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Diagnosis of lymph node tuberculosis using the GeneXpert MTB/RIF in Tunisia

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ABSTRACT

Introduction: GeneXpert MTB/RIF is a fully-automated diagnostic molecular test which simultaneously detects tuberculosis (TB) and rifampicin (RIF) drug resistance. The purpose of this study is to evaluate the performance of the GeneXpert MTB/RIF test for the detection of *Mycobacterium tuberculosis* complex (MTBC) in lymph node specimens and to show the place of *Mycobacterium bovis* as a major cause of TB lymphadenitis.

Material and methods: This study was conducted simultaneously in the National Reference Laboratory for Mycobacteria of Ariana and the Central Laboratory of Sfax, from January to December 2013. In total, 174 lymph node specimens were processed simultaneously for Ziehl–Neelsen, auramine and immuno-histochemical staining. Conventional culture on both Lowenstein–Jensen and liquid medium (Bactec MGIT 960 BD system) and the new molecular-based GeneXpert MTB/RIF assay system were performed. Positive cultures were confirmed using molecular identification (Genotype MTBC Hain Lifescience).

Results: Among the 174 samples tested, the GeneXpert detected the DNA of MTBC in 134 samples (77%). Standard bacteriological assays, including AFB microscopy and culture, were positive, respectively, in 41 (23.6%) and 79 (45.4%) specimens. *M. bovis* was isolated in 76% of positive cultures. GeneXpert sensitivity and specificity results were assessed according to smear and culture results, clinical and histological findings. The sensitivity and specificity of the Xpert assay were 87.5% (126/144) and 73.3%, respectively.

Conclusion: The implementation of the GeneXpert MTB/RIF assay may dramatically improve the rapid diagnosis of lymph node TB.

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Introduction

Tuberculosis (TB) of the lymph nodes (tuberculosis lymphadenitis) is one of the most common forms of extrapulmonary TB whose diagnosis still faces many challenges [1]. In Tunisia, the rate of TB lymphadenitis has increased. In 2012, its frequency was estimated to be 29.3% and in 2014, 35.8% of TB cases [2]. Conventional *Mycobacterium tuberculosis (M. tuberculosis)* detection techniques, based on microscopic examination of Ziehl–Neelsen or auramine-stained specimens and culture, are required for diagnostic confirmation, but they fail to provide an optimal sensitivity [3]. In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity. One of the latest systems, the GeneXpert MTB/RIF (Xpert) assay, based on nested real-time PCR and molecular beacon technology, has been shown to be rapid, with a result for TB and RIF drug resistance under 2 h [4].

The first purpose of the current study was to evaluate the performance of Xpert MTB/RIF test for the detection of *M. tuberculosis* complex (MTBC) in lymph node specimens and to compare it with conventional methods. This study also aims to show the place of *Mycobacterium bovis* (*M. bovis*) as a major cause of TB lymphadenitis.

Material and methods

Study specimens

This study was conducted simultaneously in the National Reference Laboratory for Mycobacteria of Ariana and the Central Laboratory of Sfax, from January to December 2013. This study was approved by the Ethics Committee of the A. Mami Hospital of Pneumology, Ariana, Tunisia.

Lymph node specimens (tissues and aspirates) were prospectively collected from 174 patients in different Tunisian health centers.

Lymph node TB was suspected on the basis of clinical criteria. Two specimens were collected; the first one was sent to the bacteriology laboratory and the second to the pathology laboratory. In the bacteriology lab, the sample was divided into two parts (biopsies were firstly crushed in a sterile mortar); one was used for the Xpert test and the second one was tested by direct and concentrated acid fast bacilli (AFB) microscopy and culture.

Mycobacterial culture and smear

All specimens were fully processed by digestion, decontamination and concentration. The N-acetyl-L-cysteine and sodium hydroxide method (NALC/NaOH) was used for digestion and decontamination. Thereafter, the specimens were concentrated by centrifugation at 3500*g* for 15 min and resuspended in 1 ml of sterile phosphate buffer (pH = 6.8) [5].

The processed specimen sediment was used to inoculate two Lowenstein–Jensen (LJ) slants and a Bactec MGIT 960 tube (mycobacteria growth indicator tube), which were incubated at 37 °C for 12 and 6 weeks, respectively [5,6].

Smears of processed sediment were stained with both Auramine and Ziehl–Neelsen method and examined with fluorescent and light microscopy.

Identification

Cultures were identified using TB Antigen MPT 64, standard phenotypic identification tests (Table 1) and molecular methods [7].

SD bioline TB Ag MPT64

All positive cultures were subjected to the immunochromatographic identification test that uses the mouse monoclonal anti MPT64 [8]. This test can be easily used for rapid identification of the MTBC and can differentiate between MTBC and non-tuberculous mycobacteria (NTM).

Phenotypic identification

Phenotypic identification was based on the niacin test, nitrate reduction test, susceptibility to thiophene-2-carboxylic acid hydrazide (TCH) and growth on p-nitrobenzoic acid (PNB) medium (Table 1) [9–11].

Molecular identification

For the final identification of MTBC species, the Genotype MTBC system (Hain Lifescience, Germany) was used. The assay was performed according to the instructions of the manufacturer [12].

Drug susceptibility testing (DST)

Drug susceptibility testing (DST) of first-line anti-TB drugs (RIF, isoniazid, ethambutol, pyrazinamide and streptomycin) was performed with the Bactec MGIT 960 method (MGIT 960; Becton Dickinson Diagnostic Systems).

GeneXpert MTB/RIF procedure

The reagent was added in a 2:1 ratio to decontaminated and concentrated specimen. The sample container was agitated twice during a 15-min incubation period at room temperature. Finally, 2 ml of the inactivated mixture was transferred to the Xpert test cartridge. Cartridges were inserted into the GeneXpert device, and the automatically generated results were read after 90 min [13,14].

Patient categories

Patients were categorized into 4 groups: “confirmed TB” cases (culture positive, smear negative or positive), “probable TB” cases (culture negative, but showing clinical symptoms, smear positive and histology/cytology suggestive of TB), “possible TB” cases (negative culture, but showing clinical symptoms, and negative smear and histology/cytology suggestive of TB), and “not TB” cases (only clinical symptoms) [15]. Table 2 represents a detailed algorithm used for the categorization of patients into different categories.

Table 1 – Phenotypic identification of MTBC.

	Niacin	Nitrate	PNB	TCH
M. tuberculosis	+	+	S	R
M. bovis	–	–	S	S
M. africanum	V	V	S	V
Non-tuberculous mycobacteria	–	–	R	S

S: sensitive; R: resistant; V: variable.

Table 2 – Patients categorization.

	AFB smear	Culture	Symptoms	Histology/cytology
Confirmed TB	+/–	+	+	+/–
Probable TB	+	–	+	+/–
Possible TB	–	–	+	+
Not TB	–	–	+	–/NP

NP: not performed.
For histology/cytology in case of "Probable TB", a specimen was positive if the presence of caseation necrosis and epithelioid granulomas was reported.

Results

This study included 174 patients. The male-to-female ratio was 0.47 (56/118). The median age of the patients was 32.3 years (range 3–79 years). All patients were found to be HIV negative.

AFB smears were positive for 41 cases (23.6%). Scanty AFB (less than 10 AFB) were observed in 75.6% of smear-positive specimens. Culture was considered positive if the MGIT tube and/or LJ media was positive. Contaminated MGIT tube and LJ media were considered to be negative for the Xpert assay sensitivity and specificity calculation. Histology/cytology results were not available for 33 specimens. Overall, 79 (45.4%) of the 174 specimens tested were culture positive. MTBC was isolated on MGIT and LJ medium in respectively 78 (98.7%) and 40 (50.6%) culture-positive samples. Among the 174 samples tested, the Xpert detected the DNA of MTBC in 134 samples (77%). Histopathology was positive for 121 (69.5%) specimens showing the presence of caseation and epithelioid granulomas (Fig. 1) [14].

In this study, 79 specimens (45.4%) were culture positive (55 [31.6%] being smear negative and 24 [13.8%] being smear positive); 22 (12.6%) were "probable TB" cases; 43 (24.7%) were only histologically/cytologically positive showing necrosis, caseation, or epithelioid granuloma suggestive of "possible TB" cases; and 30 (17.2%) patients had no evidence of TB and were "not TB" cases. Table 3 shows in detail the Xpert accuracy according to smear and culture results, clinical and histological findings.

Sensitivity and specificity

Xpert detected MTBC DNA in 75/79 of culture-positive specimens. The sensitivity and specificity of the Xpert assay were 94.9% and 37.9%, respectively, when compared with culture (Table 4). The sensitivity of the molecular test in smear-

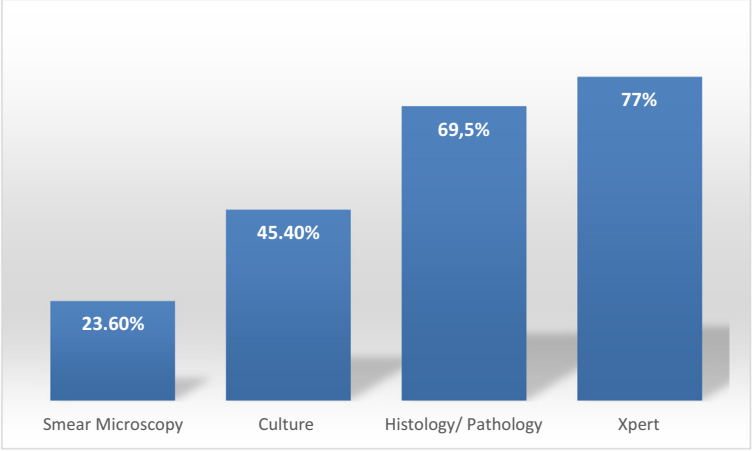

Fig. 1 – Percentage of positive smear microscopy, culture, histology/cytology and Xpert assay.

Table 3 – Xpert accuracy according to smear and culture results, clinical and histological findings.

	AFB smear	Culture	Symptoms	Histology/cytology	XP+	XP−	Total
Confirmed TB	+	+	+	+/−	24	0	24
	+	+	+	+/−	51	4	55
Probable TB	+	−	+	+	13	2	15
	+	−	+	−	6	1	7
Possible TB	−	−	+	+	32	11	43
Not TB	−	−	+	−/NP	8	22	30
Total					134	40	174

NP: not performed.
For histology/cytology a specimen was positive if the presence of caseation necrosis and epithelioid granulomas was reported.

Table 4 – Sensitivities of the Xpert test in comparison with culture method as reference standard.

Method compared to culture		% positive specimen (no positive specimen/total No. of specimens)
Xpert sensitivity	Smear positive, culture positive	100% (24/24)
	Smear negative, culture positive	92.7% (51/55)
	All culture positive	94.9% (75/79)
Xpert specificity		37.9% (36/95)

positive or -negative and culture-positive samples was respectively 100% and 92.7%.

The Xpert test detected TB in 77.6% (45/58) of patients with negative cultures and positive histology. Furthermore, the Xpert assay showed 8 positive results in “not TB” cases.

Table 5 shows the sensitivity and specificity of Xpert assay when compared with smear microscopy, culture results and histological findings. The sensitivity and specificity were, respectively, 87.5% and 73.3%. Positive predictive value (ppv) was 94%, whereas the negative predictive value (npv) was 55%.

Identification and RIF resistance detection

All the strains isolated were identified. *M. bovis* and *M. tuberculosis* were isolated in 76% (60/79) and 24% (19/79), respectively, (Fig. 2). NTM were not isolated. Positive Ag MPT64 bands were obtained for all MTBC isolates. Strain identification was confirmed by the Genotype MTBC assay. All strains were found to be susceptible to RIF in both Xpert method and conventional drug susceptibility testing [12,13]. *M. bovis* strains showed resistance to pyrazinamide. No additional resistance was found to anti-tuberculous drugs.

Discussion

In 2012, the incidence of TB in Tunisia was 31/100,000 and the incidence of lymph node TB was 4.9/100,000 [16]. In 2014, 58.74% of TB cases were extrapulmonary [2]. Tuberculous lymphadenitis accounted for 29.3% in 2012 and increased to 35.8% in 2014 [2]. Improving diagnosis tools in Tunisia seems to be necessary. Although different laboratory techniques are available, they suffer from sub-optimal sensitivity and/or specificity, and diagnosis of lymph node TB remains difficult.

Microscopy using Ziehl–Neelsen or auramine staining procedure is easy, rapid and cheap. The sensitivity varies depending on the source of the sample. It was low (23.6%) in this study. Scanty AFB were observed in 75.6% of smear-positive microscopy. In India, the sensitivity ranges from 46% to 78% [17]. This weak sensitivity can be explained by the paucibacillary nature of specimens.

Culture is the gold standard for TB diagnosis. It is time-consuming, requires biosafety measures and needs trained laboratory personnel [18]. MGIT has improved lymph node TB diagnosis. Indeed, it gave a higher yield of mycobacteria and faster results than LJ medium [6]. Although solid and liquid media were combined, culture shows a low sensitivity in

Table 5 – Sensitivity and specificity of Xpert in comparison with smear microscopy and culture results, clinical and histological[a] findings.

	TB+[b]	TB−[c]	Sensitivity	Specificity	VPP	VPN
Xpert+	126	8	87.5% (126/144)	73.3% (22/30)	94% (126/134)	55% (22/40)
Xpert−	18	22				

a For histology/cytology, a specimen was positive if the presence of caseation necrosis with or without epithelioid granulomas was reported.
b Confirmed, probable or possible TB.
c Smear microscopy (–) and culture (–) and histology/cytology (–).

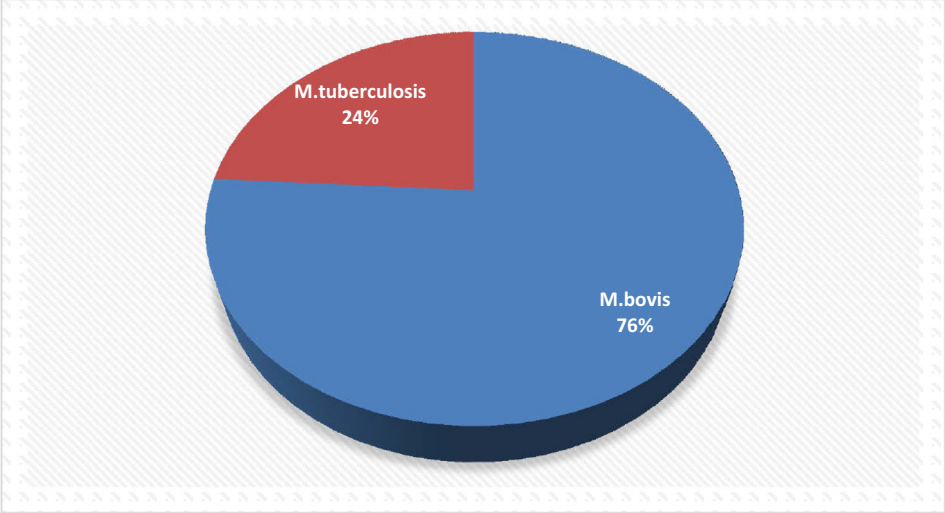

Fig. 2 – Distribution of MTBC strains.

case of lymph node specimens [15]. Culture-confirmed cases were 45.4% in this study. This may be attributed to the uneven bacilli distribution and the loss of the low number of viable bacilli during NALC-NaOH processing [6,18].

The World Health Organization (WHO) has endorsed the Xpert MTB/RIF assay as a replacement for sputum smear microscopy. For the diagnosis of patients presumed to have extrapulmonary TB, Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (lymph nodes and other tissues) [19].

This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling for the diagnosis of TB and simultaneous assessment of RIF resistance to be completed within 2 h [20].

In the present study, a high sensitivity of the Xpert test for the diagnosis of lymph node TB reported. In fact, the assay detects 94.93% (75/79) of all "confirmed TB" cases, including 92.72% (51/55) of smear-negative TB cases. It was also observed that the Xpert assay detects TB in 75.47% (40/53) of the samples from "probable TB" cases (Table 3). The Xpert sensitivity of "confirmed TB" was higher than that reported by Vadwai et al. (83%), whereas the sensitivity of "probable TB" was found to be lower (80%) [15].

The Xpert was also positive in 26.67% (8/30) of "not TB" samples (Tables 3 and 4). Out of these 8 specimens, 1 was cytologically negative, and for 7 specimens, histology/cytology was not performed. These 8 specimens were considered cytologically negative. This can explain the lack of specificity (73.33% [22/30]) reported in the study. If specimens not tested for cytology were excluded, the Xpert specificity would be dramatically improved to 95.65% (22/23). The lack of specificity can be also attributed to patients who were under antitubercular treatment when enrolled in the study. This data was not taken into account.

Few studies reported bacteriological identification of extrapulmonary and lymph node TB in Tunisia. The exact contribution of *M. bovis* to the human TB remains unknown. This study shows that *M. bovis* was the dominant causative agent of TB lymphadenitis (76%). In contrast, most studies report *M. tuberculosis* as the main etiological agent of lymph node TB [21,22]. Historically, *M. bovis* was the common cause of TB lymphadenitis, but pasteurization and bovine TB programs have virtually eliminated this source of human infection in developed countries; risk remains with the consumption of unpasteurized milk and dairy products [23]. These products are widely consumed in Tunisia where bovine TB is enzootic [24–26]. A national program for bovine TB control has been implemented since 1984. Nevertheless, the disease continues to be prevalent, mainly in the private sector where predominant small cattle herds make the veterinary control a challenge [24,26].

Finally, because of the absence of RIF-resistant strains, the performance of Xpert assay for RIF-resistance detection cannot be assessed.

Conclusion

Rapid TB tests may be the key to worldwide TB control strategies. The high sensitivity and specificity, coupled with its speed and simplicity, make the GeneXpert MTB/RIF the most useful tool in the rapid diagnosis of lymph node TB. This rapid TB diagnostic test may complement usual methods (conventional microscopy, culture and histopathology).

Conflict of interest

None.

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