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The Contribution of the Xpert® MTB/RIF Assay to the Surveillance of Drug-Resistant Tuberculosis in the Central African Republic

Alain Farra[1*], Lydie V. Danebera[2], Gilles Ngaya[3], Brice M. Yambiyo[4], Alexandre Manirakiza[4], Christian D. Mossoro-Kpinde[2]

[1]National Reference Laboratory for Tuberculosis, Institut Pasteur of Bangui, Bangui, Central African Republic
[2]Faculty of Health Sciences, University of Bangui, Bangui, Central African Republic
[3]Medical Laboratory, Institute Pasteur of Bangui, Bangui, Central African Republic
[4]Epidemiology Unit, Institute Pasteur of Bangui, Bangui, Central African Republic
Email: *farra_alain@yahoo.fr

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Abstract

Introduction: The Central African Republic is one of the 30 high Tuberculosis burden countries in the world, with an incidence of 540 cases per 100,000 population and a mortality of 91 deaths per 100,000 population. Since 2020, following WHO recommendations, the National Reference Laboratory for Tuberculosis has been using the Xpert® MTB/RIF assay as a first-line diagnostic test for the early detection of Drug Resistance Tuberculosis. The goal of this study was to evaluate the contribution of the Xpert® MTB/RIF assay to the surveillance of rifampicin resistance in new and previously treated tuberculosis cases. **Materials and Methods:** The data relative to the Xpert® MTB/RIF assay carried out on various categories of tuberculosis patients registered at the National Reference Laboratory for Tuberculosis in 2020 were analyzed retrospectively. The categories of tuberculosis patients were new cases, failed treatment cases, relapse cases, lost-to-follow-up cases and multidrug-resistant tuberculosis contact cases. **Results:** A total of 1404 tuberculosis patients were registered at the NRL-TB in 2020; the mean age was 39.2 years (2 - 90 years) and the male-to-female sex ratio was 1.16:1. Overall, 32.7% (454/1404) proved infected with tuberculosis, of which 22.5% (102/454) cases showed resistance to rifampicin. The primary resistance rate was 9.1% (27/298) and the secondary resistance rate was 46.6% (75/161). Treatment failures and relapsed cases were significantly associated with rifampicin resistance ($p < 0.005$). **Conclusion:** Large-scale use of Xpert® MTB/RIF, especially in the provinces of the Central African Republic, will help the Ministry of Health to better control Drug Resistance Tuberculosis in the country.

Keywords

Xpert® MTB/RIF Assay, Rifampicin, Surveillance, CAR

1. Introduction

Despite the various strategies set up by the World Health Organization (WHO) and extensive support provided to national Tuberculosis (TB) control programs, the emergence and spread of resistance to TB drugs have become not only a real threat to the efficient control of TB in many countries, but also represent a veritable challenge for the WHO's End TB Strategy that aims to virtually eradicate TB by 2035 [1].

In 2019, the WHO reported 10 million cases of TB worldwide, with more than 0.5 million cases of resistance to rifampicin, of which 78% were Multidrug-Resistance TB (MDR-TB) cases. Drug-Resistant TB (DR-TB) has been reported in new TB patients as well as in patients with previous TB treatment, accounting for 3.3% and 17.7% of cases, respectively [2] [3].

Although the annual number of new cases of TB is fewer than 10 cases per 100,000 population in most high-income countries, the Central African Republic (CAR) is one of the 30 high TB burden countries in the world, with an incidence of 540 cases per 100,000 population and a mortality of 91 deaths per 100,000 population [2].

Since 2010, the WHO has approved the large-scale use of the Xpert® MTB/RIF assay, first as an initial test in patients co-infected with HIV and TB and in presumptive DR-TB patients. It was later approved as a first-line test for all presumptive TB cases [4]. In the CAR, the use of the Xpert® MTB/RIF assay began in 2015, initially in patients previously treated for TB, then as of 2020 as a first-line test for all presumptive TB cases.

The goal of this study was to demonstrate the contribution of the Xpert® MTB/RIF assay to the surveillance of DR-TB in new TB cases as well as in previously treated cases registered in the CAR in 2020.

2. Materials and Methods

2.1. Type and Duration of Study

This cross-sectional study spanned one calendar year and included all patients registered at the National Reference Laboratory for TB (NRL-TB) at the Institut Pasteur of Bangui during the year 2020.

2.2. Study Population

The study population was composed of patients targeted by DR-TB surveillance in the national TB control program, namely treatment failures, relapses, recurrence after lost-to-follow-up treatment and MDR-TB contact cases, as well as presumptive TB cases.

For the most part, patient cases were referred to the NRL-TB by Diagnosis and Treatment Centers (DTCs) in Bangui. Upon request for an Xpert® MTB/RIF test, the DTC sent patient sputum samples, the results of the smear microscopy carried out at the DTC along with the test request form, which provided information on age, sex, place of residence, sample type, and case type. The HIV serology status of patients was not indicated on the request form. In some cases, sputum samples from provinces were sent to the NRL-TB via medical humanitarian organizations. These samples arrived at the NRL-TB within 48 h along with their request forms in UN 2814-certified triple-packaging transportation boxes. The sampling was exhaustive taking into account all the patients registered for the diagnosis of TB during the year 2020.

2.3. Variable Definitions

According to the WHO «Definitions and reporting framework for tuberculosis—2013 revision» (http://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf;jsessionid=1A5C34511A95ED2DE3BD874F22238F49?sequence=1). The PNLT in CAR defined the types of cases as follows:

a) Presumptive TB cases: refers to a patient who presents with symptoms or signs suggestive of TB (previously known as a TB suspect).

b) New cases (patients): patients who have never been treated for TB or have taken anti-TB drugs for less than 1 month.

c) Previously treated cases: Patients who received 1 month or more of anti-tuberculosis drugs in the past. They are classified according to the result of their treatment as follows:

- **Treatment failures (or failure patients)**: Patients who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment;
- **Relapses (patients)**: Patients who have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB;
- **Recurrence after lost-to-follow-up treatment**: Patients who have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment.

d) MDR-TB contact cases: Patients who have been in contact with a confirmed MDR patient and who may or may not have signs of tuberculosis.

2.4. Laboratory Analyses

Laboratory analyses were carried out according to the workflow established for DR-TB in the CAR (**Figure 1**).

All sputum samples were tested using the Xpert® MTB/RIF assay as well as smear microscopy. If rifampicin resistance was detected, the samples were cultured on solid Löwenstein-Jensen media incubated à 37˚C for 8 weeks and sensitivity

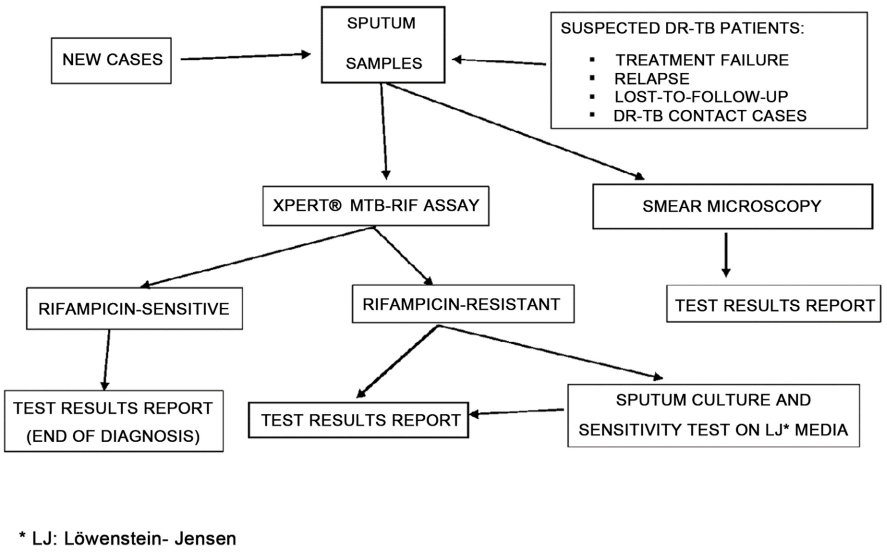

Figure 1. Diagnosis workflow for drug resistant tuberculosis at the National Reference Laboratory for Tuberculosis in Central African Republic.

tests were carried out on sputum cultures using the proportion method to compare the phenotypic and genotypic results of rifampicin resistance.

2.5. Data Collection and Analysis

The data on the request forms were compiled in a spreadsheet along with the results from the Xpert® MTB/RIF assay. Sampling was exhaustive, being based on all the patients registered during the year 2020. The socio-demographic data on the patients as well as the laboratory results were analyzed using Stat software (ver. 14). A chi-squared test was used for statistical comparisons and Odds Ratios (ORs) were calculated when the *p*-value was less than 0.05.

2.6. Ethical Considerations

This surveillance program was approved by the ethics committee of the Ministry of Health as part of the national TB control plan in the CAR (RCA PSN TB 2017-2023). In addition, analyses were carried out on anonymous data and were conducted in strict confidentiality.

3. Results

3.1. Characteristics of the Study Population

A total of 1404 patients were included in this study. The largest percentage of patients (46.1%; 647/1404) belonged to the 21 - 40 years age group, followed by the 41 - 60 years age group (38.2%; 536/1404). Only 8.9% (125/1404) of the patients were under 20 years of age and 6.8% (96/1404) were older than 60 years. Males dominated the patient population (54%; 755/1404) and the male-to-female sex ratio was 1.16:1. Virtually all patients (96.3%; 1352/1404) were from the capital Bangui; patients from the provinces accounted for only 3.7% (52/1404) of the

study population. Most patients presented new cases (83.8%, 1176/1404) and previously treated patients represented only 16.2% (228/1404) of the study population (Table 1).

3.2. Results of the Xpert® MTB/RIF Analyses

Overall, 22.3% (102/458) of the cases proved to be rifampicin-resistant cases. The primary resistance rate was 9.1% (27/298) and the secondary resistance rate was 46.6% (75/161) (Figure 2).

3.3. Comparison of the Performance of the Xpert® MTB/RIF Assay with Smear Microscopy

The Xpert® MTB/RIFassay led to the diagnosis of TB in 14 new cases that tested negative using the smear microscopy method and in 3 cases in previously treated patients, of which 2 were cases of rifampicin resistance (Table 2).

3.4. Genotypic and Phenotypic Detection of Rifampicin Resistance

In new cases and in previously treated cases, all rifampicin-resistance cases detected using the Xpert® MTB/RIF assay were also detected in the sensitivity tests using the proportion method, with no discrepancies (Table 3).

3.5. Risk Factors for Rifampicin Resistance

The study of the risk factors showed that relapses and treatment failures in particular were significantly associated with the occurrence of rifampicin resistance ($p < 0.005$). Relative to new cases, the risk of rifampicin resistance is 3.06 times higher in relapse cases and 21.15 times higher in failed treatment cases (Table 4).

Table 1. Socio-demographic characteristics of the study population.

Characteristic	Number of cases (Total: 1404)	Percentage (%)
Age group (years)		
≤20	125	8.9
21 - 40	647	46.1
41 - 60	536	38.2
≥61	96	6.8
Gender		
M	755	54
F	649	46
Residence		
Bangui (capital city)	1352	96.3
Province	52	3.7
Type of case		
New cases	1176	83.8
Previously treated cases	228	16.2

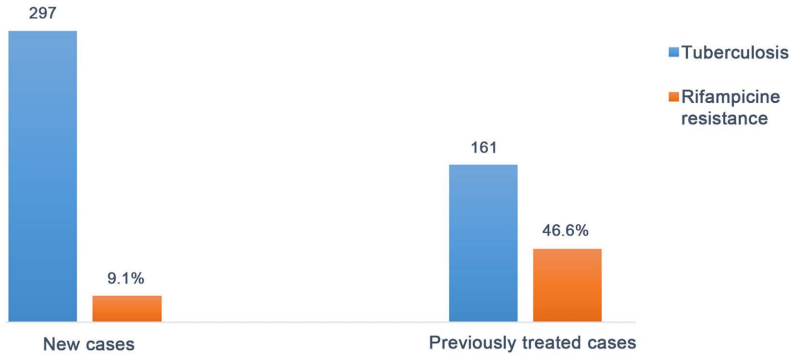

Figure 2. Prevalence of rifampicin resistance in new cases and in previously treated cases of tuberculosis.

Table 2. Performance of the Xpert® MTB/RIF assay in the detection of resistance to rifampicin compared with smear microscopy in new tuberculosis cases and in previously treated tuberculosis cases.

	Xpert-positive (%)	Xpert-negative (%)	Rifampicin resistance (%)
New cases			
Smear-positive	283 (100)	0	27 (9.5)
Smear-negative	**14 (1.6)**	879 (98.4)	0
Previously treated cases			
Smear-positive	158 (100)	0	73 (46.2)
Smear-negative	**3 (4.2)**	67 (95.8)	**2 (2.8)**

Table 3. Rifampicin resistance revealed with the Xpert® MTB/RIF assay compared with the sensitivity test using the proportion method on sputum cultures.

	Number of cases	
Type of case	**Xpert MTB/RIF assay (%)**	**Sensitivity test (%)**
New cases	27 (26.5)	27 (26.5)
Previously treated cases	75 (73.5)	75 (73.5)
Total	102 (100)	102 (100)

Table 4. Odds ratio (OR) and 95% confidence intervals (95% CI) of the occurrence of rifampicin resistance for significant variables.

	Rifampicin resistance			
	Number of cases		Odds ratio	
Characteristics	**Resistant (%)**	**Sensitive (%)**	**OR [95% CI]**	***p***
Age (years)				
<20	1 (0.01)	34 (9.5)	-	-
21 - 40	68 (68)	187 (52.23)	12.36 [1.66, 92.07]	0.01
41 - 60	31 (31)	12 (3.35)	8.43 [1.11, 64.01]	0.03
≥61	0 (0)	12 (3.35)	-	-

Continued

Gender				
F	34 (34)	133 (37.15)	-	-
M	66 (66)	225 (62.85)	-	-
Residence				
Bangui	91 (91.92)	345 (96.64)	-	-
Provinces	8 (8.08)	12 (3.36)	2.52 [1, 1.36]	0.04
Type of case				
New cases	27 (27)	270 (75.42)	-	-
Treatment failures	55 (55)	26 (7.26)	21.15 [11.47, 38.99]	0.001
Relapse cases	15 (15)	49 (13.69)	3.06 [1.51, 6.16]	0.002
Lost-to-follow-up treatment cases	3 (3)	13 (3.63)		-

4. Discussion

This study set out to assess the contribution of the Xpert® MTB/RIF assay to DR-TB surveillance in the CAR. Our study conclusively showed that the Xpert® MTB/RIF results oriented some TB patients, particularly new TB cases, directly to the second-line treatment, thereby avoiding first-line treatment failure.

DR-TB cases observed in this study represented 9.1% of new cases and 46.6% of previously treated patients, rates that are roughly three times higher than the mean reported by the WHO in 2020. Our results demonstrate that the national TB control program does not effectively treat all drug-sensitive TB cases, which subsequently exposes TB patients to resistant forms, nor does it efficiently control the spread of DR-TB in the CAR. However, a literature review shows that resistance rates are increasing globally in both types of cases, with rates higher than those observed in the CAR. The most striking example is that of Russia, with 35% resistance in new cases and 71% in previously treated cases. These high rates seriously compromise the global fight against TB and challenge the End TB Strategy's 2035 target [3] [5] [6] [7] [8].

Although all age groups showed TB cases, resistance was significantly more frequent in the 21 - 40 years and 41 - 60 years age groups. Resistance has generally been observed more frequently in these age groups, being attributed to greater exposure to outdoor environments and the high workload often encountered for these age groups, sometimes accompanied by conditions favoring infection and poor treatment follow-up [4] [5] [9].

The majority of patients with rifampicin-resistant TB were male, but this difference was notsignificant. Although men are more frequently affected by DR-TB, women often spend more time taking care of their parents who may be infected with DR-TB, which exposes them also to this form of the disease [5] [10] [11] [12].

Our results indicate that 92% of the patients with rifampicin resistance were

from Bangui and only 8% from the provinces. The low rate from the provinces likely reveals the difficulty of the national TB control program to deploy the Xpert® MTB/RIF assay in the provinces, and also to arrange the transport of sputum samples to Bangui, where the NRL-TB is located. Some studies have nevertheless shown more DR-TB cases in rural settings and have attributed the disproportion to poor access to healthcare in these regions [5].

The use of Xpert® MTB/RIF assay as a first-line diagnostic tool led to the diagnosis of 14 smear-negative TB cases among the new cases and 3 smear-negative cases in previously treated patients, of which 2 proved to have DR-TB. The Xpert® MTB/RIF assay, other than its rapidity, proved to be highly sensitive to detect patients with negative smear results, including some that had a resistant form of TB. This information is vital for proper treatment and control of DR-TB [13].

All forms of resistance to rifampicin revealed by Xpert® MTB/RIF were also detected using the sensitivity test with the proportion method. The Xpert® MTB/RIF assay clearly offers a robust alternative to cultures and sensitivity tests that sometimes require 1 to 3.5 months to obtain results [14] [15].

5. Conclusion

This study demonstrated the usefulness of Xpert® MTB/RIF assay in the surveillance of DR-TB at the NRL-TB at the Institut Pasteur of Bangui. Our results showed that, in 2020, 9.1% of new TB cases and 46.6% of previously treated TB cases were DR-TB cases, which is three times higher than the worldwide rate based on WHO data. These cases of resistance were found in the capital and in the provinces. In the fight against TB in the CAR, it is thus important for the Ministry of Health to set up and especially maintain an operational network of GeneXpert sites and the Xpert® MTB/RIF assay in the various regions of the country to better control DR-TB at the national level. Only then can the CAR hope to contribute effectively to the WHO's End TB Strategy and the eradication of the global TB epidemic by 2035.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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