of hybridization of at least one of these probes reflects the existence of at least one mutation in the RDRR and therefore the resistant character of the bacillus to RIF. Each probe makes it possible to identify a certain number of genetic mutations due to a nucleotide substitution (Fig. 2). In its design, the Xpert test can only identify mutations located in the RDRR [3]. Recent studies have highlighted the existence of mutations (V176F and I497F) outside the RDRR that are associated with resistance to RIF [3].

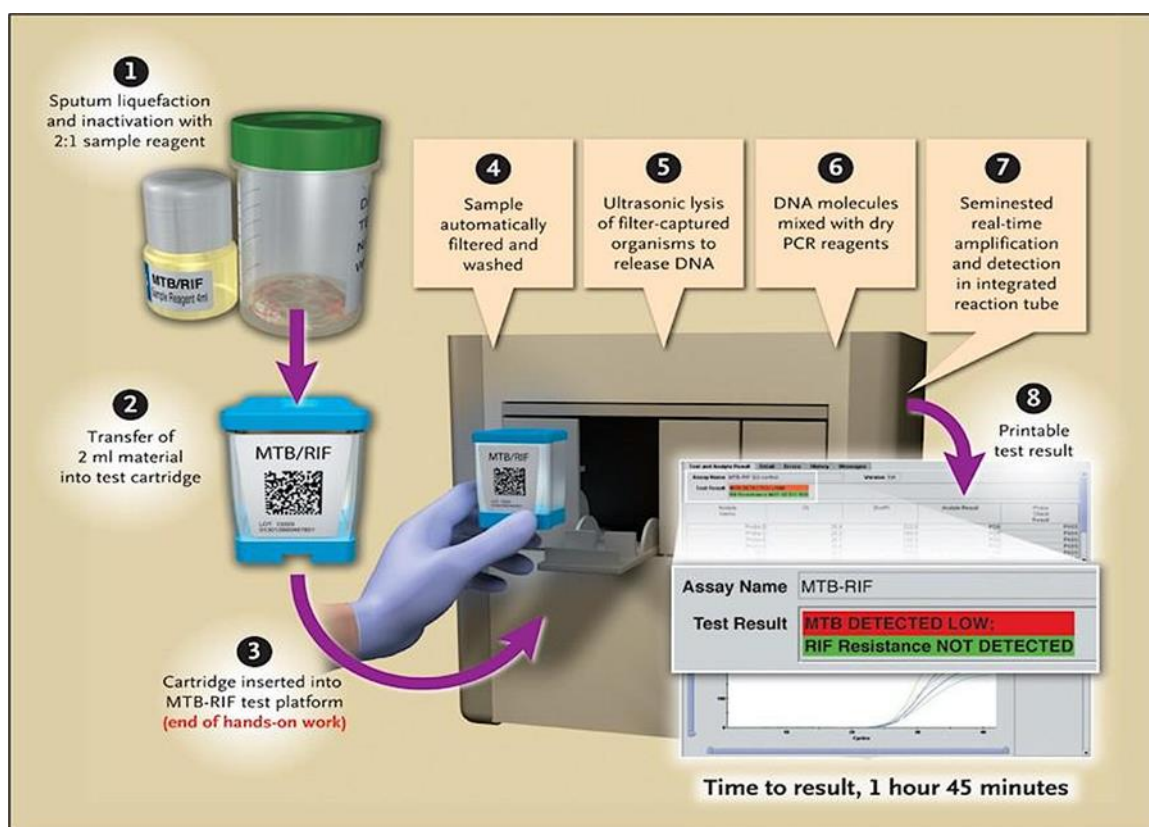


Figure 1: Procedure for performing the Xpert MTB/RIF test on a clinical sample [4].

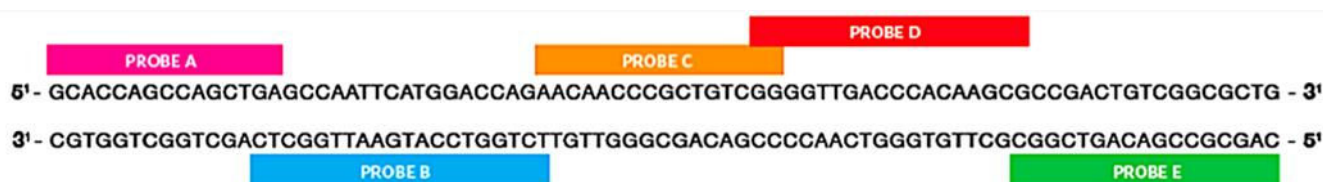


Figure 2: Representation of the 81 bp of the RDRR of the *rpoB* gene and the positions of each of the Xpert probes on said region [5].

Collection of Data

Data collection was carried out based on examination requests established by doctors (sociodemographic and clinical characteristics) and the results of laboratory analyses (biological characteristics).

All biological samples should be treated with the usual precautions of the health institution. Guidelines for sample management are available from the US Center for Disease Control and Prevention (CDC) and the Clinical and Laboratory Standards Institute (CLSI) [6,7]. The study obtained authorization from the ministry in charge of public health and that in charge of research for the collection of data and in turn, the agreement of those responsible for the CHU-RN.

Results

Sociodemographic and Clinical Characteristics

From the beginning of August to the end of December 2020, a total of 680 patients were registered with the Mycobacteria unit laboratory of the CHU-RN. A specialized unit dedicated to the microbiological and molecular examination of pulmonary tuberculosis sensitive and resistant to rifampicin. The study population was mainly represented by men (66.62%), with a sex ratio of 1.99. The age range varies from 2 to 85 years old. The median age was 35 years, which reveals the central tendency of ages. The most represented age group was 25-34 years (28.57%), followed by 35-44 years (21.89%). Table 1 present the study population according to age and sex.

Gender (N=680)	n	%
Male	453	66.62
Feminine	227	33.38
Age (years) (N=644)		
0-14	12	1.86
15-24	113	17.55
25-34	184	28.57
35-44	141	21.89
45-54	109	16.93
55-64	47	7.30
65-74	26	4.04
>75	12	1.86

Table 1: Distribution of study patients according to age and sex.

Most of the patients in the study lived in N'Djamena (95.68%) and carried out various professional activities (health, education, defense forces, commerce, engineering, etc.) and others were unemployed. Patients from other localities represented 4.32%. These localities are: Arada, Djarmaye, Dourbali, Guelendeng, Koundoul, Mandalia, Massaguet and Moussoro. Requests for examination most often came from the various CHU-RN services, but also from external centers (referred patients): 89.91% and 10.09%, respectively. Regarding clinical status, patients with screening requests represented 25% of the study population and those coming for checks represented 18.55% (N=124, clinical data available). Patients who were in relapse, resumption of treatment or treatment failure represented 31.45%, 12.10% and 12.90%, respectively.

Biological Data

Mycobacterium Tuberculosis Complex (MTBC) bacilli were detected in 51.63% (N=674) including 244 men and 104 women (sex ratio of 2.35). The majority of tuberculosis patients are young people aged 15-34 (49.14%). Children whose samples tested positive for tuberculosis were about 2% of the 348 MTBC-positive samples. 7.18% (20 males and 5 females) were resistant to rifampicin, confirming a rifampicin sensitivity of 92.53%. Among these 25 cases of rifampicin resistance, 15 patients (4.31%) were already treated and the other 10 patients represented new cases of tuberculosis (2.87%). The Gene Xpert results according to the month of inclusion are shown in Fig. 3.

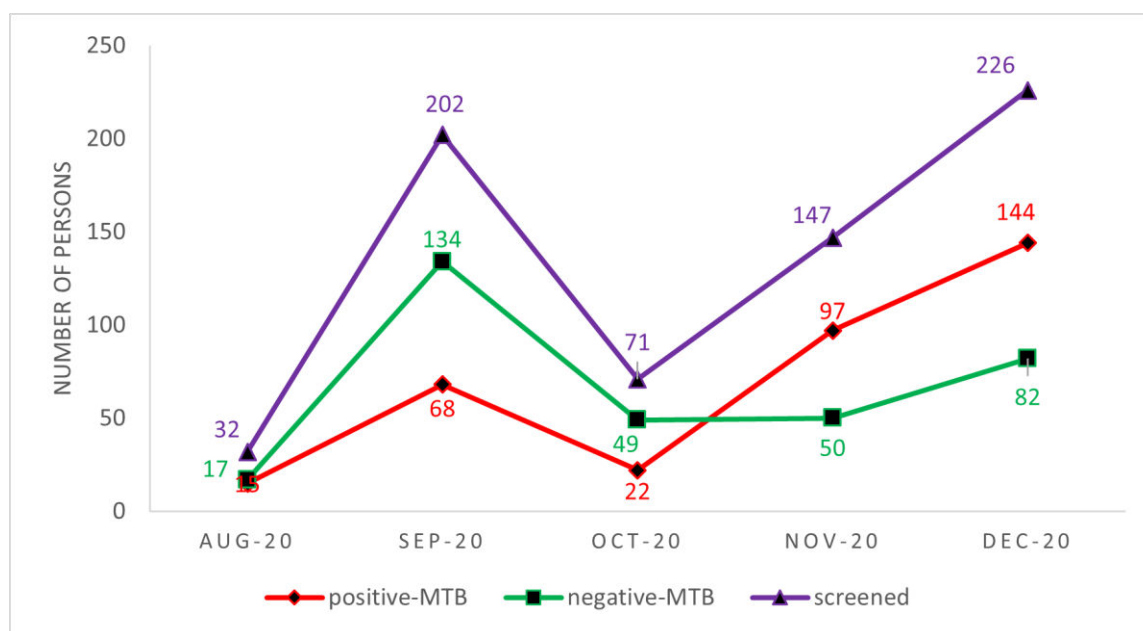


Figure 3: Number of patient samples analyzed by Gene Xpert based on months.

Discussion

The study we conducted determined the proportion of *Mycobacterium tuberculosis* complex in patients with a symptom of pulmonary tuberculosis, which was 51.63%. Pulmonary tuberculosis is more common among men than women in Chad, with a ratio of 2.35 to 1. The most affected age groups were young adults aged 15 to 34. Multi-resistance (MDR) of *Mycobacterium tuberculosis* to antibiotics is defined by simultaneous resistance to at least isoniazid or rifampicin [1], the prevalence of multi-resistance in this study was 7.20% (25 cases). Resistance is only sought in positive samples.

The study by Amor, et al., estimated the prevalence of multidrug-resistant tuberculosis in Chad at 1.9% (0.5–9.0%) of tuberculosis cases, even though no resistant strain had been reported until this study [8]. The work of O Abdelhadi, et al., reported a prevalence of multidrug-resistant tuberculosis of 2.2% in three strains, of which only one is resistant only to isoniazid and rifampicin [9]. The other two strains were also resistant to at least one first-line antibiotic. The 25 MDR strains detected in our study came mainly from patients under treatment (4.31% among TB), which reflects secondary resistance compared to 10 cases (2.87% among TB) of primary resistance in newly diagnosed patients and never processed. The WHO Global TB Report on 2019 data showed similar rates of new MDR-TB cases in Chad and treated cases of 2.5% and 14%, respectively [1]. The latter concerned overall data from Chad, including all tuberculosis patients. Furthermore, we clearly observe an increase in cases of MDR-TB from 2008 to 2020 in Chad, despite the efforts and resources that Chad has made available to health facilities for the fight against tuberculosis. Data on MDR-TB exist due to the introduction of the Xpert MTB/RIF® rapid test (Cepheid, Maurens-Scopont, France), used in most cases for the diagnosis of multi-drug resistant tuberculosis in six provinces (N'Djamena, Bongor, Moundou, Sarh, Am-timan and Abéché), but unfortunately these data are not published. According to Chad's national health development plan 3: 2018-2021, GeneXpert device coverage will gradually extend to all hospitals in provincial capitals and a strategy of decentralization of diagnosis and tuberculosis care is being implemented on a pilot basis in 30 faith-based health centers and 112 public health centers [10]. Diagnosis and treatment of pulmonary tuberculosis in Chad are often limited by limited resources and insufficient health infrastructure. This leads to delays in diagnosis, high rates of treatment non-adherence and increased drug resistance.

The study we conducted showed a very low diagnosis rate, the peak of which was observed in December 2020 with 227 patient samples analyzed, which corresponds to a third of the usual number observed in this national reference center compared to 32 samples analyzed in August 2020. This particular result highlights the question that arises when diagnosing Multidrug-Resistant Tuberculosis (MDR-TB) in the period following the COVID-19 pandemic. This is explained by the advent of the COVID-19 pandemic. Indeed, this period was marked by negligence on the part of those involved in the fight against tuberculosis, leading to gene transfers upstream manufacturing. Due to the weak technical platform, the National Reference Laboratory for Mycobacteria (LNR-TB) of the PNT is not able to carry out the activities of culturing mycobacteria and testing the sensitivity of mycobacteria to first-and second-line anti-tuberculosis drugs [10]. It is important to clarify that the use of the Gene Xpert technique remains the only means of diagnosing MDR-TB. To fight pulmonary tuberculosis in Chad, the government and international partners must pool their efforts to implement screening, diagnosis and treatment programs for the disease. This includes the establishment of specialized health centers, the training of health personnel and raising awareness among the population about the symptoms and prevention measures of pulmonary tuberculosis [11].

Conclusion

The study we conducted identified 25 cases of multidrug-resistant tuberculosis, defined as resistance of *Mycobacterium tuberculosis* to isoniazid or rifampicin. This investigation highlighted the question of Multidrug-Resistant Tuberculosis (MDR-TB) in the post COVID-19 pandemic period. DOTS strategy should include the use of Xpert MTB/RIF® technic in all provinces of Chad. This will lead to early detection of cases of TB and MDR-TB and will allow rapid treatment of patients, as recommended by the WHO for developing countries like Chad.

Conflict of Interests

The authors declare no conflict of interest regarding authorship roles or publication of article.

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Authors Contribution

All the authors have equal contribution and all the authors have read and agreed to the published version of the manuscript.

Data Availability

Not applicable

Consent for Publication

Not applicable

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