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Isolation and Identification the resistance Mycobacterium tuberculosis from the TB patients

Israa Khudhair Obayes [1*], Sarah Kassab Shandaway Al-Zamali [2], Fatima Hakim Obaid [3], Kazem Mehdi Kazem [4]
[1] Department of Diseases and Forensic Medicine, Hammurabi College of Medicine, University of Babylon, Hilla 51002, Iraq
[2] College of Nursing, Department of Medical Microbiology, Telafer University, Telafer, Iraq
[3-4] Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, Hilla 51002, Iraq

* Corresponding Author: **Israa Khudhair Obayes**

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Abstract
Background: Tuberculosis (TB) is a contagious infection that typically affects the airways. Additionally, it has the potential to disseminate to other regions of the body, such as the brain and spine. It is the result of a bacterium known as Mycobacterium tuberculosis.
Objectives: Identification and isolation of Mycobacterium tuberculosis resistance in TB patients. Material and Methods: This investigation involved the collection of sputum samples from 60 suspected tuberculosis patients, 40 of whom were male and 20 of whom were female. Their age ranged from 21 to over 61 years. A specialist clinician clinically diagnosed all tuberculosis cases. The patients in question were confined to the Babylon Center of Tuberculosis and Chest Disease from December 2022 to March 2023. Evaluation of direct acid fast bacilli smear microscopy, Gene Xpert, and Löwenstein-Jensen culturing of sputum for the diagnosis of pulmonary tuberculosis. Results: The GeneXpert was deemed the gold standard test, as it has a higher sensitivity than AFB smear microscopy in respiratory samples. The diagnosis of patients with a high clinical suspicion of PTB requires several minutes to be performed. Conclusions: Tuberculosis continues to be a significant health concern, particularly among individuals aged 21 to 30. The male is more susceptible to tuberculosis than the female. The majority of tuberculosis cases were significantly influenced by incarceration exposure. GeneXpert's molecular methodologies demonstrate exceptional sensitivity in the identification of drug-resistant MTB strains.

Keywords: Tuberculosis, mycobacterium tuberculosis, drug-resistant TB, GeneXpert

1. Introduction

Tuberculosis (TB) is widely regarded as one of the most significant infectious illnesses in the world, and the number of people who are infected with it is increasing [1]. There is a significant amount of morbidity and mortality that is caused by tuberculosis [2]. The common method of acquiring tuberculosis infection is the inhalation of droplets carrying Mycobacterium tuberculosis bacilli (MTB). The clinical symptoms of tuberculosis are a function of the host's immune status [3]. Tuberculosis is an immunological illness; in other words, it is a disease that affects the immune system. According to the World Health Organization (WHO), Iraq is one of seven countries in the Eastern Mediterranean Region that has a high burden of tuberculosis (TB). This burden includes twenty thousand cases of new and relapsed tuberculosis, which accounts for fifty-four percent of all cases. Each year, records show that four thousand deaths result from these twenty thousand cases. The following are some of the common symptoms that may be associated with tuberculosis: Night sweats, fever, chills, loss of appetite, fatigue, and diminished weight are some common symptoms associated with tuberculosis [5]. When an active tuberculosis infection manifests itself, the lungs are the most often affected organ (in ninety percent of cases) [6]. Symptoms may include chest pain and a cough that produces phlegm for an extended period of time. About 25 percent of people will not exhibit any symptoms and will continue to be asymptomatic. People will occasionally cough up blood in small quantities, and in exceedingly rare instances, the infection might erode through the pulmonary artery, resulting in catastrophic bleeding. This is a very rare occurrence.

Tuberculosis more commonly affects the higher lung lobes than the lower lobes [5]. Although tuberculosis primarily affects the lungs, its potential to spread to other organs can lead to some of the most catastrophic clinical outcomes. Extrapulmonary variants account for 15-20% of all tuberculosis cases. One out of every five cases of tuberculosis results in extrapulmonary tuberculosis [8]. EPTB can be primary (at the site of the original infection) or secondary (disseminated). Secondary infection usually happens when bacteria from the primary organ are spread through the blood or lymph, when latent tuberculosis (LTBI) is activated, when infected sputum is swallowed, or when the infection spreads locally from nearby organs [9]. The diagnosis was crucial for detecting tuberculosis infection and confirming the presence of the bacterium. This was achieved through the use of non-cost conventional procedures and molecular approaches, which demonstrated a high level of specificity and sensitivity compared to conventional methods [10]. The use of geneXpert and PCR to confirm MTB infection within a short period of time has the potential to play a role in limiting and terminating the occurrence of infection through the rapid introduction of anti-TB drugs, in addition to limiting the prevalence of multidrug-resistant MTB [11].

Materials and Methods

1. Sputum samples

The diagnosis of pulmonary TB requires a respiratory specimen constitute the most common type of specimen submitted for the culture and isolation of mycobacteria, about 2-5 ml sputum of deeply coughing patients collected at early morning in a sterile container for both microscopy and mycobacterial cultures for patients with suspected pulmonary tuberculosis. Patient's mouth, lips, tongue, or fingers should not touch the inside of the container and should rinse his/her mouth with water, the sputum should consist of thick mucoid material, not saliva [12].

2. Sputum processing

A-Principle

Sputum is a multifaceted specimen that is composed of network mucin molecules, filamentous actin, cell debris, leukocytes, inflammatory mediators, and elastin fibers. Additionally, it contains normal flora bacteria that are embedded within the matrix. The discharge of bacteria that have been trapped within the intricate sputum matrix is a result of the efficient processing of sputum samples. Therefore, the effectiveness of sputum processing protocols is a significant factor in the sensitivity of diagnostic assays for MTB [13]. The mucous is broken down in sputum specimens using a mucolytic agent, and the normal flora present in the sample is suppressed by a decontaminating agent. The normal flora would outgrow the more slowly growing species of mycobacteria, rendering their isolation from clinical samples impossible if this were not done. Initially, the sputum is homogenized and decontaminated using Modified Petroff's method, which employs 4% sodium hydroxide (NaOH) [14].

B-Method

The method of sputum processing according to [15]

3. Diagnosis of *Mycobacterium tuberculosis* in PTB

3.1 Direct microscopy identification of acid-fast MTB

A-Principle

Acid-fast Mycobacteria possess mycolic acid in their outer membrane, which renders the cells hydrophobic and impervious to incubation with aqueous-based dyes like the Gram stain. The cells are incubated with the principal stain, carbolfuchsin, and phenol is employed to facilitate the penetration of the stain into the waxy surface of acid-fast microorganisms. The residual stain is eliminated by treatment with a 1% sulfuric acid solution. Thereafter, the cell is treated with a secondary stain, methylene blue [16].

B-Method

The method of direct microscopy identification using AFB staining was implemented following reference [17].

3.2. Routine culture of MTB

A-Principle

All sputum samples showing AFB+ indicate the selection for culture to confirm the diagnosis of MTB. The present work utilized Lowenstein-Jensen (LJ) media, also known as egg-based media, which comprises eggs, salts, potato flour, amino acids, glycerol, and malachite green to develop MTB. The media was previously prepared in a slant screw cup tube by the laboratory at the center for chronic pulmonary illness. This medium is enriched with antibiotics to inhibit the proliferation of fungus, yeast, and bacteria during the extended incubation period [16].

B-Methods

The specimens cultivated based on [18].

3.3. GeneXpert MTB/RIF assay for molecular detection of MTB

A-Principle [19]

Developed by Cepheid Inc. in Sunnyvale, CA, USA, the Xpert MTB/RIF (Xpert) assay is a cartridge-based, semi-automated, rapid molecular assay that enables quick diagnosis of tuberculosis (TB) by detecting the DNA of MTB and simultaneously identifying most of the mutations that provide resistance to rifampicin (a strong indicator of multi-drug resistant TB). The Xpert assay utilizes polymerase chain reaction (PCR) to identify MTB and RIF resistance. It is designed to integrate and automate specimen processing, nucleic acid amplification, and detection of target sequences (specifically, the 81-bp rpoB gene segment linked to RIF resistance) in simple or complex specimens. Additionally, it probes for mutations associated with RIF resistance. The analysis is executed automatically and finishes in a time frame of 2 hours. The Xpert MTB/RIF test is applicable to sputum samples or processed sputum sediment samples, regardless of whether the specimen smear indicates AFB positivity or negativity. The system comprises a peripheral device, personal computer, barcode scanner, and preconfigured software for conducting tests on gathered specimens and observing the outcomes. This technology necessitates the use of disposable Gene-Xpert cartridges designed for single-use, which contain the PCR reagents. It is advisable to consider clinical specimens, including used cartridges, as potentially capable of spreading infectious pathogens. Adhere to appropriate safety measures, including donning protective disposable gloves, laboratory coats, and eye protection, and implement the safety protocols and recommendations set by your institution [10].

B-Method

The method of GeneXpert MTB/RIF assay according to [20].

Results and Discussion

1 Demographical data

1.1 Gender distribution of TB patients

Among (60) cases with suspected pulmonary tuberculosis, there were 40 (66.6%) of cases for males and the other 20(33.3%) of cases for females. This result revealed a significant increase of PTB infection in males (66%) than female (33%) as shown in Figure (1).

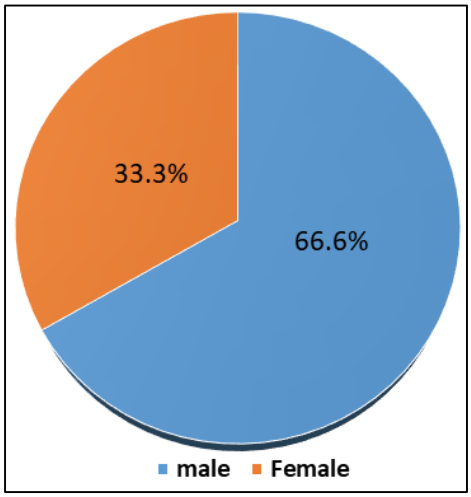

Fig 1: Gender distribution among TB patients

1.2. Age distribution among TB patients

The (60) samples are collected from suspected TB patients from different age groups from 21 to more than 51 years old, as in Table (1).

Table 1: Distribution of TB cases according to age groups of patients

Age-Groups	TB patients No. (%)	
	No. (%)	Percentage (%)
21-30	30	50
31-40	10	16.6
41-50	14	23.3
51-60	6	10
Total	60	100

1.3. Exposure of PTB patients to prison

The present study appeared that a total of 23 confirmed PTB cases there was 10 (43.4 %) only male prisoners, nine case of these was MDR MTB as shown in the table (2).

Table 2: Exposure of PTB patients to prison and the MDR evolved cases among prisoners

Gender of PTB Patients	Prisoners	MDR cases
Male	10(43.4%)	9(39.1%)*
Female	0(0%)	0(0%)
Total	10	9(39.1%)
* MDR case from prisoners, PTB: pulmonary tuberculosis, MDR: multidrug-resistant		

2. Identification of M. Tuberculosis

2.1. Diagnosis of PTB infections

A total of 60 patients, who were suspected to have pTB after showing clinical features and radiological changes for tuberculosis were submitted for laboratory diagnosis. Among these patients, there were 14 (23.3%) positive cases who were diagnosed by Ziehl–Neelsen staining, 2 (3.3%) cases were diagnosed by culture on L-J medium and 23(38.3%) cases were diagnosed by Gene Xpert.

2.1.1. Direct Microscopic Smear (AFB)

Out of the 60 cases of confirmed pulmonary tuberculosis, there were 14 (23.3%) AFB smear positive and 46(76.6 %) were AFB negative as in table (2) figure (2).

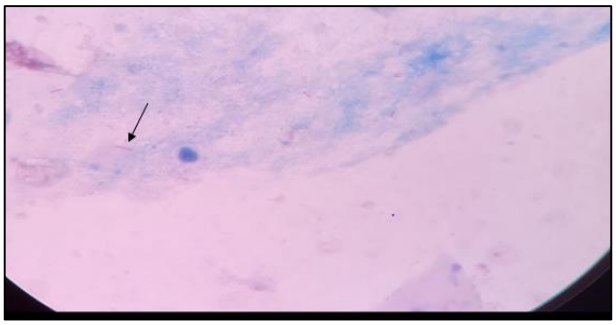

Fig 2: Bacteriological diagnosis of *Mycobacterium tuberculosis.* The field of AFB sputum smear microscopy under 100X oil immersion lens show red-purple MTB bacilli with Ziehl Neelsen stain

2.1.2. The Culture of Sputum on Solid L-J Medium

A total of 2 instances (3.3%) tested positive for culture. This finding is supported by a study conducted by Sarin *et al.*, (2010) which demonstrates that the majority of smear positive cases are culture positive. However, with therapy, the likelihood of dead bacilli increases, and in 98-100% of cases, smear positive result can be culture negative. The 28 (47%) culture negative instances of pTB in this investigation may be ascribed to the anti-TB chemotherapy administered by the physician, which enhanced the likelihood of dead Mycobacterium that could be detected by AFB staining, as shown in figure (3).

Fig 3: Growth of M. tuberculosis on L-J Medium

The present study establishes that the GeneXpert test is the gold standard for diagnosing patients with high clinical suspicion of PTB. It has a better sensitivity than AFB smear microscopy in respiratory samples, which takes several minutes to complete. As shown in table (3), the accuracy of a tuberculosis diagnostic test necessitates a rapid test with optimal specificity and sensitivity (sensitivity 100%, specificity 98.8%) and simplicity of execution, such as the molecular test GeneXpert.

Table 3: The Tests Used for Diagnosis Pulmonary Tuberculosis

Type of test	Positive Cases NO. (%)	Negative Cases NO. (%)
AFB smear	14 (23.3%)	46(76.6 %)
Culture	2 (3.3%)	58(96.6%)
Gene Xpert	23(38.3%)	37(61.6)

Discussion

The rate of males attending TB patients was found to be higher than the rate of females, according to the findings of Corroding [1]. According to the Hilla City Tuberculosis Center, which explains that because of the multiple risk factors, which include smoking, drunkenness, drug misuse, and the size of samples, tuberculosis is more prevalent among males than it is among girls. In spite of this, it is possible that biological factors, such as sex hormones, are responsible for a large portion of the disparity in susceptibility to tuberculosis that exists between males and daughters [21]. According to the findings shown in Table 1 tuberculosis was discovered in people of all age groups. Tuberculosis is most frequently seen in people who are between the ages of 21 and 30 years old, despite the fact that the field suggests that there is a higher tuberculosis infection incidence in younger age groups in comparison to other age groups. As a result of this finding, it is recommended that tuberculosis infect the active age group since this age group has direct effect over the economy of the family, the public, and the nation. This conclusion was consistent with the findings of [22], as well as those of [23], who demonstrated that there was a highly significant relationship between the age groups that were analyzed (the prevalence of tuberculosis infection was higher in younger age groups).The results of the current study were almost identical to those that were recorded by Abdul Abbas *et al*. (2018). For example, they demonstrated that the majority of tuberculosis cases in the Babylon province of Iraq occur in individuals between the ages of 18 and 35. It was found that the prevalence of jail exposure population across all of the study population was 23 samples, with a high percentage of males. This finding was in some ways consistent with the prevalence of the study, which was found to be 55.7 percent.

The current study found that the number of males who were incarcerated was much higher than the number of females; however, the interaction with convicts did not appear to differ significantly between males and females. According to the World Health Organization (WHO), the term "prison" refers to any place of detention for pre-trial and convicted prisoners. Prisons are responsible for an increase in tuberculosis incidence that is up to one hundred times higher than that of the civilian population. Prisons are responsible for up to 25 percent of tuberculosis cases and up to 24 percent of multidrug-resistant tuberculosis cases [25].

Prisons are a significant social vector for the transmission of tuberculosis and the selection of multidrug-resistant strains. This is due to a number of factors, including a higher effective contact rate in overcrowding, inadequate ventilation, inadequate medical care, delayed diagnosis, difficulties in identifying and isolating the infected prisoner during treatment, a larger population, and the availability of risk factors such as poverty [26].

For the purpose of identifying individuals who are currently experiencing active tuberculosis [27], sputum smear microscopy to detect acid-fast bacilli (AFB) is a method that is not only quick and affordable but also reasonably simple to perform. Among the most significant drawbacks of the AFB smear are the following: As a result of the moderate and variable sensitivity of the AFB smear, it is necessary to have between 6000 and 10,000 organisms per milliliter of material in order to register as a positive case [28]. Not only that, but direct microscopy is unable to differentiate between mycobacteria that cause tuberculosis and those that do not cause tuberculosis [28]. The method, notwithstanding its specificity, is not capable of identifying drug-resistant strains of tuberculosis (MDR-TB) (WHO, 2007).

According to the findings of a study conducted in the area by Abdul Abbas *et al*. (2018), sputum AFB smears exhibited a sensitivity of 76% and a specificity of 100%, respectively. The results of another screening study for AFB sensitivity and specificity carried out by [29] demonstrated that sputum AFB smears had a sensitivity of 67.5% and a specificity of 97.5%. A different piece of research, on the other hand, found that the specificity and sensitivity of AFB in comparison to culture were 89% [30].

When compared to sputum smears, sputum cultures have a significantly higher sensitivity (the threshold for culture to be positive is 100 bacilli per ml of sample). However, the growth of tuberculosis bacilli on traditional solid medium requires within six weeks, which consequently delays appropriate treatment in the absence of a confirmed diagnosis [31]. Their research came to the conclusion that the genexpert was a superior diagnostic test in comparison to smear microscopy. Furthermore, it has the potential to drastically cut down on the number of false negatives and the amount of time it takes to begin treatment [19, 32].

According to the findings presented here, the GeneXpert test is considered to be the gold standard since it has better sensitivity than the AFB smear microscopy in respiratory samples. Additionally, the GeneXpert test requires several minutes to complete diagnosis on patients who have a high clinical suspicion of PTB (Agrawal *et al*., 2016). The accuracy of a tuberculosis diagnostic test requires a test that is both rapid and easy to run, with a high level of specificity and sensitivity (sensitivity of one hundred percent and specificity of ninety-eight percent). One example of such a test is the molecular test, which is similar to GeneXpert and has been used as the gold standard test to compare with other methods for routine diagnosis [33].

Conclusions

Tuberculosis is still a significant public health concern that affects people of all ages, but particularly those between the ages of 21 and 30. The man is more likely to contract tuberculosis than the female, and vice versa. In the majority of tuberculosis cases, the prison exposure constituted a significant risk factor. When it comes to the detection of drug-resistant MTB strains, the molecular approaches developed by GeneXpert demonstrate a high level of sensitivity.

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