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MAJOR ARTICLE

Surveillance of Rifampicin Resistance With GeneXpert MTB/RIF in the National Reference Laboratory for Tuberculosis at the Institut Pasteur in Bangui, 2015–2017

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Background. The Central African Republic (CAR) has one of the heaviest burdens of tuberculosis (TB) in the world, with an incidence of 423 cases per 100 000 population. Surveillance of resistance to rifampicin with GeneXpert MTB/RIF was instituted in the National TB Reference Laboratory in 2015. The aim of this study was to evaluate, after 3 years, resistance to rifampicin, the most effective firstline drug against TB.

Methods. The surveillance database on cases of rifampicin resistance was retrospectively analyzed. The populations targeted by the National TB Programme were failure, relapse, default, and contacts of multidrug-resistant TB (MDR-TB). Statistical analyses were performed with Stata software, version 14, using chi-square tests and odds ratios.

Results. Six hundred seventeen cases were registered; 63.7% were male, 36.3% were female, and the mean age was 35.5 years (range from 2 to 81). GeneXpert MTB/RIF tests were positive in 79.1% (488/617), and resistance to rifampicin was positive in 42.2% (206/488), with 49.1% (56/114) in 2015, 34.7% (57/164) in 2016, and 44.3% (93/210) in 2017. Failure cases had the highest rate of resistance (70.4%), with a significant difference ($P < .0001$; odds ratio, 9.5; 95% confidence interval, 4.4–20.5). Resistance was observed in 40% of contacts of MDR-TB, 28.2% of the relapses and 20% of the defaults without significant difference.

Conclusions. Resistance to rifampicin is still high in the CAR and is most strongly associated with treatment failure. The Ministry of Health must to deploy GeneXpert MTB/RIF tests in the provinces to evaluate resistance to TB drugs in the country.

Keywords. Bangui; GeneXpert; resistance; rifampicine.

The emergence of strains of *Mycobacterium tuberculosis* that are resistant to drugs against tuberculosis (TB) is one of the major public health problems of the 21st century. Despite various World Health Organization (WHO) programs and national TB programs (NTPs) for controlling TB, the emergence of multidrug-resistant (MDR) strains and, even more seriously, extensively drug-resistant (XDR) TB is a challenge for the strategy End TB by 2035. According to the WHO report on the global fight against TB in 2018, 10 million new cases of TB were reported in 2017, of which 558 000 were resistant to rifampicin, the most effective firstline treatment. Of these, 82% were resistant to both rifampicin and isoniazid [1]. The WHO also reported that, in 2017, coverage of tests for rifampicin resistance was only 24% of new cases of TB and 70% of cases in people already treated for TB [1]. According to the WHO, resistance to rifampicin is predictive of MDR-TB, and patients should be given second-line treatment [2].

Although the annual incidence of TB is <10 cases per 100 000 population in most high-income countries, the Central African Republic (CAR) is among 30 high-burden countries, with an incidence rate of 423 and a mortality rate of 58 per 100 000 population [1, 3]. At the beginning of the 2010s, studies on TB drug resistance in the CAR showed 14.7% primary resistance and 40% resistance in cases of treatment failure or relapse [4, 5].

In 2015, surveillance of resistance to rifampicin in populations at risk was strengthened by the acquisition of an automated 4-module GeneXpert system by the National TB Reference Laboratory (NTRL) at the Institut Pasteur in Bangui (IPB). This unique equipment to date in the organization of the NTP is reserved exclusively for surveillance of resistance to rifampicin in retreatment patients. The target populations of the NTP are failure cases, relapse cases, default, and contact of MDR-TB [3]. As samples from suspected MDR-TB are not available from the provinces, surveillance is conducted mainly in the capital. The

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aim of this study was to use the data from GeneXpert MTB/RIF tests to determine the prevalence of resistance to rifampicin among people at risk of MRD-TB in 2015–2017.

METHODS

Study Setting

The study was conducted in Bangui. The CAR is a large (623 000 km^2), sparsely populated country, with an estimated population of 4.7 million in 2017, most of whom (61.6%) live in rural areas. The NTP has 76 Diagnostic and Treatment Centres (DTCs), with 13 in Bangui and the others distributed throughout the country. The centers are coordinated by the NTP, which provides training, supervision, quality control, and provision of laboratory reagents and consumables.

Study Type, Length, and Population

A retrospective study was conducted on data from the surveillance database on rifampicin resistance at the NTRL from 2015, when surveillance with GeneXpert MTB/RIF began, to 2017. Surveillance covered the target populations for MDR-TB: failure cases, relapse cases, default, and contact of MDR-TB.

Variable Definitions

Failure

A patient whose sputum smear or culture is positive at 5 months or later during treatment [3].

Default

A patient whose treatment was interrupted for 2 consecutive months or more.

Relapse

If the previous outcome was cured or treatment completed.

Contact of MDR-TB

All persons who have been in the patient's company, whether professional or familial, and who have presented suspicious signs of tuberculosis.

Patients

Patients were referred to the NTRL from DTCs in Bangui, bringing with them their samples and the form requesting a GeneXpert MTB/RIF test, which listed their age, sex, site of disease, type of sample, type of patient (failures, relapses, defaults, and contacts of MDR-TB), and the result of microscopy. The HIV serological status of patients was not included, as the version of the form used did not include this question. A few samples taken by nongovernmental health organizations such as Médecins Sans Frontières were received from the provinces within 24–48 hours in Cryopacks containing 2 or 3 ice packs.

Laboratory Analysis

After registration, the samples were sent to the P2 microbiological laboratory in the P2/P3 block at the IPB and opened in a biosafety cabinet. Then, a volume of sputum free of food and with no traces of blood was mixed with 2 volumes of the sample reagent of the GeneXpert MTB/RIF test (a mixture of isopropanol to decontaminate the sample and lyse the mycobacteria) in a 15-mL Falcon tube. The mixture was shaken and incubated for 15 minutes in the biosafety cabinet. Once the mixture was clear, 2 mL were removed with the pipette from the test kit and placed in the GeneXpert MTB/RIF container. Within 30 minutes, the containers were taken from the P2 laboratory to the room where the GeneXpert machine was installed for the test, which was a real-time polymerase chain reaction (PCR). Detection of *M. tuberculosis* DNA and the mutated *rpoB* gene associated with resistance to rifampicin are automatically provided by the machine at the end of the reaction, in about 1 hour and 50 minutes. Samples that were negative in the GeneXpert test but positive on microscopy were re-examined after Ziehl-Neelsen staining.

Data Collection and Analysis

The data on the GeneXpert MTB/RIF tests request forms and the results of the test were entered into Excel files, which are sent regularly to the NTP and, on demand, to our partners, the International Union against Tuberculosis and the WHO. Data were analyzed with Stata, version 14; chi^2 tests were used for statistical comparisons, and odds ratios were calculated when the P value was <.05.

RESULTS

Characteristics of the Study Population

Between 2015 and 2017, 617 specimens were tested for rifampicin resistance with GeneXpert/MTB-RIF at the NTRL, of which 87.7% were from Bangui and the remainder were from the provinces. The mean age (range) was 35.5 (2–81) years (median, 34 years); however, most were aged 31–45 years (41.2%) and 16–30 years (33.9%). Males predominated (63.9%). Most samples were from relapse cases (55.8%) or failure cases (31.6%); default cases were recorded in 10.2% of patients, and 2.4% were contacts of MDR-TB. Microscopy at the DTC indicated a positivity rate of 78.9% (Table 1).

Results of GeneXpert Tests and Microscopy at Diagnostic and Treatment Centers

The GeneXpert test for the detection of *Mycobacterium tuberculosis* was positive in 79.1% of cases; 98.7% of the positive GeneXpert tests were for patients with positive microscopy, and 1.3% of the positive GeneXpert tests were obtained in patients with negative microscopy. In 3.1%, the GeneXpert test was negative, although the result of microscopy was positive (Table 2).

Prevalence of Resistance to Rifampicin and Characteristics of Patients

During the study period, the number of patients with MDR-TB increased steadily, from 141 in 2015 to 268 in 2017. Of the

Table 1. Characteristics of the Study Population

Characteristic	No. (617)	Percentage
Age, y		
0–15	20	3.2
16–30	209	33.9
31–45	254	41.2
46–60	108	17.5
≥61	26	4.2
Sex		
Female	223	36.1
Male	394	63.9
Location		
Bangui	541	87.7
Provinces	76	12.3
Type of patient		
Failure	195	31.6
Relapse	344	55.8
Default	63	10.2
Contact of MDR-TB	15	2.4
Microscopy		
Positive	487	78.9
Negative	130	21.1

Abbreviation: MDR-TB, multidrug-resistant tuberculosis.

79.1% of cases with a positive GeneXpert/MTB-RIF test, 42.2% showed resistance to rifampicin. The rate of resistance was 49.1% in 2015, 34.7% in 2016, and 44.3% in 2017 (Figure 1).

Resistance was observed in patients of all ages, and the proportions were similar in males (40.4%) and females (45.9%) and in specimens from Bangui (43.1%) and from the provinces (34.6%). Resistance was found most frequently in failure cases (70.4%), and the difference was statistically significant ($P < .0001$). Resistance was found in 40% of contacts of MDR-TB, 28.2% of relapses cases, and 20% of default cases, with no significant difference (Table 3).

DISCUSSION

Only data on rifampicin resistance identified by the GeneXpert MTB/RIF test are reported here. According to the NTRL algorithm, specimens that are positive in this test should be cultured on Lowenstein-Jensen solid medium and tested for sensitivity to firstline drugs to identify the resistance phenotype. We were unable to include data on sensitivity in this study, however, owing to the lack of sensitivity tests in 2015 and 2016 for technical reasons. Nevertheless, the efficacy of the GeneXpert MTB/RIF test was sufficiently clear, and the WHO recommendation for its use indicates that these results can be considered sufficient in the absence of complementary conventional or molecular tests [6–8].

Table 2. Results of GeneXpert Test and Microscopy for the Detection of *M. tuberculosis*

	GeneXpert MTB/RIF Test, No. (%)			
Microscopy	Positive	Negative	Invalid	Total
Positive	482 (98.7)	4 (3.1)	1	487 (78.9)
Negative	6 (1.3)	123 (96.9)	1	130 (21.1)
Total	488 (79.1)	127 (20.6)	2 (0.3)	617 (100)

The cases of suspected resistance to rifampicin referred for verification were in patients of all ages, as found elsewhere in the region, with age ranges of 3–62 years in Morocco, 15–65 years in Togo, and 12–70 years in Chad [9–11].

A relatively small proportion of cases considered positive by GeneXpert MTB/RIF (6/130 cases, 4.6%) had been considered negative by microscopy (1.3%). The sensitivity of this test results in good diagnostic capacity, and positive results have been found in up to 75% of sputum samples found negative by microscopy [12–14]. The test also found negative results for 4 patients (3.1%) with positive microscopy reported from DTC; however, microscopic verification at the NTRL on 2 sputum samples from each of these patients indicated that they were negative. It should be noted that some patients with suspected relapse or failure cases could simply have a chronic broncho-pneumopathy, and this differential diagnosis should be considered, as 20.6% of the suspected cases of MDR-TB (127/617) were negative using the GeneXpert MTB/RIF test.

The overall prevalence of rifampicin resistance over the 3 years of the study was 42.2% (95% confidence interval [CI], 34.7–49.1), which is slightly higher than the 40% TB drug resistance reported in 2010 [5]. High rates of resistance have been reported in previously treated patients in several countries in Africa, including Ethiopia (33.2%), Mali (59%), and Lagos (66%) and Ibadan (39%) in Nigeria [15, 16]. Resistance to TB drugs is a real challenge and a threat to countries, particularly as, in addition to MDR strains, there are now pre-XDR and XDR strains, even in new patients [16–18]. Numerous studies have demonstrated the association between the development of resistance and prior exposure to TB drugs [19, 20]. Ensuring adherence to treatment is therefore important, as interrupting treatment or poor compliance can result in relapse or treatment failure [20].

The situation in the CAR is particularly alarming, as directly observed treatment (DOT) has been strongly impacted by the devastating effects of military and political conflicts on the health system. Thus, the drug dispensers who should ensure adherence have little contact with patients. Some of the DTCs visited saw only the carers of patients, who came to get their drugs. Adherence to long-term treatment of TB remains a difficult problem. Furthermore, patients with TB are stigmatized, as the disease is suspected of being associated with HIV infection. Unless patients are accompanied throughout treatment, they are tempted to stop taking their drugs after the first phase, when microscopy may be negative and they appear clinically well.

The analysis of risk factors for the acquisition of resistance showed a significant association with treatment failure. Results

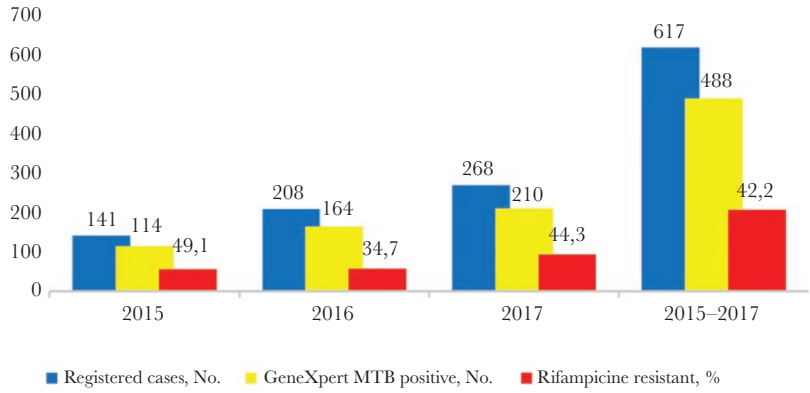

Figure 1. Results of GeneXpert MTB/RIF testing, 2015–2017.

reported from several other countries (Ethiopia, China, and India) showed that treatment failure and re-treatment failure were the most important factors for the development of resistance to rifampicin or MDR strains [21–24]. Resistance rates >75% have been reported in cases of failure of firstline treatment, which are responsible for the selection of resistant strains. It is therefore essential to perform phenotypic or genotypic sensitivity tests as soon as possible to propose an adapted therapeutic regimen [20, 25].

Other factors, such as age and sex, showed no significant association with the development of resistance as reported in other studies [26]. A major finding of our study was resistance to rifampicin in 40% of contacts of patients with MDR-TB. More and more cases of resistance are reported among contacts, with rates of 13% in Ghana and 32% in Lagos, Nigeria [16]. Urgent measures are required to protect the contacts of patients with MDR-TB, including health care workers, especially in view of the difficulty of adhering to second-line treatment.

Of the specimens sent from the provinces, 34.6% were resistant to rifampicin. Access to the interior of the country is difficult, due to the chronic armed conflict. The challenge for the NTP is now to install GeneXpert machines throughout the country, to ensure their maintenance, and to establish regional centers for the management of patients with MDR-TB. Furthermore, a system for regular shipment of suspected sputum samples to the NTRL should be put in place as soon as possible, so that complementary sensitivity tests can be conducted to make the fight against TB in the CAR more effective.

CONCLUSIONS

This surveillance study of rifampicin resistance with GeneXpert MTB/RIF in the NTRL at IPB showed a rate of resistance of 42.2% between 2015 and 2017. Treatment failure was the most important factor in the development of

Table 3. Sociodemographic Characteristics and Types of Patients With TB Resistant to Rifampicin

Characteristic	Resistant, No. (%)	Not Resistant, No. (%)	OR (95% CI)	*P*
Age, y				
0–15	6 (66.6)	3 (33.4)	-	
16–30	82 (46.1)	96 (53.9)	0.4 [0.1–1.7]	.2
31–45	81 (40.1)	121 (59.9)	0.3 [0.1–1.4]	.1
46–60	30 (36.1)	53 (63.9)	0.2 [0.1–1.2]	.08
≥61	7 (43.7)	9 (56.3)	0.4 [0.1–2.1]	.3
Sex				
Female	73 (45.9)	86 (54.1)	-	
Male	133 (40.4)	196 (59.6)	0.8 [0.5–1.2]	.2
Locality				
Bangui	188 (43.1)	248 (56.9)	1.4 [0.8–2.6]	.2
Provinces	18 (34.6)	34 (65.4)	-	
Type of patient				
Failure	119 (70.4)	50 (29.6)	9.5 [4.4–20.5]	<.0001
Relapse	73 (28.2)	186 (71.8)	1.6 [0.7–3.3]	.2
Default	10 (20.0)	40 (80.0)	-	
Contact of MRD-TB	4 (40.0)	6 (60.0)	2.6 [0.6–11.6]	. 2

Abbreviations: CI, confidence interval; MDR-TB, multidrug-resistant tuberculosis; OR, odds ratio; TB, tuberculosis.

resistant strains, and contacts of patients with MDR-TB had a high rate of resistance. The few sputum samples from the provinces of the country to the NTRL indicated circulation of strains resistant to rifampicin in the interior of the country. It is therefore imperative that the NTP not only continue to promote DOT but also extend surveillance of TB drug resistance beyond the capital, Bangui. Surveillance and management of patients with MDR-TB nationally will be required if the country wants to contribute effectively to the WHO strategy to End TB by 2035.

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Ethical considerations. The surveillance program was approved ethically by an expert committee of the Ministry of Health (order No. 0277/MSPP/CAB/DGSPP/DMPM/SMEE of August 5, 2002), and the data were used in strict confidentiality [27].

Potential conflicts of interest. The authors declare that they have no competing interests. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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