INT J TUBERC LUNG DIS 11(11):1221–1224

Anti-tuberculosis drug resistance among new and previously treated pulmonary tuberculosis patients in Cotonou, Benin

D. Affolabi,*† O. A. B. G. Adjagba,* B. Tanimomo-Kledjo,* M. Gninafon,* S. Y. Anagonou,* F. Portaels†

*** Programme National Contre la Tuberculose, Cotonou, Benin; † Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium**

SUMMARY

OBJECTIVES: To assess the current anti-tuberculosis drug resistance situation in Cotonou, at the largest anti-tuberculosis centre of Benin.

METHODS: A total of 470 isolates of *Mycobacterium tuberculosis* complex from pulmonary tuberculosis (TB) patients were analysed: 244 from new cases and 226 from previously treated cases. Drug susceptibility testing of isolates against first-line drugs was performed using the proportion method.

RESULTS: Primary multidrug resistance (MDR) depends on the patients' origin: MDR in new cases is relatively high (1.6%) when all patients are considered, but low (0.5%) and comparable to 1994 national survey results when only patients residing in Benin are considered. MDR in previously treated patients (11.1%) remains comparable to the study performed in Benin in 1994. No relation was found between human immunodeficiency virus co-infection and anti-tuberculosis drug resistance.

CONCLUSION: This study shows the great importance of correct patient identification in epidemiological surveys, where results may vary according to the population(s) studied.

KEY WORDS: drug resistance; *Mycobacterium tuberculosis*; Cotonou

TUBERCULOSIS (TB) remains a major public health problem worldwide; the World Health Organization (WHO) recommends the DOTS strategy for TB control.[1,2] The TB epidemic is nevertheless still growing and has now been complicated by the development of anti-tuberculosis drug resistance.[3] Development of drug resistance is a man-made phenomenon as a result of ineffective treatment due to poorly functioning TB control programmes, physician mismanagement or patient non-adherence.

Only a few countries in Africa have conducted anti-tuberculosis drug resistance surveys.[3] In the West African country of Benin, an anti-tuberculosis drug resistance survey was conducted in 1994–1995 covering four provinces and representing about 80% of TB patients in the country after 12 years' application of the DOTS strategy.[4] This survey showed relatively limited rates of resistance in new cases (8.4% resistance to any drug and 0.3% multidrug resistance [MDR-TB], defined as resistance to both isoniazid [INH] and rifampicin [RMP]). A decade after that study, the present study is intended to assess the situation of anti-tuberculosis drug resistance in Cotonou at the largest anti-tuberculosis centre of the country before another nationwide survey is performed.

MATERIALS AND METHODS

From 1 August 2002 to 31 December 2004, one new sputum smear-positive case in five and all sputum smear- and culture-positive patients previously treated for TB admitted to the National Pneumo-Phthisiology Hospital (NPPH) in Cotonou were prospectively included in the study. In total, 470 patients were included in the study: 226 previously treated cases and 244 new cases.

The NPPH receives patients not only from Cotonou and surrounding areas, but also from neighbouring countries of Benin. Medical history and demographic data from each patient were obtained by a physician following interview.

Culture and drug susceptibility testing

One sputum sample from each patient was decontaminated using the modified Petroff method and cultivated onto two Löwenstein-Jensen media.[5] Mycobacteria from each positive culture were identified using thiophene-2-carboxylic acid hydrazide (TCH) medium and biochemical tests (nitrate reductase, niacine and catalase at 68°C). All *Mycobacterium tuberculosis* isolates were tested for drug susceptibility against RMP, INH, streptomycin (SM) and ethambutol (EMB) using

Correspondence to: Françoise Portaels, Mycobacteriology Unit, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Tel: (+32) 3 247 63 17. Fax: (+32) 3 247 63 33. e-mail: portaels@itg.be

Article submitted 6 March 2007. Final version accepted 7 August 2007.

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.iuatld.org]

the proportion method at the following concentrations: RMP 40 μg/ml, INH 0.2 μg/ml, SM 4 μg/ml and EMB 2 μg/ml.[6] Culture and drug susceptibility testing (DST) were performed at the mycobacteria reference laboratory (LRM: Laboratoire de Référence des Mycobactéries).

Definitions

Resistance in new cases was defined as the presence of resistant strains of *M. tuberculosis* in new TB patients who had never been treated for TB for more than one month.

Resistance in previously treated patients was defined as the presence of resistant strains of *M. tuberculosis* in TB patients who had previously been treated for TB for at least one month.

A *M. tuberculosis* strain was termed multidrug-resistant (MDR) when it was resistant to at least INH and RMP.

Human immunodeficiency virus testing

A blood sample was collected anonymously from each patient for human immunodeficiency virus (HIV) testing. A rapid test (Determine HIV-1/2®, Abbott Diagnostics, Maidenhead, UK) was performed; positive samples were confirmed by enzyme-linked immunosorbent assay (ELISA) testing.

Ethical consideration

All patients included in the study gave informed consent. The study was approved by the Board of the Benin National TB Programme.

Quality control

The quality of the LRM is assured by the Mycobacteriology laboratory of the Institute of Tropical Medicine of Antwerp, as supranational laboratory. Regular proficiency testing showed excellent results, particularly for DST of RMP and INH.

RESULTS

Resistance in new cases

Of 244 strains, 61 (25%) were resistant to one or more drugs, 47 (19.2%) to one drug and 14 (5.7%) to at least two drugs. Four strains (1.6%) were MDR (Table 1). However, of these patients, three came from a neighbouring country and only one patient (0.5%) usually lived in Benin.

Resistance in previously treated cases

Of the 226 strains, 122 (54%) were resistant to one or more drugs, 69 (30.5%) were resistant to one drug, mainly SM, 54 (23.4%) were resistant to at least two drugs and 25 strains (11.1%) were MDR (Table 1).

Table 1 Prevalence of drug resistance

		Previously treated cases			
Type of resistance	New cases (*n* = 244) *n* (%)	Failure (*n* = 41) *n*	Relapse (*n* = 80) *n*	Return to treatment (*n* = 105) *n*	Total (*n* = 226) *n* (%)
Susceptible to all drugs	183 (75)	12	44	48	104 (46)
Any resistance					
H	21 (8.6)	18	21	24	63 (27.9)
S	49 (20.1)	19	22	41	82 (34.5)
R	7 (2.9)	11	13	17	41 (18.1)
E	2 (0.1)	8	4	4	18 (7.1)
Monoresistant					
H	9 (3.7)	7	7	7	21 (9.3)
S	36 (14.8)	6	10	26	42 (18.6)
R	2 (0.8)	1	1	3	5 (2.2)
E	0	1	0	0	1 (0.4)
Total	47 (32.4)	15	18	36	69
H+R resistant (MDR)					
HR	0	0	4	4	8 (3.5)
HRE	0	0	1	0	1 (0.4)
HRS	4 (1.6)	3	1	5	9 (4.0)
HRSE	0	4	2	1	7 (3.1)
Total	4	7	8	10	25 (11.1)
Other resistance					
HE	1 (0.4)	1	1	1	3 (1.3)
HS	7 (2.9)	3	5	6	14 (6.2)
HSE	0	0	0	0	0
RE	0	0	0	1	1 (0.4)
RS	1 (0.4)	1	3	2	6 (2.7)
RES	0	2	1	1	4 (1.8)
ES	1 (0.4)	0	0	0	0
Total	10 (4.1)	7	10	11	28 (12.4)

H = isoniazid; S = streptomycin; R = rifampicin; E = ethambutol; MDR = multidrug-resistant.

Table 2 Drug resistance in our study and comparison with others

Pattern of resistance	Cotonou, 2004 (present study) All patients	Cotonou, 2004 (present study) Foreign patients excluded	Benin,[4] 4 provinces, 1994	Ethiopia,[7] Addis Ababa, 1997	Cameroon,[8] West province, 1998	Equatorial Guinea,[9] 18 districts, 2001
Resistance in new cases	*n* = 244	*n* = 204	*n* = 333	*n* = 103	*n* = 437	*n* = 224
Total resistance	25%	23%	8.4%	14%	19.7%	16.9%
Multidrug resistance*	1.6%	0.5%	0.3%	0.8%	1.4%	2.2%
Resistance in previously treated cases	*n* = 226	*n* = 141	*n* = 57	*n* = 18	*n* = 129	*n* = 12
Total resistance	54%	53%	46%	11.1%	51.1%	41.6%
Multidrug resistance	11.1%	10.6%	11%	0%	13.2%	25%

* Defined as resistance to both isoniazid and rifampicin.

Of the patients harbouring MDR strains, 15 lived in Benin before contracting the disease, while the remaining 10 patients lived abroad before seeking TB treatment in Benin.

Results with and without foreign patients are compared in Table 2, which also includes the results of the Benin survey of 1994 and some recent results from other parts of Africa. The rate of MDR is 0.5% in new cases and 10.6% in previously treated cases if foreign patients are excluded (Table 2). Of the 41 strains that showed resistance to RMP, only 25 were MDR. Of the remaining 16 strains, 14 (87.5%) were from foreign patients, mostly from Nigeria.

HIV serology

Among all patients tested, 48 (10.2%) were positive for HIV serology. There is no statistically significant relation between HIV infection and resistance in new cases ($P = 0.11$) and resistance in previously treated patients ($P = 0.56$).

DISCUSSION

The present study is limited to the NPPH at Cotonou. Cotonou is the largest city in Benin, and the NPPH receives about one third of the country's TB patients. This hospital also receives patients from other countries where quality of care is not optimal. Before conducting a national survey, we evaluated anti-tuberculosis drug resistance in Cotonou. However, this survey is likely to show a worse case scenario for Benin, as the NPPH at Cotonou also functions as the referral centre.

As shown in Table 2, we observed high resistance to any drug in new cases, although we considered only patients who resided in Benin before contracting the disease (23%). This result is surprising, as the DOTS strategy is rigorously applied in Benin.[4] However, this might have been influenced by patients from non-DOTS countries who claim to live in Benin. Identification of patients is difficult because not every person has a national identification card. Furthermore, as some languages in Benin are shared by neighbouring countries, language could not be used to identify patients. For future surveys, this should be investigated further.

MDR in new cases is relatively high (1.6%) when all patients are considered, but low (0.5%) and comparable to 1994 national survey results when only patients residing in Benin are considered. This shows the great importance of correct patient identification in epidemiological surveys, where results can differ depending on the population(s) studied. After 12 years of the application of the DOTS strategy, this MDR rate remains low compared to other studies in Africa.[7–9] However, the MDR rate in new cases in our study could rise rapidly if the DOTS strategy is not properly implemented in all countries surrounding Benin.

Resistance to any drug and MDR in previously treated patients remain comparable to the study performed in Benin in 1994 and in other countries.[4,7–9] The treatment of MDR-TB generally involves the use of second-line reserve drugs such as capreomycin, cycloserine, kanamycin or fluoroquinolones, which are more expensive and difficult to obtain in developing countries.[10] The management of such cases poses a difficult problem in these countries. Efforts should therefore be made to limit the spread of MDR-TB.

Collaboration with National Tuberculosis Programmes of countries sharing borders with Benin could be established to improve management of TB cases and limit the development and spread of anti-tuberculosis drug resistance.

RMP resistance without INH resistance is unusual. The high prevalence of these strains in foreign patients could be due to uncontrolled use of RMP in their countries. Therefore, for these patients, RMP resistance could not be used as a surrogate marker for MDR-TB.

HIV seroprevalence in our study (10.2%) was five times higher than in the general population (1.9%). Benin is considered as a country with a relatively low prevalence of HIV in West Africa.[11] However, as in other studies, HIV seroprevalence among TB patients is quite high, indicating the importance of encouraging TB patients to be tested for HIV.[12,13] As found in other developing areas, we did not find any relation between HIV infection and anti-tuberculosis drug resistance.[14–16]

Acknowledgements

The authors wish to thank the staff of the LRM in Cotonou. D Affolabi is supported by a grant from the Directorate General for the Development Cooperation, Brussels, Belgium.

References

1 World Health Organization. Tuberculosis. WHO Media Centre March 2006. Geneva, Switzerland: WHO, 2006. Fact sheet no. 104 revised. http://www.who.int/mediacentre/factsheets/fs104/en/print.html. Accessed March 2007.
2 Maher D, Chaulet P, Spinaci S, Harries A D. Treatment of tuberculosis: guidelines for national programmes. 2nd ed. WHO/TB/97.220. Geneva, Switzerland: WHO, 1997.
3 World Health Organization/International Union Against Tuberculosis and Lung Disease. Anti-tuberculosis drug resistance in the world. Third global report. The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance, 1999–2002. WHO/HTM/TB/2004.343. Geneva, Switzerland: WHO, 2004.
4 Trébucq A, Anagonou S, Gninafon M, Lambregts K, Boulahbal F. Prevalence of primary and acquired resistance of *Mycobacterium tuberculosis* to anti-tuberculosis drugs in Benin after 12 years of short-course chemotherapy. Int J Tuberc Lung Dis 1999; 3: 466–470.
5 World Health Organization. Laboratory services in tuberculosis control. Part III: Culture. WHO/TB/98.258. Geneva, Switzerland: WHO, 1999.
6 Canetti G, Rist N, Grosset J. Mesure de la sensibilité du bacilli tuberculeux aux drogues antibacillaires par la méthode des proportions. Rev Tuberc Pneumol 1963; 27: 217–272.
7 Tudo G, Gonzalez-Martin J, Obama R, et al. Molecular epidemiology of tuberculosis in the Bata and Malabo districts of Equatorial Guinea. Int J Tuberc Lung Dis 2004; 8: 1458–1463.
8 Sanders M, Van Deun A, Ntakirutimana D, et al. Rifampicin mono-resistant *Mycobacterium tuberculosis* in Bujumbura, Burundi: results of a drug resistance survey. Int J Tuberc Lung Dis 2006; 10: 178–183.
9 Umubyeyi A N, Vandebriel G, Gasana M, et al. Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda. Int J Tuberc Lung Dis 2007; 11: 189–194.
10 Pablos-Méndez A, Gowda K D, Frieden R T. Controlling multidrug-resistant tuberculosis and access to expensive drugs: a rational framework. Bull World Health Organ 2002; 80: 489–495.
11 World Health Organization. HIV/AIDS epidemiological surveillance report for the WHO African Region. 2005 update. Geneva, Switzerland: WHO, 2005. http://www.who.int/hiv/AFRO2005.pdf Accessed August 2007.
12 Nkoghe D, Toung Mve M, Nnegue S, et al. HIV seroprevalence among tuberculosis patients in Nkembo Hospital, Libreville, Gabon. Bull Soc Pathol Exot 2005; 98: 121–122.
13 Daniel O J, Salako A A, Oluwole F A, Alausa O K, Oladapo O T. HIV sero-prevalence among newly diagnosed adult pulmonary tuberculosis patients in Sagamu. Niger J Med 2004; 13: 393–397.
14 Bruchfeld J, Aderaye G, Palme I B, et al. Molecular epidemiology and drug resistance of *Mycobacterium tuberculosis* isolates from Ethiopian pulmonary tuberculosis patients with and without human immunodeficiency virus infection. J Clin Microbiol 2002; 40: 1636–1643.
15 Kuaban C, Bercion R, Noeske J, Cunin P, Nkamsse P, Ngo Niobe S. Anti-tuberculosis drug resistance in the West Province of Cameroon. Int J Tuberc Lung Dis 2000; 4: 356–360.
16 Owusu-Dabo E, Adjei O, Meyer C G, et al. *Mycobacterium tuberculosis* drug resistance, Ghana. Emerg Infect Dis 2006; 12: 1171–1172.

RÉSUMÉ

OBJECTIF : Evaluer la situation actuelle de la résistance aux antituberculeux à Cotonou, dans le plus grand centre antituberculeux du Bénin.
MATERIEL ET MÉTHODES : Au total, 470 souches de *Mycobacterium tuberculosis* provenant de patients atteints de tuberculose pulmonaire ont été testées par la méthode de proportion : 224 provenant de nouveaux cas et 226 de patients admis pour un retraitement.
RÉSULTATS : Le taux de multirésistance (MDR) chez les nouveaux cas est variable suivant l'origine des patients : il est relativement élevé (1,6%) lorsqu'on considère l'ensemble des patients étudiés, mais faible (0,5%) et comparable à celui de l'enquête nationale de 1994 lorsqu'on considère seulement les patients résidant au Bénin. Chez les patients admis en retraitement, le taux de MDR (11,1%) est comparable à celui de l'enquête de 1994. Il n'a pas été observé de relation entre la co-infection avec le virus de l'immunodéficience humaine et la résistance aux antituberculeux.
CONCLUSION : Cette étude montre l'importance d'une identification correcte des patients dans une telle étude épidémiologique ; les résultats pouvant varier selon la population étudiée.

RESUMEN

OBJECTIVOS : Evaluar la situación actual de resistencia a los medicamentos antituberculosos en Cotonou, en el mayor centro antituberculoso de Benín.
MÉTODOS : Se analizaron 470 aislados del complejo *Mycobacterium tuberculosis* provenientes de pacientes con tuberculosis (TB) pulmonar : 244 casos nuevos y 226 casos tratados previamente. Se realizaron pruebas de sensibilidad a los medicamentos de primera línea mediante el método de las proporciones.
RESULTADOS : La prevalencia de multidrogorresistencia (MDR) primaria fue función del origen de los pacientes : la MDR de los casos nuevos fue relativamente alta (1,6%) cuando se tomaron en cuenta todos los pacientes, pero baja (0,5%) y comparable con los resultados de un estudio nacional de 1994, cuando se consideraron exclusivamente los pacientes residentes en Benín. La MDR en pacientes que habían recibido un tratamiento previo (11,1%) siguió siendo comparable con el estudio realizado en Benin en 1994. No se encontró una relación entre la coinfección por el virus de la inmunodeficiencia humana y la resistencia a los medicamentos antituberculosos.
CONCLUSIÓN : El presente estudio demuestra la importancia de una identificación adecuada de los pacientes en este tipo de estudios epidemiológicos, en los cuales los resultados pueden variar en función de la población o poblaciones estudiadas.