American Journal of Microbiological Research, 2017, Vol. 5, No. 2, 32-36
Available online at http://pubs.sciepub.com/ajmr/5/2/2

DOI:10.12691/ajmr-5-2-2

Prevalence of Multidrug-Resistant Tuberculosis among Smear Positive Pulmonary Tuberculosis Patients in Eastern Sudan

Muataz M. Eldirdery[1,*], Intisar E. Alrayah[2], Mona OA. Awad ElkareIm[3,*], Fatima A. Khalid[4], Asrar M A/Salam Elegail[5], Nuha Y. Ibrahim[5], Eman OM. Nour[5], Rahma H. Ali[5], HAMDAN M. HAMDAN[6], Nagi M. Awad[7], Yassir M. Mohamed Ahmed[1], Nihad M A. Elhaj[1], Atif A. Elagib[8]

[1]Department of Microbiology, Tropical Medicine Research Institute National Center for Research, Khartoum, Sudan
[2]Department of Microbiology, Tropical Medicine Research Institute National Center for Research, Khartoum, Sudan and Faculty of Applied Medical Science, Shaqra University, Shaqra, Saudi Arabia
[3]Blood Transfusion Service, National Blood Transfusion Center, Ministry of Health, Khartoum, Sudan
[4]Tuberculosis Research Center, University of Kassala, Kassala, Sudan
[5]National Tuberculosis Reference Laboratory, National Laboratory of Public Health, Khartoum Sudan
[6]Consultant of Tuberculosis control program, Khartoum, Sudan
[7]Tuberculosis control program, Khartoum, Sudan
[8]Tropical Medicine Research Institute National Center for Research, Deputy Director of National Center for Research, Khartoum, Sudan
*Corresponding author: ELDIRDERY5@HOTMAIL.COM

Abstract **Background:** The prevalence of multidrug-resistant tuberculosis (MDR-TB) is increasing throughout the world. Although previous treatment for TB is the most important risk factor for development of MDR-TB, treatment-naïve patients are also at risk due to either spontaneous mutations or transmission of drug-resistant strains. **The main objective** of this study to determine the prevalence of MDR-TB among new and retreatment cases of sputum-positive pulmonary TB in Eastern Sudan. **Methods:** A cross sectional study involving retreatment and newly diagnosed cases of sputum-positive pulmonary tuberculosis between November 2011 to October 2012 was carried out in Eastern Sudan. All sputum specimens were positive smear TB were subjected to Line Probe assay, culture and drug susceptibility test (DST) for first-line drug. MDR-TB was defined as TB caused by bacilli showing resistance to at least isoniazid and rifampicin. **Results:** A total of 109 cases of sputum-positive pulmonary tuberculosis were enrolled. Out of 109 patients screened (88 new case patients and 21 previously treated case patients); MDR TB prevalence was 4(36.36%) and 7 (63.64%) among new case-patients and previously treated case patients, respectively. The age group 31-45 years was recorded the highest frequency of MDR-TB infection 5 (45.45%). Females were more affected than Males 7 (63.6%) and 4 (36.4%) respectively. Among the MDR cases only one patient was HIV positive and it was new case. **Discussion and conclusion:** This study showed that the prevalence of MDR tuberculosis among the new and retreatment cases in Kassala State is much higher than what was reported previously. This is the first study in Geddarif state and there were no previous studies documented or were published, other studies is needed to know the really reasons of the continuously increasing of MDR TB in Geddarif and Kassala states.

Keywords*: tuberculosis, MDR, Eastern Sudan*

Cite This Article: Muataz M. Eldirdery, Intisar E. Alrayah, Mona OA. Awad ElkareIm, Fatima A. Khalid, Asrar M A/Salam Elegail, Nuha Y. Ibrahim, Eman OM. Nour, Rahma H. Ali, HAMDAN M. HAMDAN, Nagi M. Awad, Yassir M. Mohamed Ahmed, Nihad M A. Elhaj, and Atif A. Elagib, "Prevalence of Multidrug-Resistant Tuberculosis among Smear Positive Pulmonary Tuberculosis Patients in Eastern Sudan." *American Journal of Microbiological Research*, vol. 5, no. 2 (2017): 32-36. doi: 10.12691/ajmr-5-2-2.

1. Introduction

Tuberculosis (TB) is an ancient disease that has affected mankind for more than 4,000 years. It is a chronic disease caused by the bacillus *Mycobacterium tuberculosis* and spreads from person to person through air. A patient with infectious pulmonary TB can infect 10-15 persons per year [1]. Sudan alone carries 8% of the TB burden in the Eastern Mediterranean Region, (EMR). In 2012, the estimated new cases were 9.0 million and 1.5 million TB death. The morbidity and mortality rate were increased by 400,000 and 200,000 cases, respectively [2]. Multidrug-resistant tuberculosis (MDR-TB) is defined as tuberculosis that is resistant to at least two main first-line drugs Isoniazid (INH) and Rifampicin (RIF) [3]. The spread of multidrug-resistant tuberculosis (MDR TB) in

the world remains a major public health problem and an obstacle to effective global TB control [4]. Early diagnosis of tuberculosis and rapid detection of drug resistance is an urgent priority to identify patients who are not responding to the standard treatment and to avoid dissemination of resistant strains. The proportion of MDR-TB cases among new cases and previously treated cases of tuberculosis reported globally from 1994 through 2009 ranged from 0 to 28.3% and from 0 to 61.6%, respectively [5,6]. According to World Health Organization (WHO), 2015 globally, an estimated 3.3% (95% CI: 2.2–4.4%) of new cases and 20% (95%CI: 14–27%) of previously treated cases have MDR-TB; these levels have remained virtually unchanged in recent years [7]. In 2014, there were an estimated 480, 000 (range: 360, 000–600, 000) new cases of MDR-TB worldwide, and approximately 190, 000 (range: 120, 000–260, 000) deaths from MDR-TB. Among patients with pulmonary TB who were notified in 2014, an estimated 300, 000 (range: 220, 000–370, 000) had MDR-TB, more than half of these patients were in India, China and the Russian Federation. Of the 480, 000 cases of multidrug-resistant TB (MDR-TB) estimated to have occurred in 2014, only about a quarter of these – 123, 000 – were detected and reported [7]. This cross- sectional study was conducted in Tropical Medicine Research Institute in collaboration with Tuberculosis center for research Kassala University and National Reference Laboratory (NRL) Khartoum - Sudan between June 2011 to October 2012 to know the prevalence of MDR-TB in Eastern Sudan.

2. Materials and Methods

Study Design

A cross-sectional study.

Study Specimens

The present study was done in the department of National Reference Laboratory (NRL) Khartoum Sudan. One hundred and nine Strains were isolated from sputum samples of pulmonary tuberculosis (PTB) patients who attending to TB units at Eastern Sudan (Kassala and Geddarif) from November 2011 to October 2012.

Study Subjects

Inclusion Criteria

All microscopy positive patients were included in the study.

Exclusion Criteria

All microscopy Negative patients were excluded from the study.

Methods

Specimen Collection and Processing

Smear Examination

Smear-positive sputum was collected from patients at risk of MDR-TB at the National Reference TB Laboratory were consecutively screened for acid fast bacilli (AFB) using Ziehl-Neelsen (ZN) smear microscopy. All consenting smear positive patients were enrolled.

Conventional laboratory testing

Sodium Hydroxide (NaOH4%)

Sputum was processed with sodium hydroxide (NaOH4%) method for decontamination because the specimens susceptible to contamination by more rapidly growing normal flora. Any processed specimen remaining was stored at 2-8°C for the duration of the study to allow for re-testing of specimens giving discrepant results

Culture on Lowenstein-Jensen Medium

Two or three drops of the decontaminated sample were inoculated in the Lowenstein-Jensen (LJ) solid medium and were incubated at 37°C for 8 weeks [8].

Identification of Culture

Dry, rough, raised, irregular colonies with wrinkled surface, creamy white becoming yellowish or buff colored on further incubation were subjected to AFB staining and niacin test. All isolates which were positive for niacin test were identified as *Mycobacterium tuberculosis* (*M. tuberculosis*) and were subjected to drug susceptibility testing [9].

Drug Susceptibility Test

Drug susceptibility test (DST) was done for 109 isolates, LJ media contain drugs (Rifampicin, Isoniazid, Streptomycin and Ethambutol), for drug susceptibility test of *M. tuberculosis* strains was prepared as mentioned by National Tuberculosis Programme briefly; LJ media containing a drugs with the different concentrations (0.2µg/ml for INH, 40 µg/ml for RIF, 4 µg/ml for SM and 2.0 µg/ml for ETH). Two plain LJ medium were prepared without drugs as a control [10]. The suspension was prepared by transferring of a certain amount of bacterial growth from the culture by 10 µL loop into the suspension bottle (Peugeot bottles contains glass beads). Homogenization was made by vortexing the bottle, the turbidity of suspension was adjusted to McFarland standard No.1. Then four serial dilutions were made from the original suspension of each sample (10-1, 10-2 10-3 and 10-4). Two to three drops was inoculated from dilution 10-2 in media which contain drugs and 10-4 in plain media, then was incubated at 37°C, the bottles was tightening loosely for 24 hours to allow evaporation and then well tighten. Contamination was examined for 72 hours and the contaminated bottles were discharged. The first examination for growth of *M.tuberculosis* was done after two weeks from inoculation, and the second examination after month from the first examination. The resistance was calculated as the ratio of the number of colonies on the drug containing medium and those of control media, the strain was considered as resistant if the ratio is greater or equal to 1% [11].

Line probe assay

Line probe assays (LPA) have been recently approved for use in low income settings and can be used to screen smear-positive sputum specimens for resistance to Rifampicin and Isoniazid in 1-2 days. LPA testing was performed in three separate rooms, according to WHO recommendations [12]. Deoxyribonucleic acid (DNA) extraction was performed in the BSL3 laboratory, master mix preparation in a second room, and PCR and hybridization were performed in a third laboratory. Five hundred microlitres of processed sediment was used to perform the Genotype MTBDRplus (Hain Lifescience GmbH) assay, according to the manufacturer's instructions [13].

Statistical analysis

Statistical package for social science version 22 was used to analyze the data, excel have been used to generate the graphs. Categorical variables were presented in frequencies and percentages.

3. Result

The study was carried out in two states in eastern Sudan (Kassala and Geddarif) and enrolled 109 individual, all the study population were PTB patients. The study included males and females; the age was ranged from 13 to 80 years old with different infection case. The age group 16-30 years recorded the highest frequency of PTB infection were 44 (40.37%). Males were more affected than females; 64 (58.72%) and 45 (41.28%) respectively. HIV status was reported for all patients from TB control program records, 6 cases were found positive. Among MDR-TB cases there was only one case was HIV Positive and it was new cases.

Table 1. Demographic and clinical data of all study population

Character		Frequency	Percentage %
Age(Year)	<15	2	1.84
	16-30	44	40.37
	31-45	40	36.70
	46-60	15	13.76
	61-75	7	6.42
	>75	1	0.92
Gender	Female	45	41.28
	Male	64	58.72
Patient case	Defaulter	2	1.835
	Failure	3	2.752
	New	88	80.733
	Relapse	16	14.678
HIV status	Negative	103	94.49
	Positive	6	5.51

The overall prevalence of MDR-TB in Eastern Sudan

The prevalence of MDR in the eastern Sudan was 10.1%, Figure 1.

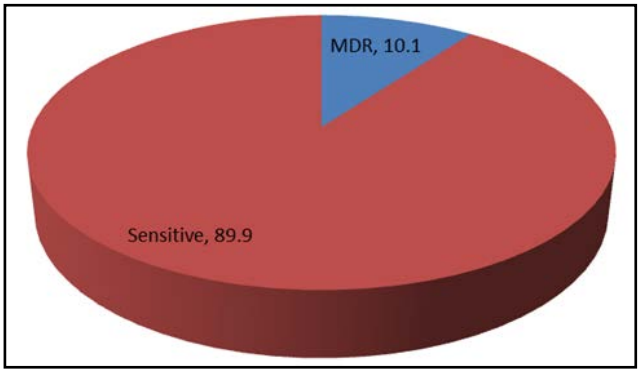

Figure 1. The overall prevalence of MDR-TB by DST and LPA

The prevalence of MDR-TB in Kassala and Geddarif state

In total of 59 sample collected from pulmonary tuberculosis PTB patient from Kassala state 8 samples (13.6%) was MDR-TB patient and 51sample (86.4%) was sensitive. In Geddarif state; 3 samples (6%) out of 50 samples was MDR-TB while 47 samples (94%) was sensitive. Figure 2.

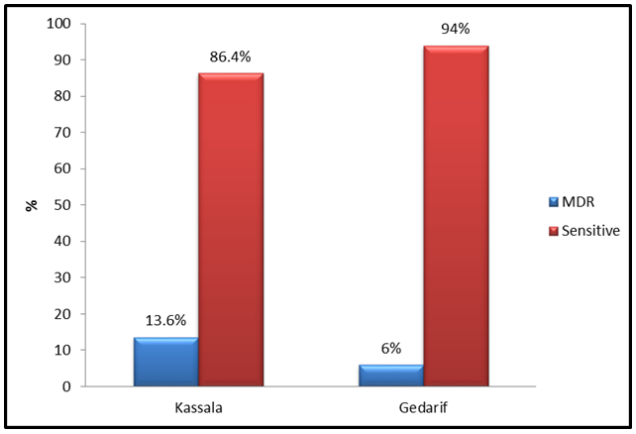

Figure 2. The prevalence of MDR-TB in Kassala and Geddarif state

Demographic and clinical data of MDR-TB cases

The study showed that the age group 31-40 years had the highest frequency of MDR- PTB infection also males was more infected than females. 45.5% of MDR patients were relapse infection case. Most of MDR-TB patients whom infected previously by PTB were completed 80% of the treatment in the previous infection despite this they became MDR-PTB. Among the MDR cases only one patient was HIV positive and it was new case.

Table 2. Demographic and clinical data of MDR-TB cases

Character		Frequency	Percentage%
Age group	16-30	3	27.27
	31-45	5	45.45
	46-60	3	27.27
Gender	Female	7	63.6
	Male	4	36.4
Patient case	Defaulter	1	9.09
	Failure	1	9.09
	New	4	36.36
	Relapse	5	45.45
Treatment	No	4	36.4
	Yes	7	63.6
HIV	Positive	1	9.1
	Negative	10	90.9

4. Discussion

Tuberculosis (TB) remains a major public health problem worldwide due to its high risk of person-to-person transmission, morbidity and mortality [14]. Multi-Drug Resistant (MDR) now constitutes an emerging threat for the control of the disease and the further spread of drug Resistance. This study covered two states in Eastern Sudan (Kassala and Geddarif), through the period from November 2011 to October 2012 to determine the prevalence of pulmonary MDRTB. Most of the PTB patients in this study were diagnosed for the first time 88

(80.73%) while 21 (19.27%) had history of PTB reflecting the active transmission of TB. The results of the study revealed that the prevalence of MDR tuberculosis in eastern Sudan 11 (10.1%). Both Kassala and al Geddarif are a border states which means the possibility of entry of the disease from neighboring countries (Ethiopia and Eritrea). Ethiopia is one of the 27 high MDR-TB countries; it is ranked 15th with more than 5000 estimated MDR-TB patients each year [15,16,17,18]. The WHO report 2015 revealed that the MDR-TB survey in Eritrea not done till now [7]. Also there are some reasons that lead to spread of Multidrug-resistant (MDR-TB), Such as meager resources that resulted in poor health services for the needy in community, the civil unrest and the displacement of population has exacerbated the health problems and favored the spread of TB disease in the different parts of the state. Also the social stigma which associated with infection by the disease. This is the first study in Geddarif state and there were no previous studies documented or published. In this study the prevalence of MDR in Kassala state was 8 (13.6%), this result is comparable to other result in previous study in Kassala Teaching Hospital, as drug susceptibility test to anti tuberculosis drugs was done for 53 isolates, 5 (9.4%) of them were MDR(19). Also this result is comparable to another study which was done later in Kassala state revealed that the multidrug resistance was 5 (21.74%) [20]. The study showed that the age group 31-40 years had the highest frequency of MDR- PTB infection, which is mostly responsible for the financial support of their families so; their illness could lead to loss of their job, affecting their lifestyle, this result agrees with study done in Kassala and Khartoum states [19,21], the study showed that the MDR-TB is high among new cases of sputum-positive pulmonary TB in Kassala in consonance with studies which have reported a high prevalence of MDR-TB among new TB cases in India [22,23]. The prevalence of MDR-TB is increasing throughout the world both among new tuberculosis cases as well as among previously-treated ones [24]. this increasing may be due to the treatment strategies for drug resistant TB or stigma In the Bedouin communities (treatment-naïve patients also at risk due to either spontaneous mutations or transmission of resistant strains) [25,26]. The prevalence of MDR TB in Geddarif state was 3 (6%) and this is the first study determined the prevalence of MDR TB in this state. On the other hand in this study out of 11 MDR TB patients 7 patients was Completed the treatment, may be the reasons mentioned above or for returning to the patient himself or the weakness of effectiveness of TB drug used. In this study 4 patients were new cases for MDR-TB and 7 patients were retreatment cases for MDR-TB.

5. Conclusion and Recommendations

This study highlights the extent of the problem of MDR-TB in Eastern Sudan, in Kassala State has high prevalence of MDR and is continuously increasing each year, and we recommend more studies to know really reasons. Also efforts should be directed towards continued surveillance for MDR-TB among newly diagnosed and relapsed TB cases.

Ethical Considerations

The study was approved by the Research Ethics Committee of Tropical Medicine Research Institute – National Center for Research, Sudan.

Acknowledgements

This study was funded by International Atomic Energy Agency (IAEA) RAF 60/40, National Center for Research and National TB Control Program. Also Extends thanks to Tropical Medicine Research Institute staff, National Reference Laboratory Staff and (Kassala and Al Geddarif) Hospitals Staff. Finally I am particularly grateful to all participant in this study and we hope to all of them fully health and wellness.

Competing Interests

The authors have no competing interests.

Abbreviations

AFB: Acid Fast Bacili
DNA: Deoxyribo Nucleic Acid
DST: Drug Susceptibility Test
EMR: Eastern Mediterranean Region
ETH: Ethambutol
IAEA: International Atomic Energy Agency
INH: Isoniazid
LJ: Lowenstein-Jensen
LPA: Line Probe Assay
MDR-TB: Multi-Drug Resistant Tuberculosis
NRL: National Reference Laboratory
PTB: Pulmonary Tuberculosis
RIF: Rifampicin
SM: Streptomycin
WHO: World Health Orgnization
ZN: Ziehl- Nielsen.

References

[1] Vasantha M, Gopi PG, Subramani R; Survival of tuberculosis patients treated under dots in a rural tuberculosis unit (TU), South India.Indian J Tuberc., 2008; 55(2): 64-69.
[2] World Health Organization (WHO), Global Tuberculosis Report 2014, Geneva, Switzerland.
[3] CDC. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs – worldwide, 2000-2004.Morb Mortal Wkly Report (2006). 55:301-305.
[4] L.P. Ormerod, Multidrug-resistant tuberculosis (MDR-TB): epidemiology, prevention and treatment, Br. Med. Bull. 73-74 (2005) 17-24.
[5] Sharma SK, Mohan A; Multidrug resistant tuberculosis. Indian J Med Res., 2004; 120(4): 354-376.
[6] Lango DL, Fauci AS, Kaser D, Hauser S L, Janeson J L, Loscalzo J; Harrison's Principles of Internal Medicine. 18thedition. NewYork, McGraw Hill, 2012: 1340-1377.
[7] World Health Organization Global Tuberculosis report 2015.
[8] World Health Organization WHO, Laboratory Services in Tuberculosis Control Culture (1998). Part III PP (57-75).

[9] Koneman EW; Mycobacteria. In Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition, Philadelphia, Lippincott Williams and Wilkins, 2006:1065-1117.

[10] National TB Control Programme. Manual of Standard Operating Procedures (SOPs). Culture of *Mycobacterium tuberculosis* and Drug Susceptibility Testing on solid Medium Intermediate Reference Laboratory for Tuberculosis - Central TB Division, (Ministry of Health & Family Welfare) (2009). New Delhi-Version No. 01.01 Date: 01/04.

[11] Sethi S, Sharma S, Sharma SK, Meharwal SK, Jindal SK and Meera S. Drug susceptibility of *Mycobacterium tuberculosis* to primary anti- tubercular drugs by nitrate reductase assay (2004). Indian J Med Res. 120, PP 468-471.

[12] World Health Organisation: Policy Statement. Molecular Line Probe Assaysfor Rapid Screening of patients at risk of mul tidrug resistant tuberculosis (MDR-TB), 2008. http://www.who.int/tb/dots/laboratory/lpa_policy.pdf.

[13] Hain Lifescience. Genotype® MTBDRplus product insert. Version http://www.hainlifescience.de/en/products/microbiology/mycobacteria/genotypemtbdrplus.html

[14] Martin G and Lazarus A. Epidemiology and Diagnosis of Tuberculosis. Recognition of At-risk Patients is Key to Detection (2000). Postgrad. Med. 108:42-44, 47-50, 53-54.

[15] Federal Ministry of Health of Ethiopia. Guideline for program and clinical management of drug resistant tuberculosis 2009. Addis Ababa, Ethiopia: FMOH;.

[16] World Health Organization. WHO report. Global Tuberculosis control 2013. Geneva: Switzerland.

[17] World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB) - 2010 global report on surveillance and Response. Geneva: Switzerland.

[18] World Health Organization. WHO report 2011. Global Tuberculosis Control. Geneva: Switzerland.

[19] Khalid F.A. and Mukhtar M. M. Epidemiology of Tuberculosis and molecular characterization of drugs resistance isolates of mycobacterium tuberculosis from kassla state, Institute of Endemic Diseases-University of Khartoum (2009). Thesis.

[20] Fatima A. Khalid, Zuhal A. Hamid b, M.M. Mukhtar. Tuberculosis drug resistance isolates from pulmonary tuberculosis patients, Kassala State, Sudan (2015). International Journal of Mycobacteriology. 4. 44-47.

[21] Mohammed E. M., Saeed N. S. Isolation, Identification and Antimicrobial Sensitivity of Mycobacterium tuberculosis from Patients with Pulmonary Infection M.V.C. University of Khartoum (2008). Thesis.

[22] Jain A, Mondal R, Prasad R, Singh K, Ahuja RC. Prevalence of multidrug resistant Mycobacterium tuberculosis in Lucknow, Uttar Pradesh. Indian J Med Res 2008; 128: 300-6.

[23] D'souza DT, Mistry NF, Vira TS, Dholakia Y, Hoffner S, Pasvol G, et al. High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. BMC Public Health 2009; 211: 1-9.

[24] World Health Organization WHO Anti-Tuberculosis Drug Resistance in the World. Report No.4. Geneva, Switzerland. The WHO / IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance2008; p. 394. WHO/HTM/TB.

[25] Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis in India. Indian J Med Res. 2004; 120:377-86. [PubMed]

[26] Snider DE, Jr, Kelly GD, Cauthen GM, Thompson NJ, Kilburn JO. Infection and disease among contacts of tuberculosis cases with drug resistant and drug susceptible bacilli. Am Rev Respir Dis. 1985; 132:125-32. [PubMed].