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Advancements in tuberculosis diagnostics: A comprehensive review of laboratory confirmation methods

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Abstract

This literature review provides a comprehensive analysis of laboratory confirmation methods for tuberculosis (TB) diagnostics. It encompasses historical perspectives and contemporary advancements, offering insights into the evolution of TB diagnosis over time. The review focuses on three primary diagnostic methods: microscopic examination, culture, and molecular techniques. Microscopic examination, initiated by Dr. Robert Koch in 1882, remains a fundamental approach in TB diagnostics. The simplicity, cost-effectiveness, and widespread applicability make it a cornerstone in preventive TB programs. Noteworthy advantages include its affordability and minimal technical qualifications, contributing to its enduring significance. Culturing methods, particularly on solid media like Lowenstein-Jensen, play a vital role in diagnosing TB. Despite the extended duration required for results, this approach boasts higher sensitivity compared to acid-fast bacilli smears. The ability to differentiate between Mycobacterium tuberculosis (MTB) and non-TB entities enhances its diagnostic precision. Recommended by the World Health Organization (WHO) for both adults and pediatric populations, these automated tests significantly expedite diagnosis by detecting MTB and rifampicin resistance simultaneously. Additionally, the paper discusses the MGIT 960 system, emphasizing its role in efficiently detecting mycobacteria in clinical specimens. The review evaluates the strengths and limitations of each diagnostic method, considering factors such as sensitivity, speed, and applicability. In conclusion, this literature review provides a comprehensive understanding of TB diagnostic methods, bridging historical practices with contemporary technologies. It serves as a valuable resource for healthcare professionals, researchers, and policymakers involved in TB control programs globally.

Keywords: Tuberculosis; Communicable disease; Diagnostics; Laboratory; Methods

1. Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains a global health challenge, impacting millions of lives each year. The fight against TB necessitates accurate and timely diagnostic methods to facilitate early detection, treatment initiation, and effective disease management. This literature review delves into the rich history and contemporary landscape of laboratory confirmation methods for TB, exploring the evolution of diagnostic techniques from microscopic examinations initiated by Dr. Robert Koch in 1882 to modern molecular advancements like the GeneXpert MTB/RIF test. In the late 19th century, Dr. Robert Koch's groundbreaking work paved the way for TB diagnostics through microscopic examination of sputum smears. This simple yet revolutionary method laid the foundation for TB control programs globally. The effectiveness of microscopic examination in identifying acid-fast bacilli

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(AFB) in sputum specimens remains unparalleled in terms of accessibility, cost-effectiveness, and efficiency [1]. This historical perspective underlines the enduring significance of basic microscopy in preventive TB efforts. The advantages of this method extend beyond its cost-effectiveness to include low technical qualifications, making it a vital tool in resource-constrained settings. [2] Culturing methods, particularly on solid media such as Lowenstein-Jensen, emerged as a pivotal diagnostic approach for TB. Though time-consuming, the culture method provides higher sensitivity compared to AFB smears and plays a crucial role in differentiating between Mycobacterium tuberculosis (MTB) and non-TB entities [3]. This differentiation is especially relevant in cases where AFB-negative results may not necessarily rule out TB. Despite the limitations associated with the extended duration required for results, the cultural method's role in enhancing diagnostic precision cannot be overstated. The molecular era witnessed significant strides in TB diagnostics, with the advent of GeneXpert MTB/RIF. Recommended by the World Health Organization (WHO) in 2010 for diagnosing TB in adults and later extended to pediatric populations in 2013. This molecular method revolutionized TB diagnosis. This molecular diagnostic tool has streamlined diagnostic workflows, offering rapid and accurate results crucial for timely treatment initiation. In addition to these established methods, the MGIT 960 system, combining solid media with broth-based techniques, stands as a notable player in efficiently detecting mycobacteria. Its significance in contemporary diagnostics lies in its ability to rapidly and accurately identify mycobacterial infections using human clinical specimens. This literature review aims toprovide a comprehensive understanding of the diagnostic methods employed in the battle against TB, evaluating their strengths, weaknesses, and contributions to global TB control efforts. As we navigate through the historical underpinnings and cutting-edge technologies, this review seeks to inform healthcare professionals, researchers, and policymakers engaged in the ongoing fight against this persistent global health threat.

2. Review Content

2.1. Pulmonary Tuberculosis

According to the Ministry of Health (2018), TB or tuberculosis is an infectious disease caused by the bacterium Mycobacterium tuberculosis. While it predominantly infects the lungs, it is possible to affect other organs, leading to organ damage such as in the brain, kidneys, and spine. The disease is contagious when individuals with TB cough, sneeze, or speak in our direction, commonly referred to as airborne infection, which involves transmission through the air. The history of Tuberculosis (TB) can be traced back 3.3 million years. In the 18th and 19th centuries, tuberculosis reached epidemic levels in Europe and North America. However, in the 20th century, the number of tuberculosis cases declined in developed countries. Nevertheless, TB remains a serious concern in developing or low- to middle-income countries and in developed countries, especially with the increasing emergence of drug-resistant strains and the HIV epidemic. [4]

2.2. Epidemiology

One of the diseases causing the most morbidity and mortality is Tuberculosis (TB). Worldwide, there are 9 million new cases of TB annually. Among these cases, 2 million new cases result in death. Out of the 9million new cases, 1 million new cases are found in children under 15 years old [5]. According to the World Health Organization (WHO), 33% of the world's population has been infected with TB. WHO also estimates that TB is the infectious disease causing the most mortality in both children and adults. TB occurs in every part of the world. In 2020, the highest number of TB cases occurred in the Southeast Asia region with 43% of new cases, followed by the African region with 25% of new cases [5].

Looking at a global perspective, in individuals diagnosed as HIV-negative, there was an increase in the number of deaths by approximately 1 million between 2019 and 2020. In 2019, WHO reported 1.2 million deaths, while in 2020, there were 1.3 million deaths. This increase also occurred in individuals diagnosed as HIV-positive. In 2019, there were 209,000 deaths, rising to 214,000 in 2020. WHO's 'Global Tuberculosis Report' published in 2021 stated that the officially categorized mortality due to tuberculosis (TB) in 2020 was 1.3 million. This figure is nearly double the deaths caused by HIV/AIDS in 2020. Mortality due to TB was significantly affected by the COVID-19 pandemic in 2020 compared to HIV/AIDS. In contrast to TB cases, deaths caused by HIV/AIDS continued to decline between 2019 and 2020. According to WHO's latest report on global estimates of deaths, tuberculosis (TB) ranks 13th among the leading causes of death worldwide and is a crucial cause among single infectious agents.

2.3. Etiology

Mycobacterium tuberculosis is the bacterium responsible for TB or Tuberculosis. This bacterium is acid-fast (AFB) and alcohol-resistant. Mycobacterium tuberculosis has obligate aerobic, facultative, and intracellular characteristics. It has a high concentration of lipids in its cell wall, making it resistant to certain antibiotics and challenging to stain with gram staining. Mycobacterium tuberculosis can adapt to extreme acidic and basic conditions. The spread of this bacterium

occurs person-to-person, through aerosolized droplet particles. The infectious droplets from TB or Tuberculosis patients range from 0.65 μm to >7.0 μm [6].

The primary portal causing most cases of tuberculosis is the lungs. Often, little or no clinical symptoms appear in the initial local infection in the peripheral lungs, mainly caused by inhaled tubercle bacilli. Symptoms such as mild fever, fatigue, and other hypersensitivity symptoms usually arise due to the immune system's response to the organism until the development of tuberculin hypersensitivity, typically around 4–6 weeks. The evidence of tuberculosis progression is not found in the majority of patients, and the process is halted by local and systemic defenses. Rupture into the pleural cavity can occur with the development of pleuritic tuberculosis with effusion. This happens because the primary focus is usually subpleural. This condition typically occurs with classic symptoms but nonspecific pleuritis. The initial spread of tuberculosis occurs through local dissemination to the hilar lymph nodes. The main clinical manifestations of tuberculosis can emerge due to hematogenous spread from the organism, resulting in both pulmonary and extrapulmonary foci. Radiographically, the spread is manifested by lymph node enlargement, subsequent calcification of both lymph nodes and parenchymal lesions. This is the classical Ghon complex suggestive not only of tuberculosis infection but also diseases like histoplasmosis.[7]

2.4. Pathogenesis

Tuberculosis (TB) spreads through the air. People can contract tuberculosis by inhaling air containing bacteria from individuals diagnosed with tuberculosis. This occurs when individuals with tuberculosis cough and speak, releasing infectious droplets and aerosolized particles into the surrounding air. These particles are very small, with a diameter of 0.5 – 5 μm [8]. When a person sneezes, they can release around 40,000 small particles [9]. Each droplet or small particle released by an infected person can cause the transmission of this disease [13]. Tuberculosis does not spread through handshakes, sharing food and drinks, or sharing toothbrushes.

2.5. Management of Pulmonary TB

According to the 2010 guidelines from the American Lung Association on the 'Management of Tuberculosis, if you have active TB disease, you may be treated with a combination of antibacterial drugs for a period of six to 12 months. The most common treatment for active TB includes isoniazid (INH) in combination with three other drugs—rifampin, pyrazinamide, and ethambutol. You might start feeling better within a few weeks of starting the medication, but TB treatment takes longer than treating other bacterial infections. It's crucial to continue taking the prescribed medication for the duration specified by your doctor to prevent a relapse, make it more challenging for the disease to reoccur, and avoid spreading it to others. Not completing the entire course of your treatment can also contribute to drug-resistant TB [10].

2.6. Diagnostic Methods for TB Laboratory Confirmation

2.6.1. Microscopic Methods

In 1882, a doctor named Robert Koch diagnosed cases of TB using microscopic examination of smears. The main factor in the TB preventive program for confirming diagnosis, evaluation, and follow-up treatment from the examination of three morning sputum specimens is microscopic examination of sputum [11]. Microscopic examination of sputum is the easiest, cheapest, efficient, specific, and can be conducted in all laboratory units. This method has advantages, such as low cost and low technical qualification [12]

2.6.2. Culture Method

To diagnose TB in suspected patients, one method that can be used is the culture method. Approximately 50 – 100 bacteria/ml of sputum are needed to cultivate bacteria. On solid media, usually Lowenstein-Jensen, mycobacterial culture has historically been performed. Although this method takes 4-6 weeks, which is a limitation in its use, it has higher sensitivity than acid-fast bacilli (AFB) smears and can detect as low as 1102 bacilli per mL. Moreover, this method can differentiate between Mycobacterium tuberculosis (MTB) and non-TB, which are challenging to distinguish under microscopic examination. Because patients with AFB-negative but culture-positive results are less contagious and have a more gradual disease progression, they are not considered dominant contributors to the ongoing epidemic. Therefore, patients with such criteria are historically given lower priority in TB control programs in developing countries [2].

2.6.3. Molecular Methods

To confirm the diagnosis of pulmonary TB in adults, WHO recommended the use of GeneXpert MTB/RIF in 2010. Then, in 2013, WHO again recommended the GeneXpert MTB/RIF test for diagnosing TB in children and extrapulmonary cases

(WHO, 2017). The molecular diagnostic method (Xpert MTB/RIF) is an advanced diagnostic tool used to automatically detect the presence of Mycobacterium tuberculosis (MTB) bacteria. To detect bacterial resistance to rifampicin, the molecular diagnostic method (Xpert MTB/RIF) can also be used.

2.6.4. MGIT 960 SYSTEM

Health facilities such as laboratories play a crucial role in quickly and accurately detecting and identifying mycobacteria using human clinical specimens, given the high incidence of tuberculosis and mycobacterial diseases. Currently, the combination of solid media with broth-based methods has become the existing reference standard for the diagnosis of mycobacterial infections. Among all liquid media methods, the semiautomated radiometric BACTEC 460 TB system is widely recognized and has become a benchmark for detecting mycobacteria. This system is also known for its undeniable limitations, such as the use of radioactive materials, complex installation processes, the risk of needlestick injuries, the potential for cross contamination, and a lack of computerized data management. Cross contamination is condition where bacteria are transferred from one place to another [13]. Therefore, a new generation has emerged, namely the MGIT 960 system. The MGIT system features small tubes that will be filled with an oxygen-charged solution, facilitating the growth of tb-causing bacteria. Subsequently, TB medication will be mixed into the solution along with TB bacteria, for example, froam a patient's sputum. The process of detecting TB bacteria can take between 6 to 10 days [14].

3. Conclusion

In conclusion, this review highlights the historical importance of microscopic examination, the continued relevance of culturing methods, and the transformative impact of molecular diagnostics in tuberculosis diagnosis. Balancing accessibility and accuracy are crucial for effective TB management. This concise overview serves as a valuable resource for global stakeholders in the ongoing fight against tuberculosis.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare there is no conflict of interest in this study.

Statement of ethical approval

This study used data from Dr. Soetomo General Academic Hospital Surabaya. This study has received permission and approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital with number 1296/LOE/301.4.2/IV/2023.

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