

Molecular diagnostics and genetic markers for rapid identification of parasitic diseases in resource-limited settings.

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Abstract

Parasitic diseases are a significant global health burden, particularly in resource-limited settings where access to advanced diagnostic tools and healthcare facilities is limited. Conventional diagnostic methods for parasitic diseases often lack sensitivity, specificity, and speed, leading to delayed diagnosis and inadequate patient management. Molecular diagnostics and genetic markers have emerged as promising approaches for rapid and accurate identification of parasitic infections in such settings. This research paper provides an overview of the current state of molecular diagnostics and the utilization of genetic markers for the detection and identification of parasitic diseases in resource-limited regions. It highlights the potential benefits, challenges, and future prospects of implementing these advanced techniques to enhance parasitic disease diagnosis, surveillance, and control.

Keywords: Parasitic diseases, Molecular Diagnostics, Resources, Technology.

Introduction

Parasitic diseases continue to impose a significant health burden on vulnerable populations residing in resource-limited settings [1]. The limitations of traditional diagnostic methods, such as microscopy and serology, have spurred the exploration of molecular diagnostic techniques, which rely on the detection of specific genetic markers of parasites [2]. This paper aims to discuss the various molecular diagnostic approaches and genetic markers employed for the rapid identification of parasitic diseases in resource-limited settings, emphasizing their potential impact on disease management and control.

Molecular diagnostics for parasitic diseases

Polymerase Chain Reaction (PCR) is a widely used molecular diagnostic technique that amplifies specific DNA sequences. It involves cycles of DNA denaturation, primer annealing, and DNA synthesis by a thermostable DNA polymerase. PCR has revolutionized the field of parasitic disease diagnosis due to its high sensitivity and specificity [3].

This variant allows for the quantification of the amplified DNA in real-time, enabling accurate measurement of parasite load. It is particularly valuable for monitoring treatment efficacy and assessing disease progression [4]. RT-PCR is employed for the detection of RNA viruses, including certain parasitic infections caused by RNA-based parasites.

Nested PCR involves two rounds of amplification, where the first round uses outer primers to generate a larger DNA

fragment, which is subsequently amplified with inner primers in the second round. This technique enhances sensitivity and specificity, making it useful for low-level parasite detection. Loop-Mediated Isothermal Amplification (LAMP) is a novel molecular diagnostic technique that enables rapid amplification of DNA under isothermal conditions. It utilizes a set of four to six primers targeting multiple regions within the target DNA sequence, forming loop structures that facilitate DNA amplification. LAMP offers several advantages for parasitic disease diagnosis in resource-limited settings [5].

Simplicity: LAMP does not require complex equipment or stringent temperature control, making it suitable for low-resource settings. **Sensitivity and Specificity:** LAMP exhibits high sensitivity and specificity, enabling the detection of low parasite loads and differentiation of closely related species. LAMP results can be visualized with the naked eye using turbidity indicators or fluorescent dyes, eliminating the need for specialized detection instruments [6].

Nucleic Acid Sequence-Based Amplification (NASBA) is an isothermal amplification technique that specifically amplifies RNA sequences. It employs three enzymes: reverse transcriptase, RNase H, and T7 RNA polymerase. NASBA offers advantages for diagnosing parasitic infections caused by RNA-based parasites:

a. RNA Targeting: NASBA allows direct amplification of RNA targets without the need for reverse transcription, making it suitable for RNA-based parasites.

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b. High Amplification Yield: NASBA generates large amounts of RNA transcripts, enabling easy detection of the amplified products.

c. Real-time Monitoring: NASBA can be coupled with real-time monitoring techniques, such as molecular beacons or fluorescent dyes, facilitating quantitative and rapid detection.

Recombinase Polymerase Amplification (RPA):

Recombinase Polymerase Amplification (RPA) is an isothermal amplification method that utilizes recombinase and single-stranded DNA binding proteins to enable DNA amplification at a constant temperature. RPA offers several advantages for molecular diagnosis of parasitic diseases in resource-limited settings:

a. Rapid Amplification: RPA amplifies DNA rapidly, often within minutes, allowing for quick diagnosis and prompt initiation of treatment.

b. Simplicity and Portability: RPA can be performed using portable, battery-powered devices, enabling point-of-care testing in remote and resource-limited areas.

c. High Sensitivity and Specificity: RPA exhibits high sensitivity and specificity, ensuring accurate detection of parasitic infections.

Next-generation sequencing (ngs) for metagenomic analysis

Next-Generation Sequencing (NGS) technologies have revolutionized the field of genomics and are increasingly being used for metagenomic analysis of parasitic infections. NGS enables high-throughput sequencing of millions of DNA fragments in parallel, allowing for comprehensive analysis of parasite genomes, detection of mixed infections, and identification of novel or rare parasite strains. NGS-based metagenomic approaches offer several advantages for parasitic disease diagnosis:

a. Comprehensive Detection: NGS can identify a broad range of parasitic pathogens in a single assay, making it useful for complex infections and cases with multiple parasite species.

b. Genomic Characterization: NGS enables detailed genomic characterization of parasites, facilitating the study of drug resistance, genetic diversity, and transmission dynamics.

c. Diagnostic Accuracy: NGS can provide highly accurate and sensitive detection, particularly in cases where conventional methods fail to identify the causative agent.

In conclusion, molecular diagnostic techniques such as PCR and its variants, LAMP, NASBA, RPA, and NGS have revolutionized the detection and identification of parasitic diseases in resource-limited settings. These methods offer enhanced sensitivity, specificity, rapidity, and portability, making them valuable tools for improving disease management, surveillance, and control in regions where traditional diagnostic methods are limited.

Genetic markers as diagnostic tools

Genetic markers play a crucial role as diagnostic tools in the identification and characterization of parasitic diseases. These markers are specific DNA sequences or genes that are associated with particular parasite species or strains. They provide valuable information for accurate and sensitive detection, differentiation, and monitoring of parasitic infections. Below are some of the key genetic markers used in molecular diagnostics for parasitic diseases:

Single-copy genes and multi-copy genes

Single-copy genes are unique sequences present in a single copy in the genome of a parasite. They are often used as genetic markers for species identification due to their species-specific characteristics. Examples of single-copy genes used as genetic markers include genes encoding surface proteins or enzymes with species-specific variations.

In contrast, multi-copy genes exist in multiple copies in the parasite genome. These genes can be used as targets for highly sensitive detection and quantification of parasites, especially in cases with low parasite loads. Some common multi-copy genes used as genetic markers include ribosomal RNA genes and mitochondrial DNA (mtDNA) genes.

Ribosomal RNA (rRNA) genes

Ribosomal RNA genes are essential components of the ribosomes involved in protein synthesis. The high copy number of rRNA genes in the genome of parasites makes them attractive targets for molecular diagnostics. The small subunit (SSU) rRNA gene and the internal transcribed spacer (ITS) regions are commonly targeted for species identification and differentiation due to their variable sequences among different parasite species [7].

Mitochondrial DNA (mtDNA) is a valuable genetic marker for identifying and distinguishing parasitic species. Mitochondria are organelles found in eukaryotic cells that contain their own DNA. mtDNA is particularly useful for identifying closely related species or differentiating various strains of the same parasite species. Its high copy number in cells makes mtDNA an excellent target for sensitive detection using PCR-based methods.

Antigen-coding genes are genes that encode proteins expressed on the surface of parasites. These proteins are involved in interactions with the host's immune system and are often targeted by the host's immune response. Antigen-coding genes can be used as diagnostic markers to detect specific antigens in clinical samples, providing evidence of an ongoing infection. Immunoassays and antigen detection tests are commonly employed to detect parasite-specific antigens in patient samples [8].

Microsatellites and minisatellites are short, repetitive DNA sequences scattered throughout the genome of parasites. These genetic markers are used in population genetics and epidemiological studies to assess the genetic diversity and transmission patterns of parasites. Microsatellite analysis provides insights into parasite populations, their sources of infection, and their potential for drug resistance [9].

Incorporating these genetic markers into molecular diagnostic assays enhances the accuracy and specificity of parasitic disease identification. The choice of the appropriate genetic marker depends on the specific diagnostic objectives, the target parasite(s), and the available resources and technology. Integrating genetic marker-based diagnostic tools with other molecular diagnostic techniques can significantly improve the detection, surveillance, and control of parasitic diseases in resource-limited settings, leading to better patient outcomes and public health interventions [2].

Applications of Molecular Diagnostics and Genetic Markers

Malaria: Identifying drug resistance and species differentiation:

Molecular diagnostics and genetic markers are invaluable tools for malaria management and control. They allow for the early detection of drug resistance, which is a critical concern in malaria-endemic regions. By targeting specific genetic markers associated with resistance to antimalarial drugs, such as mutations in the *Plasmodium falciparum* dihydrofolate reductase (*pfdhfr*) and dihydropteroate synthase (*pfdhps*) genes, researchers and healthcare professionals can monitor the emergence and spread of drug-resistant strains. Additionally, genetic markers like the *Plasmodium* species-specific 18S rRNA gene enable accurate differentiation between *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*, aiding in appropriate treatment strategies.

Leishmaniasis: Species identification and disease staging

Leishmaniasis is caused by various species of *Leishmania* parasites, each with distinct clinical presentations and treatment responses. Molecular diagnostics play a crucial role in identifying the causative *Leishmania* species through the amplification and characterization of specific genetic markers, such as kinetoplast DNA (kDNA) minicircles or the internal transcribed spacer (ITS) regions. This information is essential for selecting appropriate treatment regimens. Moreover, molecular techniques can also be used to determine the stage of the disease, aiding in the prognosis and management of patients.

Schistosomiasis: Differentiation of species and monitoring treatment efficacy

Schistosomiasis, caused by different species of *Schistosoma* parasites, requires accurate species identification to tailor treatment and surveillance efforts. Molecular diagnostics utilize specific genetic markers, such as the internal transcribed spacer (ITS) regions or mitochondrial genes, for differentiation between *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, and others. Furthermore, monitoring treatment efficacy can be achieved through quantitative PCR (qPCR) targeting parasite DNA, providing valuable information about treatment outcomes and potential drug resistance.

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, exhibits genetic diversity with various strains and

lineages present in different geographic regions. Molecular diagnostics based on genetic markers like variable surface glycoprotein (VSG) genes allow for strain identification and differentiation. This information is crucial for understanding the epidemiology and transmission dynamics of Chagas disease and can guide the development of targeted interventions [10, 11].

Soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm species, are common intestinal parasites in resource-limited settings. Molecular diagnostics using genetic markers can be employed to assess anthelmintic drug efficacy by detecting specific mutations associated with drug resistance in these parasites. Additionally, quantitative PCR (qPCR) can be used to determine parasite load in patient samples, providing insights into the effectiveness of anthelmintic treatment and guiding public health interventions [12].

In summary, molecular diagnostics and genetic markers have numerous applications in the diagnosis and management of parasitic diseases. They allow for the identification of drug resistance, species differentiation, disease staging, and monitoring treatment efficacy. The integration of these advanced techniques into routine healthcare and surveillance systems in resource-limited settings can significantly improve the accuracy and efficiency of parasitic disease control strategies, ultimately leading to better health outcomes for affected populations.

Challenges and Limitations

Cost and infrastructure constraints

One of the significant challenges in implementing molecular diagnostics and genetic markers for parasitic diseases in resource-limited settings is the cost associated with the equipment, reagents, and maintenance. Sophisticated laboratory equipment required for techniques like real-time PCR and Next-Generation Sequencing (NGS) can be expensive, making them less accessible in regions with limited financial resources. Moreover, reliable power supply and internet connectivity are essential for running and analyzing molecular tests, and the lack of adequate infrastructure can hinder the adoption of these advanced diagnostic methods.

Training and capacity-building requirements

Molecular diagnostics and the utilization of genetic markers require specialized training and expertise. Healthcare professionals and laboratory personnel need to be proficient in sample processing, nucleic acid extraction, and data analysis. Conducting accurate and reliable tests necessitates regular training and capacity-building initiatives to ensure the proficiency of the workforce. However, the availability of trained personnel can be limited in remote areas, further exacerbating the challenges of implementing these diagnostic approaches.

Sample collection, storage, and transportation issues

Proper collection, storage, and transportation of biological samples are critical for reliable molecular diagnostics.

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However, in resource-limited settings, inadequate facilities for sample collection and preservation may lead to sample degradation, compromising the accuracy of test results. Additionally, the transportation of samples from remote areas to central laboratories, where molecular testing is conducted, can be challenging due to long distances and limited access to reliable transportation.

Ethical considerations and data sharing

The use of molecular diagnostics and genetic markers in research and clinical settings raises ethical considerations regarding informed consent, privacy, and data sharing. Genetic information obtained from patients may have implications beyond the immediate diagnosis, including potential implications for family members and communities. Proper ethical guidelines and informed consent procedures must be in place to protect the rights and privacy of individuals involved in the testing process. Moreover, data sharing and collaborative research efforts are essential for improving global understanding of parasitic diseases, but issues related to data ownership and sharing may need to be addressed to foster collaboration and knowledge exchange.

Addressing these challenges and limitations is crucial for the successful integration of molecular diagnostics and genetic markers into routine healthcare practices in resource-limited settings. Solutions may involve the development of affordable and portable diagnostic devices, the establishment of regional testing hubs, investment in training programs for local healthcare professionals, improvement of sample collection and storage facilities, and the implementation of ethical frameworks for data management and sharing. By overcoming these obstacles, we can unlock the full potential of molecular diagnostics to improve the diagnosis and management of parasitic diseases in regions where they are most needed.

Point-of-care tests (POCTs):

Point-of-care tests (POCTs) are diagnostic tests that can be performed near the patient, usually at the community or primary healthcare level, without the need for specialized laboratory infrastructure. Implementing POCTs for parasitic diseases in resource-limited settings can significantly improve patient access to rapid and accurate diagnostics. These tests are often simple to use and provide quick results, enabling timely initiation of treatment and reducing the burden on centralized laboratories. Deploying affordable and user-friendly POCTs for diseases like malaria, schistosomiasis, and soil-transmitted helminths can be instrumental in improving healthcare delivery in remote areas with limited access to advanced laboratory facilities.

Mobile health (mHealth) and telemedicine

The integration of mobile health (mHealth) and telemedicine technologies can enhance the reach and impact of molecular diagnostics in resource-limited settings. Mobile phones and handheld devices can serve as platforms for real-time data collection, communication with healthcare providers, and access to diagnostic results. MHealth applications can be used to facilitate remote training and capacity-building for

healthcare personnel, support electronic data capture, and improve coordination between healthcare facilities and central laboratories. Telemedicine consultations can also help in the interpretation of test results, patient management, and treatment recommendations, connecting patients in underserved areas with specialized expertise.

Partnerships and collaborations for technology transfer

Establishing partnerships and collaborations between research institutions, governmental organizations, non-governmental organizations (NGOs), and private entities is crucial for technology transfer and knowledge exchange. In resource-limited settings, where local expertise and infrastructure may be limited, partnerships with more developed institutions can facilitate the adoption and implementation of molecular diagnostics and genetic markers. Such collaborations can involve training programs, equipment donations, and knowledge sharing to build local capacity and ensure the sustainable use of advanced diagnostic technologies.

Strengthening surveillance and epidemiological studies

Comprehensive surveillance and epidemiological studies are essential for understanding the prevalence, distribution, and transmission dynamics of parasitic diseases in resource-limited settings. Molecular diagnostics and genetic markers can contribute significantly to these efforts by providing accurate data on the parasite species, drug resistance patterns, and genetic diversity. Strengthening surveillance and conducting epidemiological studies with molecular tools can guide targeted interventions, inform public health policies, and improve disease control strategies. These studies can also identify emerging parasite strains or drug-resistant variants, enabling early responses to potential outbreaks.

By implementing these strategies, stakeholders can overcome the challenges associated with adopting molecular diagnostics and genetic markers in resource-limited settings. Embracing point-of-care tests, leveraging mobile health and telemedicine technologies, fostering partnerships for technology transfer, and investing in surveillance and epidemiological studies will contribute to more effective and equitable healthcare for populations at risk of parasitic diseases. Ultimately, the successful integration of advanced diagnostic tools can lead to improved patient outcomes, enhanced disease control efforts, and better health outcomes for vulnerable communities.

Technological advancements and emerging tools

The field of molecular diagnostics is constantly evolving, with ongoing technological advancements and the development of new tools. Future directions should focus on further simplifying and miniaturizing diagnostic devices to make them more portable and affordable for resource-limited settings. Advances in microfluidics, lab-on-a-chip technology, and isothermal amplification methods may lead to innovative point-of-care devices that require minimal infrastructure and technical expertise. Additionally, the integration of multiple diagnostic assays into single platforms or multiplexed tests can increase efficiency and reduce costs.

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Integration with existing healthcare systems

To ensure sustainability and scalability, molecular diagnostics and genetic markers should be seamlessly integrated into existing healthcare systems. This integration involves establishing standardized diagnostic protocols, training healthcare personnel, and incorporating diagnostic results into electronic health records (EHRs). Collaborative efforts between health ministries, NGOs, and international organizations are essential to streamline the adoption of these technologies, ensuring they become an integral part of routine patient care in resource-limited settings.

Harnessing artificial intelligence for data analysis

The vast amount of data generated by molecular diagnostics, especially with the use of Next-Generation Sequencing (NGS), requires advanced data analysis approaches. Artificial intelligence (AI) and machine learning algorithms can play a pivotal role in analyzing complex genomic data, identifying patterns, and predicting disease outcomes. AI-driven tools can aid in species identification, drug resistance prediction, and epidemiological modeling, thereby improving diagnostic accuracy and patient management. These technologies have the potential to optimize resource utilization and enhance decision-making in the context of parasitic disease control.

Addressing socio-cultural factors and community engagement

The successful implementation of molecular diagnostics and genetic markers in resource-limited settings also requires addressing socio-cultural factors that influence healthcare-seeking behavior and patient acceptance of new diagnostic technologies. Community engagement and awareness campaigns are crucial for educating local communities about the benefits of these advanced diagnostic approaches and addressing potential misconceptions or fears. Involving local stakeholders, such as community leaders and traditional healers, can build trust and facilitate the acceptance and adoption of molecular diagnostics. Culturally sensitive approaches that consider local practices and beliefs are essential for ensuring the equitable and effective implementation of these technologies.

In conclusion, the future of molecular diagnostics and genetic markers for parasitic diseases in resource-limited settings holds great promise. Advancements in technology, integration with existing healthcare systems, harnessing AI for data analysis, and addressing socio-cultural factors are key areas that will shape the successful implementation and impact of these diagnostic tools. By continuing to invest in research, technology development, and community engagement, we can improve the diagnosis, management, and control of parasitic diseases, ultimately advancing global health efforts and reducing the burden of these infections on vulnerable populations.

Conclusion

Molecular diagnostics and genetic markers offer great potential for revolutionizing the diagnosis and management of parasitic

diseases in resource-limited settings. Their increased adoption will lead to early detection, accurate species identification, monitoring of drug resistance, and improved surveillance. Nevertheless, overcoming the challenges associated with implementation is essential to ensure equitable access to reliable diagnostic methods and contribute to the global efforts to combat parasitic diseases.

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