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Original Article

Mycobacterium tuberculosis drug-resistance in previously treated patients in Ouagadougou, Burkina Faso

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Abstract

Background: Tuberculosis drug-resistance becomes common in sub-Saharan Africa; however, very few data are available in Burkina Faso. The aim of this study is to assess the acquired resistance of *Mycobacterium tuberculosis* complex strains identified in TB patients to four first-line drugs in Ouagadougou.
Methods: One hundred and ten (110) pulmonary tuberculosis patients with acid-fast bacilli-positive sputum and in situation of failure, relapse, or treatment abandonment were included in the study. Ninety six strains, including 92 (95.8%) *M. tuberculosis* and 4 (4.2%) *M. africanum*, were isolated from the sputum samples of these patients. Their drug susceptibility testing was performed using the proportion method. The first-line drugs tested were isoniazid (INH), streptomycin (STR), ethambutol (EMB), and rifampicin (RIF).
Results: The overall drug-resistance rate of *M. tuberculosis* was 67.4% (*n*=60), including 3.4% to one drug, 18% to two, 10.1% to three, and 35.9% to four drugs. The resistance to INH, RIF, EMB, and STR were 67.4%, 51.7%, 50.6%, and 44.9%, respectively. Two strains of *M. africanum* were resistant to all drugs. Forty-six (51.7%) strains were multidrug-resistant (resistant to at least INH and RIF).
Conclusions: In previously treated patients, the level of resistance of *M. tuberculosis* complex to commonly used anti-tuberculosis drugs is very high in Ouagadougou. Our results showed that multidrug-resistant tuberculosis could be a public health problem in Burkina Faso.

Keywords: Burkina faso, drug resistance, Ouagadougou, tuberculosis

Résumé

Arrière-plan: Tuberculose pharmacorésistance devient commun en Afrique; Toutefois, très peu de données est disponibles au Burkina Faso. L'objectif de cette étude est pour évaluer la résistance acquise de *Mycobacterium tuberculosis* complexes souches identifiées dans les tuberculeux aux quatre premiers médicaments de ligne à Ouagadougou.
Méthodes: Une centaine et dix patients de tuberculose pulmonaire avec acid-fast bacilles-positif expectorations et en situation de défaillance, abandon de rechute ou de traitement ont été inclus dans l'étude. Quatre-vingt seize souches, y compris 92 (95.8%) *M. tuberculose* et 4 (4.2%) *M. africanum*, ont été isolées à partir des échantillons de salive de ces patients. Leurs médicaments. test de sensibilité a été effectuée à l'aide de la méthode de proportion. Le premier ligne de médicaments testés ont été isoniazide (INH), streptomycine (STR), ethambutol (EMB) et rifampicine (RIF).
Résultats: Le le taux global de la résistance aux médicaments de *la tuberculose M.* était 67.4% (*n*=60), y compris de 3.4% à un médicament, 18% à deux, 10.1% à trois et 35.9% aux quatre médicaments. La résistance à l'INH, RIF, ECE et STR était 67.4%, 51.7%, 50.6% et 44.9%, respectivement. Deux souches de *M. africanum* ont été résistant à toutes les drogues. Quarante-six (51.7%) souches ont été tuberculoses résistants (résistant au moins INH et RIF).

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Conclusions: Chez les patients traités précédemment, le niveau de résistance de M . *la tuberculose* complexe aux médicaments antituberculeux couramment utilisés est très élevée à Ouagadougou. Nos résultats montrent que tuberculoses multirésistantes pourrait être un problème de santé publique au Burkina Faso.

Mots clés: La tuberculose, la résistance aux médicaments, Ouagadougou, Burkina Faso

Introduction

The resurgence of tuberculosis (TB) is a major concern at the global level. The World Health Organization (WHO) estimates that 1.7 to 2 billion people are infected with *Mycobacterium tuberculosis* and that each year, 7–9 million develop TB of which 3 million die.[1] In Africa, TB prevalence remains high due mainly to the epidemic of HIV infection and the spread of multidrug-resistant (MDR) tuberculosis.[1-5]

The drug-susceptibility testing of *M. tuberculosis* remains a significant part of the monitoring process of the TB resistance to drugs.[6] Surveillance of drug-resistance, notably by periodic assessments, can guide the decision-maker in defining the standardized regimens and for assessing its quality on the field. Thus, the level and trend of resistance enables appropriate adjustments to be made either at the organizational level or changing the treatment regimens at patients' level.

In Africa, drug-susceptibility testing for *M. tuberculosis* is rarely done. Nevertheless, some useful information has been obtained in some countries in the course of investigations conducted by WHO and the International Union against Tuberculosis and Lung Disease (IUATLD),[7] or as part of studies done locally.[8-12] For the specific case of Burkina Faso, one study conducted at the Centre Muraz in Bobo-Dioulasso in 1996, a year after the adoption of directly observed treatment (DOT) in the country, showed an 8.6% overall prevalence of resistance to TB drugs in new cases.[13] Since then, no other study or evaluation has been conducted despite the fact that clinical treatment failures which suggest the existence of drug-resistant strains are recorded increasingly. Such a gap in information could complicate the implementation of any successful drug control program.

The aim of this study was to assess the prevalence of *M. tuberculosis* resistance to antibiotics in patients experiencing failure, relapse, and treatment abandonment in order to assess the quality of treatment provided in Burkina Faso.

Materials and Methods

Setting

The study was carried out in the laboratory of the National Tuberculosis Centre (NTC) in Ouagadougou, Burkina Faso. All bacteriological examinations were run in this laboratory where new TB cases are detected and where the bacteriological response to therapy of TB patients is monitored. It strongly supports the activities of the National Tuberculosis Programme (NTP).

Patients and ethical consideration

From April 2005 to February 2006, 110 patients experiencing failures, relapses, or treatment abandonment for at least 1 month and with acid fast bacilli (AFB) positive sputum smears were consecutively enrolled in a cross-sectional study. The aim of the study was explained to each participant in his language of communication. After obtaining informed consent, a standard questionnaire was completed for each patient to collect demographic data and the history of the disease such as: previous anti-TB treatment, treatment duration with or without injection (streptomycin), recognition of the anti-TB drugs on behalf of the patient, previous pulmonary radiographs and examinations of sputa.

Bacteriological study

Microscopic examination and strains isolation.

Sputum smears were prepared and stained by the Ziehl-Neelsen hot method, as recommended by IUATLD.[14] One AFB-positive sample from each patient was used for the culture.

The sputum samples were decontaminated with 4% NaOH, according to the Petroff method,[15,16] centrifuged, and the sediments transferred onto Loewenstein-Jensen (LJ) media, LJ supplemented with pyruvate (LJ+pyruvate), and LJ containing 5 mg/L hydrazide of the thiophene-2-carboxylic acid (TCH) (LJ+TCH). These culture media were incubated at 37°C and observed on days 3 and 7 to detect contaminations and/or fast growth of atypical mycobacteria and subsequently every week to note the growth rate and the morphology of the colonies.

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Identification and drug-susceptibility test

Isolates were identified by acid fast staining, colony growth time, resistance to TCH, culture abundance in LJ+pyruvate media, and activity to usual biochemical assays (niacin, nitrate reductase, catalase activity at 22°C and 70°C).[17]

The antibiotics were tested according to the method of Canetti, Rist, and Grosset.[18] The concentrations used were 0.2 μg/mL for isoniazid (INH), 40 μg/mL for rifampicin (RIF), 4 μg/mL for streptomycin (STR), and 2 μg/mL for ethambutol (EMB). A strain was declared resistant to an antibiotic if the bacterial growth on medium with the drug was ≥1% compared to the control or sensitive when their growth was <1%. Drug resistance was acquired if the patient was previously treated with antituberculosis drugs.

A *M. tuberculosis* H37 ATCC 27294 strain was used for quality control in DST. Proficiency testing for culture and identification were done in collaboration with the National reference Center for Mycobacteria in Borstel (Parkallee Borstel Germany): 10 strains of *M. tuberculosis* Complex and 10 of atypical mycobacteria strains were used for this control.

Statistical analysis

The data were recorded and analyzed on SPSS version 12.0. The results were interpreted using standard chi-square test (χ^2). A significant threshold was $P<0.05$.

Results

Demographic characteristics of patients

One hundred and ten previously treated patients were included in the study, with 73 (66.5%) cases of failure, 25 (22.7%) relapses, and 12 (10.9%) cases of treatment abandonment at least for 1 month. The failures occurred at the fifth month in 24 (21.8%) patients, at 7/8th month in 7 (7.3%) patients, and in 41 (37.3%) failures after two courses of treatment (the chronics). The age and gender distributions are presented in Figure 1. The sex ratio was 2.4, with 74 (67.3%) men and 36 (32.7%) women. The age range was 14–76 years old, with a mean of 38.3 ± 11.4 years.

Mycobacterium resistance to the drugs tested

All the 110 patients were AFB-positive, while the culture was positive for 105 (95.5%), negative for 1 (0.9%), and contaminated for 4 (3.6%) patients. Among the 105 strains identified, there were 92 (87.6%) *M. tuberculosis*, 4 (3.8%) *M. africanum*, and 9 (8.6%) non-tuberculosis *Mycobacterium* which were excluded.

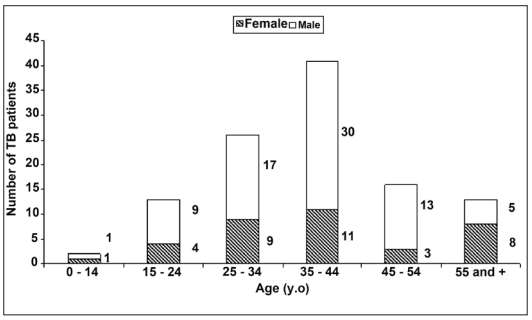

Figure 1: Age and gender distribution of TB patients

Three isolates of *M. tuberculosis* were excluded because of insufficient number of colonies. Drug-susceptibility testing to INH, RIF, EMB, and STR was performed with 89 *M. tuberculosis* and 4 *M. africanum* strains. The results showed that 29 (32.6%) *M. tuberculosis* strains were sensitive to all antibiotics tested and 60 (67.4%) were resistant. Monodrug-resistance was 67.4% to INH, 51.6% to RIF, 44.9% to STR, and 50.5% to EMB. However, 32 (35.9%) strains were MDR for INH and RIF. The other resistance details are presented in Table 1. Two of the four *M. africanum* isolates were resistant to all antibiotics tested.

Table 1: Acquired drug-resistance of *Mycobacterium tuberculosis*

Antibiotics	Number	%
Total number of strains tested	89	
Total number of susceptible strains	29	32.6
Resistance to any drug	60	67.4
H	60	67.4
R	46	51.7
E	45	50.6
S	40	44.9
Monoresistance	3	3.4
H	3	3.4
R	0	0
E	0	0
S	0	0
Multiresistance (MDR)	46	51.6
H+R	8	9.0
H+R+E	5	5.6
H+R+S	1	1.1
H+R+E+S	32	35.9
Other patterns	11	12.4
H+E	5	5.6
H+S	3	3.4
H+E+S	3	3.4
R+E	0	0
R+S	0	0
R+E+S	0	0
E+S	0	0
Susceptible to all drugs	29	32.6
Number of drug-resistant strains	60	67.4
Resistant to one drug	3	3.4
Resistant to two drugs	16	18.0
Resistant to three drugs	9	10.1
Resistant to four drugs	32	35.9

H, isoniazid; R, rifampicin; E, ethambutol ; S, streptomycin

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Table 2: H, R, E, S, and HRES acquired resistance of *M. tuberculosis* according to TB history in patients

	H	R	E	S	H+R+E+S
Failures on 5th month	20 (33.3)	13 (28.3)	14 (31.1)	10 (25)	6 (18.8)
Failures 7/8th month	4 (6.7)	4 (8.7)	4 (8.9)	2 (5)	2 (6.3)
Chronics	30 (50)	28 (60.9)	26 (57.8)	24 (60)	23 (71.9)
Relapses	4 (6.7)	1 (2.2)	1 (1.2)	2 (5)	1 (3.1)
Abandon ≥ 1 month	2 (3.3)		0	0	2 (5)
Total	60 (100)	46 (100)	45 (100)	40 (100)	32 (100)

H, isoniazid; R, rifampicin; E, ethambutol; S, streptomycin.

Resistance of *M. tuberculosis* to INH, RIF, EMB, and STR separately or combined was higher in chronic cases than among cases of relapse and treatment abandonment [Table 2]. Chronic TB patients were more resistant to RIF and STR than those with failures at the 5th and 7/8th months (P=0.031 and 0.042, respectively) and also more resistant to any drug than the cases with relapse and abandonment of less than 1 month (P=0.0001).

Discussion

In this study and generally in sub-Saharan Africa, TB is more prevalent in patients aged 20 to 45 years, with a clear male prevalence.[19] In fact, young adults, and especially male adults, are the most economically productive and are committed in various activities from where the transmission of the tuberculosis bacillus can occur easily.[19] These reasons could also explain why there is more failure and relapse in this group.

The prevalence of *M. tuberculosis* drug-resistance in this study was 67.4%. Lower rates have been described in Nepal, Italy, Estonia,[14] in Mexico,[20] and in Prague[21] (40.9%, 47.2%, 58.1%, 65%, and 52%, respectively). Higher rates were found in Egypt (68.2%), Russian Federation (73.3%), Kazakhstan (82.1%),[20] Ivory Coast (79.0%),[19] and Japan (80.0%).[22]

The drug-resistance rate was higher in chronic cases (failure after two courses of treatment) than in other patients (failure after 5th or 7/8th month and the relapses). It seems that the proportion of patients with resistant bacilli becomes dominant after failure of two treatment courses. It is probable that the provision of a second course of treatment as recommended by WHO will fail in the treatment of these chronic cases because when a strain is resistant to both INH and RIF, the chance of successful therapy is low.[23]

The chronic cases could have been detected if the culture and drug susceptibility tests were performed after the failure of the first course of treatment. Unfortunately, *in vitro* surveillance of *M. tuberculosis* resistance to drugs is not effective in the country, despite the current need. Presently, a further study is ongoing to detect XDR tuberculosis in these strains.

The MDR observed among patients in a situation of failure (particularly the chronics), relapse, and abandonment of treatment may reflect the quality of care or their poor compliance. This is an important public health problem considering the high level of resistance to drug used in the country. Monitoring of drug-resistant *M. tuberculosis* should be enhanced by periodic surveys to assess trends in resistance and take corrective action when necessary.

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References

1. Dye C. Global epidemiology of tuberculosis. Lancet 2006;367:938-40.
2. Center for Disease Control. Primary resistance to antituberculosis drugs, United States. MMWR Morb Mortal Wkly Rep 1983;32:521-3.
3. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 1993;328:521-32.
4. Bloch AB, Cauthen GM, Onorato IM, Dansbury KG, Kelly GD, Driver CR, *et al.* Nationwide survey of drug-resistant tuberculosis in the United States. JAMA 1994;271:665-71.
5. Grosset J. Fréquence et gravité actuelles de la résistance de *Mycobacterium tuberculosis* aux antibiotiques. Ann Inst Pasteur 1993;4:196-202.
6. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 1994-1997. Anti-tuberculosis drug resistance in the world. Geneva: WHO Tuberculosis Global Programme, 1997.
7. WHO/IUATLD. Anti-tuberculosis drug resistance in the world: Third global report/the WHO/IUATLD Global Project on anti-tuberculosis drug resistance surveillance 1999-2002.

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8. Dosso M, Bonard D, Msellati P, Bamba A, Doulhourou C, Vincent V, *et al*. Primary resistance to antituberculosis drugs: A national survey conducted in Côte d'Ivoire. Int J Tuber Lung Dis 1999;3:805-9.
9. Kuaban C, Bercion R, Jifon G, Cunin P, Blackett KN. Acquired anti-tuberculosis drug resistance in Yaounde, Cameroon. Int J Tuberc Lung Dis 2000;4:427-32.
10. Warndorff DK, Yates M, Ngwira B, Chagaluka S, Jenkins PA, Drobniewski F, *et al*. Trends in antituberculosis drug resistance in Karonga, Malawi, 1986-1998. Int J Tuber Lung Dis 2000;4:752-7.
11. Demissie M, Gebeyehu M, Berhane Y. Primary resistance to antituberculosis drugs in Addis Ababa, Ethiopia. Int J Tuberc Lung Dis 1997;1:64-7.
12. Owusu-Dabo E, Adjei O, Meyer CG, Horstmann RD, Enimil A, Kruppa TF, *et al*. *Mycobacterium tuberculosis* drug resistance, Ghana. Emerg Inf Dis 2006;12:1171-2.
13. Ledru S, Cauchoix B, Yaméogo M, Zoubga A, Lamandé-Chiron J, Portaels F, *et al*. Impact of short-course therapy on tuberculosis drug resistance in South-West Burkina Faso. Tuberc Lung Dis 1996;77:429-36.
14. Enarson DA, Rieder HL, Arnadottir T. Managements of tuberculosis: Guide for low income countries. 5th ed. Paris: WHO/IUATLD; 2000.
15. Petroff SA. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J Exp Med 1915;21:38-42.
16. Keilty RA. A final report on the cultivation of the tubercle bacillus from the sputum by the method of Petroff. J Exp Med 1916;24:41-8.
17. El Helali N, Vergez P. Identification des mycobactéries. Feuillets de Biologie 1993;34:5-19.
18. Canetti G, Rist N, Grosset J. Mesure de la sensibilité du bacille tuberculeux aux drogues antibacillaires par la méthode des proportions. Rev Tuberc Pneumol 1963;27:217-72.
19. Kouassi B, Horo K, N'douba KA, Koffi N, Ngom A, Aka-Danguy E, *et al*. Profil épidémiologique et microbiologique des malades tuberculeux en situation d'échec ou de rechute à Abidjan. Bull Soc Exot 2004;97:336-7.
20. Ramaswamy SV, Dou SJ, Rendon A, Yang Z, Cave MD, Graviss EA.Genotypic analysis of multidrug- resistant *Mycobacterium tuberculosis* isolates from Monterrey, Mexico. J Med Microbial 2004;53:107-13.
21. Saribas S. Resistance problems in *Mycobacterium tuberculosis*: Evaluation of the resistance of 166 *M. tuberculosis* strains against four major drugs. Euro Soc Clin Microbiol Infect Dis 2004;902:1287.
22. Quy HT, Lan NT, Borgdorff MW, Grosset J, Linh PD, Tung LB, *et al*. Drug resistance among failure and relapse cases of tuberculosis: Is the standard re-treatment regimen adequate? In J Tuberc Lung Dis 2003;7:631-6.
23. Senneville E. Traitement des patients porteurs de souches résistantes. Med Mal Infect 1995;25:369-76.

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