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Genotypic Characterization of Multi Drug-Resistant *Mycobacterium tuberculosis* Strains Isolated from Pulmonary Tuberculosis Patients, Sudan

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A B S T R A C T

Multidrug-resistant tuberculosis (MDR-TB) is caused by *Mycobacterium tuberculosis* resisting at least isoniazid and rifampicin. MDR is a major public health threat in Sudan. This is a descriptive cross-sectional and hospital-based study conducted in Kosti teaching hospital, during the period from December 2019, to December 2021, aimed to determine the prevalence of Multi-Drug resistance among TB patients and to find the most risk factors associated with generating TB resistance, using Xpert MTB/RIF assay. One thousand sputum samples from suspected TB patients were applied on Xpert MTB/RIF automated sample processing and used for ZN stain. Blood samples from the same subjects were used for the detection of HIV infection by ICT. By gene Xpert method, MTB was detected in 218 subjects. Remarkably, out of 218MTB detected by Xpert MTB/RIF assay, 13/218 (5.9%) were positive for MDR. Participants who live in rural had higher rates of MDR 8(2.4%) than urban areas 5(0.8%) with a significant statistical association. The frequency of MDR is significantly lower in subjects aged 21-40 years compared to others. It is also lower in those who showed a negative result for bacillary level but higher in subjects who were positive for ZN stain 4(2.9%), with P less than 0.05. There was an emerging MDR-TB in White Nile State that needs prompt response via early diagnosis and management of TB patients along with social awareness.

Introduction

Multidrug-resistant tuberculosis (MDR-TB), defined as tuberculosis resistant to atleast the two main first-line anti-TB drugs isoniazid (INH) and rifampin (RIF), occurred as a threat to TB control (Hu *et al.*, 2017). MDR-TB is a public health problem in resource-limited countries like Sudan. The World Health Organization (WHO) believes over 10 million people globally fell ill with tuberculosis (TB). In 2017 and 2018, the number of TB cases was seven million (WHO, 2019). MDR-TB continues to be a global public health concern with approximately 580,000 cases worldwide and mortality worse than most cancers (WHO, 2019). In 2015, approximately 480,000 MDR-TB new cases were notified with 100,000 incidents registered as rifampicin-resistant (RR) worldwide, with 250,000 deaths due to MDR/RR-TB (WHO, 2016). In addition, there were approximately 500,000 new cases of RR -TB of which 78% were MDR-TB In2018 (WHO, 2019). Previously, the WHO believed that only about 25– 30% of MDR-TB cases were detected and only approximately 25% of patients accessed second-line medications globally (WHO, 2014). In some cases, even more, severe drug-resistant TB may develop into Extensively Drug-Resistant TB, (XDR-TB), which is a form of multidrug-resistant TB; it has been reported in 117 countries worldwide (WHO, 2018). In 2017, it was reported that there was six hindered MDR/RR-TB among Sudanese patients with pulmonary TB. Moreover, it was estimated that 3.5% of new TB cases and 18% of previously treated cases are MDR/RR-TB cases (WHO, 2018).

Early diagnosis and rapid detection of drug resistance are essential for controlling the public spread of drug-resistant tuberculosis (TB) and are important for the timely identification of suitable TB treatment. Generally, drug resistance is detected by culture and drug susceptibility testing (DST). However, these procedures are difficult and take several weeks to months to complete, due to the slow growth rate of MTB. The progress of rapid molecular procedures, which can be performed within 1 or 2 days, such as Xpert MTB/RIF provides results within 2 hours or else culture-based and Line probe assays (LPAs) (WHO, 2018). Molecular methods can detect particular DNA mutations, which are related to resistance to specific anti-TB drugs. Molecular tests for detecting drug resistance to rifampicin alone or in combination with isoniazid have been recommended for use by WHO Xpert MTB/RIF and the next-generation assay Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA). Xpert MTB/RIF are fully automated nucleic acid amplification assays that detect MTB and mutations affecting the rifampicin resistance determining region (RRDR) of the rpoB gene (Cabibbe *et al.*, 2017). The GeneXpert is use for the diagnosis of TB and rapid detection of RIF resistance in clinical specimens (CDC, 2014; Kabir *et al.*, 2021) by PCR, amplifies 81 bp of the MTB rpoB gene, and subsequently probes this region for mutations that are associated with RIF resistance (Blakemore *et al.*,2010). The study aimed to detect the frequency of MDR-MTB strains Isolated from Sudanese patients with Pulmonary Tuberculosis.

Materials and Methods

This was a descriptive cross-sectional hospital study conducted during the period from December 2019 to December 2021 at the TB unit in Kosti Teaching Hospital.

Data collection

Participants' socio-demographic and clinical presentation information (such as relapse and loss of follow-up) were collected by researchers using a structured questionnaire.

Laboratory analysis

Samples collection

Under standard bio-safety procedures, sputum samples were collected from each individual and decontaminated by adding a double volume of 4% sodium hydroxide. Sputum samples were used for

the detection of MTB (By ZN stain and gene xpert) and drug resistance (gene xpert). Moreover, 2-5 ml of the venous blood sample was also collected from each participant. Subsequently, serum was obtained by centrifugation at 3000 rpm for 5 minutes and tested for Human immunodeficiency virus (HIV) infection by rapid immunochromatographic test (Acon, USA).

Method of Ziehl-Neelsen Stain

The smears of the specimen were prepared and fixed by heating.

Carbolfuschin was poured over the smear and heated gently until fumes appeared and allowed to stand for 5 minutes, then washed off with water.

Acid alcohol 3% was poured waited for one minute and repeated this step until the slide appeared light pink. Washed off with water.

Methylene blue was added for two minutes and then washed with water.

The smear dried in air and was examined under an oil immersion lens.

The acid-fast bacilli stained pink, straight, or slightly curved rods, at times having a beaded appearance. The background appears blue due to methylene blue.

The results were reported as negative when no acid-fast bacilli (AFB) was seen in at least 100 microscopic fields.

GeneXpert assay

Liquefaction and deactivation of specimens

One ml of sputum specimen was transferred to a screw-capped tube plus two ml of sample reagent was added, and then shacked vigorously 10 to 20 times, incubated for 10 minutes, then mixed agin and incubated for 5 minutes.

Loading of the sample

Each Xpert MTB/RIF cartridge is labeled with the sample identity (case number)**.** Two ml of sample was transferred slowly into the sample chamber of the Xpert MTB/RIF cartridge, and then the cartridge is loaded into the GeneXpert DX instrument, waited until the system was finished (1h and 52 min), and lastly. The results were read and interpreted according to a load of bacilli and rifampicin resistance profile (Abdul Hakeem *et al.,* 2013).

Data analysis

Version 22SPSS software was used in the data analysis. Pearson Chi-squared test and Fisher exact tests were used to assessing the variation between variables. A P-value less than 0.05 was educated as significant.

Results and Discussion

One thousand sputum specimens were processed by Xpert® MTB/RIF assay. MTB was detected in 218 specimens. The frequency of MDR among suspected pulmonary TB patients was 13 (1.3%). Out of 218 MTB, 13 (5.9%) were MDR-MTB strains (Table 1).

According to the residents, participants who live in rural had a higher rate of MDR 8(2.4%) than urban areas 5(0.8%) with a significant statistical association. The frequency of MDR is significantly lower in subjects aged 21-40 years compared to others. It is also lower in those who showed a negative result for bacillary level but higher in subjects whereas it positive for ZN stain 4 (2.9%) with, P less than 0.05 (Table 2).

The reported prevalence of MDR-MTB among suspected TB patients in the current study was 1.3% which is lower than other studies, conducted in Sudan (Enan, 2018; Nour *et al.,* 2015; Adam *et al.,* 2017). In contrast, it is higher than the study done previously in the White Nile state (Elsafi *et al.,* 2020).

Table.1 Rate of MDR strains

Variable	Rate of MDR in MTB: N=220		Frequency of MDR in suspected TB patients	
	Number	Percentage	Number	Percentage
Non-MDR	207	94.1%	207	20.7
MDR	13	5.9%	13	1.3
Negative	-	-	780	78

Table.2 Relation of MDR MTB strains with socio-demographic features

Variable	Rate of MDR			
	Rate of MDR in MTB: N (%)	P value	Rate of MDR in suspected TB patients: N (%)	P value
Gender Male **Female**	8(5.5) 5(6.8)	0.765	8(1.3) 5(1.2)	 0.059
Residence Rural **Urban**	8(9.3) 5(3.7)	0.087	8(2.4) 5(0.8)	0.039
Age 1-20 **21-40** **41-60** **61-80**	3(9.4) 4(3.4) 4(7.5) 2(10.5)	0.216	3(1.6) 4(1) 4(1.6) 2(1.3)	 <0.000
Case New case **Relapse** **Loss of flow up**	8(5) 4(9.5) 1(5.3)	0.497	8(1.1) 4(2) 1(1.4)	 0.619
HIV Positive **Negative** **Unknown**	1(6.3) 3(12.5) 9(5.5)	0.493	1(1.4) 3(3.1) 9(1.2)	 0.715
Bacillary level negative **Very low** **Low** **Medium** **High**	0(0) 5(9.3) 5(8.8) 2(3.4) 1(5.9)	0.277	0(0) 5(9.3) 5(9.) 2(3.4) 1(5.9)	 <0.000
ZN stain negative **Positive**	9(8.6) 4(3.5)	0.110	9(1) 4(2.9)	<0.000

Comparatively, the prevalence rate of MDR is lower than in Ethiopia as determined in a meta-analysis study (Girum *et al.,* 2018). The rate of MDR in the present study is also lower than in several international studies (Sharma *et al.,* 2011; Mekonnen *et al.,* 2015). This difference could be variations in sample sizes, study populations, and different geographical areas. In this study, there was no-significant relation between gender and MDR-MTB, which is similar to the finding of a study done in Shendi (Enan, 2018), and southwest Nigeria (Daniel and Osman, 2011), where gender was not significantly associated with MDR-TB.

In this study, MDR –MTB is significantly more common in rural (2.4%in) than urban areas (0.8%), which is in line with another study that found geography had a relationship with the MDR (Liu *et al*., 2015). In this study, the rate of MDR is higher in subjects with HIV-pulmonary TB co-infection than

none, which is comparable to a study from Vietnam that showed the rate of acquired MDR-TB was lower in HIV co-infected TB patients than HIV-negative patients (21) but it is disagree with data reported by the World Health Organization (WHO), which found that HIV-positive TB patients had a significantly higher rate of MDR-TB disease than HIV-negative TB patients (Dean *et al.*, 2014). This variation and disagreement in result may be due to sampling size or may be due to differences in the methods used for identification of MDR-TB or HIV from specimens, or may be due to the difference in a geographical area.

The present study revealed a high prevalence of MDR among relapse cases was reported, which is close to finding of Ullah *et al.*, (2020) study. This gives an indication of the presence of a serious problem attributed to either mismanagement of TB patients, wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment with drugs. The current study showed the highest prevalence of MDR-MTB in 1 to 20 and 41-60 age groups years, which disagree with a study previously (Elsafi *et al.*, 2020).

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