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Comparative Analysis of Molecular Diagnostic Techniques for Pulmonary Tuberculosis: A Literature Review

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ABSTRACT

Pulmonary tuberculosis is a global health problem that requires early and precise diagnosis for effective control. In this literature review, we compare several molecular diagnostic techniques used in the detection of pulmonary tuberculosis, such as Polymerase Chain Reaction (PCR), Whole Genome Sequencing (WGS), and Next Generation Sequencing (NGS), as well as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based Diagnostic Techniques. Polymerase Chain Reaction stands out for its high sensitivity, although time-consuming and high operational costs. Meanwhile, whole genome sequencing and next generation sequencing have detailed tuberculosis (TB) strain identification capabilities but have high costs and limited availability. On the other hand, CRISPR-based diagnostic techniques offer speed and low cost but are still in the advanced stages of development. Challenges in implementing new techniques include technical barriers, logistics, and improving sensitivity and specificity. Suggestions for future research include the development of more effective, faster, and affordable techniques, especially in developing portable diagnostic tests for accessibility of pulmonary tuberculosis diagnosis in various regions.

1. Introduction

Pulmonary tuberculosis (pulmonary TB) remains a significant global health problem despite intensive efforts to control the disease. According to the latest statistics from the World Health Organization (WHO), pulmonary TB remains one of the leading causes of death from infectious diseases all over the world, with millions of new cases reported each year. In 2023, the prevalence of TB is estimated at 10.8 million people worldwide, including 6.0 million men, 3.6 million women, and 1.3 million children.[1–3] In Indonesia, the situation is not much different, with prevalence rates that are also quite high, especially in areas with high population density and limited accessibility of health services.[4,5]

The importance of early diagnosis in controlling pulmonary TB cannot be doubted. According to the increasing prevalence of TB, rapid and precise diagnosis of pulmonary TB can help restrict the disease's spread and enable patients to receive the right therapy right away. [6,7] However, diagnosing pulmonary TB is not always easy, especially in subclinical cases or in patients with atypical symptoms.[8,9] Current tests might miss these cases due to lower sensitivity, drug resistance detection gaps, and operational constraints, leading to delayed diagnosis and treatment. This is why the development of more sensitive, specific, and efficient diagnostic methods is so important in global efforts to reduce the burden of pulmonary TB.

The aim of this literature review is to comprehensively review the current molecular diagnostic methods used in the detection of pulmonary TB. In this review, we will compare the effectiveness and efficiency of these various molecular diagnostic methods, taking into account factors such as sensitivity, specificity, cost, time required for results, and ability to detect drug-resistant TB variants. Thus, it is hoped that this review will provide a deeper understanding of the latest developments in the diagnosis of pulmonary TB using molecular approaches, as well as provide useful insights for the development of diagnostic and control strategies for pulmonary TB in the future.

2. Methods

The methodology used in this literature review includes two important aspects: data sources and

literature selection criteria. The primary data sources used in this review are leading medical and scientific databases such as PubMed, ScienceDirect, Google Scholar, and Web of Science. These various databases provide broad access to relevant literature on molecular diagnostics for pulmonary TB from various points of view. To ensure the quality and relevance of the literature included in the review, strict selection criteria were used. The selected articles must be published in accredited scientific journals and have gone through a peer-review process. Additionally, articles should specifically address molecular diagnostic methods such as Polymerase Chain Reaction (PCR), Loop-mediated Isothermal Amplification (LAMP), Whole Genome Sequencing (WGS) and Next Generation Sequencing (NGS), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based Diagnostic Techniques, and Chip-based Technologies and Microarrays. Empirical data about the effectiveness, sensitivity, specificity, advantages, and disadvantages of each diagnostic method are also important criteria. Articles that are too old (more than 10 years) or irrelevant to recent developments in molecular diagnostics of pulmonary TB will be excluded from the review. By applying these criteria, it is hoped that this review can provide an in-depth and up-to-date analysis of molecular diagnostic methods for pulmonary TB, which can be a useful guide for health practitioners and researchers in this field.

3. Pathogenesis and Epidemiology of Pulmonary TB

Pulmonary tuberculosis (pulmonary TB) is caused by *Mycobacterium tuberculosis* infection, a bacterium that attacks the human respiratory system.[10] The pulmonary TB infection cycle begins when the bacteria enter the respiratory tract through infected air, usually via droplets from active TB sufferers. The mechanism for spreading of *M. tuberculosis* occurs through inhalation of bacteria that are inhaled into the respiratory tract, then the bacteria infect macrophage cells in the lungs.[11,12]

After entering macrophage cells, *M. tuberculosis* evades the immune system and survives in these cells. The bacteria can remain latent in the body for years without showing clinical symptoms, becoming latent TB.[13,14] However, if the immune system is weakened, for example, due to stress or other illnesses, bacteria can multiply and cause active TB with symptoms such as chronic cough, fever, weight loss, and others.[15–17]

The infection of *M. tuberculosis* begins when tiny aerosol particles containing the bacteria are inhaled by a person and land in the lower lungs of a new host. Importantly, in around 95% of cases the primary infection is asymptomatic, and it can either resolve spontaneously or result in a latent form where the bacteria persist without causing disease. The bacteria then attract infected macrophages to the lung's surface, where they help move the bacteria to deeper tissues by passing through the lung epithelium.[18,19]

In the latent phase, the immune system controls *M. tuberculosis* propagation, forming granulomas—organized structures containing immune cells that can trap the bacteria. While most people with latent TB do not go on to develop active disease, about 5–10 percent eventually reactivate, especially those whose immune systems are compromised. An organized collection of differentiated macrophages and other immune cells known as a granuloma is created when a fresh round of macrophage recruitment to the initial infected macrophage is started. In this phase, the granuloma spreads infection by enabling bacteria to infect the just arrived macrophages. The granuloma can limit bacterial growth as adaptive immunity grows. Nonetheless, in a variety of situations, the infected granuloma macrophages may die, creating a necrotic core that aids in the development of the bacterium and its spread to the subsequent host.[20]

Because *M. tuberculosis* inhibits phagosome-lysosome as well as performs apoptosis escape, it can persist and replicate, and other *M. tuberculosis* can escape and infect further cells. Active pulmonary TB results from failure of the immune response, allowing unrestricted replication of *M. tuberculosis* and symptomatic disease with cough, weight loss, fever, and night sweats. This stage is infectious and if not treated can cause extreme damage to the lungs. .[20]

The epidemiology of pulmonary TB has greatly increased. With a projected 10.6 million cases of TB and 1.3 million fatalities from the disease worldwide as of 2023, TB is the second most common infectious killer after COVID-19. India, China, Pakistan, and Indonesia had the greatest cumulative incidence. This epidemiology is influenced by socioeconomic factors, access to health services, and the prevalence of TB in the surrounding environment.[21] Areas with high population density, poor sanitation conditions, and limited access to TB treatment tend to have higher rates of TB cases. Low-income groups are disproportionately affected by TB because of overcrowding, inadequate nutrition, and restricted access to healthcare. Delays in diagnosis and treatment are another effect of poverty because people may put off getting medical care because of the expense or lack of knowledge, which can result in continuous transmission among communities. Tuberculosis spreads more easily in high-density environments, especially in urban slums. The danger of airborne tuberculosis transmission is increased by cramped living quarters and inadequate ventilation. Since inadequate sanitation and hygiene are linked to overall ill health and an increased risk of infection, they also contribute to the incidence of tuberculosis.[22,23]

4. Basic Principles of Molecular Diagnostics

Molecular diagnostics for pulmonary TB are based on molecular technologies that enable direct detection of *M. tuberculosis* DNA or RNA in clinical samples. These technologies include methods such as

PCR, LAMP, WGS, and NGS, CRISPR-based Diagnostic Techniques, and Chip-based Technologies and Microarrays. The basic principle of molecular diagnostics is the amplification of specific genetic targets of *M. tuberculosis*, such as the IS6110 gene or the hsp65 gene, using special primers.[24–26] Through a process amplification, the number of genetic targets that can be detected increases significantly, so that even very small amounts of bacteria can be detected. The results of this amplification are then analyzed to determine the presence of *M. tuberculosis* in the sample.

The main advantages of molecular diagnostics are their speed and high sensitivity.[27] These methods are able to detect pulmonary TB more quickly than conventional methods such as bacterial culture, allowing earlier diagnosis and more effective treatment. In addition, molecular diagnostics can also be used to detect drug resistance, allowing more targeted treatment according to the sensitivity profile of the detected bacteria.[27]

5. Molecular Diagnostic Techniques

a. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is a very sensitive and specific DNA amplification technique. The working principle of PCR involves several thermal stages, including denaturation of target DNA, primer hybridization, and elongation by the DNA polymerase enzyme.[28,29] Variants include RT-PCR (Reverse Transcription PCR) which is used to detect RNA, and qPCR (quantitative PCR) which allows the quantification of target DNA. In TB diagnostics, PCR is used to detect *M. tuberculosis* DNA in clinical samples such as sputum, cerebrospinal fluid, or other body tissues, with high sensitivity and fast result times.[30,31]

b. Whole Genome Sequencing (WGS) and NGS (Next Generation Sequencing)

The techniques of WGS and NGS are capable for identifying TB strains and drug resistance in great detail. Whole Genome Sequencing (WGS) comprehensively sequences entire bacterial genomes, enabling analysis of genetic mutations associated with drug resistance. Next Generation Sequencing (NGS) speeds up the sequencing process by enabling the sequencing of several DNA fragments in parallel.[32] This technique provides more complete and in-depth genetic information, important in understanding TB epidemiology and optimizing treatment with appropriate antibiotics.

c. CRISPR-based Diagnostic Techniques

CRISPR is used to detect specific DNA from *M. tuberculosis* with high sensitivity. CRISPR-based diagnostic techniques offer the potential for rapid and accurate diagnosis of pulmonary TB.[33,34] The combination of CRISPR with other techniques such as Loop-mediated Isothermal Amplification (LAMP) allows the development of simple and rapid diagnostic tests that can be used in the field or in areas with limited resources.[35,36]

d. Chip-based Technologies and Microarrays

Chip and microarray-based technologies are used in rapid detection and genetic profiling of TB. Microarrays are platforms that enable the simultaneous detection of multiple genetic targets, whereas chip-based technologies such as DNA chips or biochips enable analysis of the genetic complexity of *M. tuberculosis*. This technique is often used in research to understand the genetic diversity of TB and support the development of more sophisticated diagnostic methods.[30,31,37]

e. LAM (Lipoarabinomannan) Assay

LAM (Lipoarabinomannan) Assay is a test used especially in HIV patients to detect LAM antigen from *M. tuberculosis* cell walls in urine.[2,38] This test has important utility in detecting pulmonary TB in patients with immunodeficiency status, due to its relatively high sensitivity in this population. However, its weakness is the lack of specificity, especially in non-HIV patients. People with a greater immunological response typically respond less well to the LAM test. Due to severe TB infections, HIV-positive patients with low CD4 counts excrete more LAM in their urine than non-HIV patients or those with higher CD4 levels. It is more difficult for the LAM test to differentiate TB from other illnesses in non-immunocompromised individuals because to this decreased concentration. People with a greater immunological response typically respond less well to the LAM test. Due to severe TB infections, HIV-positive patients with low CD4 counts excrete more LAM in their urine than non-HIV patients or those with higher CD4 levels. It is more difficult for the LAM test to differentiate TB from other illnesses in non-immunocompromised individuals because to this decreased concentration.[32]

6. Comparison of Diagnostic Methods

a. Accuracy and Speed

Polymerase Chain Reaction (PCR) is a very accurate method for detecting DNA of *M. tuberculosis*, with sensitivity reaching one copy of the bacterial genome. However, the weakness of PCR is that it requires a relatively long time because the DNA amplification process is time-consuming.[37,39] In contrast, WGS and NGS have a high level of accuracy in identifying TB strains and drug resistance mutations, although they require more time due to sequencing the entire bacterial genome and complex data analysis.[40,41] On the other hand, CRISPR-based Diagnostic Techniques offer high accuracy and faster time, suitable for diagnostic applications that require fast results, making it an attractive alternative to with PCR and WGS.[36,42]

b. Cost and Availability

Polymerase Chain Reaction (PCR) has lower costs compared to WGS and NGS, but still requires special laboratory equipment and expensive reagents. For PCR testing of M. tuberculosis, BSL-2 labs with enhanced biosafety measures are commonly used.

However, if handling live cultures or conducting procedures that might produce infectious aerosols, then a BSL-3 setting would be necessary.[43,44] Although commonly available in medical and research laboratories, the infrastructure and equipment required are not always available in every healthcare facility. On the other hand, the cost of WGS and NGS are relatively high due to sophisticated technology and complex data analysis, especially in areas with limited resources.[45] CRISPR-based Diagnostic Techniques, which are not cheap, have the potential to be developed into portable diagnostic tests that are suitable for use in the field or in areas with limited access to laboratory infrastructure.[34]

c. Advantages and Disadvantages

Polymerase Chain Reaction (PCR) has the advantages of high sensitivity, good specificity, and the ability to detect drug resistance.[46] However, the main drawback is the time required for results and high operational costs. On the other hand, Whole Genome Sequencing and Next Generation Sequencing have advantages in identifying TB strains in great detail and detecting drug resistance mutations, but the costs are high, time required, and availability is limited in areas with limited resources is a drawback. CRISPR-based Diagnostic Techniques offer speed, accuracy, and lower costs, as well as the potential for the development of portable diagnostic tests, although they are still in further stages of development and validation, and are dependent on the continued development of CRISPR technology. Achieving a high degree of repeatability, improving sensitivity for extremely low quantities of bacterial or viral genetic material, and guaranteeing specificity to avoid false positives are the main concerns. Additionally, there are still issues with standards and regulations, especially with regard to global scalability. Moving from the experimental phases to widespread clinical adoption of many CRISPR diagnoses still presents logistical challenges, particularly outside of well-equipped research facilities.[37,39]

7. Challenges and Obstacles

a. Issues in the Implementation of New Techniques

Implementation of new molecular diagnostic techniques faces numerous technical and logistical obstacles. One of them is the need for adequate laboratory infrastructure, including the special equipment and technical knowledge required to operate it. When establishing a biological testing laboratory for tuberculosis (TB), specific safety and medical requirements must be adhered to in order to protect laboratory personnel, patients, and the public from the risks associated with *Mycobacterium tuberculosis*, which is an airborne pathogen. The laboratories need to validate the method according to some requirement validation guidelines. In addition, the availability of quality reagents and chemicals is also an important factor in successful implementation. The availability of human resources trained to carry out this technique is also a challenge, especially in areas with limited resources. Integration of new technologies into existing health systems also requires good coordination between various related parties, such as health service providers, governments, and research institutions.[47]

Table 1. Accuracy, speed, cost, availability, advantages and disadvantages for the five molecular diagnostic techniques for pulmonary TB

Criteria	PCR	WGS and NGS	CRISPR-based	Chip-based Tech. & Micr.	LAM
Accuracy	Tall	Tall	Tall	Tall	Tall
Speed	Relatively Slow	Slow	Fast	Slow	Fast
Cost	Tall	Tall	Tall	Tall	Low
Availability	Common in laboratories	Common in laboratories	In development	Limited to the laboratory	Limited to the laboratory
Advantages	High sensitivity and good specificity, used for RNA detection (RT-PCR) and DNA quantification (qPCR), available in medical and research laboratories	Identification of TB strains and drug resistance mutations in detail. It is important to understand TB epidemiology and appropriate treatment with antibiotics	High sensitivity in detecting *M. tuberculosis specific DNA*, Potential for rapid and accurate diagnosis of pulmonary TB, Lower costs, development of portable diagnostic tests	Rapid detection and genetic profiling of TB via chip or microarray platforms, supporting the development of more advanced diagnostic methods	High sensitivity in detecting pulmonary TB in HIV patients, fast results, and lower costs
Disadvantages	Requires a relatively long time for results, high operational costs, requires special laboratory infrastructure and equipment	High cost, relatively long time required, Limited availability in areas with limited resources	Still in the stage of further development and validation	Relatively long time required, high costs, limited availability in areas with limited resources	Less specific for non-HIV patients, it cannot be used generally for all TB patients

b. Sensitivity and Specificity

A major challenge in improving the sensitivity and specificity of molecular diagnostic techniques is ensuring that the tests can detect targets accurately without providing false positive or false negative results. Increased sensitivity means the test is able to detect even very small amounts of the target being sought, while specificity refers to the ability of the test to specifically identify the target without providing a false response to the substance or other substance. Factors that can influence sensitivity and specificity include sample quality, appropriate sampling process, specific primer or probe design, and careful validation and testing of the diagnostic method used. With these challenges in mind, the development and validation of molecular diagnostic techniques continues to ensure quality and accuracy in the diagnosis of diseases such as pulmonary tuberculosis.[42]

8. Conclusion

From a review of molecular diagnostic techniques for pulmonary tuberculosis, it can be concluded that each technique has advantages and disadvantages that need to be considered. Polymerase Chain Reaction (PCR) has high sensitivity but requires a relatively long time and quite high operational costs. Whole Genome Sequencing (WGS) and Next Generation Sequencing (NGS) are able to identify TB strains in detail but their costs are high and their availability is still limited. CRISPR-based Diagnostic Techniques offer speed, low cost, and potential for the development of portable diagnostic tests, but are still in the stages of further development and validation.

For future research, it is recommended to continue to develop more effective, rapid and affordable molecular diagnostic techniques for pulmonary tuberculosis. It is also recommended to continue and or explore other techniques deeply. Further development of CRISPR-based Diagnostic Techniques technology could be a major focus, including further validation against various strains of *M. tuberculosis* and improving the sensitivity and specificity of the test. Apart from that, research can also be focused on developing diagnostic tests that can be used in the field with limited infrastructure, thereby increasing the accessibility of pulmonary TB diagnosis in various regions. Collaboration between research institutions, industry and government is also urgently needed to accelerate the development and implementation of innovative and effective molecular diagnostic techniques in controlling pulmonary tuberculosis.

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