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Full Length Research Paper

Primary and secondary resistance to first-line anti-tuberculosis medications at the Institute Pasteur Bangui, Central African Republic

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The emergence and spread of anti-tuberculosis-resistant strains of *Mycobacterium tuberculosis* presently represents a real challenge to the WHO's End TB by 2035 strategy. Through this study, we wanted to determine the current prevalence of primary and secondary resistance to first-line anti-tuberculosis drugs at the Pasteur Institute in Bangui. Cultures and sensitivity tests were carried out for 6 months (July - December 2018) in 225 consenting tuberculosis patients. The prevalence was 4.1% in new cases and 25.2% in retreatment cases. Previous exposure to treatment has been the risk factor for the development of drug resistance. The resistance profile showed that 73.2% of patients were multidrug resistant (MDR) and 34.2% of retreatment patients presented resistance to all first-line anti-tuberculosis drugs; moreover, of the three new cases with resistance to anti-TB medicines, two showed multiple resistance, associating rifampicin and isoniazid. Surveillance of resistance to anti-tuberculosis drugs at the national level is necessary because it will allow better control of tuberculosis in the Central African Republic.

Key words: News cases, retreatement, MDR-TB, Risk factor, Bangui.

INTRODUCTION

Since Robert Koch discovered the tuberculosis (TB)-causing bacillus in 1882, tuberculosis has continued to be and remains a public health problem around the world. In 2018, the World Health Organization (WHO) reported 10 million tuberculosis cases in the world, and 1.2 HIV-negative people died. Rifampicin-resistant tuberculosis (RR-TB) cases were estimated at 484,000, of which 78% were multidrug resistant tuberculosis (MDR-TB) and 214,000 deaths (WHO, 2019). The worldwide prevalence rate of MDR-TB was estimated at 3.4% in new patients and 18% in previously treated cases; still higher rates have been reported from Russia, with 25% for new

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cases, and more than 50% for relapse cases.

Drug-resistance thus appears to be a major issue in the control of pulmonary TB, because if a patient has MDR-TB, the disease cannot be treated with either of the main anti-TB medicines, rifampicin and isoniazid (Abate et al., 2014). The two main causes of the development of drug-resistance are attributed to non-observance of the prescribed treatment and the use of inadequate treatment regimens (WHO, 2019). For TB patients, the main risk factors for drug resistance are prior treatment with anti-TB medicines and contact with a person with MDR-TB (Chen et al., 2013; Mekonnen et al*.,* 2015).

The emergence and spread of MDR-TB constitute a real challenge for the WHO End TB Strategy, because they compromise not only all the work of the national TB control programs around the world, but also threaten to destabilize the worldwide efforts to eradicate TB (Sabeel et al., 2017).The prevalence of MDR-TB is increasing throughout the world, in both new cases and in relapse cases, and only 50% of MDR-TB patients have been successfully treated (WHO, 2019; Abate et al*.,* 2014). The public health danger posed by an MDR-TB patient cannot be underestimated. The Central African Republic (CAR) is one of the countries with a high tuberculosis burden with an incidence of 540/100 000 inhabitants (WHO, 2019) but to date**,** only partial data is available on MDR-TB prevalence on a national level. Limited studies carried out in 2010 and 2011 on primary and secondary resistance, respectively, reported 0.4% of primary resistance and 40% in relapse cases (Minime-Lingoupou et al., 2010, 2015). The object of this study, carried out at the National Reference Laboratory for TB (NRL-TB) at the Institut Pasteur in Bangui (IPB), CAR, was to review the current levels of primary and secondary resistance to first-line anti-TB medicines.

MATERIALS AND METHODS

Study type, duration and location

This cross-sectional study was carried out for 6 months (1 July to 31 December 2018) by the NRL-TB (IPB).

Study population

The study population was composed of all the patients referred to the NRL-TB for a tuberculosis exam. These patients included new TB cases or retreatment patients due to failed treatment, relapse or default.

Inclusion criteria

All patients who consented to participate in the study were included in the study.

Exclusion criteria

All patients whose sputum smear was negative or who had not given consent were excluded from the study.

Data collection

A pre-defined questionnaire provided socio-demographic information on the included patients, such as patient age, sex, geographic origin and TB case type. Information on alcohol and tobacco use was also collected.

Ethical considerations

This project of study was submitted to the National Scientific Committee in Charge of Validating Study Protocols and Results for the CAR and received approval on 5 July 2018 (registration no. 14). The data were analyzed anonymously to preserve patient identity.

Laboratory analyses

Cultures

Cultures were carried out in the Biosafety Level 2+ (BSL-2+) laboratory at the NRL-TB. This laboratory operates under negative pressure and has two class II biosafety cabinets (BSCs), one for cultures and the other for drug susceptibility tests. Under the BSC for cultures, sputum samples were decontaminated with NaOH using the Petroff method and then seeded on Lowenstein-Jensen (LJ) media, with two plates per sample. The tubes were then incubated at 37°C and observed weekly for up to 8 weeks before pronouncing the final result, because some mycobacterial strains may be difficult to culture. For internal quality control, growth and sterility tests were carried out on each new batch, and external quality control was carried out by the National Institute for Communicable Diseases, a national public health institution of South Africa and obtained a performance score of 100%.

Reporting of results

In the absence of colonies, the result was declared negative; between 1 and 49 colonies, the result was reported as countable colonies; between 50 and 100 colonies, positive 1+,between 100 and 200 colonies, positive 2+ and more than 200 colonies, positive 3+.

Identification

The identification test for the *Mycobacterium tuberculosis* complex was carried out by screening for the MPT64 antigen using a rapid immunochromatographic test (SD Bioline) according to the manufacturer's instructions. The appearance of only the control line indicated a negative test and the appearance of a band on the T line of the test indicated positive identification of the causative agent of TB.

Drug susceptibility testing

Susceptibility tests were carried out in the BSL-2+ laboratory using the proportion method, because the NRL-TB does not have liquid media. The antibiotic concentrations per tube were as follows: 0.2 µg/ml isoniazid and 1 µg/ml isoniazid; 40 µg/ml rifampicin; 2 µg/ml ethambutol and 4 µg/ml streptomycin. After 4 to 6 weeks of incubation at 37°C, results were read. Internal quality controls for drug susceptibility testing were carried out for each batch of LJ

Table 1. Results from mycobacterial cultures and diagnosis sensitivity on samples from the included patients (n= 228).

Variable	Microscopy		
	Positive (%)	Negative (%)	Contaminated (%)
Culture	225 (98.7%)	0	3 (1.3%)
Sensitivity	225 (100)	0	0

media by using two reference strains provided by the Supranational TB Reference Laboratory in Anvers, Belgium: drug-susceptible strain 7187 and rifampicin-, isoniazid-, ethambutol- and streptomycin-resistant strain 6025. External quality controls were carried out in 2018 as for the culture controls by the South African National Institute for Communicable Diseases for the four anti-TB drugs (rifampicin, isoniazid, ethambutol and streptomycin) with an acceptable score of 81%.

Data analysis

The study data were recorded in a Epi Info™ database and analyzed using Stata 14 software.

RESULTS

Characteristics of the study population

The average age of the included patients was 40 years, with the extremes of 9 and 71 years. The 21-40 years age class was the most represented with 64.9% of the included patients, followed by the 41-60 years age class with 27.1%. At 65.7%, men made up the majority of the study population, which had a sex-ratio (M:F) of 1.9:1. For geographic origin, 80.9% of the patients were from Bangui, and only 19.1% were from the provinces. TB case types were mostly relapses (40.9%), followed by new cases (32.9%) and treatment failures (18.4%). Default represented only 8.4% of the study population. Regarding alcohol and tobacco consumption, 51.3% of the patients consumed alcohol, 24.9% were regular smokers and 22.2% of patients were alcohol users and smokers.

Laboratory analyses

Of the 228 patients, sputum smears were positive for 98.7% (225/228). Three cultures (1.3%) were contaminated; the diagnosis sensitivity tests on the 225 positive samples were 100% conclusive (Table 1).

Prevalence of resistance to anti-TB medicines

The prevalence of resistance among the new cases was 4.1% (3/74), but in the retreatment patients, prevalence was six times higher with a rate of 25.2% (38/151) (Figures 1 and 2).

Distribution of patients according to their resistance profile

Most of the resistance cases involved resistance to rifampicin (35/41, 85.4%); 73.2% (30/41) of the patients were MDR-TB cases, showing resistance to both rifampicin and isoniazid. Importantly, 31.7% (13/38) retreatment patients presented resistance to all of the first-line anti-TB treatments; moreover, of the three new cases with resistance to anti-TB medicines, two showed multiple resistance, associating rifampicin and isoniazid (Table 2).

Profile of patients with resistant anti-TB strains

Age

Resistance was most frequently observed in the 41-60 years age class, in 19.7% of the age class, followed by the <20 years age group and the 21-40 years age group, with 18.2 and 17.8%, respectively, but this difference was not significant (Chi-square test, χ^2 = 0.1757; p= 0.981; Table 3).

Sex

Resistant strains were observed more frequently in men than in women (19.1 vs. 16.7%), with no significant difference (χ^2=0.1939; p = 0.660; Table 3).

Geographical origin

There were no significant differences between the capital Bangui, where 18.1% of patients harbored resistant strains, and provincial towns, which showed 18.6% resistant cases (χ^2 = 0.0052; p = 0.942; Table 3).

Alcohol and tobacco consumption

13.1% of patients who consumed alcohol presented

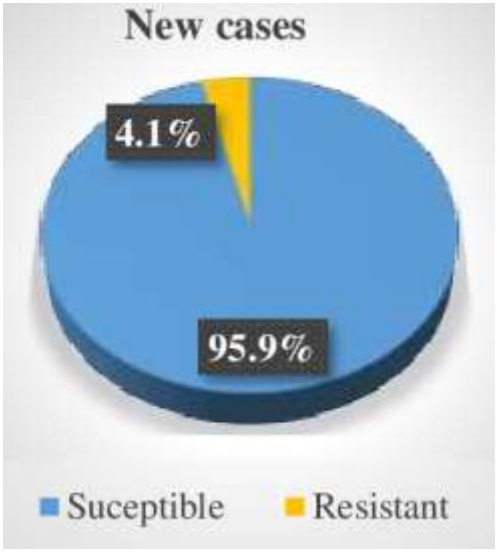

Figure 1. Prevalence of resistance to anti-tuberculosis medicines in new cases.

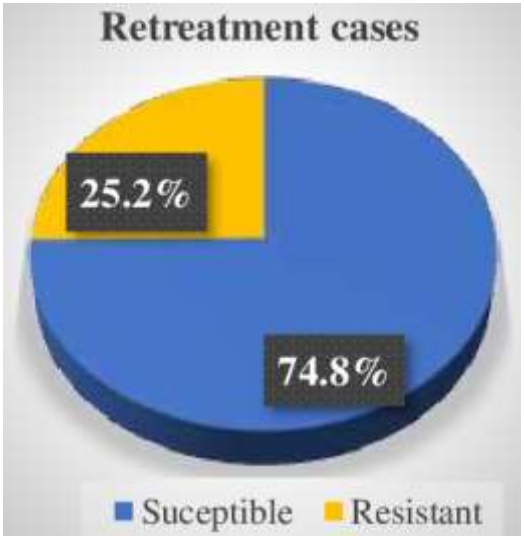

Figure 2. Prevalence of resistance to anti-tuberculous medicines in retreatment cases.

resistance, in non-alcohol drinkers, the proportion is higher (23.6%). Regarding tobacco use, a higher rate of resistance was also observed in non-smokers (23.6%) than in smokers (12.5%). Finally, non-alcoholic tobacco users also showed more resistance (20.6%) than alcoholic tobacco users (10%) (Table 3).

Case type

Retreatment patients presented resistant strains significantly more frequently than new cases did (25.2% vs. 4.1%; χ^2=14.8; p =0.001; Table 3).

DISCUSSION

This study aimed to determine primary and secondary resistance in TB patients registered at the NRL-TB (IPB). Although the study population is not representative of the CAR population, the techniques used and the quality controls carried out by supranational TB laboratories ensure that the results are robust and can be used as a baseline for a national-level study.

Although the reported prevalence rates (4.1% in new cases and 25.2% in retreatment cases) remain within the limits of those reported by the WHO on a worldwide scale, primary resistance jumped from 0.4% in 2011 to 4.1% in 2018, a 10-fold increase (Minime-Lingoupou et al., 2015). The increase in the rate of resistance in new cases is of high concern, because it reflects the difficulties national programs encounter to control the spread of MDR-TB. Today, MDR-TB is no longer solely an issue for retreatment patients, primary TB infections now also show non-negligible rates of MDR-TB. Therefore, drug susceptibility tests should be carried out before choosing a treatment regimen (Mekonnen et al., 2015; Abdella et al., 2015). The automated diagnostic Xpert MTB-RIF test is now particularly useful in this context, because TB can be diagnosed and its resistance status determined within one day, which is not possible for conventional methods (Boehme et al., 2010 , 2011; WHO, 2014). Although lower than that in 2010, the secondary resistance rate shows that retreatment patients are still more exposed to resistant strains than first-time TB patients (Chen et al., 2013; Mekonnen et al., 2015). Resistance in secondary cases has always been clearly higher than in primary cases, as reported in several studies, with initial exposure to anti-TB treatment being the main risk factor. It is therefore still necessary to monitor adherence to the initial treatment regimen to limit the risk of resistance (Hamusse et al., 2016; Mulisa et al., 2015).

This study demonstrated that most resistance cases involved rifampicin (35/41, 85.4%); 73.2% of the patients were MDR-TB cases, showing resistance to rifampicin and isoniazid. Moreover, 31.7% (13/41) presented resistance to all four tested first-line anti-TB medicines at the NRL-TB (Table 2). In a Nigerian study, similar resistance rates were reported, with 76.4% of MDR-TB and 56.4% resistant to all four first-line medicines (Olusoji and Eltayeb, 2016). The ever-more disturbing increase in resistance to rifampicin and in MDR-TB cases are arguments for the large-scale use of Xpert MTB-RIF in resource-limited countries. This test can rapidly detect rifampicin resistance, leading to the prompt initiation of a second-line treatment. Conventional culture and drug susceptibility tests still require contained laboratories with

Table 2. Resistance profiles observed during the study.

Variable	Number of resistant cases			
	New cases (%)	Retreatment cases (%)	Total (%)	
R	0	5 (100)	5 (12.2)	
RH	2 (13.3)	15 (86.7)	17 (41.5)	35/41 (85.4)
RHEZ	0	13 (100)	13 (31.7)	
Other	1 (16.7)	5 (83.3)	6 (14.6)	
Total	3 (7.3)	38 (92.7)	41 (100)	

R, rifampicin; H, isoniazid; E, ethambutol; Z, pyrazinamide.

Table 3. Profile of patients infected with mycobacterial strains resistant to anti-tuberculosis medicines.

Characteristic	Resistance		Chi-2	P
	No (%)	Yes (%)		
Sex				
F	65 (83.3)	13 (16.7)	0.1939	0.660
M	119 (80.9)	28 (19.1)		
Age class (year)				
<20	9 (81.8)	2 (18.2)	0.1757	0.981
21-40	120(82.2)	26 (17.8)		
41-60	49(80.3)	12 (19.7)		
>60	6 (85.7)	1 (14.3)		
Geographic origin				
Bangui	149 (81.9)	33 (18.1)	0.0052	0.942
Province	35 (81.4)	8 (18.6)		
Alcohol consumption				
Yes	100 (86.9)	15 (13.1)	4.2335	**0.040**
No	84 (76.4)	26 (23.6)		
Smoker				
Yes	49 (87.5)	7 (12.5)	1.6382	0.201
No	135 (79.9)	34 (20.1)		
Alcohol and tobacco use				
Yes	45 (90.0)	5 (10.0)	2.9165	0.088
No	139 (79.4)	36 (20.6)		
Case type				
New case	71 (95.9)	3 (4.1)		
Retreatment case	113 (74.8)	38 (25.2)	14.8	**0.001**

a biosafety level whose cost remains out of reach for developing countries (Olusoji and Eltayeb, 2016). Today, the clear evidence of MDR-TB in new patients should encourage national TB control programs to strive to contain this resistance in the coming decades. The WHO's backing for the use of Xpert MTB-RIF as a first-line, rapid diagnosis tool is an important step, but developing countries must have the means to deploy the diagnosis method (Metcalfe et al., 2016; Steingart et al., 2014; Pimkina et al., 2015).

Resistance was most frequently observed in the 41-60 years age group, but with no link to the disease (Lomtadze et al., 2009). Men were generally more affected than women, this may be an effect of differential

exposure of men and women to TB due to differences in their social and professional activities (Sangaré et al., 2010). In this study, 2.5% of patients with resistant TB were from outside the capital city. This low recruitment rate reflects the logistic problems that affect the transfer of sputum specimens from provincial centers to the NRL-TB, located in the capital, and the only laboratory in the country that can carry out mycobacterial cultures and susceptibility tests.

In this study too, there appeared to be a significant difference between non-alcohol drinkers and alcohol drinkers (P: 0.040) which may have seemed paradoxical. In fact, neither alcohol nor cigarettes were found to be risk factors in this study. The significance for alcohol in favor of non-drinkers is probably due to confusion bias since the stratified study in patients who do not smoke showed that non-alcoholics were in the majority (23.08%) against (15.38%) without significant difference (p = 0.22). Likewise in smoking patients, non-alcoholics were also in the majority (33.33%) against 10% with no significant difference (p = 0.1). However, the studies carried out in Brazil and in Spain have shown that alcohol and smoking are undeniable risk factors for mycobacterial resistance to anti-TB medicines, with smoking increasing the risk for MDR-TB by a factor of 3 (Barroso et al., 2003; Suárez-García et al., 2009).

Overall, resistance was observed more frequently in retreatment cases than in new cases, a difference that was statistically significant. Acquiring resistance after anti-TB treatment has been described in the literature and the antecedent of having already taken anti-TB medicines can multiply the risk of MDR-TB by a factor of 5 (Misombo-Kalabela et al., 2016).

Conclusion

Resistance to anti-TB drugs remains a major challenge for national TB control programs. Our demonstration of MDR-TB in new TB cases, reported elsewhere throughout the world, implies that more WHO-recommended drug susceptibility testing is needed, using for example Xpert MTB/RIF tests. Moreover, previous exposure to anti-TB treatment, and alcohol and tobacco use remain the main risk factors for contracting resistant TB. Therefore, efforts are needed to deploy the 'directly observed treatment, short-course (DOTS)' strategy strictly and to foster awareness to promote change in behavior. Partners in development and in the fight to end TB are called upon to contribute more to national efforts to contain the spread of multi-resistant tuberculosis bacilli.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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