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ORIGINAL ARTICLE

Prevalence of Multidrug-Resistant Tuberculosis Using Phenotypic Drug Susceptibility Testing and GeneXpert MTB/RIF with Characterization of Non-tuberculous Mycobacteria Using MALDI-TOF

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ABSTRACT

Background*: Multidrug-resistant tuberculosis (MDR-TB) and infections by non-tuberculous mycobacteria (NTM) are diseases of major public health concern.* ***Objective****: The aim of the present work is to study the prevalence and patterns of MDR-TB as well as the characterization of isolated NTM species.* ***Methodology****: All samples (1069) were subjected to smear microscopy, culture on Lowenstein-Jensen (LJ) media, and phenotypic drug susceptibility testing (DST) of MTB to isoniazid (INH), rifampin (RF), streptomycin (S), and ethambutol (E). GeneXpert was used for direct detection of MTB and RF resistance. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS) was utilized for characterizing isolated NTM species.* ***Results****: M.tuberculosis (MTB) was isolated at a rate of 95.3% (1019/1069). MDR-TB was detected at rate of 7.16% with significant patterns for INH + RF + S + E (46.5%) and INR + RF (24.6%) (P-value <0.001). RF resistance was detected at a rate of 27.2% by GeneXpert. Seven NTM species (0.6%) were isolated in culture of which M.porcinum and M.fortuitum had confident identification by MALDI-TOF (score ≥1.8).* ***Conclusion****: MDR-TB rate was found to be 7.16% with significant dominance for INH + RF + S + E and INR + RF resistance patterns, while NTM rate was 0.6%.*

INTRODUCTION

Tuberculosis (TB) is a high public health threat airborne disease caused by *M.tuberculosis* (MTB)[1]. TB is ranked among the top 10 leading causes of death all over the world. According to the WHO, TB infects one-third of the world population. Globally, TB-infected patients were estimated at 10 million in 2019, and death from TB occurred in 1.4 million[2]. TB is considered more endemic in developing countries despite the challenges of underreporting[3]. In 2019, TB infection in Egypt was estimated at a rate of 12 (10–13) per 100,000 population[2].

The evolving resistance of MTB to anti-TB drugs has adverse health and economic consequences[4]. One of the implicated factors is defective laboratory diagnostic capacities for MTB detection and drug susceptibility testing (DST)[5]. Multidrug-resistant TB (MDR-TB) is defined as resistance to at least isoniazid (INH) and rifampin (RF), the most effective first-line anti-TB drugs, where RF is taken as a surrogate marker for MDR-TB[5]. In 2019, about 465,000 people were infected with MDR-TB worldwide with a poor treatment success rate of 57%[2].

Non-inferior to TB are the infections caused by non-tuberculous mycobacteria (NTM) or so called mycobacteria other than TB (MOTT)[6]. NTM are inhabitants in environment and can cause different types of infections[6]. Differentiating NTM from TB is considered crucial as both have nearly close clinical features but different therapeutic regimens[6].

Laboratory diagnosis plays a pivotal role in diagnosis and control of mycobacterial infections. Phenotypic culture and DST are considered the standard methods for diagnosis; however, are time-consuming and laborious[7]. This urged the introduction of molecular tests to diagnostic field which have been recently endorsed by the WHO as an asset in TB diagnosis[8]. GeneXpert MTB/RIF assay is one of the rapid and sensitive molecular platforms for detecting MTB and RF resistance[7]. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS) is a proteomic technology that has been introduced as a reliable tool for identifying MTB

and NTM with favorable agreement with molecular techniques[6].

Information about the prevalence and patterns of MDR-TB is crucial in establishing effective TB therapeutic regimens[9]. Notably, data on prevalence of MDR-TB as well as NTM are still limited in literature[3]. In Egypt, the real burden of TB may be underestimated by deficient laboratory capacities in many country regions[1]. To this end, we aimed in the present work, first to study the prevalence and patterns of MDR-TB using gold standard phenotypic culture and DST as well as GeneXpert MTB/RIF assay and second to characterize the isolated NTM species using MALDI-TOF MS.

METHODOLOGY

The current study was conducted with a total of 1069 samples of patients who either were new cases suspected of having TB or treated cases with previous history of anti-TB drug intake. These samples were delivered to the Mycobacteriology Unit of Central Public Health Laboratories (CPHL) over a period of 1 year from July 2019 to June 2020. All samples were received in sterile leak-proof containers and processed following standard biosafety measures. This study received an approval by the CPHL and was conducted with samples routinely sent to the laboratory. History of previous anti-TB drug intake was retrieved from the laboratory electronic records.

Smear Microscopy:

Direct smears were prepared from all samples and stained through the use of Ziehl-Neelsen (ZN) technique to be examined microscopically for acid fast bacilli per high power field x1000[10].

Specimen Preparation:

Non-sterile samples were decontaminated using NALC/ NaOH solution of 1% *N*-acetyl-L-cysteine (NALC), 4% sodium hydroxide (NaOH), and 2.9% sodium citrate. The solution was properly shaken with the specimen in screw capped centrifuge tubes and was concentrated by centrifugation at 15,000 x g (13,000 rpm) for 5 minutes. After discarding the supernatant, sterile phosphate buffer (1.5 ml) was added in order to suspend the concentrated sediment and be ready for LJ culture and Xpert assay[11].

GeneXpert®MTB/RIF for Detection of MTB and RF Resistance:

All samples were directly tested using Xpert MTB/RIF assay (Cepheid, Sunnyvale, USA) for simultaneous detection of MTB and RF resistance. Xpert assay is a fully automated real-time (semi-nested) PCR system capable of detecting *rpoB* gene (192 base pair genetic determinant of RF resistance). The test procedure was carried out following the manufacturer's guidelines. Briefly, the provided reagent was added to the processed specimen in a ratio of 3:1 followed by manual agitation 5–10 times during an incubation period of 15 minutes at room temperature. A volume of 2 ml was taken from sample-reagent mixture and transferred to sample port of Xpert cartridge that was inserted in the Xpert device, and the results were automatically generated after 90 minutes. The results were interpreted as positive or negative for detection of MTB and RF resistance.

Isolation of Mycobacteria and Phenotypic DST of MTB:

Tubes of Lowenstein-Jensen (LJ) media for culture were manually prepared and quality checked for sterility and performance. LJ tubes were inoculated by a volume of 0.2 ml of the prepared specimens and incubated at 37^{o}C to be routinely inspected weekly over a period of 2 months for mycobacterial growth[12]. Isolated mycobacteria were identified based on the rate of growth, colony morphology, ZN smear, and standard biochemical tests. MTB was differentiated from MOTT using para-nitrobenzoic acid (PNB) added to LJ medium which inhibits MTB but allows growth of MOTT[12]. Isolated MTB was tested for susceptibility to first-line anti-TB drugs by the 1% proportion method using drug containing media with added critical concentrations of INH (0.2 µg/ml), RF (4.0 µg/ml), S (4 µg/ml), and E (2 µg/ml). Test procedure was carried out, and the results were interpreted in accordance with the Clinical and Laboratory Standard Institute (CLSI). Any isolate with 1% (critical proportion) of bacilli growth in media with any of the 4 drugs is considered resistant to that drug[13].

MALDI-TOF MS for Identification of MOTT:

The isolated NTM were identified by the use of MALDI-TOF mass spectrometry **(**Bruker Microflex LT Biotyper, Bruker Daltonics, Bremen, Germany) following the protocol of the manufacturer. The identification process of NTM by MALDI-TOF MS involves steps in the form of inactivation, extraction, and analysis, and it is based on generated unique fingerprints for the extracted proteins. Following extraction, an aliquot is placed onto a steel plate and covered with a chemical matrix. The plate is loaded into the device where proteins are ionized using laser, and the ions are separated based on the ratio of the mass to the charge[14]. The resulting proteomic profiles were analyzed as per the database of v3.0 Mycobacteria Library Software (Bruker Daltonics, Bremen, Germany). Reliable identification was estimated from the *m/z* ratio and logarithmic score ranging from 0 to 3 based on the best match between spectra from clinical samples and in the database. According to the Bruker system, the "high confidence genus and species identification" has been established at a score value of ≥1.8 and the "low confidence species identification" at a score value of ≥1.6–1.79[15].

Statistical Analysis:

Statistical analyses of data were carried out using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and SPSS statistics version 20.0 (IBM Inc., Armonk, NY) for Windows (Microsoft Corp., Redmond, WA). Statistically significant difference between study groups was at *P* values less than 0.05.

RESULTS

The present study was conducted on a total number of 1069 samples distributed as pulmonary (1039, 97.19%) and extrapulmonary (30, 2.8%) samples. Positive MTB growth was detected by gold standard LJ culture method in 1019 out of 1069 samples with a prevalence of 95.3%, among which pulmonary and extrapulmonary samples constituted proportions of 989/1019 and 30/1019 with rates of 97.05% and 2.94%, respectively. As illustrated in figure (1), among the total 1019 MTB culture +ve samples, 237 (207 pulmonary + 30 extrapulmonary) (23.2%) were for new cases who were clinically suspected of tuberculosis with no history of previous anti-TB drug intake, while 782 (76.7%) were for previously treated cases. The present study encountered MOTT isolates from 7 pulmonary samples for patients who had no history of anti-TB drugs with a rate of 0.65%.

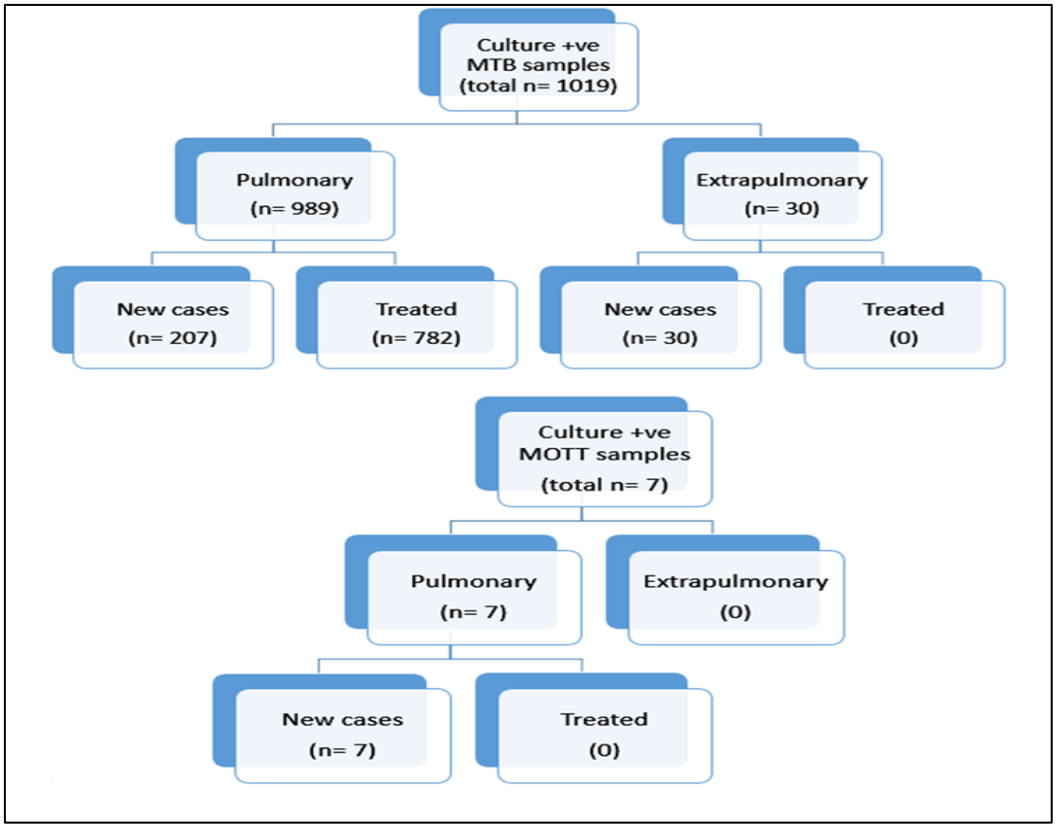

Fig. 1: Distribution of MTB and MOTT isolated by LJ culture method among pulmonary and extrapulmonary samples for new and treated cases

ZN smear microscopy exhibited positive acid fast bacilli (AFB) in a total of 77 samples (70 MTB + 7 MOTT). Among the 1019 MTB culture positive samples, 70 were culture +ve/smear +ve (59 pulmonary + 11 extrapulmonary), while 949 were culture +ve/smear –ve, showing 100% specificity and 68.6% sensitivity with sensitivities of 5.9% and 36.6% among pulmonary and extrapulmonary MTB culture +ve samples, respectively (table 1). For MOTT, all 7 culture +ve MOTT samples were smear positive with 100% sensitivity and specificity.

Table 1: Results of MTB detection using ZN microscopy and GeneXpert compared to gold standard LJ culture.

LJ culture	Total samples n=1069 (pulmonary n=1039 + extrapulmonary n=30)			
	ZN microscopy		GeneXpert	
	Smear +ve (n=70)	Smear –ve (n=999)	MTB +ve (n=1006)	MTB-ve (n=63)
MTB +ve (n=1019)	70	949	998	21
MTB –ve (n=50)	0	50	8	42
Sensitivity	68.8%		97.9%	
Specificity	100%		84%	
NPV	5.0%		66.6%	
PPV	100%		99.2%	

LJ: Lowenstein-Jensen, **MTB**: *M.tuberculosis*, **ZN**: Ziehl-Neelsen, **NPV**: negative predictive value, and **PPV**: positive predictive value.

MTB was directly detected by GeneXpert in 1006 out of 1069 tested samples with a rate of 94%. As indicated in table (1), among the total 1019 culture positive samples, 998 were culture +ve/GeneXpert +ve (978/989 pulmonary versus 20/30 extrapulmonary), while 21 were culture +ve /GeneXpert –ve, showing a recorded total sensitivity of 97.9% (95% CI: 0.96–0.98). The sensitivity of Xpert for MTB detection was shown to be 98.8% and 66.6% in pulmonary versus extrapulmonary samples, respectively. Xpert presented positive MTB detection in all 70 smear +ve/culture +ve samples with 100% sensitivity and in 939 out of 949 smear –ve/culture +ve samples with 98.9% sensitivity. GeneXpert and culture were concomitantly negative for MTB in 42 samples, while 8 samples were Xpert +ve/culture –ve, showing 84% Xpert specificity (95% CI: 0.723–0.923). GeneXpert showed good agreement with culture method (97.2%, kappa index: 0.72).

As presented in table (2), the phenotypic DST detected total drug resistance (resistance to at least one anti-TB drug) in 391 out of 1019 MTB isolates with a rate of 38.37% and detected multidrug resistance (resistance to at least INH and RF) in 73 with a rate of 7.16%. As displayed in figure (2), MDR-TB was detected at a rate of 4.2% (10/237) among MTB isolates from new cases and at a rate of 8.05% (63/782) among MTB isolates from treated cases (95% CI: 0.25–0.99) with a *P*-value <0.05. All MDR-TB isolates were detected from pulmonary source, while extrapulmonary MTB isolates had other patterns of resistance that did not include RF. MDR-TB defined isolates had various resistance patterns in the form of INH + RF + S + E (34/73; 46.5%), INH + RF (18/73; 24.6%), INH + RF + S (15/73; 20.5%), and INH + RF + E (6/73; 8.2%), showing a significant difference with a *P*-value <0.001. Resistance patterns other than MDR are illustrated in table (2) and figure (2). Combined resistance to >1 anti-TB drug was detected in 32/237 MTB isolates of new cases versus 126/782 MTB isolates of treated cases with rates of 13.5% and 16.11%, respectively.

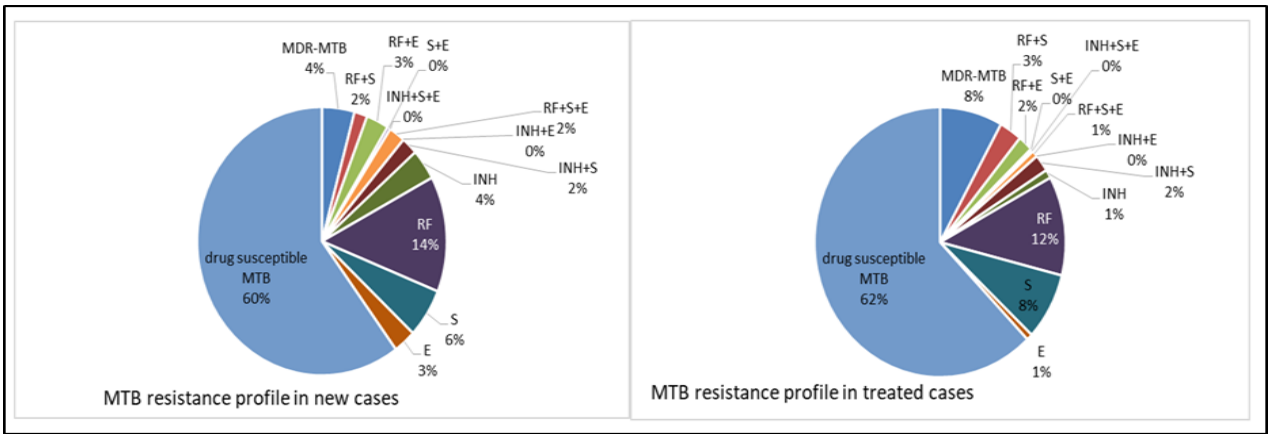

Fig. 2: Resistance patterns of MTB against first-line anti-TB drugs among new versus treated cases.

Table 2: Resistance profile of isolated MTB to first-line anti-TB drugs by phenotypic DST

Resistance profile to anti-TB drugs	Total culture +ve MTB samples (n=1019)					*P- value*
	MTB +ve new cases (n=237)			MTB +ve treated cases (n=782)	Total N (%)	
	Pulmonary (n=207) N (%)	Extrapulmonary (n=30) N (%)	Total N (%)	Pulmonary* (n=782) N (%)		
INH	5 (2.4%)	4 (13.3%)	9 (3.7%)	8 (1.02%)	17 (1.66%)	0.008$^{\Delta}$
RF	33 (15.9%)	0	33(13.9%)	93 (11.89%)	126(12.3%)	0.47
S	12 (5.79%)	2 (6.6%)	14 (5.9%)	63 (8.05%)	77 (7.5%)	0.27
E	2 (0.96%)	5 (16.6%)	7 (2.9%)	6 (0.76%)	13 (1.27%)	0.008$^{\Delta}$
INH + RF	2 (0.96%)	0	2 (0.84%)	16 (2.04%)	18 (1.76%)	0.22
INH + S	5 (2.4%)	0	5 (2.1%)	16 (2.04%)	21 (2.06%)	1.00
INH + E	0	0	0 (0%)	0	0	0
RF + S	4 (1.93%)	0	4 (1.68%)	23 (2.9%)	27 (0.26%)	0.29
RF + E	7 (3.3%)	0	7 (2.9%)	15 (1.91%)	22 (2.15%)	0.33
S + E	1 (0.48%)	0	1 (0.42%)	1 (0.12%)	2 (0.19%)	1.00
INH + RF + S	2 (0.96%)	0	2 (0.84%)	13 (1.66%)	15 (1.47%)	0.35
INH + RF + E	1(0.48%)	0	1 (0.42%)	5 (0.639%)	6 (0.58%)	0.69
INH + S + E	0	0	0 (0%)	2 (0.25%)	2 (0.19%)	1.00
RF + S + E	5 (2.4%)	0	5 (2.1%)	6 (0.76%)	11 (1.07%)	0.07
INH + RF + S + E	5 (2.4%)	0	5 (2.1%)	29 (3.7%)	34 (3.33%)	0.23
MDR-TB**	10 (4.8%)	0	10 (4.2%)	63 (8.05%)	73 (7.16%)	0.04$^{\Delta}$

(*): all samples from treated cases were pulmonary, INH: isoniazid, RF: rifampin, S: streptomycin, E: ethambutol, **(**)**: MDR-TB: MTB resistant to at least INH and RF of first-line anti-TB drugs, and **($^{\Delta}$)**: significant *P*-value <0.05.

RF, INH, S, and E were involved in 259, 113, 189, and 90 out of 391 drug-resistant MTB (DR-MTB) isolates with contribution rates of 66.2%, 28.9%, 48.3%, and 23%, respectively, showing a significant difference with a *P*-value <0.001. The phenotypic DST showed RF mono-resistance in 126 out of 1019 MTB isolates with a rate of 12.3% distributed as 33/237 (13.9%) and 93/782 (11.8%) among MTB isolates of new cases and treated cases, respectively. The 259 total RF-resistant (RR-MTB) isolates were distributed as 126 RF-mono-resistant (48.6%) and 133 isolates with combined resistance to RF and other anti-TB drugs (51.3%) of which 60 were resistant to RF and drugs other than INH as detailed in table (2).

Rifampin resistance was detected by GeneXpert in 274 samples with MDR rate of 27.2% among 1006 Xpert MTB +ve samples and a rate of 26.8% among 1019 culture +ve MTB samples. All RR-MTB detected by Xpert was from pulmonary samples. As demonstrated in table 3, positive RR-MTB was concomitantly detected by both Xpert and LJ phenotypic DST in 254 samples. Discordant results for RF resistance were indicated in 25 samples in the form of 20 Xpert +ve/LJ –ve and 5 Xpert –ve/LJ +ve. The eight samples that were MTB positive by Xpert, however not grown in culture, showed no RF resistance. Regarding RF resistance, GeneXpert recorded 98% sensitivity (95% CI: 0.95–0.99) and 97.3% specificity (95% CI: 0.95–0.98) compared to the gold standard phenotypic DST done for the 1019 culture +ve MTB samples with a perfect categorical agreement (97.5%, Kappa index: 0.93).

Table 3: Results of RF susceptibility testing of MTB by Xpert compared to phenotypic DST by LJ.

No. of samples (n=1069)	MTB detection		RF susceptibility result	
	LJ	Xpert	LJ	Xpert
5	+	+	R	S
254	+	+	R	R
20	+	+	S	R
719	+	+	S	S
42	-	-	-	-
21	+	-	S	-
8	-	+	-	S

R: resistant, **S**: sensitive, **(+)**: positive detection, and **(-)**: negative detection.

Regarding NTM detection, LJ culture yielded 7 NTM isolates among total enrolled 1069 samples, with a rate of 0.6%. Species were identified by MALDI-TOF as described in table (4) and figure (3), where 2 isolates (28.5%) had high confidence species level of identification (score ≥1.8) and 5 isolates (71.4%) were secure for genus but with low confidence species identification (score ≥1.6–1.79).

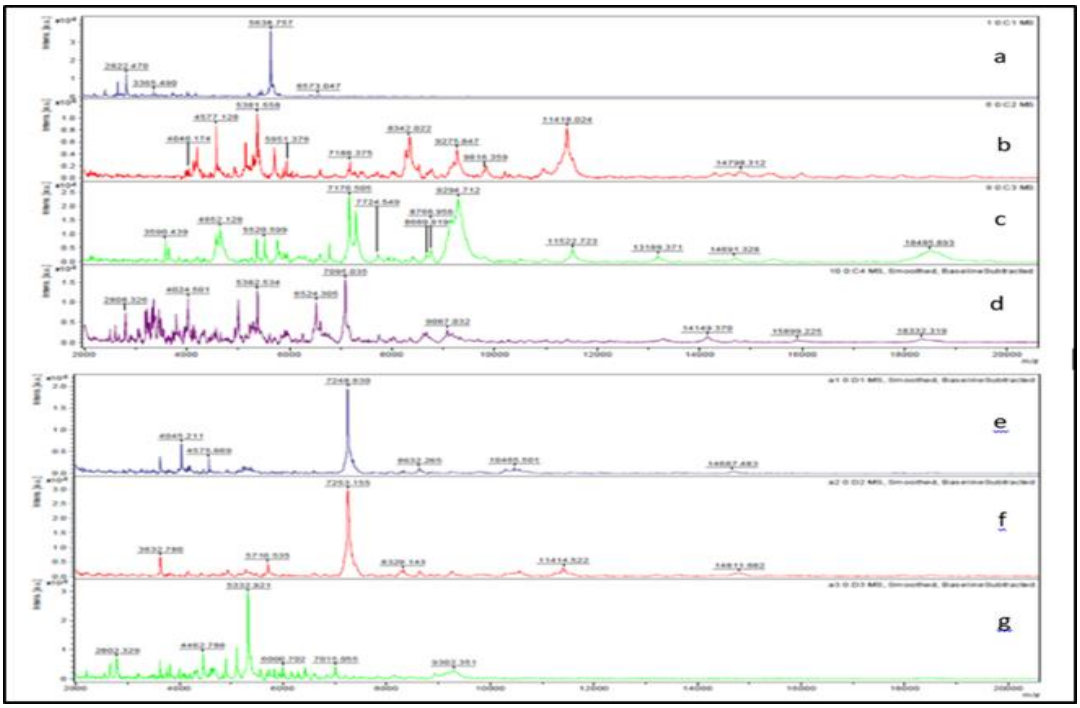

Fig. 3: Types of MOTT species identified by MALDI-TOF MS. (a): *M.abscessus*, (b): *M.fortuitum*, (c): *M.neoaurum*, (d): *M.goodii*, (e): *M.gordonae*, (f): *M.porcinum*, (g): *M.bacteremicum*

Table 4: Characterization of NTM isolates by MALDI-TOF MS.

NTM isolates (n=7)	Score
Mycobacterium abscessus ssp. *massiliense*	1.72
Mycobacterium fortuitum	1.95
Mycobacterium neoaurum	1.65
Mycobacterium goodii	1.63
Mycobacterium gordonae	1.64
Mycobacterium porcinum	1.82
Mycobacterium bacteremicum	1.69

DISCUSSION

Mycobacterial infections caused by MTB and NTM are of high global threat to public health especially with increasing rates of drug resistance. The present study exhibited positive culture for MTB in 1019 out of 1069 samples with a rate of 95.3%. This rate was comparable to the rates reported in other studies that were conducted in our country with a range of 73–93%[16, 17]. According to the WHO, over 95% of TB cases occur in developing countries as a result of low socioeconomic standards. The African region recorded TB infection at a rate of 25%. Higher rates of MTB were reported in countries belonging to the Asian region[2]. This was supported by a study in India (one of the 30 high burden countries) which reported culture positive MTB rate of 99.8%[18]. In contrast, lower rates of MTB detection (40% and 19.4%) were reported by other studies[19, 20]. According to the WHO, reports of the lowest MTB rates were found from developed countries in America (2.9%) and Europe (2.5%). Variable MTB rates in different studies might be attributed to variability in TB burden among different geographical regions, proportion of new to treated cases and the MTB detection method[2].

TB is mainly pulmonary, however can affect extrapulmonary sites. Our study showed the dominance of pulmonary over extrapulmonary samples with rates of 97% and 2.8% among total enrolled samples and rates of 97.05% and 2.94% among culture positive MTB samples, respectively. This was consistent with another study in Egypt that found pulmonary and

extrapulmonary samples at rates of 95% and 5%, respectively[19]. Likely, this was reported in many other studies in literature with rates ranging from 4.5% to 53.8%[21-23, 24].

Although being cost-effective, smear microscopy is challenged by low sensitivity and specificity[19]. In our study, smear microscopy showed 68.6% sensitivity and 100% specificity, which was consistent with several reports for ZN sensitivity and specificity with ranges of 32–94% and 94–100%, respectively[17, 25].

In our study, GeneXpert exhibited MTB positivity rate of 94% compared to 95.3% by the gold standard culture method with 97.9% sensitivity and 84% specificity and 97.2% agreement. This was comparable to several studies conducted from Egypt and other countries which reported Xpert sensitivity and specificity ranges of 90–100% and 73.3–100%, respectively[16, 17, 19, 21, 26]. In our study, Xpert was fully sensitive among smear positive/culture MTB positive samples but had lower sensitivity (98.9%) among smear negative samples, complying with reports by several studies and explained by the different bacillary load[16, 17, 20]. Relatively lower specificity in our study can be explained by higher proportion of samples for treated cases than new cases, as Xpert can detect non-cultivable dead bacilli.

Drug resistance comprises a challenging obstacle in TB eradication. In the present study, MDR-TB was detected at a rate of 7.16% among 1019 MTB isolates which was close to reported rates of 5.5% and 10.9% in other studies from Egypt[19, 21]. This was comparable to MDR-TB rates of 5.4–12% reported from other countries[18, 27]. Higher MDR-TB rates were reported by studies that were conducted in high TB burden countries or that included high proportion of treated cases[4, 28]. Our study observed significant higher incidence of MDR-TB among treated cases (8.05%) than new cases (4.2%) with a *P*-value <0.05, which complies with several reports in other studies[7, 9, 27]. This is supported by the WHO report in Egypt of higher MDR-TB rate among treated cases than new cases[2].

MDR-TB showed various patterns in the present study with dominant resistance to the 4 anti-TB drugs INH + RF + S + E (46.5%), followed by INH + RF (24.6%), INH + RF + S (20.5%), and INH + RF + E (8.2%). Likely, in several studies, most MDR-TB isolates were resistant to 3-4 anti-TB drugs as the dominant pattern[4, 9]. The diversity in MDR pattern can be explained by heterogeneous mycobacterial resistance mutations[4]. This supports the WHO recommendations for starting MDR-TB treatment with 4 and not to depend on 1 or 2 first-line anti-TB drugs till full susceptibility report is available[9].

Rifampin had a significant contribution (66.2%) among total MTB resistant isolates compared to 28.9%, 48.3%, and 23% for INH, S, and E, respectively. The majority of total RR-MTB isolates 133/259 (51.3%) showed associated resistance with other anti-TB drugs supporting the universal consensus of considering RF as a surrogate marker for multidrug resistance in most of the rapid DST diagnostics[9]. Notably, RF mono-resistance in our study was detected in nearly half of the total RR-MTB isolates (48.6%) which justifies other controversial opinions about not relying on RF in defining MDR-TB and recommending performing full DST[9].

RF resistance was detected by GeneXpert at a rate of 27.2% among total 1006 Xpert +ve samples with a sensitivity and specificity of 98% and 97.3%, respectively which is comparable to previous reported ranges of 86-100% and 95-100%, respectively, which reflects the superiority of Xpert in rapid detection of MTB and RF resistance[7, 16]. Yet, missed detection of RF resistance by Xpert may be explained by mutant genes other than *rpoB* gene or the capability of Xpert probe hybridization. False positive results of RF resistance may occur due to non-functional *rpoB* genes[7].

NTM is considered of non-inferior clinical significance to MTB although diagnosis is still deficient[29]. The present study recovered 7 NTM isolates with a rate of 0.6%, which is consistent with a rate of 1.2% reported by another study in India[30]. However, higher NTM rates were reported by other studies which might be due to different geographical areas, sample size, and method of detection[31].

Identification of NTM species is important to establish their clinical significance[30, 31]. In the current study, MALDI-TOF identified *M.abscessus*, *M.fortuitum*, and *M.gordonae* which are considered among the commonly encountered species in clinical practice[14]. Other NTM species were reported from other countries reflecting non-uniform NTM distribution worldwide[30]. *M neoaurum*, *M.porcinum*, *M.goodii*, and *M.bacteremicum* were identified in our study and in literature as less frequently encountered clinical NTM species[32].

CONCLUSION

MDR-TB and NTM infections are of major public health concern. The present study indicated MDR-TB rate of 7.16% with dominant patterns for INH + RF + S + E and INH + RF. Seven NTM were isolated (0.6%) and MALDI-TOF had a considerable role in species identification. Upgrade of laboratory capacities is mandatory to improve infection diagnosis and control.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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