

Burden of Multidrug Resistant *Mycobacterium tuberculosis* Among New Cases in Al-Madinah Al-Monawarah, Saudi Arabia



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Abstract: Background: The pattern of *Mycobacterium tuberculosis* susceptibility to first line drugs and multidrug resistance in Al-Madinah Al-Munawarah, a seasonally overcrowded area during Hajj and Omrah, is not well studied.

Objective: This study aimed to investigate anti-tuberculosis drug resistance and its distribution among new cases in Al-Madinah Al-Monawarah.

Methods: Study subjects included 622 patients with first time confirmed TB referred to the central tuberculosis laboratory in Al-Madinah between January 2012 and December 2014.

Results: Out of the 622 isolates, 99 (15.9%) were Mycobacteria Other Than Tuberculosis (MOTTs) and 25 (4.0%), three of which (12%) were children under five years of age, revealed multidrug resistance (MDR). Monoresistance to isoniazid (H) was (1.8%), to rifampin (R) was (1.4%), to streptomycin (S) was (1.9 %) to ethambutol (E) was (1.1 %) and to pyrazinamide (Z) was (2.1%).

Conclusion: Being among the new cases, multidrug resistant tuberculosis (MDR TB) is supposed to be caused by strains which are originally multidrug resistant. Neither nationality nor gender was found to be associated with MDR TB. Since 12% of MDR cases were among children, a probability of primary infection with MDR strains is to be considered. Moreover, mass gathering during Hajj and Omrah seasons does not seem to increase the burden of MDR in the region. However, further investigation is needed to molecularly characterize MDR isolates and their phylogenetics and geographical origin.

Keywords: MDR TB, Al-madinah Almonawarah, Genexpert, BACTEC MGIT 960, mycobacterium, anti-tuberculosis drug, phylogenetics.

INTRODUCTION

World health organization (WHO) declared tuberculosis (TB) as a global emergency in the

mid-1990s. Third of the world population was estimated to be infected with *Mycobacterium tuberculosis* in 1996 with eight million new cases, and three million deaths annually all over the world. However, 8 800 000 new infections and 1 100 000 deaths worldwide were reported by the WHO in 2011. The WHO estimates reported 3.5% MDR cases among new TB infections and 20.5% in previously treated cases [1]. Multidrug-resistant tu-

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ARTICLE HISTORY

Received: August 25, 2016
Revised: October 18, 2016
Accepted: October 19, 2016

DOI:
10.2174/18715265176661611161042
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erculosis (MDR-TB) is the form of TB caused by bacteria that do not respond to the two most powerful, first-line (or standard) anti-TB drugs, isoniazid and rifampicin [1].

In the Kingdom of Saudi Arabia (KSA), the latest reports revealed a prevalence rate of TB ranging from 8.5% in the central region (Riyadh) to 23.1% and 38% in Hail for locals and in the Makkah region for non Saudis, respectively [2]. However, healthcare improvements have reduced the incidence, but not the prevalence levels [3], which is probably the result of events in which there are mass gatherings such as the Hajj and Omarah linked to this region of the world specifically [4].

According to the WHO, the confirmed percentage of MDR in KSA in 2013 was 0.51 % and 5.5 % in new and previously treated cases, respectively¹. Studies carried out in the KSA showed proportions of resistance ranging between 14% to 20% and 1% to 44% to first-line drugs and MDR-TB, respectively [5-7].

MATERIALS AND METHODS

Study type, population and sampling: This is a longitudinal study. Patients (n = 622) with confirmed tuberculosis (all were culture positive), who were referred to the central tuberculosis laboratory in Al-Madinah Al-Monawarah in the period from Jan 2012 to Dec 2014 were included in this study after being given their informed consents. The central tuberculosis laboratory is considered by the ministry of health to be the health authority responsible for tuberculosis cases diagnosed in the health care units of Al-Madinah region with its seven governorates which include Al Madinah, Al Hunakiyah, Mahd Al Thahab, Al-'Ula, Badr, Khaybar and Yanbu Al Bahar. All the cases were newly diagnosed and no subject with re-infection or relapse were included. Basic data were collected by using a standard data questionnaire eliciting information about patient demographics, age, gender, and country of origin.

Sputum specimens were collected from each enrolled pulmonary tuberculosis patient and processed by standard procedures. In patients with extra pulmonary tuberculosis, fine needle aspirates were collected and in few cases gastric lavage samples were collected from infants.

Isolation and Identification of Mycobacterium

Ziehl-Neelsen stain: ZN staining was performed on all collected specimens as follows; smears of the specimen were fixed by absolute alcohol. Carbol fuchsin was poured over smear and heated gently until fumes appear then allowed to stand for 5 minutes, then washed off with water. Sulphuric acid (20%) was poured, kept for one minute and washed off with water. Then methylene blue was added, let for two minutes, again washed with water. Finally, air dry was allowed and slides were then examined under oil immersion lens for acid fast bacilli.

DRUG SUSCEPTIBILITY TESTING BY USING BACTEC MGIT 960

Specimens from the 622 TB patients were cultured in the MGIT 960 system (Becton Dickinson Microbiology System, USA). Ninety-nine of the specimens revealed Non Tuberculous Mycobacteria (NTM) and the rest 523 were *Mycobacterium tuberculosis* cultures and they were introduced to the M960 system for Drug Susceptibility Testing (DST), as directed by the manufacturer. Final drug concentrations used were as follows; E 5.0 mg/ml, S 1.0 mg/ml, R 1.0 mg/ml, H 0.1 mg/ml and 25mg/ml for Z. For each isolate, a growth tube (growth supplement without drug) was included as a control for the determination of the relative growth ratio and the M960 system was used for reporting the results of susceptibility testing.

GenXpert MTB/RIF Assay

The MTB/RIF assay was done according to Blakemore, *et al.*, (2010) [8]. Briefly, three volumes of sample reagent were added to one volume of sample and the container was shaken and kept at room temperature for 15 minutes then 2 ml of the mixture was transferred to the test cartridge. Samples with suspicious results, that their results are different by culture from that by GenXpert or vice versa, were subjected to recheck.

GenXpert negative samples were subjected for further identification by conventional methods including ZN staining and the follow up of cultures on Lowenstein Jensen (LJ) medium for identification of Mycobacteria other than Tuberculosis (MOTTs) [10].

Data Analysis

The Statistical Package for Social Sciences (SPSS) statistical software package, version 16 (SPSS Inc., Chicago, IL, USA) was applied for the data analysis. Descriptive statistics were used to describe the basic features of the data in this study. Differences in categorical variables between groups were compared and analyzed using the chi-square test. A two-sided P-value < 0.05 was considered statistically significant.

RESULTS

Study Population

During the period from January 2012 to December 2014, 622 of the patients referred to tuberculosis reference laboratory in Al-Madinah Al-Monawara were enrolled. Diagnosis was undertaken by ZN smear, culture and PCR. Table (1) shows the demographic data of the patients and the smear result, while Fig. (1) shows the results of the different methods used in diagnosis. They were all culture positive, and 99 of the cultured isolates (15.9 %) were identified as mycobacteria other than tuberculosis (MOTTs) as they were found negative in GenXpert and rapid growers. Table (2).

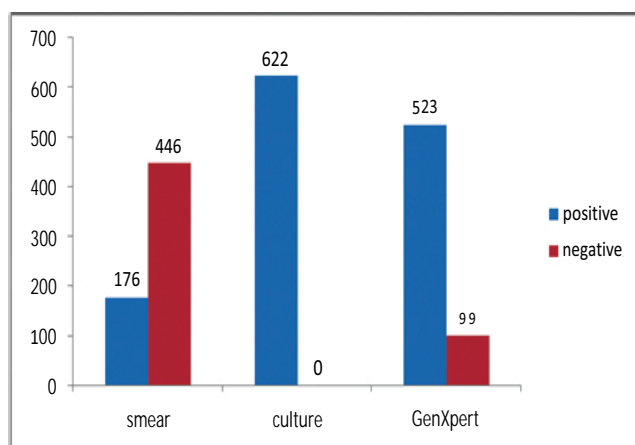


Fig. (1). Results of different diagnostic method.

There were 393 males and 229 females with a male/female ratio of 1.7 which gives significantly lower number of females compared to males ($P = 0.000$). The study population was divided into 4 age groups; age group of ≤ 15 consisting of 68 (10.9 %), age group 16-29 with 185 (29.7%) and

171 patients in the age group 30-44 (27.5%) while the age group ≥ 45 composed of the rest 198 patients (31.8 %). The difference between the age groups in their susceptibility to the infection was highly significant ($P = 0.000$). Regarding nationality, 343 (55.1%) were Saudis and the rest 279 (44.9%) were residents from different nationalities, Asians, Africans and Arabians, with a significant difference between Saudi and non Saudi residents ($P = 0.015$). ZN staining revealed positive smear in 176 (28.3%) cases. The sensitivity of ZN smear was significantly lower than culture ($P = 0.004$). Extrapulmonary tuberculosis (EPTB) was found in 99 (15.9 %) of the cases which was significantly lower than pulmonary tuberculosis ($P = 0.000$). The distribution of extrapulmonary cases among study population is shown in Table (3). Samples for extrapulmonary TB included fine needle lymph node aspirates, pus as well as gastric lavages. 48 (48.5%) of extra pulmonary tuberculosis patients are from the age group of 15 years old or less, 31 of whom (64.6%) are neonates of age range 1 month-1 year. Regarding the other age groups, 5 patients in age group 2 (16-29 years); that is, 26.3%, had diabetes mellitus. In age group 3 (30-44 years), one of the females (7.1%) had rheumatic arthritis and one male (7.1%) had liver cirrhosis. In age group 4 (≥ 45), three males (16.7%) were also reported as diabetic, while the other three (16.7%) were older than 85 years. In addition, nine of the extrapulmonary TB cases (9.1%) were due to MOTTs (Table 3).

All the study population was new TB cases and none of them had a history of re-infection, relapse or drug failure.

Drug Susceptibility Testing

Out of the 622 cases included in this study, monodrug resistance to first line drugs was as follows; 13 (2.1%) isolates were resistant to pyrazinamide, 7 (1.1%) were resistant to ethambutol, 9 (1.4%) were resistant to rifampin, 11 (1.8%) were resistant to isoniazid and 12 (1.9%) were resistant to streptomycin. MDR was found in 25 (4.0 %) of the isolates and poly-drug resistance (resistance to more than one of the first-line drugs but not MDR) was also identified in 11 (1.8%) of the isolates. Table (4) shows the resistance patterns to pyrazinamide, isoniazid, rifampin, ethambutol, and streptomycin.

Table (5) presents the distribution of MDR among the study population considering gender, age group, nationality and smear result. Interestingly, one of the MDR isolates was obtained from a Saudi male with five months of age who has pulmonary tuberculosis while two MDR isolates were also from Saudi male children with extrapulmonary TB who were 2 and 4 years old as shown in Table (6), showing a detailed demographic and drug susceptibility data of the MDR cases.

Table 1. Age, gender, nationality, disease site and smear result of the study subjects.

Character		No. %	
Sex	Male	393	63.2
	Female	229	36.8
Age	15 ≥	68	10.9
	16-29	185	29.7
	30-44	171	27.5
	45 ≤	198	31.8
Nationality	SA	343	55.1
	Foreigner	279	44.9
Smear result	Positive	176	28.3
	Negative	446	71.7
Site of disease	Pulmonary	523	84.1
	Extra pulmonary	99	15.9

Table 2. Distribution of MOTTs among study subjects.

Character		No. %	
Sex	Male	61	61.6
	Female	38	38.4
Age	15 ≥	8	8.1
	16-29	14	14.1
	30-44	30	30.3
	45 ≤	47	47.5
Nationality	SA	54	54.5
	Foreigner	45	45.5
Smear result	Positive	15	15.2
	Negative	84	84.8
Site of disease	Pulmonary	90	90.9
	Extra pulmonary	9	9.1

Table 3. Distribution of Extrapulmonary TB among study subjects.

Character		No. %	
Sex	Male	67	67.7
	Female	32	32.3
Age	15 ≥	48	48.5
	16-29	14	19.2
	30-44	18	14.1
	45 ≤	18	18.2
Nationality	SA	80	80.8
	Foreigner	19	19.2
Smear result	Positive	38	38.4
	Negative	61	61.6

Table 4. Drug susceptibility pattern of *M. tuberculosis* to the different first line anti-TB drugs in Almadinah almonawarah (Jan 2012-Dec 2013).

Drug	No.	%
Z	13	2.1
E	7	1.1
R	9	1.4
H	11	1.8
S	12	1.9
R+H	17	2.7
Z+H	4	0.6
Z+S	2	0.3
H+S	3	0.5
R+H+S	1	0.2
Z+H+S	1	0.2
Z+E+S	1	0.2
Z+E+R+H	1	0.2
Z+R+H	2	0.3
Z+R+H+S	2	0.3
Z+E+R+H+S	2	0.3
Total Z	28	4.5
Total E	11	1.8
Total R	34	5.6
Total H	44	7.1
Total S	24	3.9
Poly resistant	11	1.8
Multidrug resistant	25	4.0

Table 5. Distribution of MDR TB among study subjects.

Character		No. %	
Sex	Male	21	84.0
	Female	4	16.0
Age	15 ≥	3	12.0
	16-29	10	40.0
	30-44	8	32.0
	45 ≤	4	16.0
Nationality	SA	13	52.0
	Foreigner	12	48.0
Smear result	Positive	7	28.0
	Negative	18	72.0
Site of disease	Pulmonary	22	88.0
	Extra pulmonary	3	12.0

DISCUSSION

Infection control of tuberculosis positive cases and patient management are both dependant on early and accurate detection of MDR-TB [10].

The urgent need for a rapid and easily applicable method of MDR detection is raised by the increase in the global incidence of MDR TB [11]. Increased prevalence of drug resistant tuberculosis is critical to tuberculosis control because the number of effective drugs is going to be more limited and there is also a possibility of the emergence of extensive drug resistance (XDR) [12, 13].

In this study, all the study population included were those diagnosed as new cases with positive

Table 6. Demographic data , bacteriological findings and drug susceptibility results of MDR cases.

No.	Age (in years)	Sex	Nationality	type of specimen	Type of TB	smear	Culture	dst S	dst H	dst R	dst E	dst Z
1	17	F	NSA	SPUTUM	P	POS	POS	R	R	R	S	S
2	22	M	NSA	SPUTUM	P	POS	POS	R	R	R	R	R
3	21	M	SA	SPUTUM	P	NEG	POS	S	R	R	S	S
4	27	M	NSA	SPUTUM	P	POS	POS	R	R	R	S	R
5	2	M	SA	FNA*	EP	NEG	POS	S	R	R	S	S
6	30	M	SA	SPUTUM	P	NEG	POS	S	R	R	S	NA**
7	23	M	NSA	SPUTUM	P	NEG	POS	S	R	R	S	NA
8	49	M	NSA	SPUTUM	P	NEG	POS	S	R	R	S	R
9	80	M	NSA	SPUTUM	P	NEG	POS	S	R	R	S	S
10	4	M	SA	PUS	EP	NEG	POS	S	R	R	S	S
11	33	M	SA	SPUTUM	P	NEG	POS	S	R	R	S	S
12	39	M	SA	SPUTUM	P	NEG	POS	S	R	R	S	S
13	38	M	SA	SPUTUM	P	NEG	POS	S	R	R	S	S
14	52	M	SA	SPUTUM	P	NA	POS	S	R	R	S	S
15	40	M	N SA	SPUTUM	P	POS	POS	S	R	R	R	R
16	23	F	N SA	SPUTUM	P	POS	POS	R	R	R	R	R
17	30	M	SA	SPUTUM	P	NA	POS	S	R	R	S	S
18	34	F	N SA	SPUTUM	P	NA	POS	S	R	R	S	S
19	23	F		SPUTUM	P	POS	POS	S	R	R	S	S
20	34	M	SA	SPUTUM	P	NA	POS	S	R	R	S	S
21	27	M	SA	SPUTUM	P	POS	POS	S	R	R	S	S
22	21	M	SA	SPUTUM	P	NA	POS	S	R	R	S	S
23	25	M	N SA	SPUTUM	P	POS	POS	R	R	R	S	R
24	5 MONT	M	S	FNA	EP	POS	POS	S	R	R	S	S
25	32	M	N SA	SPUTUM	P	POS	POS	S	R	R	S	S

* Fine Needle Aspirate

** Not Available

cultures in the years 2012, 2013 and 2014. Of the total grown cultures, 15.0% were MOTTs. This result partially agrees with previous studies in KSA which found that NTM account for 9% of the total mycobacterial infection [14]. However, another study from KSA revealed even higher NTM rate (24.5%) from the total positive mycobacterial cultures [15] is nearly double the percentage represented in this study. Another recent study from KSA revealed that in a total of 95 NTM isolates, 67.1% were from clinically relevant respiratory cases, and 9.7% were respiratory colonizers. Moreover, chronic obstructive pulmonary disorder and history of previous tuberculosis infection were found to be the major risk factors [16]. In a review study from the middle east it was concluded that the number of NTM isolates has significantly increased in the Middle East and the authors suggested the increasing concern about NTM diseases as a reason [17]. In Greece, a retrospective study revealed a ratio of 0.59 of NTM isolates to *M. tuberculosis* in all hospitalized patients which means that more than half of isolates are NTM [18]. Thus, the ratio reported here is within the local and regional rates.

The male /female ratio was found to be 1.7. This comes into agreement with studies from other places; Borgdorff *et al.* (2000) [19], found greater numbers of TB cases among men than women in almost all cases studied in areas of Southeast Asia, the Western Pacific and US [20, 21]. The ratio is even higher in other parts of the world as it was found to be 5.1:1 in Vietnam [22].

Regarding age, in this study young adults represented the highest rate of infection. This comes into agreement with previous studies [23].

Out of the total number of cases included in this study, 15.9 % were presented as extrapulmonary tuberculosis. A previous study from KSA found that extrapulmonary TB is present in 57.5% of all tuberculosis cases in a Teaching hospital in Al Riyadh through the period from 2001 to 2007 [24]. However, in a more recent study also from KSA, the proportion of extrapulmonary TB was found to be 32% in 2011 which is higher than global figures [25] with intra-thoracic and extra-thoracic lymph nodes being the most common sites of infection [26]. Extrapulmonary TB represented 28.2% of all reported TB cases in 1997 [27]. An older study revealed 63% extrapulmonary cases out of the to-

tal number of cases in the period from 1991-2000 in Riyadh [28] and 57% in Alkhobar, in the Eastern Province of Saudi Arabia from 1997-2005 [29]. Regarding the co morbidities associated with extrapulmonary tuberculosis in this study, diabetes mellitus, liver cirrhosis and rheumatoid arthritis were recorded. However, the most obvious risk factor was age, as almost half of EPTB patients are children, most of whom were neonates, a group of population defined as immuno compromised. There is a big disagreement between several previous studies regarding the risk factors associated with EPTB, while some authors proved that age, gender, HIV co infection and liver disease as independent risk factors with alcoholism being a high risk factor [30], others found that gender and age are associated with the disease while alcoholism, smoking, contact with pulmonary tuberculosis patients and BCG vaccination has a protective role [31]. Moreover, in this study, a considerable percentage of EPTB cases were due to MOTTs (9.1%), However, this is not an odd phenomena since higher percentages of EPTB in other parts of the world, such as India, were found to be caused by MOTTs [32]. Thus, the percentage of extrapulmonary TB reported in this study is relatively low. This may be due to the fact that most of the patients are pilgrims.

Regarding the nationality of this study subjects, 55.1% were Saudis. This is different from a previous study conducted in the Eastern province of KSA where the percentage of immigrant was 59.9% of the total TB patients, while the Saudis represented only 40.1%, with a detectable impact of immigrants onto the transmission of tuberculosis in the area [33]. Another study also found that the TB incidence rate in non-Saudis is nearly double the rate in Saudis in a study period of ten years extending from 2000 to 2009 [34]. Thus, the close rate of TB prevalence among Saudis and non Saudis may be due to the close contact between the different nationalities in Al-Madinah which is a target area for large population during Hajj and Omra seasons. Moreover, the fact that the nature of work of most resident foreigners is being house labors such as drivers, house maids and nannies, a type of jobs that requires much closer personal contact, and this can explain the high rate of EPTB in the children group.

In this study, MDR was found to be 4.0%. The most recent estimates by WHO revealed 3.5% of

MDR TB in new cases [1]. A study in 2014 showed a percentage of 4.3% of MDR in KSA [35]. Moreover, the results of the first nationwide drug surveillance survey, conducted with a representative population of nationals and immigrant patients, declared a MDR ratio of 4% [36]. However, another study conducted with a nationwide collection of isolates in KSA showed discrepancies of 2.3% in defining MDR-TB between diagnostic laboratories around the country [37]. In this regard, the pattern of MDR in Al-Madinah seems to be similar to what is present in the other parts of the country [35, 36] as well as what is declared internationally [1]. The difference to what was reported by other parts of KSA may be due to the discrepancies previously described [37]. A review study, which defined MDR as the resistance to two or more of any of the first line TB drugs, was undertaken in KSA for the years 1979 through 1998, revealed MDR range between (1.5% - 44%) [6]. However, the difference in MDR definition between our study and the above mentioned study in addition to the variable time zone of the two studies, may explain this disagreement.

The MDR percentage in this study is somewhat similar to the percentage of MDR TB in the western region of KSA which was found to be 4.9% according to the findings of the first national survey [36]. In this study, monoresistance to streptomycin was found to be 1.9%, to isoniazid 1.8%, rifampin 1.4%, ethambutol 1.1% and 2.1% to pyrazinamide. These are all lower than what was recorded by the first national survey [36] of MDR in KSA which were as follows; (8.1%) for streptomycin, (5.4%) for isoniazid (1%) for rifampin and (0.8%) for ethambutol. However, several options are still available for the treatment of MDR TB, of which one of the most recent is the trial concerning the use of *Mycobacterium vaccae* vaccine which revealed promising results as an adjunctive therapy [38]. However, (MDR TB) was observed in 4.0% of our study population which is relatively higher than the 1.8% which was reported in the new TB cases by Al-Hajoj *et al.*, (2013) [36] in the above mentioned study. However, a proportion of 14.9% of MDR TB in Saudi Arabia was detected in 1996 [39].

Drug susceptibility pattern to *M. tuberculosis* in KSA was also studied by Al-Awaidy *et al.*, (1997) [40], who found an overall resistance of 12.4%, while MDR was reported in 0.8% of their study

subjects with variable percentages of resistance to one and to two or more first line drugs other than RIF and INH. They observed that isoniazid compose for the highest monoresistance rate [40]. This result agrees with our result regarding isoniazid but the result in our study for MDR is still higher.

In KSA, MDR was observed in 5.7% of all isolates through a period of forty years. The overall resistance rate to at least one antibacterial drug was similar in isolates grown between 1979-1991 (18.5%) and 1989-2000 (18.3%). There were higher resistance rates in the Western and Southern regions of the country [41].

The special feature of Al-Madinah Al-Monawrah is that it is one of the two most crowded areas during Hajj and Omra. Previous studies found that tuberculosis is the most common reason for pneumonia that leads to admission to hospital during Hajj [42] and that was supposed to be due to environmental conditions, crowding and physical exercise during the event in addition to the fact that most of the pilgrims are of middle age and most of them have other co-morbidities which increase their susceptibility to TB or the possibility of reactivation of latent infection [43]. Another study confirmed increased prevalence of TB cases in areas close to the pilgrimage and with a higher proportion in non-Saudi patients [44].

All patients included in this study are new TB cases, the WHO global estimates revealed 3.5% of MDR among new cases and 20.5% among previously treated TB cases. This finding comes into agreement with the result of our study that revealed 4.0% resistance rate in our isolates which are all from new cases. Moreover, strain lineages associations of significant values were reported in a study from Saudi Arabia regarding the admixed phylogenetic distribution of *Mycobacterium tuberculosis* that are drug resistant in KSA [45], with the probability of latent infection reactivation endogenously and reinfection with another strain exogenously [46]. In this study, this possibility is not far from the expectations since most of the non Saudi patients are from areas of high TB burden. The presence of MDR in isolates from patients newly infected may be explained by the possibility of catching infection with a strain which is almost resistant. This suggestion could be strengthened by the fact that one of the 25 MDR isolates was obtained from a 5 months aged neonate with extrap-

ulmonary TB and two other MDR isolates were from Saudi male children of less than 5 years of age who were also presented with extrapulmonary TB.

CONCLUSION

In conclusion, although the ratio of MDR in Al Madina is similar to its ratio all over KSA, the prevalence of TB infection in Saudi nationals is significantly higher than in non Saudi residents. Thus, further studies to determine the molecular epidemiology of the disease and the phylogenetic lineages of the causative isolates are needed to know the origin of the most prevalent *Mycobacterium tuberculosis*, as well as MDR isolates in this special region. Moreover, the risk of contact with people with active TB or even history of TB infection should be considered as some of the patients, including MDR carriers, were children. According to NIH consensus, evidence of contact with adults with TB is a parameter of classification of pediatric TB cases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the staff of the Central Tuberculosis Laboratory in Al-Madina Al-Monawara for facilitating the achievement of this project.

FUNDING

This research is funded by the Deanship of Scientific Research, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia (Grant number 435/6133).

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