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Concordance between microscopic and molecular detection of *Mycobacterium tuberculosis* in hospitalized patients with suspected pulmonary tuberculosis in Peltier General Hospital, Djibouti

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Abstract

Background: Tuberculosis (TB) remains a significant public health issue, and Djibouti is still one of the nations with a high TB burden, with a forecasted incidence rate of 224 cases per 100,000 people in 2020.

Objectives: To compare the Ziehl-Neelsen (ZN) stain direct smear microscopy with the GeneXpert (Xpert MTB/RIF assay) as the gold standard for diagnosing patients with suspected pulmonary tuberculosis (PTB).

Material and method: A health facility-based cross-sectional study was conducted on hospitalized patients at Peltier General Hospital from March to July 2022. A total of 153 samples (including bronchoalveolar lavage (BAL) and sputum) collected from presumptive TB patients were tested.

Results: Out of the 153 samples analyzed by GeneXpert and ZN smear microscopy, 29 (19.0%) were detected by GeneXpert and 23 (15.0%) were positive by ZN smear microscopy.

ZN smear microscopy had sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) that were 79.31%, 100%, 100%, and 95.38%, respectively.

Conclusion: This study concluded that ZN smear had a slightly poor sensitivity for determining the presence of PTB; consequently, we continue to advise GeneXpert testing whenever it is possible to prevent prolonged morbidity in those affected and to prevent the direct as well as indirect expenses of missing the diagnosis of smear-negative PTB.

Keywords: Pulmonary tuberculosis; Peltier General Hospital; Djibouti; Ziehl-Neelsen stain; GeneXpert MTB/RIF assay

1 Introduction

Tuberculosis remains a significant public health issue. This is caused by the *Mycobacterium tuberculosis* complex (MTBc), a group of mycobacteria that are quite large, rod-shaped, aerobic, acid-fast, and facultative intracellular organisms [1].

The *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti, M. pinnipedii*, *M. microti*, and *M. caprae* species are among the seven that make up the complex. A quarter of the world's population has *M. tuberculosis* infection, while a smaller percentage has TB disease brought on by *M. bovis* and *M. africanum* [2].

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Tuberculosis is an airborne infection that typically affects and scars the upper lobes of the lung; this is called pulmonary tuberculosis (PTB), but it can also affect other sites of the human body (extrapulmonary TB). When a person with PTB sneezes or coughs, contagious infectious droplets that contain TB organisms are discharged, which are then inhaled by other people [3].

PTB is one of the top 10 causes of death worldwide, other than the Human Immunodeficiency Virus (HIV), and the leading cause of death from a single infectious agent. The reasons behind this unyielding infection are due to inaccessibility or a lack of diagnostic tools that carry higher precision and an over-reliance on clinical judgment [1].

According to the World Health Organization (WHO), globally, 10.4 million people got infected with TB in 2019, resulting in 1.7 million deaths (including 0.4 million co-infected with HIV) [1]. WHO reported in 2020 that most TB cases were in the regions of South-East Asia (43%), Africa (25%), and the Western Pacific (18%); however, smaller proportions of cases were in the Eastern Mediterranean (8.3%), America (3.0%), and Europe (2.3%) [1].

The Republic of Djibouti is a country located in the East Horn of Africa with a total population of approximately 1,010,161 people. Djibouti is still one of the nations with a high TB burden, with a forecasted incidence rate of 224 cases per 100,000 people in 2020 [4].

TB is a disease for which the use of vaccines has shown no efficacy; hence, the best control mechanisms to reduce transmission rates are early diagnosis and prompt treatment. The clinical manifestation of the disease and the identification of the bacilli in clinical samples are the two key factors that determine the diagnosis of TB. Chest X-rays, smear microscopy, culture, nucleic acid amplification assays, and immunological approaches can all be used to diagnose TB [5, 6].

In spite of the fact that AFB microscopy is the primary method for the diagnosis of PTB in low- and middle-income countries and that it is mainly used to monitor the progress of the treatment and confirm cure achievement [7], ZN is used for AFB smear microscopy, and it is a simple, convenient, and inexpensive technique. However, it has poor sensitivity and needs a concentration of 10,000 colony-forming units per mL to be seen as positive under a microscope [7].

The GeneXpert MTB/RIF is a nucleic acid amplification assay that amplifies a specific region of the rpoB gene in *M. tuberculosis* and Rifampicin (RIF) resistance-related mutations [8]. It gives results within 2 hours with higher sensitivity and diagnostic accuracy for both pulmonary and extrapulmonary TB, particularly in individuals at high risk for tuberculosis, such as people with suspected HIV-associated TB and children [9, 10].

Therefore, the present study is undertaken to compare ZN smear microscopy with the GeneXpert (Xpert MTB/RIF assay) as the gold standard for diagnosing patients with suspected PTB.

2 Material and methods

2.1 Study design and setting

A health facility-based cross-sectional study was carried out over a period of five months, from March 2022 to July 2022. It enrolled 153 hospitalized patients with suspected PTB attending the Department of Pediatrics and the Infectious and Tropical Diseases Department at Peltier General Hospital, Djibouti.

2.1.1 Study subject

All presumptive PTB inpatients exhibiting clinical symptoms of TB, such as fever, chronic cough lasting more than two weeks, weight loss, fatigue, hemoptysis, loss of appetite, and night sweats, were included, whereas extrapulmonary TB patients and outpatients were excluded from this study. Clinical and demographic data like age, sex, history of lung diseases, and HIV serostatus were collected from patient hospital records.

2.2 Laboratory Methods

According to the laboratory's transportation protocol, each sample of sputum or BAL from a single patient is delivered to the lab. One section of these samples was examined using GeneXpert, and the other section was used for ZN smear microscopy.

2.2.1 GeneXpert MTB/RIF assay

Testing was conducted using GeneXpert (Cepheid, USA) in accordance with the manufacturer's recommendations [11]. Untreated sputum or BAL was mixed with sample reagent at a 2:1 ratio, vortexed for 30 seconds to ensure that all bacteria were resuspended, and then incubated at room temperature for 15 minutes before being shaken once more. A Pasteur pipette was then used to transfer two ml of the solution to the Xpert cartridge, which was then loaded into the Xpert machine for analysis. The GeneXpert MTB/RIF assay results show if MTB was found in the samples or not.

2.2.2 Smear microscopy

Direct smears were made from the mucopurulent part, depending on the nature of the sample. The staining bridge was used to serially arrange the smears, with the smear side facing up. Filtered 0.1% carbol fuchsin was then used to flood the smears. The smears were then steam stained for five minutes, rinsed with water, and drained. They received a 3-minute decolorization process with 25% hydrochloric acid, followed by a water rinse and drain. They were then stained with a 0.1% methylene blue solution for 1 minute and then washed with water. After allowing the smear to dry naturally, it was viewed under a microscope using an oil immersion (100x) objective. Slides with red-colored AFB were judged positive, while those without any AFB were viewed negatively [12].

2.3 Data analysis

The Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY: IBM Corp.) and the MedCalc program version 12.4.0 were used to analyze the data. A p-value of less than 0.05 was considered statistically significant.

Sensitivity, specificity, PPV, and NPV were calculated using the standard formulae for ZN smear microscopy using GeneXpert as the gold standard.

3 Results

3.1 Socio-demographic and Prevalence of PTB

Table 1 Distribution of Socio-Demographic and Clinical Characteristics by GeneXpert

Socio-demographic	GeneXpert		Total (n=153)	Test of significance
	Positive 29 (19%)	Negative124 (81%)		
	No. (%)	No. (%)	No. (%)	
Distribution by Gender				P=0.296
Male	19 (65.5%)	68 (54.8%)	87 (56.9%)	
Female	10 (34.5%)	56 (45.2%)	66 (43.1%)	
Distribution by Age group(years)				P=0.006
less than 10	1 (3.4%)	21 (16.9%)	22 (14.4%)	
10-20	0 (0.0%)	11 (8.9%)	11 (7.2%)	
21-30	1 (3.4%)	5 (4.0%)	6 (3.9%)	
31- 40	6 (20.7%)	43 (34.7%)	49 (32.0%)	
above than 41	21 (72.4%)	44 (35.5%)	65 (42.5%)	
Distribution by risk factors				p=0.000
Diabetes mellitus	14 (48.3%)	2 (1.6%)	16 (10.5%)	
kidney disease	2 (6.9%)	0 (0.0%)	2 (1.3%)	
HIV	1 (3.4%)	0 (0.0%)	1 (0.7%)	
Lung infection	14 (41.4%)	122 (98.4%)	134 (87.6%)	

A total of 153 respiratory samples, comprised of sputum (n = 128) and BAL (n = 25), were tested in this study. Out of 153 patients, 87 (56.9%) were males and 66 (43.1%) were females. The prevalence of PTB detected by GeneXpert among enrolled cases was 29 (19.0%). In the present study, there was a slight male predominance among GeneXpert positive patients (65.5%), and most of them were in the age group above 41 years (72.4%). Regarding risk factors, most patients with positive PTB were diabetic patients (48.3%); however, 98.4% of patients with a history of lung disease other than TB, like chronic obstructive pulmonary disease (COPD), pneumonia, pneumoconiosis, and bronchiectasis, were negative by GeneXpert. These risk factors were found to significantly increase the risk of TB (P < 0:00). (Table. 1).

3.2 Clinical presentation

Among the most occurring clinical features, cough, chest pain, hemoptysis and night sweat were more evident in positive PTB cases and were significantly associated too (p=0.022, p=0.002, p=0.003, p=0.033). However, other clinical features, such as fever and noticeable weight loss, were found to be non-specific presentations (Table 2).

Table 2 Characterization of PTB Cases in Terms of Clinical Presentation

Clinical presentation	GeneXpert		Test of significance	ZN		Test of significance	Total n=153
	Positive	Negative		Positive	Negative		
	29 (19.0%)	124 (81.0%)		23 (15.0%)	130 (85.0%)		
Fever Yes	7 (21.2%)	26(78.8%)	p=0.709	6 (18.2%)	27 (81.8%)	p=0.568	33
No	22 (18.3%)	98 (81.7%)		17 (14.2%)	103 (85.8%)		120
Cough Yes	28 (22.4%)	97 (77.6%)	p=0.022	22 (17.6%)	103 (82.4%)	p=0.060	125
No	1 (3.6%)	27 (96.4%)		1 (3.6%)	27 (96.4%)		28
Weight loss Yes	10 (37.0%)	17 (63.0%)	p=0.008	7 (25.9%)	20 (74.1%)	p=0.081	27
No	19 (15.1%)	107 (84.9%)		16 (12.7%)	110 (87.3%)		126
Chest pain Yes	28 (25.0%)	84 (75.0%)	p=0.002	22 (19.6%)	90 (80.4%)	p=0.008	112
No	1 (2.4%)	40 (97.6%)		1 (2.4%)	40 (97.6%)		41
Hemoptysis Yes	2 (100.0%)	0 (0.0%)	p=0.003	1 (50.0%)	1 (50.0%)	p=0.164	2
No	27 (17.9%)	124 (82.1%)		22 (14.6%)	129 (85.4%)		151
Night sweat Yes	2 (66.7%)	1 (33.3%)	p=0.033	2 (66.7%)	1 (33.3%)	p=0.011	3
No	27 (18.0%)	123 (82.0%)		21 (14.0%)	129 (86.0%)		150

3.3 Performance of ZN Smear Microscopy versus GeneXpert MTB/RIF Assay Resultsas Gold Standard

Out of the 153 samples analyzed by GeneXpert and ZN smear microscopy, 23 (15.0%) werepositive by ZN smear microscopy; 6 (3.9%) were positive by GeneXpert but negative by ZNsmear microscopy; and 124 (81.0%) were negative by both methods **(Table 3)**.

Table 3 Performances of ZN-Stained Direct Smear Microscopy versus GeneXpert MTB/RIF Assay

N smear microscopy	GeneXpert		Total
	Positive	Negative	
	No. (%)	No. (%)	No. (%)
Positive	23 (15.0%)	0 (0.0%)	23 (15.0%)
Negative	6 (3.9%)	124 (81.0%)	130 (85.0%)
Total	29 (19.0%)	124 (81.0%)	153 (100.0%)

3.4 Prevalence of Rifampicin-Resistant (RR) TB

Of the 29 confirmed PTB cases by GeneXpert, one (3.4%) was resistant to rifampicin, and two (6.9%) were intermediate. The proportion of RR-TB was one (3.4%) among males, and no RR-TB was detected in females. Among the various age categories, 3.4% of individuals over the age of 41 had RR-TB. Among RR-TB cases, 3.4% were coinfected with HIV **(Table 4)**.

Table 4 Distribution of Rifampicin Resistance According to Patients Characteristics

Patients Characteristics	Rifampicin			Total
	Sensitive	Intermediate	Resistance	
	26 (89.7%)	2 (6.9%)	1 (3.4%)	29 (100.0%)
Distribution by Gender				
Male	16 (55.2%)	2 (6.9%)	1 (3.4%)	19 (65.2%)
Female	10 (34.5%)	0 (0.0%)	0 (0.0%)	10 (34.5%)
Distribution by Age group(years)				
less than 10	1 (3.4%)	0 (0.0%)	0 (0.0%)	1 (3.4%)
10-20	1 (3.4%)	0 (0.0%)	0 (0.0%)	1 (3.4%)
	1 3.4%)	0 (0.0%)	0 (0.0%)	1 3.4%)
31- 40	5 (17.2%)	0 (0.0%)	0 (0.0%)	5 (17.2%)
above than 41	18 (62.1%)	2 (6.9%)	1 (3.4%)	21 (72.2%)
Distribution by risk factors				
Diabetic melltius	12 (48.3%)	2 (1.6%)	0 (0.0%)	14 (48.3%)
kidney disease	2 (6.9%)	0 (0.0%)	0 (0.0%)	2 (6.9%)
HIV	0 (0.0%)	0 (0.0%)	1 (3.4%)	1 (3.4%)
Lung infection	12 (41.4%)	0 (0.0%)	0 (0.0%)	12 (41.4%)

3.5 Sensitivity, Specificity, Positive and Negative Predictive Value of ZN- Technique

In this study, the ZN-sensitivity, technique specificity, PPV, and NPV were calculated in comparison to the standard GeneXpert MTB/RIF. ZN-smear microscopy had sensitivity, specificity, PPV, and NPV of 79.31%, 100%, 100%, and 95.38%, respectively. The negative LK ratio was 0.21, while the positive LK ratio was unlimited. For ZN smear microscopy, the area under the curve (AUC) was 0.897. The test method's diagnostic yield and accuracy were 15.03 and 96.08, respectively. Statistically positive agreement was found between ZN smear microscopy and the gold standard with a significant kappa coefficient ($k = 0.861$, $P < 0.001$).

4 Discussion

TB remains a major worldwide health problem. In our study, the prevalence of TB among enrolled cases was 29 (19.0%), which is low as compared to the previous reports done in Djibouti (45.5%) [13], Egypt (42% [14], and Nepal (23.61%) [3], but higher than the studies done in Ethiopia (11.2%) [15] and Eritrea (7.8%) [16]. This discrepancy in the prevalence of TB could be a result of the difference in community and geographical locations, as well as the duration of the study. The smaller sample size used in the present study was also a possible reason for the higher prevalence of TB than in the above study.

There was a gender-related difference in TB, with a higher prevalence in males (65.5%). This agreed with previous reports conducted by the WHO [1], Ethiopia [15], Nepal [3], Egypt [14], and Pakistan [17]. This might be because men are more exposed to harmful external influences like smoking and alcoholism and have different social and health-seeking behaviors [18, 19].

On the contrary, Mavenyengwa et al. (2018) from Namibia and Mwesige et al. (2022) from Uganda reported that there were more females with TB infections than males [20, 21]. A study from Brazil in 2001 reported that females in their age group (5–14 years) had a higher risk of TB infection compared with younger males [22], because they perform more activities within the home; another experimental explanation could be that estrogen is protective against TB infection by increasing the Th1 immune response, production of cytokines such as TNF and IFN, and macrophage activity that facilitate control of TB. Since younger girls are mostly prepubertal and the suggested protective effect of estrogen has not yet become dominant, they could be more susceptible to infection [23].

The age group above 41 years was the one with the highest percentage of TB cases in the current study. This was in line with earlier research from Pakistan [17] and Nepal [3], where all TB cases were reported in the older age category. Rather than recent transmission, the greater TB. The prevalence in the older population may be explained by the reactivation of endogenous TB in conjunction with a weakened immune system [17].

In contrast, a different study from Ethiopia found that the age range of 16 to 30 years had the highest proportion of GeneXpert positive cases [15], and another study from Uganda found that the age range of 20 to 29 years showed the largest proportion of PTB positive individuals [21]. This may be because young individuals are more mobile and exposed to the external environment, which increases their risk of contracting the TB bacilli.

In our study, the clinical features that showed statistically significant differences between the groups were cough, chest pain, hemoptysis, and night sweat. This agreed with a study from Nepal in 2019 that reported cough and chest pain were most commonly occurring and significantly associated [3]. Moreover, Teng et al. from Shandong 2022 [24] found that hemoptysis was strongly associated with PTB.

GeneXpert also detects rifampicin resistance. The burden of DR-TB is increasing in many countries, and in Djibouti, the frequency of TB is explained by poverty and crowding. Despite free access to health care and the Directly Observed Treatment (DOT) strategy, compliance with treatment is not perfect and facilitates post-treatment resistance. In this study, the prevalence of rifampicin-resistant TB was 3.4%, which agreed with previous studies conducted in Kenya (3.7%) [25] and Zambia (5.9%) [26]. Higher percentages were conducted in Djibouti (46%) [13], China (17.6%) [27], and Ethiopia (15.8%] [15]. The fact that this study was conducted in an area with a lower prevalence of TB patients could explain the differences in RR-TB. Moreover, most Djiboutians are diagnosed and treated at the Paul Faure tuberculosis center.

Smear microscopy has been useful in diagnosing PTB in numerous investigations, with high specificity but variable sensitivity (20–80%) [7, 28].

ZN smear microscopy in the current investigation exhibited limited sensitivity but good specificity, PPV, and NPV (79.31%, 100.0%, 100.0%, and 95.38%) using the GeneXpert MTB/RIF assay as the reference test. Similar specificity, PPV, and NPV were found in studies conducted in Pakistan, albeit with a marginally lower sensitivity [28].

The sensitivity of a ZN smear may vary between different geographical regions and within the same region between different laboratories, which is unlikely to occur with nucleic acid-based methods.

In our investigation, the probability of PTB being present in the event of a positive test result was unlimited for ZN smear microscopy. The ZN smear microscopy minimizes but does not completely rule out the probability of a negative PTB because the negative ratio of likelihood was 0.21.

ZN smear microscopy is still used to explore the degree of patients' infectivity; the infectious dosage for TB is less than ten bacilli. In the current study, ZN smear microscopy missed 6 (3.9%) cases when compared with the GeneXpert MTB/RIF as the reference standard. Similar findings were also obtained by other authors [20, 28]. Due to its larger detection threshold than the GeneXpert MTB/RIF assay, 5,000 versus 136 bacilli/ml of the samples AFB smear microscopy's inferior sensitivity can be explained [7]. Missed PTB diagnosis causes patients' morbidity due to the fact that 10–20% of PTB cases with smear-negative results can still transmit the disease [9].

A sensitive and precise diagnostic for smear-negative PTB is the GeneXpert MTB/RIF assay. Even though the GeneXpert MTB/RIF assay is more expensive than ZN smear microscopy, we still advise GeneXpert testing when it is possible to do so in order to prevent prolonged morbidity in the affected people and to prevent the direct and indirect costs of a missed diagnosis of smear-negative PTB.

5 Conclusion

With the evidence from the results, this study concluded that ZN smear had a slightly poor sensitivity for determining the presence of PTB; consequently, we continue to advise GeneXpert testing whenever it is possible to prevent prolonged morbidity in those affected and to prevent the direct as well as indirect expenses of missing the diagnosis of smear-negative PTB. Furthermore, the results of this study strongly indicate that GeneXpert is a helpful addition to the TB toolbox because it is a quick assay that may be used in conjunction with clinical data to make first-line PTB therapy decisions. This can lead to improvements in TB control campaigns.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This study was approved by Peltier General Hospital's ethics board No. 3511/HGP1/DGAT1/2022.

Statement of informed consent

Informed consent was obtained from all subjects involved in the study.

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