Original article

Experience of an Egyptian tertiary laboratory in the diagnosis of tuberculous and non-tuberculous mycobacterial infections: A Five-year retrospective study

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A R T I C L E   I N F O

A B S T R A C T

Background: Tuberculosis (TB) is one of the significant health concerns in Egypt, necessitating rapid, affordable, and accurate diagnosis to aid in its control. This study presents data collected over five years (2017-2021) to explore different diagnostic TB methods in our lab. **Methods:** A total of 33700 non-repetitive samples were subjected to different diagnostic methods; 30000 were stained by Ziehl–Neelsen (ZN), of which 2819 were cultured on Löwenstein-Janssen (LJ) media, and 456 were analyzed by GeneXpert; 3700 samples were tested by interferon-gamma release assay (IGRA) Quantiferon. Moreover, 3650 patients underwent the tuberculin skin test (TST). **Results:** GeneXpert displayed the highest positivity (23.9%), while ZN smear microscopy was the lowest (4.8%). The detection capability between pulmonary and extrapulmonary samples was not statistically significant using LJ culture but significant using GeneXpert MTB/RIF. Rifampicin resistance was 17.4% among the GeneXpert *Mycobacterium tuberculosis* (MTB)-positive samples. **Conclusions:** GeneXpert MTB/RIF assay is a user-friendly, rapid, and efficient method for identifying pulmonary and extrapulmonary TB with the simultaneous detection of rifampicin-resistant strains. However, culture and smear microscopy remain the most dominant diagnostic methods in developing countries due to their low cost and relatively acceptable specificity.

Introduction

Tuberculosis (TB) is caused by the contagious acid-fast bacillus Mycobacterium tuberculosis (M. tuberculosis, MTB), a disease typically possible to prevent and treat. After the coronavirus disease 2019 (COVID-19), TB was the second most common infectious agent-related cause of death worldwide in 2022, accounting for an estimated 1.30 million deaths—nearly twice as many as human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) deaths. More than 10 million individuals acquire TB yearly [1].

It is believed that a quarter of the world's population has contracted TB. About 90% of all cases of TB occur in adults, with men accounting for

a more significant proportion of cases than women [2]. Malnutrition, smoking, alcohol intake disorders, diabetes, low body mass index, poverty, and other socioeconomic factors are all linked to TB occurrence [3]. Egypt is ranked among the countries with low to moderate-level TB incidence. According to the TB country profiles, the total (new and relapsed) rate of TB incidence per 100,000 people in Egypt was 9.8 in 2022 [4].

Only patients with relevant clinical symptoms are generally considered to undergo TB diagnosis. A fast, simple, accurate, and inexpensive diagnostic method is critical for controlling TB infections, preventing community dissemination, and prompt patient management with effective clinical outcomes [5]. Early TB diagnosis in medical practice continues to be challenging, particularly for patients with pediatric TB, HIV co-infected TB, as well as extrapulmonary TB (EPTB), which can affect any region of the body apart from the lungs with atypical clinical presentations [6].

Fundamentals of TB diagnosis in many parts of the globe continue to be based on microscopy and culture. The Ziehl–Neelsen (ZN) staining method is the primary method used in nations with limited resources. Nevertheless, this technique fails to detect a substantial number of cases, particularly when used solely [7]. Culture is the gold-standard reference method for diagnosing mycobacterial infections due to its low detection limit [8]. However, the slow-growing nature of mycobacteria and the requirement for biosafety level three (BSL-3), unavailable in many laboratories, render culture challenging [9].

Fortunately, molecular techniques were later developed as quick diagnostic tools for TB. Compared to conventional culture and microscopy procedures, the introduction of nucleic acid amplification tests (NAAT) has considerably minimized the turnaround time for TB diagnosis [10]. The Xpert MTB/RIF assay, approved by the Food and Drug Administration (FDA), specializes in TB diagnosis and is designed to identify drug-sensitive and rifampicin-resistant TB (RR-TB) strains straight from various clinical specimens. The test offers high sensitivity (> 90%) with only a two-hour turnaround time. It is based on a nested real-time PCR (RT-PCR) amplifying the rpoB gene, which is the main target for detecting rifampicin resistance [11].

Approximately one-third to one-quarter of the world's population carry latent TB infections (LTBI) [12]. These individuals have the potential to reactivate into TB patients under several host immune-suppressive circumstances. Limited techniques are available for diagnosing LTBI cases, unlike active TB. The interferon-gamma release assay (IGRA) and the tuberculin skin test (TST) are the two screening procedures most often utilized for LTBI [13].

Significant regional variations are found in strain distribution as well as the frequency and prevalence of non-tuberculous mycobacteria (NTM) among patients. NTM infections are primarily contracted via contaminated environmental sources, although they can also spread from person to person [14]. Since both MTB and NTM species reveal positive results for the traditional smear acid-fast staining, differentiating between the two in clinical specimens is a considerable difficulty that often yields misleading results. Consequently, in many TB-endemic countries, NTM incidence is underestimated [15].

In this study, we aim to present our data for five years (2017-2021) regarding the performance and results of different diagnostic methods for tuberculous and non-tuberculous mycobacterial infections in different types of clinical specimens presented to our laboratory.

Materials and Methods

Laboratory setup and sampling

This retrospective study collected data from January 2017 to December 2021 from a tertiary microbiology lab in Alexandria, Egypt. The lab is well-equipped with appropriate TB diagnosis facilities and standard biosafety precautions regarding specimen handling, processing, and inoculation. The lab received samples not only from the Alexandria governorate but also from various surrounding geographical regions. The following tests were carried out for mycobacterial diagnosis, summarized in **figure (1)**:

a) Microscopy: Using Ziehl-Neelsen (ZN) acid-fast stain (30000 samples, with an average of ~500 per month)

b) Culture: Löwenstein-Janssen (LJ) culture media for MTB and NTM (2819 samples).

c) Molecular Methods: GeneXpert MTB/RIF assay (456 samples)

d) Immunoassays: - IGRA QuantiFERON®-TB Gold In-Tube test (QFT-GIT) (3700 samples)

- Tuberculin Skin Test (TST) (3650 patients)

All samples sent for culture and GeneXpert MTB/RIF assay were screened using ZN smear microscopy. However, the simultaneous performance of smear staining, culture, and GeneXpert analysis for all admitted samples was a limitation, as the choice of the test was primarily according to the physicians' requests, in addition to the financial burden per case.

Microscopical examination

Smears were prepared and stained using ZN stain for microscopy according to the standard procedures for each sample type. Red-colored acid-fast bacilli were viewed as positive [16].

Culture

Samples from sterile sites were directly processed. Non-sterile clinical specimens were processed and decontaminated by the conventional N-acetyl-L-cysteine-NaOH (NALC-NaOH) method [17].

Culture was carried out on two types of culture media slants, LJ media and LJ media supplemented with p-nitrobenzoic acid (pNBA), to discriminate between the typical MTB complex and the NTM. Growth of MTB is inhibited in the presence of pNBA, while NTM is resistant [18]. Only after eight weeks of no growth at 37 °C was the culture recognized as negative. Colony morphology, pigmentation, and growth date were recorded for each isolate.

Immunodiagnostic tests

Two immunodiagnostic tests are available in our lab for testing MTB: the TST and the IGRA QuantiFERON®-TB Gold In-Tube test (QFT-GIT). TST was performed using a purified protein derivative (5 TU/0.1ml, VACSERA, Egypt) as previously described [19]. For QFT-GIT, venous blood samples were drawn into vacutainer tubes and processed according to the manufacturer's instructions (QIAGEN, Germany). IFN-γ levels (IU/ml) were estimated using an ELISA reader (ELx808, BioTek, USA) according to the kit used; results were considered positive if the value of the TB Antigen minus Nil control was $\geq$0.35 IU/ml and $\geq$25% of the nil value.

Xpert MTB/RIF

GeneXpert testing was performed according to the manufacturer's instructions [20]. Samples reporting "error," "Invalid," or "no result" were excluded from the study.

Statistical analysis

Data were collectively recorded in a master table using a Microsoft Excel spreadsheet, followed by analysis with SPSS software package version 20.0. The significance of the obtained results was judged at the 5% level. Equations calculated test sensitivity, specificity, and predictive values.

Results

During this five-year retrospective study (2017-2021), 30000 samples were screened by ZN stain (26760 pulmonary; 3240 extrapulmonary), out of which 2819 samples (1974 pulmonary; 845 extrapulmonary) were subjected to culture, while only 456 (377 pulmonary; 79 extrapulmonary) were tested by GeneXpert. Also, during this period, 3700 samples and 3650 suspected TB cases were tested using IGRA Quantiferon assay and TST, of which 15.1% and 21.9% were positive, respectively. GeneXpert displayed the highest TB positivity (23.9%), whereas ZN smear microscopy had the lowest positivity (4.8%) **(Table 1)**.

The distribution of pulmonary and extrapulmonary samples collected for microscopy **(Table 2)** and culture **(Table 3)** have shown that pulmonary samples represented 89.2% and 70% of the total samples, respectively, with sputum as the most common pulmonary sample type. Among the overall pulmonary samples subjected to ZN microscopy, 4.5% were positive, with lung abscesses and pulmonary tissues having the highest positive rates. On the other hand, 7.5% of extrapulmonary samples subjected to microscopy were positive, with lymph node tissues having the highest positive rates **(Table 2)**. An increasing trend of percent TB culture-positive samples with respect to the total samples collected was noticed after 2019 **(Figure 2)**.

ZN smear microscopy was positive in 66.4% of culture-positive samples. No statistical significance was found in the LJ culture detection capability between pulmonary and extrapulmonary samples (p= 0.838) **(Table 3)**. Amongst culture-positive samples, the contribution of MTB was 86.7%, 89.1%, 88.5%, 95%, and 96.7% in the respective five years of study **(Figure 3)**.

By taking culture as the reference standard TB diagnostic method, 225 samples (7.98%) were considered as ZN smear true positive (positive ZN, positive culture), 21 (0.75%) were false positive (positive ZN, negative culture), 114 (4.04%) were false negative (negative ZN, positive culture), whereas 2459 (87.23%) were true negative (negative ZN, negative culture), with ZN smear microscopy overall performance of 64.76% sensitivity, 99.18% specificity, 90.3% PPV 95.6% NPV **(Table 4)**.

The GeneXpert assay results revealed that 6 (5.5%) of GeneXpert positive samples were ZN negative, whereas 8 (2.3%) of GeneXpert negative samples were positive for ZN smear microscopy **(Table 5a)**. GeneXpert positive results were highest among sputum samples (35%), followed by CSF (30.4%), lymph node tissues (25%), bronchoalveolar lavage (BAL) (24.6%), lung abscess (20%), and pleural fluid (17.7%). Resistance to rifampicin was found among 20.5% and 7.7% of pulmonary and extrapulmonary GeneXpert-positive samples, respectively. Resistance was detected in 27% of sputum samples, followed by 15.2% of BAL samples. Pleural fluid was the only extrapulmonary sample type that recorded rifampicin resistance (14.3%) **(Table 5b)**. GeneXpert's TB genetic detection ability was significantly better among pulmonary samples ($p= 0.003$).

Table 1. Number of samples incorporated within each TB test (2017-2021).

Test \ Year	2017	2018	2019	2020	2021	Total no. of positive samples	% Positive
ZN Smear Microscopy (n= 30000)	5162	6040	7405	5390	6003	1441	**4.8%**
Tuberculin skin test (n= 3650)	1172	953	800	221	504	800	**21.9%**
QuantiFERON (n= 3700)	549	793	968	536	854	560	**15.1%**
GeneXpert (n= 456)	53	65	86	96	156	109	**23.9%**
LJ culture media (n= 2819)	416	597	716	586	504	339	**12.03%**

Table 2. Distribution of Pulmonary and Extrapulmonary samples among those subjected to Ziehl-Neelsen smear microscopy (2017-2021).

	No. of tested samples (n=30000)	No. of positive samples (n= 1441)	%
A) Pulmonary samples	**n= 26760**	**n= 1197**	**4.5**
Sputum	22134	929	4.2
BAL and Mini BAL	3817	213	5.6
Others (lung abscess, pulmonary tissue)	809	55	6.8
B) Extra pulmonary samples	**n= 3240**	**n= 244**	**7.5**
Pleural fluids and tissue	1802	159	8.8
Lymph node tissue	500	56	11.2
Biological fluids (CSF, ascetic fluid, etc.)	793	24	3.0
Bone tissue	145	5	3.4

Table 3. Pulmonary & Extrapulmonary samples among the total collected samples, and among positive culture samples.

	2017 (n = 416)		2018 (n = 597)		2019 (n = 716)		2020 (n = 586)		2021 (n = 504)		Total (n = 2819)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A- Pulmonary	284	68.3	451	75.5	534	74.6	381	65	324	64.3	1974	70.0
Extrapulmonary	132	31.7	146	24.5	182	25.4	205	35	180	35.7	845	30.0
B- Positive culture	**45**	**10.8**	**46**	**7.7**	**87**	**12.2**	**100**	**17.1**	**61**	**21.1**	**339**	**12.0**
Pulmonary	32	71.0	37	80.4	58	66.6	67	67.0	45	73.7	239	70.5
Extrapulmonary	13	29.0	9	19.6	29	33.4	33	33.0	16	26.3	100	29.5
ZN Positive	28	62.2	20	43.5	61	70.1	66	66.0	50	82.0	225	66.4

Table 4. (A) ZN smear microscopy and LJ culture media test results (2017 – 2021); (B) Sensitivity, Specificity, and predictive values of ZN smear microscopy with culture as the reference standard method (95% CI).

	2017 (n = 416)	2018 (n = 597)	2019 (n = 716)	2020 (n = 586)	2021 (n = 504)	Total (n = 2819)	
						No.	%
A- Positive ZN							
Positive culture	28	20	61	66	50	225	7.98
Negative culture	1	6	5	5	4	21	0.75
Negative ZN							
Positive culture	17	26	26	34	11	114	4.04
Negative culture	370	545	624	481	439	2459	87.23
Total	**416**	**597**	**716**	**586**	**504**	**2819**	**100.0**
B- Performance Parameters							
Sensitivity	62.2%	43.5%	70.1%	66.0%	82.0%	64.76%	
Specificity	99.7%	98.9%	99.2%	99.0%	99.1%	99.18%	
Positive predictive value (PPV)	96.6%	76.9%	92.4%	93.0%	92.6%	90.3%	
Negative predictive value (NPV)	95.6%	95.4%	96.0%	93.4%	97.6%	95.6%	

Table 5a. GeneXpert versus ZN smear microscopy test results

ZN Smear Microscopy	GeneXpert	
	Positive	Negative
Positive	103	8
Negative	6[*]	339
Total	**109**	**347**

*: 4 Pulmonary, 2 Extrapulmonary samples

Table 5b. GeneXpert assay results: Mycobacterial and rifampicin resistance positivity.

Sample type	No. of Tested Samples	Positive No. (%)	Resistance RIF No. (%)
BAL	187	46 (24.6%)	7 (15.2%)
Sputum	106	37 (35%)	10 (27.0%)
Total (Pulmonary)	**293**	**83 (28.3%)**	**17 (20.5%)**
Pleural fluid	79	14 (17.7%)	2 (14.3%)
Lymphnode tissue	16	4 (25.0%)	0
CSF	23	7 (30.4%)	0
Blood	9	0	0
Ascitic fluid	18	0	0
Abscess	5	1 (20.0%)	0
Urine	5	0	0
Synovial fluid	3	0	0
Pericardial fluid	5	0	0
Total (Extrapulmonary)	**163**	**26 (16.0%)**	**2 (7.7%)**
Total	**456**	**109 (23.9%)**	**19 (17.4%)**

Figure 1. Different tests were carried out for TB diagnosis with the corresponding sample and patient numbers.
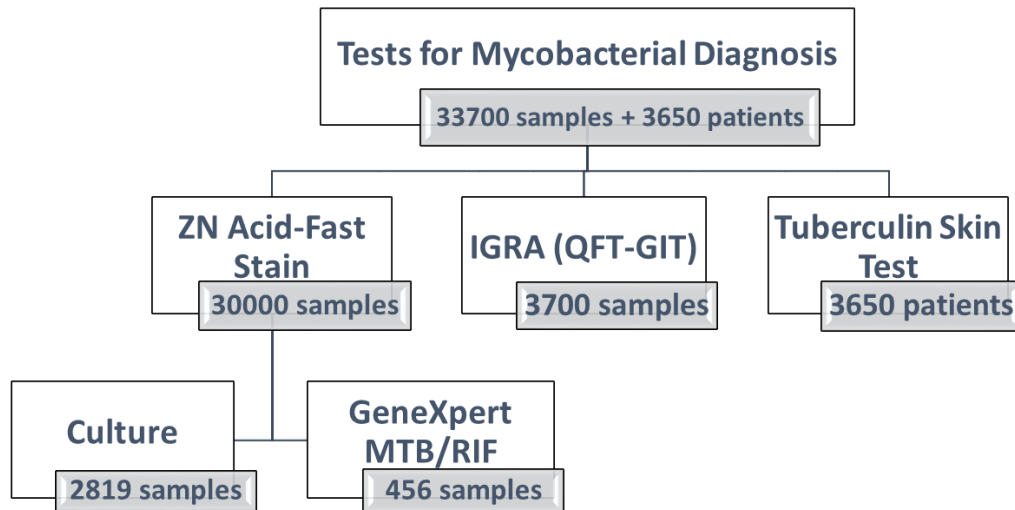
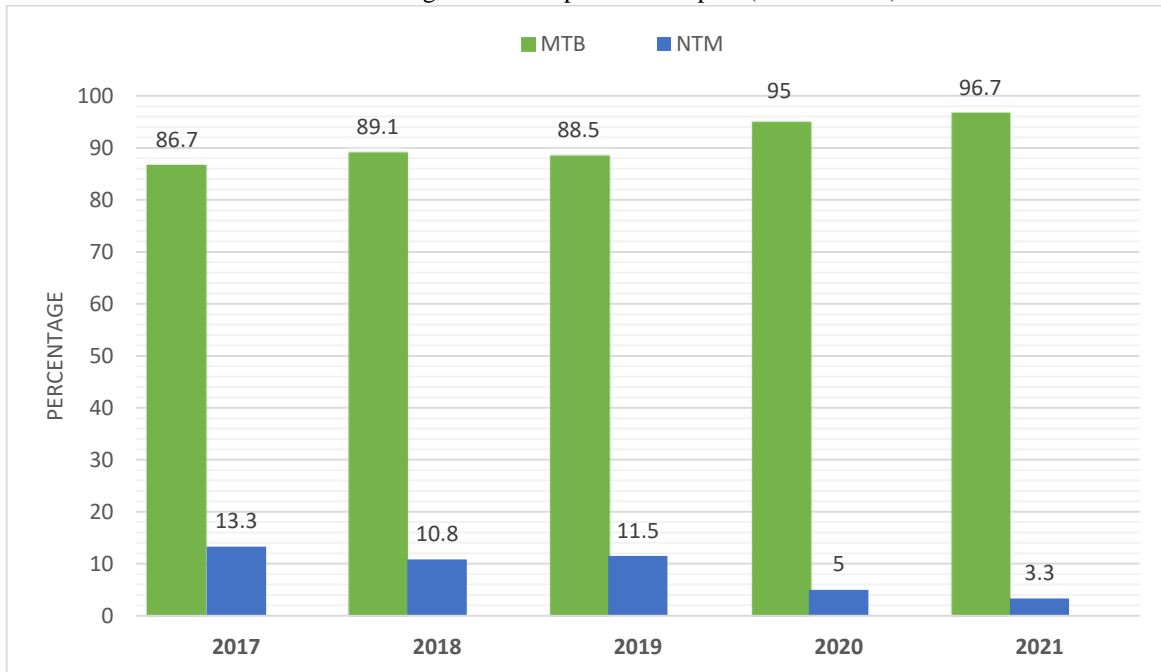**Figure 2.** Distribution trend of total collected samples and positive culture samples amongst them (2017-2021).
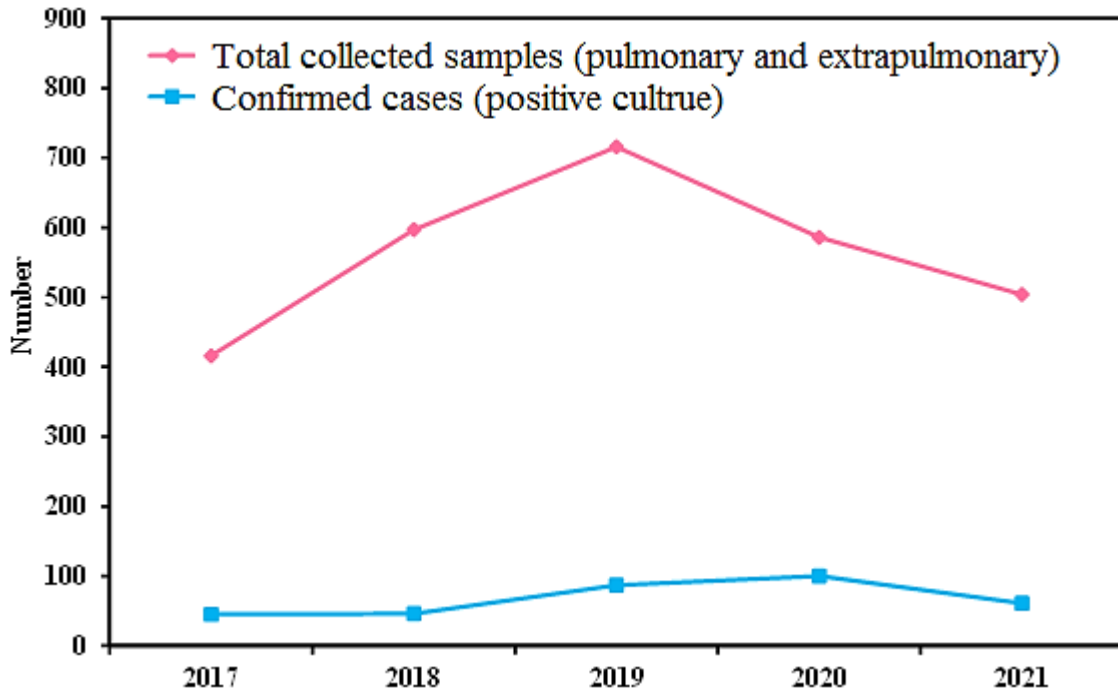
Figure 3. Distribution of MTB & NTM among LJ–culture positive samples (2017 – 2021).

Discussion

Tuberculosis is regarded as one of Egypt's most serious healthcare challenges [21]. Rapid and efficient diagnosis is essential for quick interventions and proper disease management and control. Classical methods, including microscopy and culture, still play the foundational role in TB diagnostics; however, they may suffer some limitations. Culture, for example, is the gold standard technique, but it is laborious and time-consuming, as the results may take several weeks to be revealed [22].

In the current study, two tests were used to detect suspected cases of latent TB, the TST as well as QuantiFERON®-TB Gold, with positive cases of 21.9% and 15.1% respectively. Both tests were carried out for different patients according to physicians' requests, so their results are non-comparable. **So. et al.** (2017) [23] reported 34.2% and 28.9%, while **Anwar. et al.** (2019) [24] reported 34.6% and 9.1% respective test positivities. A study conducted by **Abdulkareem et al.** (2020) [25] revealed that 19.85% and 24.05% of the enrolled cases were TST and Quantiferon test positive, respectively, results that are comparable to ours. Other studies outlined significantly higher positivity rates, with a percentage positivity of up to 55.6% for TST and 54% for Quantiferon [26,27], which may reflect the high-risk group populations that were incorporated.

Direct smear microscopy using ZN stain is a highly specific, quick, and inexpensive method for mycobacterial diagnosis. However, this method suffers variable sensitivities (20–80%), influenced by several factors such as the sample quality, mycobacterial count per sample, disease prevalence, and the experience of lab personnel. Moreover, microscopy is unable to distinguish between mycobacterial species, which poses a concern, particularly in children and those with impaired immune systems [22]. Previous studies reported a ranging sensitivity of 22.2-72.7%, specificity of 70.5-100%, PPV of 46.2-100% and NPV of 51.4-88.7% [5,28,29]. In our study, ZN smear microscopy showed a sensitivity of 64.76%, excellent specificity of 99.18%, and a PPV of 90.3%. The NPV of 95.6% in our study is higher than the studies mentioned, indicating lower TB prevalence in our region compared to them.

During this 5-year retrospective study, culture for mycobacteria was positive in 12.1% and 11.8% of pulmonary and extrapulmonary samples, respectively. Another Egyptian study reported that LJ culture was positive in 18.9% and 17.4% of pulmonary and extrapulmonary samples, respectively [30]. **Elbrolosy et al.** (2021) reported that 27.2% and 28.6% were culture-positive among pulmonary and extrapulmonary samples [28]. In line with our results, both of them did not find any statistical difference in the LJ culture's ability to detect MTB among pulmonary and extrapulmonary samples.

NTM are widely distributed diverse pathogenic organisms that exist in nature [31], resulting in serious infections in both immunocompetent and immunocompromised individuals. It has been previously reported that in the Middle East, NTM is causing a growing concern [32], with 2.4% to 20.8% prevalence reported in the African region [33]. Our findings are consistent with the regional prevalence range, and we additionally noticed an overall decreasing trend of atypical mycobacteria, from 13.3% in 2017 to 3.3% in 2021. A recent Egyptian study reported that the overall prevalence of NTM amongst studied patients was 7% using phenotypic detection methods [34]. More focused studies are required to highlight the prevalence rates of NTM in Egypt.

The WHO has endorsed fully automated real-time semi-nested PCR systems, such as the GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) and the newer version, the GeneXpert Ultra [35]. The GeneXpert MTB/RIF has superior sensitivity and specificity in both pulmonary and extrapulmonary specimens when compared to smear microscopy, as it can identify MTB and detect mutations linked to rifampicin resistance simultaneously, rendering it a valuable tool for the identification of MDR-TB strains [36].

Out of 109 samples positive for GeneXpert in this study, microscopy failed to identify six samples. This observation may be attributed to variable sample processing and lab personnel experience. Moreover, 8 out of 347 GeneXpert negative samples were ZN positive, which suggests that these samples could be NTM, as GeneXpert detects MTB only. It is generally recommended in such cases to perform real-time PCR (RT-PCR) to detect NTM.

GeneXpert was TB positive in 83 (28.3%) pulmonary samples and 26 (16%) extrapulmonary samples in our study. Two other studies from Iran

and Egypt reported that GeneXpert was positive in 13% and 19.6% of pulmonary samples and 9.4% and 19.3% of extrapulmonary samples, respectively [30,37]. We also compared the pulmonary and extrapulmonary capability of the GeneXpert assay, where a statistically significant difference (p=0.003) in the GeneXpert detection rate was documented. **Hefzy et al.** [30], on the other hand, reported no significant difference (*p*=0.887).

We detected rifampicin resistance in 20.5% of pulmonary samples and 7.7% of extrapulmonary samples, with an overall resistance of 17.4% among total MTB-positive samples. This is consistent with **Elbrolosy et al.** (2021) [28], who reported an overall rifampicin resistance of 17.9%. Several studies indicated lower resistance rates of 5.7%, 7.7%, and 8.6% [30,37,38], while others reported much higher rifampicin resistance rates 68.5% [39]. Elevated rifampicin resistance rates are alarming, with the most significant risk factor documented for this phenomenon is patient incomplete anti-TB regimens, which suppress the growth of drug-susceptible bacilli only, giving an appropriate chance for the development of MDR-TB mutants [40].

The present work detected an overall respective positivity of 4.8%, 12.03%, and 23.9% for ZN smear microscopy, LJ culture, and GeneXpert. Studies from Egypt and India reported 13.6%, 18.5%, 19.5% positivity [30], and 8.2%, 22%, 24.7% positivity [5] among the same tests, respectively. Other studies investigating the capabilities of ZN microscopy and culture revealed 20.6%, 41.3% [41], and 7.29%, 11.74% [42] positive rates respectively, with the latter results similar to our study.

It is worth mentioning that a number of samples subjected to GeneXpert and ZN smear microscopy were also tested using LJ culture. All culture-positive samples were also positive by GeneXpert except for one (pleural fluid), which was GeneXpert, as well as ZN stain negative. An explanation for this is that the count was too low in the sample to be detected by microscopy and GeneXpert, showing that a patient having MTB or NTM may still experience negative GeneXpert results [43]. It has been reported that the diagnostic utility of GeneXpert in pleural fluid is limited, with poor sensitivity rates, and that a negative GeneXpert does not exclude extrapulmonary TB diagnosis [44].

In this study, we noted that the number of samples obtained for culture and microscopy decreased in 2020 and 2021 compared to 2019. A possible explanation is the COVID-19 lockdown, in which there was public fear of acquiring the viral infection, in addition to the shortfall of transportation and general services. All such factors may have hindered individuals from attending laboratories and healthcare facilities during the pandemic. The WHO reported a significant reduction in the reported number of diagnosed cases (-18%) and in the number of individuals provided with rifampicin-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB) treatments (-17%) [45]. Although the total number of samples collected in this study decreased in 2020 and 2021, the percentage of culture-positive samples was enhanced noticeably. This could be attributed to the fact that patients were not sent for diagnosis except when TB was highly suspected during the lockdown.

Conclusions

The overall increasing number of admitted samples for TB testing during the study period may reflect an actual increase in TB prevalence in Egypt. The COVID-19 pandemic negatively affected access to TB diagnosis and treatment, as well as TB burden and drug resistance. Despite notable advancements in recent years, mycobacterial infections remain challenging to diagnose microbiologically. GeneXpert is a superior method for identifying MTB among different specimens because of its rapidity and ability to detect rifampicin-resistant strains. Nevertheless, culture is still irreplaceable and remains the most reliable and well-established method used in developing countries for its high sensitivity and low cost. It is necessary to implement the assurances made at the UN high-level TB meeting in 2023 into practice in order to halt the global TB epidemic.

Ethics approval and consent to participate

This article does not contain any studies with human participants or live vertebrates and/or higher invertebrates.

Competing interests

The authors report no conflicts of interest.

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Authors' contributions

Asmaa A. Ramadan: data curation, formal analysis, methodology, investigation, writing—original draft;

Sherine Shawky: conceptualization, supervision, writing—review and editing;

Arsany Ibrahem: methodology, investigation, writing—review and editing;

Mohammed A. El-Kholy: conceptualization, data curation, formal analysis, methodology, investigation, writing—review and editing.

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