Brief Original Article

High prevalence of multidrug resistant tuberculosis in Djibouti: a retrospective study

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Abstract

Introduction: The Republic of Djibouti is an African country that exhibits one of the highest incidence rate of tuberculosis in the world. The aim of this study was to evaluate the prevalence of multidrug-resistant tuberculosis among new cases.
Methodology: We studied retrospectively every tuberculosis case diagnosed over a 12-month period in patients hospitalized at the French Military Hospital of Bouffard. During this period, 1,274 samples from 675 patients were tested.
Results: We isolated 266 mycobacteria corresponding to 180 cases of tuberculosis. Thirty-three were fully susceptible and 57% met the tuberculosis criteria, with 46% primary resistance. No extensively-drug-resistant tuberculosis was found.
Conclusion: Our results highlight a major concern about the situation in this part of the world.

Key words: Mycobacterium tuberculosis; drug-resistant tuberculosis; Africa

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Introduction

Emerging multidrug-resistant (MDR) *Mycobacterium tuberculosis* (TB) is one of the major concern of the Health policy [1]. According to the World Health Organization 650,000 people are infected by MDR-TB worldwide and 12 million suffer from tuberculosis. In Africa, 1.9% of new cases and 9.4% of diagnosed and treated patients are infected by a MDR strain [2]. The Republic of Djibouti, with a population of approximately 800,000 people, is a country located in the Horn of Africa that exhibits one of the highest incidence rates of tuberculosis in the world close to 300 new cases per 100,000 people per year. In 2011, a single-center study conducted in Djibouti [3] highlighted a very high level of MDR-TB in this country and few cases of extensively drug-resistant (XDR) TB. To go further into this debate, we decided to conduct a clinical and bacteriological study whose aim was to evaluate the prevalence of MDR-TB in patients admitted to the French Military Hospital of Bouffard (HMCB), Djibouti.

Study

We performed a single-center study between April 2010 and April 2011. Every sample sent for examination to HMCB laboratory for tuberculosis testing was included. For each case, we collected data on age, sex, geographical origin, HIV status and, if administered, previous tuberculosis treatment. No follow-up was available. Scientific and ethical approval was obtained from the HMCB ethic committee. Informed consent was obtained from all patients.

Direct smear examination after Ziehl-Nielssen staining was systematically performed. Cultures were done on Lowenstein-Jensen and Coletsos medium during 60 days. Isolates were sent to the Armed Forces Mycobacterium Reference Laboratory of the Military Medical Center Percy (France) for identification and drug susceptibility testing. Identification was based on staining, MPT-64 detection (SD-Bioline-TB AgMPT64, Eurobio Laboratoires, Courtaboeuf, France) and conventional biochemical procedures. For non tuberculosis mycobacterium, the identification was based on conventional biochemical tests, culture

criteria [4] and molecular hybridation (Genotype-MTBC, Hain lifescience, Nehren, Germany).

Isolate susceptibility was performed in liquid medium (Bactec-M-GIT960, Becton Dickinson-Franklin Lakes, USA) for isoniazid, rifampicin, pyrazimamide and streptomycin. When resistance was detected, susceptibility to second-line drugs (aminoglycosid and fluoroquinolones) was performed by molecular hybridation (Genotype kit-MTBRplus, Hain-Lifescience, Nehren, Germany) according to manufacturer's recommendations. Multi-drug resistance was defined by resistance to rifampicin and isoniazid, and extensively drug-resistance when the strain was MDR-TB and resistant to any of the fluoroquinolones and to at least one of three injectable second-line drugs.

Results

During this 12-month-long study, 1,274 samples from 675 patients were sent to the laboratory. One hundred (8%) were positive at smear examination and 266 (21%) yielded isolates after culture. Finally, 180 cases were diagnosed as tuberculosis infection based on clinical, radiological and biological findings. Sample data, smear examination and culture results are summarized in Table 1. Prior to culture, acid-fast bacilli were found in 46% of pulmonary tuberculosis with sputum, in 24% of pulmonary tuberculosis without sputum and in 33% of ganglionic tuberculosis. Among those 180 patients, 101 benefited of further analysis in France (56%, representing 257 samples). Unfortunately, the remaining samples could not be analyzed due to transport difficulties.

The average age was 32 (3-83), 57% of patients were men (n = 58), 94% was Djiboutian. Forty six percent (n = 47), were hospitalized at the French Military Hospital of Bouffard, 10% (n = 10) in the Djiboutian National Tuberculosis Center of Paul Faure and 33% (n = 34) were treated at home. The identification of the sample revealed 89% of infection by *M. tuberculosis* (n = 90), 6% of *M. canetti* and 5% of non tuberculosis mycobacteria (*M. chelonae* n = 1,

Table 1: Samples received at Bouffard's laboratory: characteristics, culture results and smears examination.

	All samples *(n = 1274 ,675 patients)*	**POSITIVE CULTURES** *(n = 266, 101 patients)*	**SMEAR EXAMINATION** *n (%/positive culture)**
Sputum	704	169 (23,7%**)	77 (45,5%)
BAL/TA/PDA	6		
Gastric aspiration	449	79 (17,6%)	19 (24%)
Adenopathies	22	9 (40,9%)	3 (33,3%)
Cerebral Spinal Fluid	39	3 (7,7%)	1
Urines	2	0	0
Pleural effusion	13	2 (15,4%)	0
Ascitic fluid	11	1 (9%)	0

BAL: bronchoalveolar lavage, TA: tracheal aspiration, PDA: protected distal aspiration, *: positive culture i.e. n = 266, **: % represents % within positive cultures.

Table 2: Antibiotics susceptibility of isolated *Mycobacterium tuberculosis* (n = 88)

	First line therapy				***Second line therapy***			***Patients***
	R	**H**	**E**	**Z**	**S**	**A**	**F**	**n = 88**
Susceptible	s	s	s	s	s	s	s	33
One resistance	s	R	s	s	s	s	s	3
More than one resistance	s	R	s	R	s	s	s	1
	s	s	s	R	R	s	s	1
MDR-TB	R	R	s	s	s	s	s	10
	R	R	s	R	s	s	s	6
	R	R	R	R	s	s	s	13
	R	R	R	s	s	s	s	1
	R	R	s	R	R	s	s	2
	R	R	R	R	R	s	s	5
	R	R	s	s	s	R	s	1
	R	R	s	R	s	R	s	2
	R	R	R	R	s	R	s	1
	R	R	s	R	R	R	s	5
	R	R	R	R	R	R	s	4
XDR-TB	R	R	R	R	R	R	R	0

R= Rifampicin, H=Isoniazid, E= Ethambutol, Z= Pyrazinamide, S= Streptomycin, A= Aminosides, F= fluoroquinolones. In the table, R= resistant, s=susceptible.

M. fortuitum n = 2, *M. peregrinum* n = 1)

A drug susceptibility testing was available for 98% of patients (88/90) with infection due to *M. tuberculosis*. Susceptibility results are summarized in Table 2. Thirty-three patients (38%) had an infection with susceptible mycobacteria, five strains harbored a single resistance to isoniazid (n = 3), and 51 isolates (56,8%) were MDR-TB. No XDR-TB was identified.

Past medical history, especially anti-tuberculosis therapy was reported in 44 patients, (ten from the National Tuberculosis Center and 34 from the HMCB). Twenty (45%, four positive for HIV) were infected by a susceptible tuberculosis isolate, and 24 (55%, four positive for HIV) had a MDR-TB. Among the MDR strains, a previous anti-tuberculosis treatment was reported in 13 patients (54%), this suggests that 46% of the MDR TB isolated in our study (n = 11) could not be related to a previous exposition to anti-tuberculosis drug and we can hypothesize that these patients had been contaminated by spreading MDR strains

Discussion

Drug resistance of *M. tuberculosis* is a world health concern. Our study analyzed samples from each patient infected by *M. tuberculosis* during a period of twelve months at the French Military Hospital of Bouffard in Djibouti where both French and Djiboutian people are treated. This population cannot be considered as a representative sample of the entire population but results from this study revealed an emerging health problem. In contrast with the 2011 WHO report [1] our work showed a worrisome high level of MDR-TB responsible for disease in the majority of patients (57%). However, the WHO report included only four African countries (Congo, Ethiopia, Nigeria and South Africa) that cannot be compared to Djibouti. The MDR-TB proportion in this study strengthens results found by Olle-Goig *et al*. [3] (75% MDR-TB and 11% XDR-TB) but seems to be more objective since they only tested samples yielded from hospitalized patients, some with a long history of hospitalization, whereas a considerable part of our patients were treated at home. If compared to the first study of tuberculosis drugs susceptibility performed in 2002 [5], the increasing proportion of MDR-TB observed in less than ten years (from 2,4% in 2002 to 56,7% in 2011) is really frightening.

However, we are aware of the limitations of our results. First, this is a retrospective study and we could not evaluate the incidence of infection by MDR strains. Secondly, even if 94% of our patients were Djiboutian, nearly half had to support the daily hospitalization cost, that cannot be afforded by all patients and has lead to an obvious bias. We have also gathered data from patients moving from the National Tuberculosis Center to the French Military Hospital and this could lead to an overestimation of the MDR rate. In fact, when analyzing samples collected from this center, we found an average of 80% of MDR-TB infections, as reported by Olle-Goig *et al*. Thus, out of the 79 remaining patients, 43 (54%) were infected by a MDR strain.

In Djibouti, frequency of tuberculosis is explained by poverty and crowding. Despite free access to health care and DOT (Directly Observed Treatment) strategy, the compliance to treatment is not perfect and facilitates post-treatment resistance. Moreover, the majority of Djiboutians are diagnosed and treated at Paul Faure tuberculosis center and until summer 2012, this center only performed smear microscopy as diagnostic tool without culture and treatment relied exclusively on first-line drugs. This medical process can explain transmission with MDR TB to "under-treated" people without isolation devices. Since summer 2012, the National Tuberculosis Center started to perform rapid test for infection and susceptibility to rifampicin based on polymerase chain reaction (PCR) and culture. A real hope to control the spreading of MDR TB is emerging from this improvement, but some limitations persist since physicians have to select the most critical patients due to the cost and the limited availability of technicians with the required skills [6].

Despite limitations, the results of this study illustrate the work of the only laboratory performing drug susceptibility testing on tuberculosis samples in Djibouti between 2010 and 2011. Rapid changes in diagnosis and treatment of tuberculosis cases are required to limit infection spreading. Moreover, a genotypic study is necessary to understand the emergence and spread of MDR-TB.

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