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Drug Resistance of *Mycobacterium tuberculosis* Complex among Newly Diagnosed Tuberculosis Cases in Burkina Faso

La résistance aux médicaments de Mycobacterium tuberculosis Complex parmi les nouveaux cas de tuberculose diagnostiqués au Burkina Faso

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ABSTRACT

BACKGROUND: **In Burkina Faso, there is no recent data about the level of drug resistance in *Mycobacterium tuberculosis* strains among newly diagnosed tuberculosis cases.**

OBJECTIVE: **To provide an update of the primary drug resistance of mycobacterium tuberculosis among patients in Burkina faso.**

METHODS: ***Mycobacterium* strains were identified in 323 newly diagnosed tuberculosis patients between April 2005 and September 2006, and their susceptibility to isoniazid, rifampicin, streptomycin, and ethambutol was determined according to the proportions method. Among these patients, 243 accepted voluntarily to be tested for antibodies to HIV.**

RESULTS: **The age range of the patients was 11 and 75 years and included 221 (68.4%) males and 102 (21.6%) females. The isolates included 314 (97.2%) *M. tuberculosis*, eight (0.3%) *M. africanum* and one *M. bovis*. Thirty-nine (12.4%) of the *M. tuberculosis* strains were resistant, with 7.3% resistant to one drug, 2.9% to two drugs, 0.3% to three drugs and 1.9% to four drugs. In total 3.2% of the isolates were multidrug-resistant (MDR). One isolate of *M. africanum* was resistant to all drugs while the single strain of *M. bovis* was sensitive to all the drugs. Among the 243 patients tested for HIV 77 were positive. However, there was no relationship between drug resistance and gender, age group or HIV serostatus of the patients.**

CONCLUSION: **The resistance rate of *M. tuberculosis* strains to all the four drugs tested (12.4%) and the rate of MDR (3.2%) are high. These results demand an increased effort by the National Tuberculosis Program to limit the spread of MDR strains of tuberculosis. WAJM 2009; 28(6): 353–357.**

Keywords: **Tuberculosis, primary resistance, MDR, Burkina Faso.**

RÉSUMÉ

CONTEXTE: *Au Burkina Faso, il n'existe pas de données récentes sur le niveau de résistance aux médicaments dans les souches de Mycobacterium tuberculosis, la tuberculose parmi les cas nouvellement diagnostiqués.*

OBJECTIF: *Assurer une mise à jour de la résistance primaire de Mycobacterium tuberculosis chez les patients dans le Burkina Faso.*

MÉTHODES: *Les souches de Mycobacterium ont été identifiées dans 323 patients atteints de tuberculose nouvellement diagnostiqués entre avril 2005 et Septembre 2006, et leur sensibilité à l'isoniazide, la rifampicine, la streptomycine et l'éthambutol a été déterminée selon la méthode des proportions. Parmi ces patients, 243 ont accepté volontairement d'être testé pour les anticorps au VIH.*

RÉSULTATS: *La tranche d'âge des patients était de 11 et 75 ans et inscrite sur 221 (68,4%) hommes et 102 (21,6%) femmes. Les isolats inclus 314 (97,2%) M. tuberculosis, huit (0,3%) M. africanum et un M. bovis. Trente-neuf (12,4%) des souches de M. tuberculosis étaient résistants, avec 7,3% résistant à un médicament, de 2,9% à deux médicaments, 0,3% à trois médicaments et de 1,9% à quatre médicaments. Au total, 3,2% des isolats étaient multirésistants (MDR). Un isolat de M. africanum était résistante à tous les médicaments alors que la seule souche de M. bovis est sensible à toutes les drogues. Parmi les 243 patients testés pour le VIH 77 étaient positifs. Toutefois, il n'y avait aucune relation entre la résistance aux médicaments et par sexe, tranche d'âge ou de statut sérologique VIH des patients.*

CONCLUSION: *Le taux de résistance des souches de M. tuberculosis à l'ensemble des quatre médicaments testés (12,4%) et le taux de tuberculose multirésistante (3,2%) sont élevés. Ces résultats doivent être un effort accru par le Programme National contre la Tuberculose de limiter la propagation de souches multirésistantes de la tuberculose.* ***WAJM 2009; 28 (6): 353–357.***

Mots-clés: *Tuberculose, la résistance primaire, multirésistance aux médicaments, au Burkina Faso.*

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Abbreviations: DOTS, Directly Observed Therapy Short-course; **DST,** Drug susceptibility testing; **E,** Ethambutol; **H,** Isoniazid; **HIV,** Human immunodeficiency virus; **IUATLD,** International Union Against Tuberculosis and Lung Disease; **LJ,** Löwenstein-Jensen; **MDR,** Multi-drug resistance; **NTP,** National Tuberculosis Programme; **R,** Rifampicin; **S,** Streptomycin; **TB,** Tuberculosis; **TCH,** Hydrazine of thiophene-2-carboxylic acid; **UNAIDS,** The Joint United Nations Programme on HIV/AIDS; **WHO,** World Health Organization.

INTRODUCTION

Tuberculosis (TB) became a curable disease with the discovery of effective anti-TB drugs. The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUTLD) recommend Directly Observed Therapy Short-course (DOTS) for its treatment.[1] Nevertheless, the TB organism is becoming increasingly resistant to the first line drugs.[2] The WHO estimates that each year nearly 450,000 new cases of multidrug-resistant (MDR) tuberculosis occur world wide.[3] Recently, a cumulative rate of 23.5% of MDR *M. tuberculosis* was reported in 13 African countries and Afghanistan.[4] Such a situation has serious consequences in settings with limited access to second line drugs.[5] Drug resistance arises in areas with poor TB control programmes due to the improper use of drugs in the treatment of drug-susceptible TB patients. Health care workers should ensure that patients complete the whole course of treatment.[6,7]

Unfortunately, in Africa where the highest number of TB cases are found, only a few countries are able to perform culture and drug-susceptibility testing (DST) of *M.* tuberculosis, therefore necessitating periodic studies. In Burkina Faso, drug resistance in different clinical settings is not tested systematically,. The last epidemiological study on DST in new cases of TB in Burkina Faso was done from April 1992 to April 1994.[8] More than ten years after, we undertook this study to update the data on the level of drug resistance in *Mycobacterium tuberculosis* complex strains among newly diagnosed TB cases in Burkina Faso.We also assessed the relationship between resistance to TB drugs and the HIV serostatus among TB patients.

SUBJECTS, MATERIALS, AND METHODS

Centres included in this study were the National Centre for the Fight against Tuberculosis in Ouagadougou, the Regional Hospital in Dori, the Medical Centre in Gorom-Gorom, the Regional Centre for the Fight against Tuberculosis and University Hospital Centre Souro Sanon, these last two are located in Bobo-Dioulasso. These centres are situated in the Centre, North and South-west of the country, respectively. They represent the largest urban centres for TB diagnosis and treatment and diagnose annually, about 85% of all TB patients in the country.

Patients and Ethical Consideration

This cross-sectional study was performed between April 2005 and September 2006. All the subjects were TB-suspected patients presenting at the participating centres , and complaining of cough for more than two weeks. The objective of the study was explained to each patient. A standard questionnaire was completed for each patient, collecting demographic information and the history of the disease; such as treatment history, possible long duration treatment with or without injection (streptomycin), recognition of the anti-tuberculosis drugs, chest radiographs and sputum microscopy done in the past. This facilitated the classification of patients into the group of newly diagnosed cases. Only the patients, who gave a written informed and voluntary consent, were identified with at least a positive microscopy and had the drug-susceptibility testing performed, were enrolled. A total of 323 new TB cases were included in the study. Patients who were previously treated at any centres were excluded.

Bacteriological Studies

Direct microscopy was done on patient sputum in each diagnostic centre before transferring them to our laboratory for culture. Smears were prepared and stained by hot Ziehl-Neelsen method as recommended by the IUATLD.[1] The sputum samples used for culture were first decontaminated by NaOH 4%, according to the Petroff method,[9] then inoculated onto Löwenstein-Jensen (LJ), LJ supplemented with pyruvate (LJ+Pyruvate) and LJ containing five µg/mL hydrazide of thiophene-2-carboxylic acid (LJ+TCH) media. These media were incubated at 37°C and inspected on the third and seventh days for contamination or growth of rapid growing atypical mycobacteria. The growth rate and morphology of the colonies of the isolates were also monitored weekly. A culture was declared negative if there was no growth after 10 weeks. The isolates were identified by acid-fast stain, growth rate, morphology, resistance to TCH, growth on LJ+Pyruvate and reactions to usual biochemistry tests (niacin, nitrate reductase, catalase activity at 22°C and 70°C).[10]

For the drug-susceptibility testing, the standardized indirect method of the proportions was used.[11] Three dilutions (10^{-1}, 10^{-3}, 10^{-5}) were used, starting from the initial bacillary suspension which was inoculated to the media with antibiotics and to control media without antibiotics. Four major drugs were tested at the following concentrations: 0.2µg/mL for isoniazid (H), 40µg/mL for rifampicin (R), 4µg/mL for streptomycin (S), and 2µg/mL for ethambutol (E). A strain was declared resistant if the bacterial growth with antibiotic was ≥1% compared to the control and sensitive if the bacterial growth with antibiotic was <1%. Resistance was primary if the patient was not in contact with anti-tuberculosis drugs or was for a period of less than one month.

The *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 ans 10 atypical mycobacteria strains were used for this control).

HIV Testing

A blood sample was collected from patients for HIV testing after voluntary counselling and obtaining their consent, according to the national standard recommended by the Ministry of Health. Two rapid tests were performed according to the UNAIDS/WHO recommendations:[12] the blood sample was first tested using the Determine™ HIV-1/2 (Abbott Diagnosis). Any sample that was non-reactive on the first assay was considered HIV-negative. The positive samples to the first assay were tested with Immuno-Comb®II BiSpot HIV 1/2 assay (PBS Orgenics) to discriminate between HIV-1, HIV-2 and dual-reactivity. The HIV-

positive patients were referred to the specialized treatment services for follow-up.

Statistical Analysis

Data were entered into Excel and analyzed using SPSS 15.0. Chi-square test ($\div^2$) was used to interpret the results of association between drug resistance and gender, age range and HIV status of the TB patients. The statistical significance threshold was $p< 0.05$.

RESULTS

Characteristics of the Patients

Three hundred and twenty-three new patients were enrolled; they included 221 (68.4%) males and 102 (31.6%) females, with a *sex ratio* (M/F) of 2.17:1. The average age was 36.7±13.1 years [11-75 years]; 51 (15.8%) patients were aged less than 25 years, 191 (59.1%) from 25 to 44 years and 81 (25.1%) more than 44 years. Six (1.8%) patients were foreigners.

Drug Resistance in Mycobacterium Strains

A strain of *Mycobacterium* was isolated and identified in each patient. The drug-susceptibility testing (DST) was run for all the 323 strains, including 314 *M. tuberculosis*, eight *M. africanum* and one *M. bovis*. The primary resistance of *M. tuberculosis* strains to anti-TB drugs is shown in Table 1. Among the 314 isolates of *M. tuberculosis*, 275 (87.6%) were susceptible to all drugs tested while 39 (12.4%) were drug-resistant. An overall resistance rate of 3.8% was found to isoniazid (H) alone, 3.2% to streptomycin (S) alone and 0.3% to ethambutol (E), while none was detected to rifampicin (R). The analysis revealed 3.2% of multidrug-resistant (MDR) at least to isoniazid and rifampicin and 1.9% other patterns. One strain of *M. africanum* was resistant to each drug, while no resistance was found with the *M. bovis* strain. The inclusion rate of the patients in the study was higher in Ouagadougou (28.4%) than in the three other regions. The drug-resistance rate by region is shown in Table 2. Among these new TB cases, there was no significant statistical association between drug resistance and sex *(p=0.818)*, or drug resistance and age group (*p=0.38)*.

Table 1: Drug resistance of *Mycobacterium tuberculosis* among 314 new tuberculosis cases

Drugs	Number	%
Susceptible to all drugs	275	87.6
Global primary resistance	**39**	**12.4**
Total primary resistance to each drug		
H	27	8.6
R	11	3.5
E	11	3.5
S	19	6.1
Monoresistance	**23**	**7.3**
H	12	3.8
R	0	–
E	1	0.3
S	10	3.2
Multiresistance	**10**	**3.2**
H+R	3	1.0
H+R+E	1	0.3
H+R+S	0	–
H+R+E+S	6	1.9
Other Patterns	**6**	**1.9**
H+E	3	1.0
H+S	2	0.6
H+E+S	0	–
R+E	0	–
R+S	1	0.3
R+E+S	0	–
E+S	0	–
Number of drug resistance to		
1 drug	23	7.3
2 drugs	9	2.9
3 drugs	1	0.3
4 drugs	6	1.9

The eight *M. africanum* and 1 *M. bovis* strains are not included in this table.
H: isoniazid; R: rifampicin; E: ethambutol; S: streptomycin.

Table 2: Drug resistance in *M. tuberculosis* strains by region

Region	Resistance to any Drug N(%)		
	Yes	No	Total
Ouagadougou	29 (9.2)	196 (62.4)	225 (71.6)
Bobo-Dioulasso	3 (1.0)	39 (12.4)	42 (13.4)
Gorom-Gorom	4 (1.2)	27 (8.6)	31 (9.9)
Dori	3 (1.0)	13 (4.2)	16 (5.1)
Total	**39 (12.4)**	**275 (87.6)**	**314 (100.0)**

HIV Status of the TB Patients

Among the 314 patients, 243 (77.4%) consented for HIV testing: 77 (31.7%) of them were HIV-positive, including 68 HIV-1, 6 HIV-2 and 3 HIV-1+2 dually reactive. *M. tuberculosis* drug-resistant strains were found in 10 (13%) HIV-positive patients *versus* 17 (10.2%) in HIV-negative patients; this difference was not statistically significant in both serological groups, *P= 0.531* (Likelihood Ratio).

DISCUSSION

Our study showed a rate of 12.4% drug-resistant *M. tuberculosis* strains and 3.2% of MDR-TB among new cases (Table 1). The patients in this study declared that they had never been previously treated, implying that they were most likely infected with drug resistant strains. The majority of the patients (71.6%) in this study were from the city of Ouagadougou, a possible limitation to our study as this might not reflect the real drug resistance problem in Burkina Faso. Nevertheless, since 28.4% (89/314) were referred from three regions of the country (Table 2), the results presented here indicate a problem of drug resistance in general and an emerging problem of MDR-TB in particular. The MDR-TB rate in Bobo-Dioulasso, the second largest city in Burkina Faso in 1992-1994 was 1.8%.[8] Some of the reasons for the drug resistance observed could be that the treatment success rate in new smear positive cases has not improved ever since the NTP was initiated in 1995. Another factor to be noted was the introduction of the private health sector in the detection and treatment of TB which operates differently from the public health sector. In fact such insufficiencies constitute factors which generate and worsen the problem of drug resistance.[13] The *M. tuberculosis* resistance to isoniazid and streptomycin can be related to the wide use of these drugs by the NTPs in developing countries due to their availability and their moderate cost.[14] In Burkina Faso, streptomycin is largely used to treat many other infectious diseases.

The multi resistance rate in this study appears as an important challenge for the NTP authorities in Burkina Faso. The fact that 3.2% of TB patients developed resistance at least to H and R constitutes a major threat to the NTP. To specifically confront drug-resistant TB and save lives, NTP should immediately improve its ability to rapidly diagnose all TB cases and treating them until cured, which is the best way to prevent the development of drug resistance. Surveillance of TB drug resistance is of major importance for TB control and is an indicator for the quality of the TB treatment.[15]

The comparison of our results with those of other studies showed that the resistance rate to isoniazid (3.8%) was lower than the 7.2% obtained in Malawi,[16] but higher than the 3.1% obtained in Ivory Coast.[17] The resistance rate to streptomycin (3.2%) although high, was lower than the 4.3% found in South Africa,[18] 11.6% in Maputo (Mozambique),[19] 8.5% in Madagascar[20] and 4.4% in Botswana. However, it was similar to the 3.1% found in Algeria.[1921] The mono-resistance rate to ethambutol is 1% vs. none in South Africa[18] and in Maputo.[19] No strain of *M. tuberculosis* resistant to rifampicin alone was found: a similar result was observed in Hoogli.[4] The MDR rate (3.2%) was low compared to the 5.3% reported in Ivory Coast,[17] 8.7% in Ghana.[22] It was higher than the 1.1% reported in Algeria and 2.2% in Egypt.[21] In our study population, it was likely that the inclusion of three patients treated for less than one month as new cases contributed to the increase in the resistance rate to isoniazid as well as to rifampicin as proposed by others.[23] The global resistance of *M tuberculosis* to anti-TB drugs (12.4%) in our study was lower than the 23.5% found in Ghana.[22] As Ledru *et al.*[8], in their study in Bobo-Dioulasso, we did not find a relationship between HIV serostatus and drug resistance.

Conclusion

The level of resistance in *M. tuberculosis* strains to all the four drugs tested, the resistance to any drugs (12.4%) and the rate of MDR (3.2%) were high. In the countries where the WHO recommendations are well applied in "new cases", the global resistance (or resistance to any drugs) and the MDR rates should be below 10% and 3% respectively.[24] Efforts must be made to limit MDR-TB spread in Burkina Faso. There is a need to prevent its further development and to find alternative drugs for the existing cases. The results showed that there was no significant statistical association between drug resistance and gender, age group, and HIV serostatus in these new TB cases.

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