Characterization of *Mycobacterium tuberculosis* isolated from cancer patients with suspected tuberculosis infection in Egypt: identification, prevalence, risk factors and resistance pattern

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Abstract

Data are sparse on *Mycobacterium tuberculosis* infection among patients with cancer in Egypt. We sought to detect the presence of tuberculosis (TB) disease among patients with malignant conditions and suspected TB and to study the main risk factors. Also, we compared different diagnostic procedures and detected the antimicrobial susceptibility of *M. tuberculosis* isolates against rifampin and isoniazid. One hundred patients were included in this study, all of them had malignant conditions and were suspected by the clinicians of having TB. Identification of *M. tuberculosis* in different specimens was performed by smear microscopy, followed by Lowenstein–Jensen medium and *Mycobacterium* growth indicator tube (MGIT) cultures and artus® real-time PCR. In addition, an indirect MGIT anti-TB susceptibility test was carried out against rifampin and isoniazid. A total of 76% of studied cases were found to be TB positive. The frequencies of TB-positive cases in the bronchogenic, haematological and solid tumour malignancy groups were 21%, 25% and 30%, respectively. Significant differences between pulmonary and extrapulmonary TB in different malignancy groups were recorded. Real-time PCR showed the highest overall diagnostic efficiency. Multidrug-resistance of *M. tuberculosis* to both rifampin and isoniazid was detected in 28.6% of examined isolates. Infection in cancer patients with TB was significantly more often recorded among elderly patients and those suffering from poverty. Pulmonary TB is more common than extrapulmonary TB in patients with malignancy. Real-time PCR is the most accurate and rapid method for TB diagnosis. MGIT-rifampin resistance may be used as a reliable marker for detection of multidrug-resistant TB. Diagnosis and instituting treatment course for active or latent TB infection are crucial before starting anticancer therapy.

Keywords: Cancer, isoniazid, mycobacterium growth indicator tube, real-time PCR, rifampin, tuberculosis
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Introduction

Although Egypt is not one of the 22 countries listed by WHO for their high levels of tuberculosis (TB) infection, TB is a considerable public health problem in Egypt [1]. The inflammation and fibrosis associated with TB could promote malignancy. This was mainly attributed to blocking of lymphatics by pulmonary scarring and fibrosis causing a delay in clearance of activated leucocytes and consequently an enhancement of metastatic cell deposition within fibrotic areas [2–4].

On the other hand, malnutrition and immunosuppression caused by cancer were reported as possible primary causes of contraction or reactivation of TB infection. Recently, Wu *et al.* [5] have described cancers as risk factors for TB. Although a causal mechanism is not proven, lung cancer risk, especially adenocarcinoma, was reported to be increased with TB especially if associated with chronic obstructive pulmonary disease or smoking-related cancers [4]. The possibility of co-infection with TB was reported in patients with a malignancy, especially those with lung cancer [6].

Finally, clinicians should be aware of the similarities between TB and cancer, especially lung cancer [7–9]. Some patients with chronic and slowly progressive pulmonary TB were mistakenly thought to have lung cancer, such as bronchoalveolar carcinoma or pulmonary non-Hodgkin's lymphoma [10,11]. Due to the similarity between some clinical and radiographic aspects of TB with various diseases, extrapulmonary TB may be misdiagnosed as cancer.

Documentation of resistance among TB bacilli is globally escalating [12,13]. However, there is a shortage of reports concerning antimycobacterial resistance and prevalence of *M. tuberculosis* isolates from cancer patients in hospitals in Egypt. Consequently, this work aimed to measure the relative frequency of TB disease in patients with malignant conditions and to study other TB risk factors in Egypt during the period 2007–2010. Moreover, the study aimed to compare different diagnostic procedures as well as to detect the antimicrobial susceptibility patterns of *M. tuberculosis* isolates against rifampin and isoniazid.

Patients and Methods

Patients' specimens

Sputum, pus, ascetic fluids and bronchoalveolar lavage were collected as per the clinician's recommendations from 100 patients with cancer who were suspected of having TB by the clinicians at the National Cancer Institute of Egypt during the period 2007–2010. Patients filled in and signed a questionnaire that included demographic data, past medical history and agreement of inclusion in the study.

Microbial identification

Mycobacterium tuberculosis strains were identified using microscopic examination of smears stained by the Ziehl–Neelsen technique. Decontaminated samples were inoculated into Lowenstein Jensen Medium (LJ) (Becton Dickinson, Diagnostic Instrument Systems, Inc., Franklin Lakes, NJ, USA) as well as Mycobacterium growth indicator tube (MGIT) (BBL, Becton Dickinson, Diagnostic Instrument Systems, Inc., Franklin Lakes, NJ, USA) for the purpose of confirmation and comparison of their efficacy [14].

Real-time PCR

DNA extraction was performed on the decontaminated samples using a QIAamp DNA minikit (Qiagen Co., Valencia, CA, USA). It was designed to amplify the region encoding the gene for 16S rRNA within the *Mycobacterium* genome and to detect all *M. tuberculosis* complex organisms, including *M. tuberculosis*, *M. africanum*, *M. bovis* and *M. microti*. The specific amplicons (a 159 bp region of the mycobacterial genome) were detected directly using the 5′-nuclease (TaqMan) probe labelled with FAM dye [15] and JOE dye for detection of Internal Control (*M. tuberculosis*™ IC). Amplification was carried out using an artus® *M. tuberculosis*™ PCR Kit (Qiagen) on Step One Applied Biosystems thermal cycle, and manufacturers' protocols were followed [16].

Results revealed positive detection of *M. tuberculosis* whether solid cultures, liquid cultures or real-time PCR were compared.

Indirect anti-TB MGIT susceptibility test

Due to the low bacillary load in specimens, as indicated by Ziehl–Neelsen (ZN) smears, indirect anti-TB MGIT susceptibility test (indirect AST) was performed to detect the isolates' resistance pattern.

Isoniazid (1 mg/mL) stock aqueous solution and rifampin (250 mg/mL) stock solution in dimethylsulphoxide : water (1:1) mixture were prepared. Tubes containing rifampin or isoniazid at concentrations of 1.0 and 0.1 mg/mL, respectively, and one growth control (GC) tube were prepared for each inoculum [17,18].

All MGIT tubes were incubated at 37°C and were checked daily for growth indication from the third to the twelfth day via 365-nm UV trans-illuminator. Each isolate was considered susceptible if the drug-containing tube did not fluoresce within 2 days of positivity in the GC tube. A resistant strain was considered if the drug-containing tube showed growth on the day of GC positivity or within 2 days.

Statistical analysis

Categorical data were summarized as a percentage. Comparisons among different groups were performed using GRAPH PAD INSTAT by chi-square and two-sided p-value and $p < 0.05$ was considered significant.

Results

Frequency of TB in clinical specimens isolated from suspected patients

The frequencies of TB-positive cases in bronchogenic, haematological and solid tumour malignancy groups were 21%, 25% and 30%, respectively. Positive TB was detected in 76 of the 100 cancer patients. During the 3 years of the study, TB-positive cases represented 65.9%, 86.4% and 78.9%, respectively. There was a significant difference ($p < 0.05$) between pulmonary TB (PTB) and extrapulmonary TB (ETB), (Table 1).

Mean age of TB-positive patients with malignancy was 47 ± 17.6 years (mean ± SD). Although, 24 (31.5%) TB-positive patients with malignancy were older than 55 years, there

TABLE 1. Frequency of tuberculosis (TB) -positive cases with different malignancies during the study period

TB site	Malignant condition	1st year *n* = 44 (100%)	2nd year *n* = 37 (100%)	3rd year *n* = 19 (100%)
PTB	Bronchogenic	5 (11.3)	11 (29.7)	3 (15.8)
	Haematological	4 (9.1)	8 (21.6)	5 (26.3)
	Solid tumours	5 (11.3)	4 (10.8)	3 (15.7)
ETB	Bronchogenic	2 (4.5)	0 (0)	0 (0)
	Haematological	4 (9.1)	2 (5.4)	2 (10.5)
	Solid tumours	9 (20.4)	7 (18.9)	2 (10.5)

1. Relation between positive PTB and ETB $\chi^2 = 7.661$, p <0.05*
2. Relation between PTB and ETB in different malignancy groups $\chi^2 = 13.901$, p <0.05*
3. Relation between different types of malignancy $\chi^2 = 1.696$, p >0.05

*Significant.
PTB, pulmonary TB; ETB, extrapulmonary TB.

was a significant increase (p <0.05) in contraction of TB infection among elderly patients. Concerning gender, there was not a significant difference between male and female TB-positive patients.

Poverty was found as a risk factor for all TB-positive cases, where patients are on a low or fixed income, live in remote areas, these factors can lead to difficult access to the medical care needed to diagnose and treat TB. There is coexistence between TB infection and malignancies in all cases. Other studied risk factors include a number of diseases such as hepatosplenic disorders (i.e. splenectomy, liver cirrhosis, hepatitis C virus, hepato-splenomegaly), anaemia, diabetes mellitus and hypertension; these were recorded in frequencies of 6.6%, 6.6%, 3.9% and 3.9% of TB-positive cases, respectively.

Our study reported smoking in 3.9% of TB-positive patients. Past TB history was recorded in 5.2% of TB-positive cases. However, there was no significant correlation statistically (p >0.05) between the presence of primary or recurrent TB infection and TB history. Additionally, there was no significant difference (p >0.05) between the diagnosis of PTB and ETB cases with regards to patient age, gender and demographic characteristics (Table 2).

In the current study, the proportion of males to females regarding the sputum ZN smear was statistically significant (p <0.05), where ZN-smear-positive female and male sputa were 70.9% and 92.5%, respectively of total TB-positive sputum (Table 3). However, no statistically significant difference was detected between different age groups regarding the sputum ZN smear positivity, a significant difference (p <0.05) was only recorded for the older age group >65 years. The present study was based on testing the following specimens: sputum, pus, ascetic fluid and bronchoalveolar lavage, their TB diagnostic efficiencies were 75.8%, 76.6%, 100% and 66.6%, respectively. On the other hand, there are neither significant difference (p >0.05) in TB diagnosis between different types of specimens nor between sputum and other specimen types (Table 4).

TABLE 2. Prevalence of PTB and ETB in different patients' demographic characteristics

TB disease demographic characteristics	ETB *n* = 28	PTB *n* = 48	p-value
Age (mean ± SD) years	42.4 ± 14.2	49.4 ± 18.6	$\chi^2 = 0.534$; p >0.05
Gender			
Female	17 (60.7%)	25 (52.1%)	$\chi^2 = 0.423$; p >0.05
Male	11 (39.3%)	23 (47.9%)	
TB risk factors (%)			
Diabetes mellitus	0	3 (6.3%)	p >0.05
Hypertension	0	3 (6.3%)	p >0.05
Smoking	1 (3.6%)	2 (4.2%)	p >0.05
Renal disorder	0	2 (4.2%)	p >0.05
Hepatosplenic disease	2 (7.1%)	3 (6.3%)	p >0.05
Old age > 55 year	7 (25%)	17 (35.4%)	p >0.05
Young age <14 year	0	3 (6.3%)	p >0.05
Anaemia	1 (3.6%)	4 (8.3%	p >0.05
TB history	1 (3.6%)	3 (6.3%)	p >0.05
Close contact with TB case	0	1 (2.1%)	p >0.05
Congenital CV disorder	1 (3.6%)	0	p >0.05
Occupation	1 (3.6%)	0	p >0.05

TB, tuberculosis; PTB, pulmonary TB; ETB, extrapulmonary TB; CV, cardiovascular.

Evaluation of used diagnostic methods

TABLE 3. Relation between genders and smear-positive sputum samples in different age groups

Gender	Female		Male		
ZN smear	Positive (%)	Negative (%)	Positive (%)	Negative (%)	p-value
Age groups (years)					
0–14	3 (13.6)	0	3 (12)	0	>0.05
15–24	0	0	3 (12)	2	>0.05
25–34	0	0	3 (12)	0	>0.05
35–44	5 (22.8)	2	3 (12)	0	>0.05
45–54	8 (36.3)	2	7 (28)	0	>0.05
55–64	3 (13.7)	0	3 (12)	0	>0.05
>65	3 (13.6)	5	3 (12)	0	<0.05*
Total	22 (100)	9	25 (100)	2	<0.05*
Frequency of ZN smear positivity	70.9		92.5		

*Significant.

TABLE 4. Relation between type of specimens and prevalence of tuberculosis (TB)

Specimen	ZN result	TB positive (%)	TB negative (%)	Total TB positive	Efficiency= ($\sum^2$ TB positive/*n*) *100 (%)	χ^2, p-value between all types at
Sputum *n* = 58	Smear-positive	32 (55.2)	3 (5.2)	44	75.8	χ^2 = 6.478, p >0.05
	Smear-negative	12 (20.7)	11 (19)			
Pus *n* = 30	Smear-positive	20 (66.7)	3 (10)	23	76.6	χ^2, p-value between specimen and sputum
	Smear-negative	3 (10)	4 (13.3)			
Ascetic fluid *n* = 3	Smear-positive	3 (100)	0	3	100	χ^2 = 0.032, p >0.05
	Smear-negative	0	0			
BAL *n* = 9	Smear-positive	6 (66.7)	2 (22.2)	6	66.6	
	Smear-negative	32 (55.2)	3 (5.2)			

ZN, Zeil–Neelsen; $\sum^2$, algebraic sum; BAL, bronchoalveolar lavage.

Different diagnostic methods used were evaluated with regards to diagnostic efficiencies and recovery rates. The highest overall diagnostic efficiency of the methods used was achieved using all three methods LJ, MGIT and in the PTB group. The highest diagnostic efficiency in ETB was in smears positive using a combination of MGIT and artus® real-time PCR. However, PCR was able to detect TB solely in one case of smear-positive PTB and three cases of smear-negative ETB.

The overall proportion of the total TB isolates (number of confirmed positive cultures by MGIT or LJ/total number of positive cultures by both methods) that were positive by MGIT was 85% compared with 80.8% for the reference standard, LJ. Of all culture-positive specimens, 81.7% of smear-positive and 100% of smear-negative specimens were positive by MGIT whereas LJ recovery rates were 93.3% and 23% for smear-positive and smear-negative specimens, respectively. These data indicate that MGIT liquid medium was significantly more sensitive than the LJ solid culture medium in smear-negative specimens ($p < 0.05$). In contrast, there was no statistical difference (i.e. $p > 0.05$) in sensitivity between the two culture media in smear-positive and total samples (Table 5).

The overall proportion of total TB isolates (number of confirmed positive cultures by artus® real-time PCR or LJ/total number of positive cultures by either method) that were positive by PCR was 98.7%, compared with 77.6% for LJ. Of all culture-positive specimens, 98.4% of smear-positive and 100% of smear-negative specimens were positive by PCR whereas LJ recovery rates were 91.8% and 20% for smear-positive and smear-negative specimens, respectively. These data indicate that artus® real-time PCR was significantly ($p < 0.05$) more sensitive than the LJ solid culture medium in smear-negative samples. There was no significant difference in smear-positive and total samples (Table 6).

Comparison between MGIT and artus® real-time PCR was performed using the chi-square method (Table 7). Real-time PCR showed more significant ($p < 0.05$) sensitivity of total samples than MGIT using LJ as the reference standard. Additionally, negative predictive value (NPV) of PCR versus LJ was more significant than that for MGIT versus LJ ($p < 0.05$).

TABLE 5. Comparison between recovery rates of LJ and MGIT to all positive cultures

ZN smear	MGIT/LJ+	MGIT+/LJ−	MGIT−/LJ+	MGIT/LJ−	Total positive cultures	MGIT+	MGIT recovery rate (%)	LJ+	LJ recovery rate (%)	χ^2, p
Smear positive *n* = 69	45	4	11	9	60	49	81.7	56	93.3	0.769, >0.05
Smear negative *n* = 31	3	10	0	18	13	13	100	3	23	48, <0.05*
Total *n* = 100	48	14	1	27	73	62	85	59	80.8	0.106, >0.05

*Significant; χ^2, chi-square; PTB, pulmonary tuberculosis; ETB, extra-pulmonary tuberculosis; LJ, Lowenstein–Jensen; MGIT, *Mycobacterium* growth indicator tube; real-time PCR, polymerase chain reaction.

TABLE 6. Comparison between recovery rates of positive LJ cultures and artus® real-time PCR

ZN smear	LJ/PCR+	PCR+/LJ−	PCR−/LJ+	LJ/PCR−	Total positive PCR and LJ	PCR+	PCR recovery rate (%)	LJ+	LJ recovery rate (%)	χ^2, p
Smear-positive *n* = 69	55	5	1	8	61	60	98.4	56	91.8	0.229, >0.05
Smear-negative *n* = 31	3	12	0	16	15	15	100	3	20	53, <0.05*
Total *n* = 100	58	17	1	24	76	75	98.7	59	77.6	2.5, >0.05

*Significant; χ^2, chi-square; PTB, pulmonary tuberculosis; ETB, extra-pulmonary tuberculosis; LJ, Lowenstein–Jensen; real-time PCR, polymerase chain reaction.

TABLE 7. Comparison between sensitivity, specificity and predictive values of MGIT and PCR using LJ as gold standard

ZN smear	Parameter	Statistical contrasts between MGIT and artus® PCR
Positive *n* = 69	Sensitivity	χ^2 = 9.33; p <0.05*
	Specificity	χ^2 = 0.16; p >0.05
	PPV	χ^2 = 0.001; p >0.05
	NPV	χ^2 = 4.92; p <0.05*
Negative *n* = 31	Sensitivity	NA
	Specificity	χ^2 = 0.299; p >0.05
	PPV	χ^2 = 0.039; p >0.05
	NPV	NA
Total samples *n* = 100	Sensitivity	χ^2 = 9.3; p <0.05*
	Specificity	χ^2 = 0.46; p >0.05
	PPV	χ^2 = 0.0001; p >0.05
	NPV	χ^2 = 6.08; p <0.05*

*Significant; χ^2, chi-square; LJ, Lowenstein–Jensen; MGIT, mycobacterial growth indicator tube; artus® real-time PCR, polymerase chain reaction; ZN, Ziehl–Neelsen; PPV, positive predictive value; NPV, negative predictive value; NA, not applicable.

Antituberculosis susceptibility test results

An indirect anti-TB sensitivity (AST) test against rifampin and isoniazid using MGIT was performed on culture-positive isolates; 69.4% of isolates with respect to total positive culture isolates were from PTB cases and 30.6% of isolates were from ETB cases. Of all tested isolates 51% were sensitive to rifampin and isoniazid, 20.4% were resistant to isoniazid only, and 28.6% isolates were resistant to both drugs. Our study detected a significant difference (p <0.05) between different malignancy groups and presence of isolates resistant to rifampin or isoniazid. The different malignancy groups included bronchogenic, haematological and solid tumours other than bronchogenic cancers. The relation between the AST results in PTB and ETB groups showed that there was only significant resistance (p <0.05) to isoniazid only (Table 8 and Fig. 1).

Discussion and Conclusion

Cancer is the second leading cause of death after coronary artery disease [19]. Tuberculosis and cancer share similarities that lead to a serious challenge in their diagnosis [6,20]. The WHO estimated the burden of TB disease globally in 2008, 2009 and 2010; the average incidence rate of TB in Egypt (all forms of TB) was estimated at 19 per 100 000. Our study was carried out during 2007–2010 and the study population comprised cancer patients (*n* = 100) who were suspected by the clinicians of having TB. A total of 76 patients were confirmed by the study protocol to have TB infection. Although detection of TB was not performed in a wide range of patients with cancer, it is clear that these patients comprise a considerable proportion of the estimated TB-positive cases detected in Egypt by WHO [21].

In the present study, the frequencies of TB-positive cases in bronchogenic, haematological and solid tumour malignancy groups were 21%, 25% and 30%, respectively. Therefore, coexistence of TB with cancer was more common in the solid tumour malignancy group, followed by the haematological malignancy group. This finding is in agreement with those of previous studies [20,22,23].

There was no significant difference in TB detection among female and male patients in our study although a higher male ratio was reported previously [20]. According to the most recent 16th global report on tuberculosis, published in 2011 by WHO, an excess of male pulmonary TB cases was detected in all regions of the world including Egypt [21]. It was suggested that there is sex bias in TB surveys [24]. This was attributed to the difficult access for female patients to healthcare units, as well as to the poor quality of sputum

TABLE 8. Antituberculosis susceptibility of *Mycobacterium* isolates

		Associated malignant condition			
TB infection site (%)	AST result	Bronchogenic (%)	Haematological (%)	Solid tumours other than bronchogenic (%)	Total (%)
PTB (69.4)	Sensitive	6.1	14.3	10.2	30.6
	Resistance to INH and RIF	4.1	4.1	10.2	18.4
	Resistance to INH only	16.3	4.1	0	20.4
ETB (30.6)	Sensitive	2	8.2	10.2	20.4
	Resistance to INH and RIF	0	0	10.2	10.2
	Resistance to INH only	0	0	0	0

χ^2, p-value of AST results between different types of malignancy

Sensitive	Resistance to INH and RIF	Resistance to INH only
χ^2 = 2.716; p >0.05	χ^2 = 7.361; *p <0.05	χ^2 = 8.095; *p <0.05
χ^2, p-value of AST results between pulmonary and extrapulmonary positive TB		
χ^2 = 0.731; p >0.05	χ^2 = 0.691; p >0.05	χ^2 = 8.526; *p <0.05

TB, tuberculosis; PTB, pulmonary TB; ETB, extrapulmonary TB; AST, anti-tuberculosis susceptibility test; INH, isoniazid; RIF, rifampin.
χ^2, p-value of AST results between pulmonary and extra-pulmonary positive TB, *Significant.

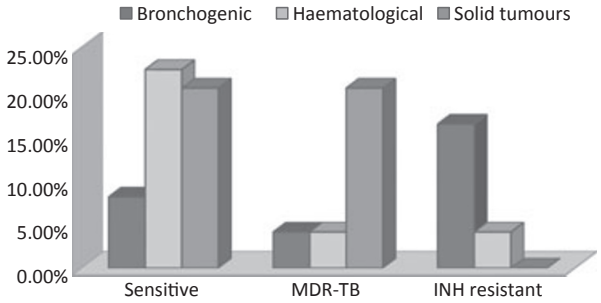

FIG. 1. Anti-tuberculosis susceptibility test (AST) in cases with different malignant conditions. MDR-TB, multidrug-resistant tuberculosis; INH, isoniazid.

samples collected from women in some regions. This was proven in the current study; where frequencies of ZN smear-positive female and male sputa were 62.5% and 90.4%, respectively, which was statistically significant. Poor sample quality would have a negative effect on the laboratory diagnosis of TB [25]. Age distribution among TB-positive patients was also interesting in the present work, the mean age of TB-positive cases detected was 47 ± 17.6 years (mean ± SD). The only significant difference in TB smear-positive sputum was observed in the older age group, >65 years.

The current study recorded a significant difference between PTB and ETB in the different malignancy groups in the ratios; 9.5:1 in bronchogenic malignancy, 2.1:1 in haematological malignancies and 1:1.5 in the other solid malignant tumours group. These results are in accordance with previous reports [20].

The study showed that TB infection is related to the studied risk factors, which included smoking, poverty and a number of diseases such as hepatosplenic disorders, anaemia, diabetes mellitus and hypertension. A similar retrospective study on TB risk factors was carried out in southern Taiwan [26–28].

The inflammation and diagnostic procedures associated with TB could promote malignancy. The development of breast carcinoma was thought to be associated with the multiple chest X-rays needed for the follow-up of a previous TB infection [29]. Similarly in the present work, one case of breast cancer had a history of TB, had developed recurrent TB infection and been subjected to repeated diagnosis. In addition, three cases with bladder cancer had reported recurrent TB infections; two had PTB and one had ETB. The relationship between TB disease and bladder cancer was previously evaluated [30,31], although the relevant mechanisms are still unknown.

Two laboratory diagnostic methods were evaluated against the reference standard LJ medium—artus® tuberculosis real-time PCR and manual MGIT liquid culture medium. The diagnostic efficiency of each laboratory method was calculated for all patients. The highest overall diagnostic efficiency of the methods was achieved using all of three methods: LJ, MGIT and real-time PCR. The highest diagnostic efficiency in ETB was in smear-positive patients using a combination of MGIT and real-time PCR. However, real-time PCR was able to detect TB in only one case of smear-positive PTB and three cases of smear-negative ETB. Similarly, the usefulness of the Rotor Gene (artus® *M. tuberculosis* RG) PCR assay was previously described [32].

Rapid and accurate diagnosis of ETB is necessary, although it has a greater impact on patient management than on limiting the spread of infection [33]. This is not often an easy task, because symptoms vary according to the infected organs and patients may have few or even no classic signs and symptoms. The recovery rates of artus® real-time PCR, MGIT and LJ were compared in our study. MGIT was significantly more sensitive than LJ in recovering *M. tuberculosis* from smear-negative specimens, which was also concluded by other studies [34,35]. Comparison between the recovery rates of artus® real-time PCR and LJ showed higher recovery (98.7%) for PCR than for LJ (77.6%).

Sensitivity and specificity of artus® real-time PCR were estimated as 96.55% and 59%, respectively. In another study, the sensitivity and specificity of the ABI prism artus® *M. tuberculosis* assay using culture as a standard were reported as 97.8% and 85.1%, respectively [15]. Considering LJ as the reference standard in the present study, artus® real-time PCR had significantly higher sensitivity in both the smear-positive and the total samples groups than the MGIT results. Agreement between LJ and PCR was previously reviewed [36].

Comparison of the cost of the laboratory diagnosis revealed that the artus® real-time PCR method using artus® TM tuberculosis had the highest financial cost. However, time elapsed to get the result was considerably the lowest. Regarding the cost of infection spread as the resutl of a delay in TB diagnosis, artus® real-time PCR is considered the most cost-effective method.

The AST were performed on culture-positive TB isolates. The MGIT system has been thoroughly evaluated for AST of *M. tuberculosis* towards anti-tuberculosis drugs and a good concordance with the reference standard proportion method was reported [37–39]. In the present study, the bacillary loads in specimens were low as indicated by ZN smears, so an indirect test was performed. The test was carried out for two of the most important first-line drugs; rifampin and isoniazid. Indirect AST using MGIT was previously reported to have excellent agreement with the proportion method [39].

In the present work, bronchogenic malignancies' patients had the highest rate of isoniazid-resistant TB isolates. On the other hand, there was a significantly larger percentage of multidrug-resistant TB (MDR-TB) isolates (resistant to both isoniazid and rifampin) among patients with solid tumours other than the bronchogenic group.

Consequently, the TB resistance pattern is not homogeneous among isolates from different malignancy groups. However, we reported a significantly higher prevalence of MDR-TB isolates in PTB than in ETB with ratios of 18.4% and 10.2%, respectively. Differences in drug-resistance profiles of *M. tuberculosis* isolates causing PTB and ETB were previously reported [40], so an investigation for drug resistance in immunocompromised patients, such as those with cancer, is recommended before starting anti-TB therapy [41].

MDR-TB isolates were detected in all patients with a history of TB. This can be attributed to inadequate case management, patient non-compliance with treatment, or inadequate drug regimens [42–44]. Therefore, drug resistance should be suspected in patients with anti-TB treatment history or treatment failure. It was of particular importance to note in this study the significant number of new TB cases with MDR-TB isolates. Recently, an exponential increase in the rate of MDR-TB among new cases has been reported. Global trends in rates of MDR-TB are unclear among new cases and need continuous surveillance [21,45].

Interestingly, no TB isolate in the present study was resistant to rifampin only. These results were previously reported [46], where mono-resistance to rifampin was rarely detected and nearly all rifampin-resistant isolates were MDR-TB. This result highly recommends the use of rifampin resistance as a reliable marker for MDR-TB in the population.

Overall, drug resistance could be observed much earlier by the MGIT method (mean, 5 days) than by the proportion method (mean, 16 days). Other studies reported rapid detection of isoniazid-resistant and rifampin-resistant *M. tuberculosis* strains using the MGIT susceptibility system [46–48].

Widespread use and misuse of first-line and second-line anti-TB drugs has led to acquired resistance [43]. Consequently, MDR-TB isolates have developed that increase TB's worldwide morbidity and mortality.

Our study concluded that specimen quality is the key in accurate diagnosis of TB, so education of patients about the proper sputum collection procedure is important for optimum specimen quality. Anti-tubercular susceptibility is essential for proper TB treatment. Indirect anti-tubercular susceptibility testing using MGIT is an easy, fast and helpful tool to test the susceptibility of TB isolates. MGIT-rifampin resistance may be used as a reliable surrogate marker for detection of MDR-TB. Inclusion of MGIT liquid culture in routine laboratory work is recommended. A real-time PCR method using artus® TM tuberculosis, was the most cost-effective method for TB diagnosis and is recommended before starting anticancer therapy. Repeated national surveillance on immunocompromised patients should be strengthened so as to stop the spread of MDR-TB.

Transparency Declaration

The authors declare that they have no potential conflicts of interest. All authors read and approved the final manuscript.

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