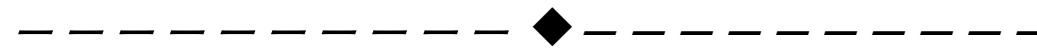
Molecular Detection of Leprosy: A Literature Review

Hersa Firda Kartika[1], Tutty Ariani[2]
[1,2]Universitas Andalas, Padang, Indonesia
Email: tuttyariani@med.unand.ac.id

Abstract

Leprosy is an infectious disease caused by the bacterium *Mycobacterium leprae*, which can lead to permanent damage to the skin, nerves, and tissues. Early diagnosis is crucial to prevent further complications. Molecular technology has emerged as a significant tool in enhancing the accuracy and speed of leprosy diagnosis. By employing techniques such as Polymerase Chain Reaction (PCR) and genetic analysis, bacterial DNA detection can be performed even at the early stages of infection, when clinical symptoms are not yet apparent. Additionally, this technology allows for the identification of different bacterial strains, providing insights into the epidemiology and transmission patterns of the disease. The application of molecular technology also has the potential to improve the monitoring of treatment effectiveness and resistance of therapy regiments. Although challenges remain in accessibility and implementation of this technology in countries with high prevalence, innovations in molecular diagnosis offer new hope for the control and eradication of leprosy globally. Further research and investment in healthcare infrastructure are essential to maximize the potential of this technology in the diagnosis and management of leprosy.

Keywords: *Leprosy, PCR, Resistance*.



A. INTRODUCTION

Leprosy or Hansen Disease (HD) is a chronic infectious illness caused by *Mycobacterium leprae*. Despite substantial progress in controlling the disease, its incidence remains a public health concern, particularly in countries with under-resourced health systems. Data from World Health Organization (WHO) indicates that thousands of new cases are still detected each year, with the highest burden in tropical and subtropical regions. In early 2012, the prevalence of this disease was 0.34 per 10,000 population (Sarode et al., 2020; Barua 2017). Affected individuals often experience social stigma and discrimination, which can further diminish their quality of life (Sharma & Singh, 2022).

Molecular diagnostic technologies have become a major focus in dermatology. Methods such as Polymerase Chain Reaction (PCR) offer advantages in detecting infectious agents, with higher sensitivity and specificity than conventional approaches such as culture or microscopic examination. By enabling the direct identification of pathogen deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from clinical specimens, molecular testing supports faster and more accurate diagnosis, which is highly valuable for clinical decision-making. This rapid turnaround is particularly important in emergency settings where prompt management is required, and it also helps identify resistance to specific antimicrobial drugs (Gama et al., 2020; Lopes-Luz et al., 2023).

IJSOC © 2026

Early detection rates for leprosy remain low in several countries, which can facilitate ongoing transmission, while drug resistance continues to be a significant concern. With increasing population mobility and urbanization, the risk of spreading resistant strains is likely to rise further. One key advantage of molecular testing is its ability to identify cases that are difficult to diagnose using traditional methods. By applying molecular techniques, diagnosis can be made more rapidly, enabling earlier intervention and reducing the risk of long-term complications (Adams, 2021).

In the context of research about resistance, molecular detection not only aids in identifying resistant strains but also in monitoring the genetic evolution of pathogens in response to antimicrobial drug use. Understanding genetic resistance patterns can guide the development of more targeted treatment strategies and provide valuable information for public health policies aimed at controlling leprosy. The role of molecular detection in the study of leprosy's disease resistance offers significant potential to improve treatment outcomes and minimize the impact of the disease. Further research in this field will not only enhance our understanding of resistance mechanisms but also provide a stronger foundation for the development of more innovative prevention and treatment strategies (Adams, 2021; Matsuoka, 2010).

Research on the sensitivity of molecular tests such as Reverse Transcription Polymerase Chain Reaction (RT-PCR), Multiplex Polymerase Chain Reaction (M-PCR), and PCR in multibacillary (MB) and paucibacillary (PB) leprosy cases has demonstrated significant results, depending on the sample collection site, such as urine, blood, and tissue specimens. Quantitative Polymerase Chain Reaction (qPCR) is known for its high sensitivity in detecting the viability of *M. leprae*, particularly in MB leprosy patients who have undergone therapy. In one study, 86.7% of patients showed positive viability despite having a zero morphological index (Devita et al., 2019). Meanwhile, M-PCR offers the advantage of targeting multiple genes in a single reaction, thus enhancing the efficiency and effectiveness of bacterial detection, although its specificity still needs improvement (Pathak et al., 2019).

B. METHOD

This study is a qualitative research with a descriptive approach that aims to review and describe the role of molecular technology in the field of leprosy diagnosis. This study was prepared by analyzing various relevant current scientific sources, both in the form of clinical research results, scientific publications, and literature reviews that have been internationally accredited. A descriptive approach is used to provide a comprehensive overview of the mechanism of action of molecular technology, its role in diagnosis leprosy, and the effectiveness of various molecular examination technique, such as Polymerase Chain Reaction (PCR), Reverse Transcription Polymerase Chain Reaction (RT-PCR), and Multiplex Polymerase Chain Reaction (M-PCR) to diagnosis leprosy and identifying therapy resistance.

This methodology was chosen because it is in accordance with the objective of exploratory research, which is to describe existing phenomena based on empirical

data from previous research. This study emphasizes on the analysis of the theoretical and clinical relationship between the molecular technology and the pathogenesis of leprosy.

This research was carried out at the Dermatology, Venereology, and Aesthetics Study Program, Faculty of Medicine, Andalas University/Dr. M. Djamil Padang Hospital, in 2024. The selection of this location was based on the availability of academic facilities that support literature research activities and the topic's relevance to the field of dermatology specialty. The research was conducted in the period from July to December 2023, with stages including the collection of scientific references, literature analysis, and the preparation of integrated study results in the form of scientific references.

The research implementation process is carried out systematically by utilizing access to scientific databases such as PubMed, ScienceDirect, and Google Scholar, in order to obtain valid and up-to-date scientific literature on molecular technologies and molecular technologies in the context of leprosy. This study focuses on the molecular mechanisms of *Mycobacterium leprae* detection, particularly through the application of molecular techniques such as PCR, qPCR, and M-PCR, and their impact on the diagnosis and management of leprosy. The scope of discussion includes a basic understanding of molecular detection methods, the structure and function of *M. leprae* DNA and RNA, and an overview of the molecular techniques used to identify the pathogen in clinical samples.

In addition, the study also explores the effectiveness and sensitivity of these molecular diagnostic tools in detecting multibacillary (MB) and paucibacillary (PB) forms of leprosy, with a focus on how these methods aid in early diagnosis and monitoring drug resistance. The safety aspect of early molecular detection is also discussed, considering its potential to reduce the risk of complications, improve treatment outcomes, and decrease the spread of the disease.

As such, this study presents a comprehensive review that not only examines the basic molecular aspects of leprosy detection but also translates these findings into a clinical context, highlighting the potential application of molecular diagnostics as an innovative approach in leprosy management. The data sources for this study are derived from secondary scientific literature, including journal articles, textbooks, clinical trial reports, as well as the results of systematic reviews and meta-analyses published between 2019 and 2024. This time frame was selected to ensure the inclusion of the most current and relevant data on molecular detection techniques for leprosy, particularly in relation to the use of PCR, qPCR, and M-PCR for early diagnosis and monitoring of *Mycobacterium leprae*.

The research population includes all studies and publications that focus on the molecular mechanisms of leprosy detection, the application of molecular diagnostic methods, and their role in improving the diagnosis and treatment of leprosy. A selection process was conducted based on methodological rigor, clinical relevance, and the validity of the research findings. This study does not utilize a direct patient

sample, but rather reviews a comprehensive population of scientific data that meets established academic criteria.

The unit of analysis in this study consists of clinical and preclinical research data that have been published with high scientific credibility. The analysis was conducted thematically to identify patterns of similarities and differences in the findings across various studies, aiming to provide a clear overview of the state of molecular diagnostic techniques for leprosy.

C. RESULTS AND DISCUSSION

The basic of molecular testing focuses on techniques that enable the analysis of genetic components of infection-causing pathogens. One of the most commonly used techniques is Polymerase Chain Reaction (PCR), which is capable of amplifying specific DNA or RNA from clinical samples. With this technique, even small amounts of genetic material can be amplified, making it easily detectable. PCR is highly effective in identifying various pathogens, including bacteria, viruses, and fungi, which are often difficult to detect using conventional culture methods. The speed and sensitivity of this technique make it an essential tool in the diagnosis of infectious dermatological diseases (Kupsch & Gräser, 2021; Mota et al., 2022).

The application of molecular testing in dermatology not only accelerates the diagnostic process but also enhances the ability to manage diseases more proactively. With accurate and rapid information, healthcare providers can promptly respond with appropriate therapy, reducing the risk of complications and improving patients' quality of life. In an era where antibiotic resistance is becoming a serious issue, a better understanding of infection patterns and pathogen characteristics through molecular testing is increasingly important. Therefore, molecular testing serves not only as a diagnostic tool but also as a foundation for the development of better health policies in the management of infectious dermatological diseases (Kupsch & Gräser, 2021).

Traditional methods that are specific but have low sensitivity for detecting *M. leprae*, such as Slit Skin Smear (SSS) examination and Fite Faraco (FF) staining for detecting *M. leprae* in skin biopsies, have been compared with newer molecular tools that are more sensitive for detecting *M. leprae* DNA. These diagnostic methods, such as qPCR and LAMP, utilize various gene targets and a wide range of biological specimens, including skin biopsies, SSS, whole peripheral blood, saliva, as well as swabs from the mouth and nose (Lopes-Luz et al., 2023).

Recent studies demonstrate that molecular methods, particularly qPCR targeting the Rlep gene, consistently yield higher sensitivity and detection rates compared with conventional techniques. For example, qPCR has been shown to detect *M. leprae* DNA in urine samples with positivity rates of up to 100% and in blood samples exceeding 96%, substantially outperforming traditional smear microscopy, especially in cases with low bacillary loads or in smear-negative patients. qPCR also maintains high specificity, indicating its potential as a complementary tool for accurate diagnosis across different clinical forms of leprosy. These findings highlight that while conventional methods remain useful, especially in resource-limited

settings, molecular diagnostics provide significantly improved detection capabilities that can aid early diagnosis and treatment monitoring (Diana & Harish, 2024).

Polymerasae Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a highly sensitive and specific molecular method for amplifying and identifying nucleic acids from a particular organism. This technique enables the rapid and specific amplification of DNA or RNA, increasing the amount of target genetic material in clinical samples by up to millions of times. With this ability, various efforts have been made to detect *M. leprae* DNA in clinical samples from Hansen's disease patients. The clinical samples used include not only skin biopsies but also various types of specimens such as Slit Skin Smears (SSS), nerve branches, urine, oral and nasal swabs, saliva, and blood. Specific and sensitive targets are crucial elements in developing diagnostic tools using PCR-based methods (Hashem, Khodair & Abd El-Samee, 2020; Siwakoti et al., 2016).

The PCR process consists of several key stages: denaturation, annealing, and extension. First, the DNA or RNA sample is heated to separate the double helix into two strands. Next, the temperature is lowered to allow primers, which are short DNA sequences, to bind specifically to the target region of the single-stranded DNA. In the final stage, the temperature is raised again to enable the DNA polymerase enzyme to extend the primers and form new copies of the target DNA. This process is repeated for several cycles, generally 25 to 40 times, producing many copies of the desired DNA in a short period (Martinez et al., 2014).

In addition to the general principles of PCR outlined above, the design and selection of specific primer pairs targeting *M. leprae* genetic sequences are crucial for achieving high sensitivity and specificity in diagnostic assays. Several gene targets have been reported in the literature, with primers developed for repetitive elements such as the RLEP sequence, which is present in multiple copies in the *M. leprae* genome and has been widely used due to its high amplification efficiency. In a recent study, a newly designed primer pair (LYON1/LYON2) targeting the RLEP region demonstrated markedly greater sensitivity in detecting the pathogen compared to conventional primer sets, producing positive results in a substantially higher proportion of clinical samples (Machado et al., 2020).

The comparison of PCR sensitivity with Slit Skin Smear (SSS) was studied by Mostafa et al. (2020). The study was conducted on 30 untreated Hansen's disease patients (9 cases of multibacillary (MB) and 21 cases of paucibacillary (PB)), with both SSS examination and blood sample collection for PCR testing. The results showed a negative Acid-Fast Bacilli (AFB) finding in the SSS test for 20 cases, giving a positivity rate of 33%. Positive PCR results were found in 20 patients (67%), which confirmed the diagnosis in 11 of the 20 patients with negative SSS results. This study concluded that PCR testing for Hansen's disease diagnosis has a higher sensitivity than SSS and is able to detect many false-negative cases (Hashem, Khodair & Abd El-Samee, 2020).

Although PCR offers many benefits, some challenges are encountered. Cross-contamination can affect results, so strict laboratory protocols must be followed.

Additionally, the interpretation of PCR results should be done carefully, as the presence of pathogen DNA or RNA does not necessarily indicate an active infection. Therefore, it is important for healthcare professionals to combine PCR results with other clinical information to make an accurate diagnosis. With ongoing technological advancements, PCR remains a highly relevant and useful method in the detection and management of infectious diseases (Tatipally, Srikantam & Kasetty, 2018).

Quantitative Polymerase Chain Reaction (qPCR)

Quantitative PCR (qPCR) testing has emerged as a more selective diagnostic method and has increasingly replaced conventional PCR due to its higher sensitivity and specificity. The advantage of qPCR lies in its ability to not only amplify target DNA sequences but also measure the quantity of *M. leprae* DNA present in the sample. Various types of clinical samples, including skin scrapings, skin biopsies, nasal swabs, and blood, have been explored for detecting *M. leprae* DNA by targeting different genes. One of the most commonly used gene targets for qPCR in leprosy diagnosis is the specific RLEP gene, as well as 16S rRNA and combinations of Rlep/16S rRNA. The RLEP gene is present in multiple copies within the *M. leprae* genome. The high copy number of the RLEP gene allows qPCR testing to achieve high sensitivity and specificity, even in paucibacillary cases (Diana & Harish, 2024).

The main difference between conventional PCR and qPCR lies in the analysis method and quantification capability. Conventional PCR focuses on the technique used to amplify DNA fragments, but the results are typically analyzed after the amplification process is complete, often through gel electrophoresis. In this method, researchers must wait until all cycles are finished before determining the presence of the desired DNA. In contrast, qPCR allows for real-time monitoring of DNA amplification during the analysis process. This qPCR technique provides quantitative information about the amount of DNA in the sample from the beginning of amplification. This not only speeds up the analysis time, reducing it from several hours to just a few minutes, but also enhances the sensitivity and specificity of DNA detection. Furthermore, qPCR reduces the risk of contamination since no additional analysis steps are required after the amplification process is completed (Sarath, Joseph & Jamir, 2023; Manta et al., 2019).

Design and selection of primers are critical to ensure the specificity, efficiency, and accuracy of target DNA amplification. Primers in qPCR are short, single-stranded oligonucleotides designed to be complementary to the sequences flanking the target region. Their design is influenced by several factors, including optimal melting temperatures (Tm), appropriate GC content, and avoidance of secondary structures such as hairpins or primer dimers. Recent advancements have introduced bioinformatics tools and databases for the systematic design of primers, which enhance the reproducibility and robustness of assays across various organisms. These tools also assist in genome-wide primer selection strategies, ensuring efficient amplification and accurate quantification. Furthermore, careful primer design is essential not only for targeting the gene of interest but also for selecting appropriate

reference genes for normalization. This normalization ensures reliable and consistent relative or absolute quantification, which is particularly important in gene expression studies and genetic analysis. Effective primer design is fundamental in reducing non-specific amplification and minimizing experimental errors, thereby improving the overall quality and precision of qPCR results (Li et al., 2025).

Study by Manta et al. (2019) examined Household Contacts (HHC) from 25 individuals showing skin lesions resembling leprosy. During sample collection, 8 individuals were confirmed to have leprosy (50% of whom were qPCR-positive), while 17 individuals were diagnosed with other skin diseases (6% qPCR-positive). Skin biopsy analysis revealed a sensitivity of 0.50 and a specificity of 0.94 for qPCR. In addition, 955 healthy HHCs were followed for a minimum of 3 years, and skin scrapings were collected from the earlobe for qPCR detection. The use of qPCR combined with household contact screening can aid in early diagnosis and monitoring the progression of MH (Manta et al., 2019).

Multiplex Polymerase Chain Reaction (M-PCR)

Multiplex PCR (M-PCR) is a technique that allows for the simultaneous amplification of multiple DNA targets in a single reaction using different specific primer pairs. In the context of Mycobacterium leprae (MH) diagnosis, this technique offers significant advantages over conventional PCR methods, as it can detect multiple disease-causing genes at once. With M-PCR, the identification of various strains of M. leprae can be performed, along with monitoring drug resistance, all in one procedure. This not only improves the efficiency of the diagnostic process but also accelerates the time required for diagnosis, making it a valuable tool in clinical settings (Pathak et al., 2019).

Samples recommended for M-PCR testing include ear lobe SSS specimens, which can display early symptoms of leprosy. Additionally, skin lesion specimens can provide insights into the distribution of *M.leprae* bacteria in the body. However, skin biopsy is considered the most sensitive sample type, as it can directly show the presence of bacteria in skin tissue, leading to more accurate results. By using the appropriate gene targets and strategically chosen sampling sites, PCR testing can enhance the ability to diagnose MH with high sensitivity and specificity (Dwivedi, Sharma & Singh, 2023).

One of the advantages of M-PCR is its ability to enhance both sensitivity and specificity in detecting *Mycobacterium leprae*. Several studies have shown that M-PCR can achieve sensitivity up to 90% and specificity around 95% in detecting infections in patients with clinical symptoms or those who have been exposed. This high sensitivity is particularly important in cases of negative SSS, where the bacteria may not be detected using conventional methods. With the ability to detect very low levels of bacteria, M-PCR can aid in early diagnosis, which is crucial in preventing further complications (Pathak et al., 2019).

The application of M-PCR in MH diagnosis is not only clinically beneficial but also practical. This method can be performed in smaller laboratory settings, making it

more accessible to healthcare facilities in endemic areas. With the availability of various non-isotopic detection methods supporting M-PCR, the diagnostic process becomes faster and more efficient, accelerating treatment for patients in need (Pathak et al., 2019).

Nested Polymerase Chain Reaction (nested PCR)

Nested Polymerase Chain Reaction (nested PCR) is a variation of conventional PCR that enhances its sensitivity and specificity by using additional primers during the second amplification step. This technique involves two consecutive amplification stages: an outer PCR and an inner PCR. The initial step uses external primers designed to amplify a broad region of the target genome in the sample. The product from the outer PCR is then used as a template for the second stage, where more specific internal primers are employed to amplify a narrower and more precise region of the target DNA. This dual-step amplification process significantly increases the detection sensitivity, making nested PCR particularly useful for detecting low amounts of DNA in complex samples or in cases where conventional PCR may fail to provide reliable results (Green & Sambrook, 2019).

A study by Wen et al. (2013) evaluated the sensitivity and specificity of nested PCR for detecting *M. leprae* DNA from whole blood samples. The whole blood samples were analyzed using nested PCR amplification in 49 multibacillary (MB) leprosy patients, 30 paucibacillary (PB) patients, 96 household contacts (HHC), 18 tuberculosis (TB) patients, and 35 healthy controls. The results showed that *M. leprae* DNA was detected in 95.92% (47/49) of MB patients, 70% (21/30) of PB patients, and 6.25% (6/96) of HHCs, but was not detected in any of the 18 TB patients or 35 normal controls. Based on these findings, nested PCR is recommended as a diagnostic tool for the early detection of leprosy (Wen et al., 2013).

Another application of nested PCR was demonstrated in a study by Chen et al. (2019), which involved 76 specimens, including skin biopsies (n = 64), formalin-fixed and paraffin-embedded (FFPE) samples (n = 11), and skin slit smears (SSS) (n = 1) from multibacillary (MB, n = 70) and paucibacillary (PB, n = 6) leprosy patients. Mutations in the drug-resistant determining regions (DRDR) of the *rpoB*, *folP1*, and *gyrA* genes, associated with resistance to rifampicin, dapsone, and quinolones, were detected through PCR following WHO recommendations, as well as nested PCR and TaqMan SNP Genotyping Assay developed in this study. Compared to conventional PCR, nested PCR improved the sensitivity of detecting *rpoB* (from 51.39% to 78.94% in MB patients and from 0.00% to 50.00% in PB patients), *gyrA* (from 75.00% to 80.26% in MB patients and from 50.00% to 66.67% in PB patients), and *folP1* (from 5.26% to 84.21% in MB patients and from 0.00% to 66.67% in PB patients). Based on these findings, it can be concluded that the nested PCR method, combined with the TaqMan SNP Genotyping Assay, is a fast and highly sensitive technique for detecting drug resistance in leprosy cases (Chen et al., 2019).

Recent studies on nested PCR assays have emphasized the importance of strategic primer design to maximize both diagnostic sensitivity and specificity in

molecular detection applications. In nested PCR, two distinct sets of primers—outer and inner—are systematically designed to target conserved genomic regions in the first amplification step and then amplify a more specific internal segment in the second step, respectively. Primer design typically begins with *in silico* analysis using sequence databases and bioinformatic tools (such as NCBI BLAST and Primer-BLAST) to identify conserved and unique regions of the target gene, ensuring high specificity against related non-target sequences. By aligning multiple reference sequences and selecting regions with minimal variability, researchers can design outer primers that broadly capture the target locus while inner primers focus on a highly specific fragment, significantly reducing non-specific amplification and background noise. Conditional parameters such as melting temperature (Tm), GC content, and amplicon size are optimized so that both primer sets function efficiently under defined thermocycling conditions. Empirical evaluations in recent nested PCR implementations have shown that this two-tiered primer strategy enhances the detection limit (i.e., lower copy number thresholds) and overall reliability of assays in complex clinical specimens (Musa et al., 2024).

Application of Molecular Technologies in Detection of Leprosy Treatment Resistance

Drug resistance in *M.leprae* is generally caused by mutations in specific regions of chromosomal genes that encode drug targets, known as Drug-Resistant Determining Regions (DRDR). These mutations play a critical role in determining the effectiveness of therapy, making a thorough understanding of the mechanisms of resistance essential. Molecular methods targeting DRDRs of specific drug targets have been validated and applied in endemic countries as part of the WHO's drug resistance monitoring initiative. This monitoring helps in identifying resistant strains, adjusting treatment protocols, and ensuring the efficacy of available therapies, which is vital for controlling leprosy and preventing the spread of resistant strains (Maladan et al., 2018; Deps, 2023).

Conventional resistance detection in *M.leprae* cannot be performed using diffusion or dilution methods because this bacterium cannot be cultured on artificial media. Prior to the development of molecular biology techniques, testing was conducted by inoculating *M. leprae* into mice, which were then treated with drugs to observe the reduction in bacterial load. This process required at least six months to determine the presence of resistance, making it ineffective for timely diagnosis. Additionally, this method is complex, time-consuming, and can only be performed in specialized laboratories, making it impractical for routine clinical use (Li et al., 2022).

Validated targets for leprosy treatment include *folP1* (dapsone), *rpoB* (rifampicin), and *gyrA* (ofloxacin), through the identification of mutations in the Drug-Resistant Determining Regions (DRDR) of these targets using specific primers. Recommended methods for detecting these mutations include PCR combined with Sanger sequencing, the commercial GenoType LepraeDR DNA strip test, or whole genome sequencing. Currently, no validated targets have been identified for other

leprosy drugs such as clofazimine, minocycline, and clarithromycin (Deps, 2023; Li et al., 2022).

The principle of detection using molecular biology involves analyzing the nucleotide sequence of genes encoding enzymes where multidrug therapy (MDT) drugs act. For example, dapsone inhibits the enzyme dihydropteroate synthase, which is encoded by the *folP* gene. The normal nucleotide sequence of the *folP* gene is available in sequence databases. By comparing the nucleotide sequence obtained from sequencing the *M. leprae* isolate with the normal sequence, we can identify any mutations that have occurred. These nucleotide changes lead to alterations in amino acids, which, in turn, modify the resulting enzyme. As a result, dapsone can no longer effectively inhibit the mutated dihydropteroate synthase enzyme. Similarly, mutations in the *rpoB* gene cause resistance to rifampicin because the enzyme targeted by the drug also undergoes changes. Several research reports indicate that the frequency of *M. leprae* mutations related to MDT resistance ranges from 2% to 5% (Lukito et al., 2024; Zivarifar et al., 2024).

Multiplex PCR (M-PCR) is another highly beneficial method for simultaneously detecting multiple targets in a single reaction. With its ability to amplify various resistance-related genes in one step, M-PCR can significantly improve the efficiency of diagnosis. For example, M-PCR can be used to detect mutations in several resistance genes, including those associated with rifampicin and dapsone, allowing for faster and more comprehensive identification of resistant strains. This multiplex approach reduces the need for multiple separate PCR tests, streamlining the process and enabling broader surveillance of drug resistance in *Mycobacterium leprae* (Li et al., 2022).

D. CONCLUSION

Molecular detection in leprosy is a crucial approach for accurate diagnosis and disease control. The selection of appropriate samples, such as nasal swabs, skin lesion biopsies, and blood, plays a key role in enhancing the sensitivity and specificity of detection results. Techniques like conventional PCR, qPCR, nested PCR, and M-PCR each offer distinct advantages. qPCR provides high sensitivity and the ability to quantify DNA, while M-PCR allows for the detection of multiple gene targets in a single test, thus improving detection efficiency. Conventional PCR remains an effective foundational method for DNA amplification. In addition to diagnosing leprosy, molecular detection is also valuable for identifying drug resistance, which aids in selecting more appropriate therapeutic strategies. By utilizing these techniques and selecting the right samples, early diagnosis of leprosy can be performed more effectively, supporting broader disease prevention and control efforts.

REFERENCES

1. Sarode, G., Sarode, S., Anand, R., Patil, S., Jafer, M., Baeshen, H., & Awan, K. H. (2020). Epidemiological aspects of leprosy. *Disease-a-Month*, *66*(7), 100899.

2. Barua, S. (2017). Global leprosy situation: historical perspective, achievements, challenges and future steps. *IAL textbook of leprosyp*, *45-56*.
3. Sharma, M., & Singh, P. (2022). Advances in the diagnosis of leprosy. *Frontiers in Tropical Diseases*, *3*, 893653.
4. Gama, R. S., Leite, L. A., Colombo, L. T., & Fraga, L. A. D. O. (2020). Prospects for new leprosy diagnostic tools, a narrative review considering ELISA and PCR assays. *Revista da Sociedade Brasileira de Medicina Tropical*, *53*, e20200197.
5. Lopes-Luz, L., Saavedra, D. P., Fogaça, M. B. T., Bührer-Sékula, S., & Stefani, M. M. D. A. (2023). Challenges and advances in serological and molecular tests to aid leprosy diagnosis. *Experimental Biology and Medicine*, *248*(22), 2083-2094.
6. Adams, L. B. (2021). Susceptibility and resistance in leprosy: Studies in the mouse model. *Immunological Reviews*, *301*(1), 157-174.
7. Matsuoka, M. (2010). Drug resistance in leprosy. *Japanese journal of infectious diseases*, *63*(1), 1-7.
8. Devita, A., Ibrahim, F., Menaldi, S. L. S. W., Budianti, A., & Yasmon, A. (2019). Detection of Mycobacterium leprae using real-time PCR in paucibacillary leprosy patients with negative acid-fast bacilli smears. *Medical Journal of Indonesia*, *28*(4), 351-7.
9. Pathak, V. K., Singh, I., Turankar, R. P., Lavania, M., Ahuja, M., Singh, V., & Sengupta, U. (2019). Utility of multiplex PCR for early diagnosis and household contact surveillance for leprosy. *Diagnostic microbiology and infectious disease*, *95*(3), 114855.
10. Kupsch, C., & Gräser, Y. (2021). The potential of molecular diagnostics in routine dermatology. In *Dermatophytes and Dermatophytoses* (pp. 339-353). Cham: Springer International Publishing.
11. Mota, D. S., Guimarães, J. M., Gandarilla, A. M. D., Filho, J. C. B. S., Brito, W. R., & Mariúba, L. A. M. (2022). Recombinase polymerase amplification in the molecular diagnosis of microbiological targets and its applications. *Canadian Journal of Microbiology*, *68*(6), 383-402.
12. Diana, D., & Harish, M. C. (2024). qPCR detection of Mycobacterium leprae DNA in urine samples of leprosy patients using the Rlep gene target. *Frontiers in Molecular Biosciences*, *11*, 1435679.
13. Hashem, O., Khodair, H. M., & Abd El-Samee, H. S. (2020). Polymerase chain reaction versus slit skin smear in diagnosis of leprosy; a cross-sectional study. *International Journal of Medical Arts*, *2*(3), 650-654.
14. Siwakoti, S., Rai, K., Bhattarai, N. R., Agarwal, S., & Khanal, B. (2016). Evaluation of polymerase chain reaction (PCR) with slit skin smear examination (SSS) to confirm clinical diagnosis of leprosy in eastern Nepal. *PLoS neglected tropical diseases*, *10*(12), e0005220.
15. Martinez, A. N., Talhari, C., Moraes, M. O., & Talhari, S. (2014). PCR-based techniques for leprosy diagnosis: from the laboratory to the clinic. *PLoS Neglected Tropical Diseases*, *8*(4), e2655.

16. Machado, A. S., Lyon, S., Rocha-Silva, F., Assunção, C. B., Hernandez, M. N., Jorge, D. S., ... & Caligiorne, R. B. (2020). Novel PCR primers for improved detection of Mycobacterium leprae and diagnosis of leprosy. *Journal of applied microbiology*, *128*(6), 1814-1819.
17. Tatipally, S., Srikantam, A., & Kasetty, S. (2018). Polymerase chain reaction (PCR) as a potential point of care laboratory test for leprosy diagnosis—a systematic review. *Tropical medicine and infectious disease*, *3*(4), 107.
18. Sarath, I. M., Joseph, N. M., & Jamir, I. (2023). Quantitative real-time polymerase chain reaction for detection of Mycobacterium leprae DNA in tissue specimens from patients with leprosy. *The American Journal of Tropical Medicine and Hygiene*, *109*(2), 345.
19. Manta, F. S., Barbieri, R. R., Moreira, S. J., Santos, P. T., Nery, J. A., Duppre, N. C., ... & Moraes, M. O. (2019). Quantitative PCR for leprosy diagnosis and monitoring in household contacts: A follow-up study, 2011–2018. *Scientific reports*, *9*(1), 16675.
20. Li, X., Meng, B., Zhang, Z., Wei, L., Chang, W., Wang, Y., ... & Lu, K. (2025). qPrimerDB 2.0: an updated comprehensive gene-specific qPCR primer database for 1172 organisms. *Nucleic Acids Research*, *53*(D1), D205-D210.
21. Dwivedi, P., Sharma, M., & Singh, P. (2023). Multiplex PCR-based RFLP assay for early identification of prevalent Mycobacterium leprae genotypes. *Diagnostic Microbiology and Infectious Disease*, *107*(4), 116084.
22. Green, M. R., & Sambrook, J. (2019). Nested polymerase chain reaction (PCR). *Cold Spring Harbor Protocols*, *2019*(2), pdb-prot095182.
23. Wen, Y., Xing, Y., Yuan, L. C., Liu, J., Zhang, Y., & Li, H. Y. (2013). Whole-blood nested-PCR amplification of M. leprae-specific DNA for early diagnosis of leprosy. *The American Journal of Tropical Medicine and Hygiene*, *88*(5), 918.
24. Chen, X., He, J., Liu, J., You, Y., Yuan, L., & Wen, Y. (2019). Nested PCR and the TaqMan SNP Genotyping Assay enhanced the sensitivity of drug resistance testing of Mycobacterium leprae using clinical specimens of leprosy patients. *PLoS Neglected Tropical Diseases*, *13*(12), e0007946.
25. Musa, S., Hemberle, T., Bensch, S., Palinauskas, V., Baltrūnaitė, L., Woog, F., & Mackenstedt, U. (2024). Raising the bar: genus-specific nested PCR improves detection and lineage identification of avian haemosporidian parasites. *Frontiers in cellular and infection microbiology*, *14*, 1385599.
26. Maladan, Y., Lestari, C. W., Tanjung, R., Cahyani, V. D., & Rokhmad, M. F. (2018). Molecular detection mutation of rpoB gene Mycobacterium leprae in relapse and default of leprosy patient in jayapura city, papua. *Health Sci J Indones*, *9*(1), 31-36.
27. Deps, P. D. (Ed.). (2023). *Hansen's disease: A complete clinical guide*. Springer Nature.
28. Li, X., Li, G., Yang, J., Jin, G., Shao, Y., Li, Y., ... & Zhang, L. (2022). Drug resistance (dapsone, rifampicin, ofloxacin) and resistance-related gene mutation features in leprosy patients: a systematic review and meta-analysis. *International Journal of Molecular Sciences*, *23*(20), 12443.

29. Lukito, A. A., Paralaga, I. B. K. B., & Supradnyan, I. K. H. (2024). The growing threat of antimicrobial resistance in Mycobacterium leprae: A literature review. *Intisari Sains Medis*, *15*(3), 1324-1332.
30. Zivarifar, H., Ahrari, F., & Karbalaei, M. (2024). Computational investigation of the global prevalence of multidrug resistant Mycobacterium leprae: A systematic review and meta-analysis. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *37*, 100495.

IJSOC © 2026