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Drug Resistance Patterns of *Mycobacterium tuberculosis* Isolates from Patients with Pulmonary Tuberculosis in the Sudan

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Abstract
Purpose: *The antimicrobial susceptibility patterns of 200 isolates of Mycobacterium tuberculosis, recovered from patients with pulmonary tuberculosis (PTB) in the Sudan, was determined against the first-line anti-tuberculosis drugs.*
Methods: *Isolates were recovered from smear-positive sputum samples and confirmed biochemically and by PCR. Drug resistance of the isolates to isoniazid (INH), rifampicin (RIF), streptomycin (STM) and ethambutol (EMB) was measured using Lowenstein-Jensen proportion method.*
Results: *Only 67 (33.5%) isolates were found sensitive to all drugs. Thirty-five (17.5%) isolates were resistant to INH, 31 (15.5%) to RIF, 43 (21.5%) to STM and 24 (12%) to EMB. Twenty-one (10.5%) isolates were multidrug-resistant (at least to both INH and RIF), of which 9 (4.5%) were resistant to the four drugs. Five (2.5%) isolates were resistant to different combinations of three drugs.*
Conclusion: *This study demonstrated a higher prevalence of multidrug resistance amongst PTB isolates of M. tuberculosis as compared to the previous reports from the Sudan. It ensures the need to monitor the changes of drug resistance in PTB in various states in order to timely implement appropriate treatment regimen.*
Keywords: *Multidrug resistance, Mycobacterium tuberculosis, Pulmonary tuberculosis, Sudan.*

I. Introduction

Tuberculosis (TB) represents one of the most important bacterial diseases that affects humans globally. It comes second only to HIV/AIDS as a cause of death. The estimates counted for 9 million incident cases and 1.5 million deaths [1]. Most cases (95%) were reported in developing countries. Although the disease is curable, the emergence of *M. tuberculosis* resistant strains complicates treatment. Two dangerous forms of the disease are currently exist, the multidrug-resistant TB (MDR TB) and the extensively drug-resistant TB (XDR TB). These forms of TB represent a real threat to human life particularly in regions where many TB patients are also infected with HIV [2]. Their response to the standard six month treatment with first-line anti-TB drugs is poor and they can take much more time to treat with drugs that are less effective [1]. Among TB patients notified in 2013, an estimated 480 000 had MDR-TB. The delay in detection of these dangerous forms of TB poses a serious threat to TB control in the world.

In the Sudan, TB is a major public health problem [3]. The disease is considered as a leading cause of health service utilization in the ambulatory service, ranking 4, the most frequent reason for hospital admission, constituting 11.6% of all cases admitted in hospitals, and the most frequent cause of hospital deaths, constituting 16% of all patients who died in hospitals. The estimated incidence of new smear-positive cases was 107 per 100,000 in 2008 and the estimated prevalence of all TB forms was 402 cases per 100,000 [4]. MDR TB among all new cases was 1.8% while that among previously treated cases was 9.8% in 2007.

The present study was carried out to determine the prevalence of MDR PTB among patients attending the main TB clinics in Khartoum State to provide an updated data that will be beneficial for the selection of effective treatment regimens to cure TB in the country.

II. Methods

2.1 Bacterial Isolates

Two-hundred clinical isolates of *M. tuberculosis* were recovered from smear-positive sputum samples collected from patients with suspected pulmonary tuberculosis at three TB clinics in Khartoum State. Culture and isolation method has been shown elsewhere [5]. All isolates were purified by repeated subculture on Lowenstein Jensen (L.J.) medium, stored into sterile cryotubes containing 1% skimmed milk and kept in a deep freezer at -70 °C. Isolates were identified by their growth pattern and biochemical performance [6] and

confirmed by PCR [7]; details were shown elsewhere [5]. The patients were between 5 and 70 years old and came to the TB clinics from different regions of the country, mainly poorer ones. The majority of them were new cases (218, 66.3%), and others were previously treated cases (62, 18.8%), relapse cases (32, 9.7%) and treatment failure cases (17, 5.0%).

2.2 Drug Sensitivity Test

The test was performed using L.J. proportion method according to WHO/IUATLD [8]. Four antituberculosis agents, namely isoniazid (INH), rifampicin (RIF), streptomycin (STM) and ethambutol (EMB) were used in the test. Drugs were adjusted in the L.J. medium to final concentrations of 0.2 µg/ml for INH, 40 µg/ml for RIF, 4 µg/ml for STR, and 2 µg/ml for ETM. For every batch of medium and every round of susceptibility testing, two MDR strains and 4 mono-resistant strains were used as controls to ensure reproducibility. A representative sample from a recent sub-culture was placed into a sterile McCartney bottle containing 1.0 mL sterile H_2O plus ten glass beads. The mixture was homogenized on a Vortex mixer for approximately one minute with brief interruptions and then the opacity of the suspension was adjusted by the addition of sterile distilled H_2O to McFarland standard No. 1 (original suspension). Two dilutions, 10^{-2} and 10^{-4} were used from the original suspension of each isolate. One slope of control medium and two slopes of each drug containing medium were inoculated with a loopful (0.01 ml) of each dilution. The drug susceptibility test results were recorded after 3-4 weeks of incubation at 37 °C. The number of colony forming units growing on the medium containing the drug was compared with the number on the control one. The proportion of resistant cells in the total viable population of the original inoculum was then calculated and expressed as a percentage. Resistance was defined as growth on drug containing medium greater than 1% of the growth on drug free control medium.

III. Results

Results of drug sensitivity testing are shown in Fig. 1. All of the controls yielded reproducible results. Among the tested *Mycobacterium*, 67 (33.5%) isolates were found sensitive to the tested drugs, while 133 (66.5 %) showed mono- or multidrug resistance. Thirty-five (17.5%) isolates were resistant to INH, 31 (15.5%) to RIF, 43 (21.5%) to STM and 24 (12%) to EMB. Twelve (6%) isolates showed growth in INH and RIF and 9 (4.5%) were resistant to the four drugs, while five (2.5%) isolates were resistant to different combinations of three drugs.

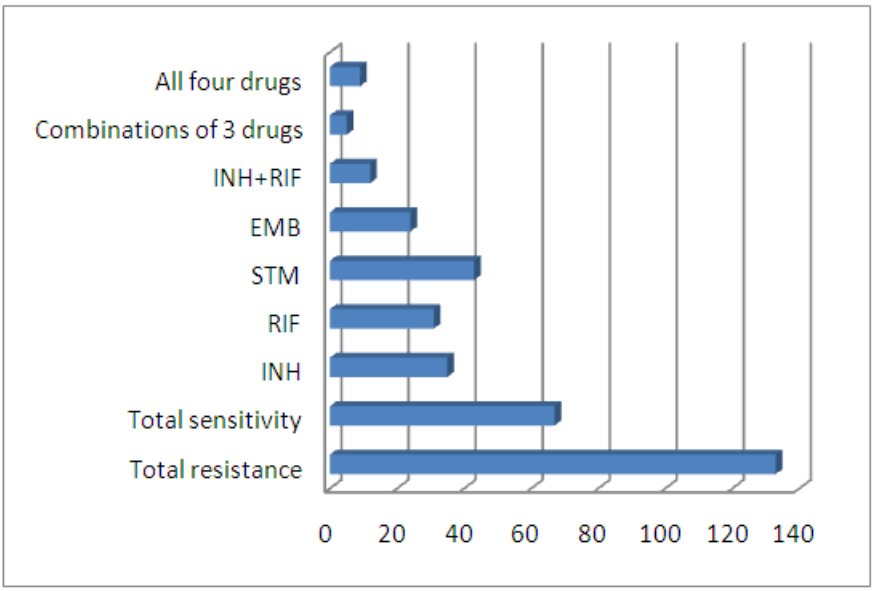

Fig. 1. Resistance of *Mycobacterium tuberculosis* isolates from PTB patients to anti-TB drugs

IV. Discussion

The emergence of MDR-TB has re-focused attention on TB as a disease of continuing significance in the world. It has been reported that 250,000 – 400,000 cases of multidrug-resistant TB (MDR TB) emerge every year [9]. Frequent detection of *M. tuberculosis* resistant strains is essential for the selection of an effective treatment and subsequent control of TB regionally and internationally. In this study, L.J. medium proportion method was used to detect drug resistance in *M. tuberculosis* isolates.

The results of the study revealed the presence of drug resistance to all tested drugs and drug mono-resistant isolates represent almost two-thirds (66.5%) of the total isolates. In previous studies in the Sudan, the percentage of resistance to the same drugs was 46% [10] and 51.7% [11]. Thus, the finding of the current study indicated that the percentage of resistance is increasing and it represents a real threat to control programs of the disease in the country. Similar studies conducted in Kenya [12] and Ethiopia [13] reported a far less percentage of resistance to at least one drug (30% and 23%, respectively), with a higher degree of resistance against isoniazid in Kenya study. Mono-drug resistance to isoniazid and rifampicin is increasing rapidly; only 12% and 8% of the isolates were found resistant to INH and RIF, respectively in previous studies in the Sudan [14; 15] compared to 17.5% and 15.5% in the current study. Isolates that cause MDR TB (resistant to combination of INH and RIF) counted for 6% of total isolates. It is really high percentage compared to results of a previous report in which the incidence of MDR TB counted for only 1.8% in 2007 [4]. Detection of multi-drug resistant isolates to combinations of 3 and 4 drugs (2.5% and 4.5%, respectively) may point to presence of XDR TB, a disease caused by bacteria that are resistant to isoniazid and rifampicin as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). In general the increased drug resistance may be attributed to widespread use and misuse of these drugs.

IV. Conclusion

This study concluded that resistance of TB bacilli to first line anti-TB drugs is continuously increasing in the Sudan and local efforts should be exerted to restrict the spread of these resistant *M. tuberculosis* strains by prescription of the best drug combinations and proper handling of them by affected patients.

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