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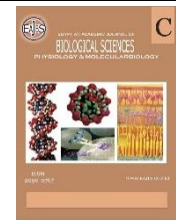
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Molecular Diagnostics for Infectious Diseases: Implications for Diagnosis and Management

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ABSTRACT

Molecular diagnostics is an excellent tool used by healthcare management for infectious diseases to detect and manage infections. As such, many researchers have addressed the ideas surrounding the molecular diagnosis. This review explores the role of molecular diagnostics in managing and diagnosing infectious diseases, highlighting its potential to prevent catastrophic outcomes. The literature surrounding the historical aspects and the importance of molecular diagnostics in modern healthcare can help explain the role of the tool and how it can improve the patient's outcome and help the therapeutic decisions. In this literature review, databases are searched using relevant keywords to gather information on the role of molecular diagnostics in managing and diagnosing infectious diseases. The review highlights the significance of molecular diagnostics in detecting resistant strains and guiding evidence-based antimicrobial treatment to combat antimicrobial resistance. Furthermore, the review discusses how adopting new diagnostic approaches as standard practice in clinical departments introduces various subjects. The findings demonstrate that implementing molecular diagnostics in clinical settings reduces mortality rates, improves healthcare efficiency, and enhances public surveillance.

INTRODUCTION

Infectious diseases represent a significant concern for public health across the globe. The emergence of new infectious agents and the re-emergence of known pathogens further intensify the urgency to manage and control these diseases effectively. Healthcare organizations and public health sectors are increasingly concerned about the detrimental implications of infectious diseases on individuals, communities, and healthcare systems. Therefore, it is imperative to establish early and precise identification of pathogens behind the disease for subsequent effective patient care (Islam & Iqbal, 2020; Hanson *et al.*, 2020). Also, there is an urgent requirement for effective disease management and outbreak control measures. (MacLean *et al.*, 2020). Conventional diagnostic methods have proven to be effective. However, they have significant challenges as they are slow in generating results. At the same time, they are susceptible, and in combination, they cannot identify novel organisms and recently emerged resistance (Afzal, 2020; Wang *et al.*, 2021; Feng *et al.*, 2020; Roberts *et al.*, 2021).

While diagnosing infectious diseases previously relied on microscopy and culture-based procedures that needed laboratory support, molecular diagnostics has undergone a significant transformation due to advancements in technology and techniques. This literature review intends to assess the progress and influence of molecular diagnostics on infectious disease recognition and management.

Molecular diagnostics includes a variety of methods for identifying and characterizing proteins, nucleic acids, and biological indicators that are used for the detection of infectious diseases (Acharya *et al.*, 2020; Hanson *et al.*, 2020; Tribolet *et al.*, 2020; Jain *et al.*, 2021). These techniques encompass high-throughput techniques such as microarray, Polymerase Chain Reaction (PCR), and nucleic sequence analysis. These approaches show various features such as screening of multiple diseases at the same time, sensitivity, and specificity. The role of molecular diagnostics in diagnosing infectious diseases is paramount and should not be underestimated. This advanced approach enables the generation of rapid results, significantly reducing the time required to control and manage contagious diseases effectively. By detecting specific genetic material or markers associated with the causative agents of these diseases, molecular diagnostics allows for early and accurate identification, leading to prompt treatment and containment measures. As a result, the spread of infectious diseases can be mitigated more efficiently, contributing to improved public health outcomes.

The specific genetic sequences or biomarkers utilized in the molecular mechanisms of infectious agents rely on targeted molecular assays. They also allow for the timely diagnosis of the agents, including situations where conventional methods cannot fulfill them. This is an essential aspect of outbreak detection, especially detecting them early on. The idea then enables health workers to immediately take control measures to prevent the further

spread of disease. Also, the role of molecular diagnostics in guiding therapeutic decisions, especially in antibiotic resistance genes or genetic marker detection. Genetic markers associated with being immune to the drug can be identified through molecular assays (Bergeron *et al.*, 2000; Huletsky *et al.*, 2005). The illness can be treated more significantly using targeted drug therapy because of this. Proper drug administration and the reduction of potential failure of the treatment procedure can be achieved. Moreover, the revolution of molecular point-of-care testing has become a new milestone in the development of molecular diagnostics, making fast diagnoses available at the bedside or in resource-limited settings (Peeling *et al.*, 2014). This ability to install therapy quickly can be liberating, refilling patients with renewed hope and easing the burden on the health infrastructure.

Method:

The search strategy consisted of a combination of recent studies retrieved from several databases such as PubMed/MEDLINE and Google Scholar. Keyword terms used include ("molecular diagnostics," "infectious diseases," "PCR," "nucleic acid sequencing," and "microarrays") used in the search results. The research using molecular tools to diagnose, control, and monitor infection by pathogens was considered. English language and peer-reviewed publications were solely considered in the entries. All these factors were crucial aspects of the research. There were no conference speeches, editors' statements, or commentaries for the exclusion.

Molecular Diagnostic Techniques:

Applying molecular diagnostics techniques has drastically changed the stage of diagnosing infections associated with pathogens. The changes have also been incorporated to improve speed, sensitization, reliability, and sensitivity detection. PCR is one of the most frequent molecular approaches, and it is based on the detection and copying of DNA sites. A cyclic repetition of reaction is used for this purpose (Mann *et*

al., 2021; Maljkovic Berry *et al.*, 2020; Rodrigues & Nosanchuk, 2021). The methodology is an essential part of these DNA applications, and it could then be used to determine specific DNA sequences (Woods *et al.*, 2021; Grassly *et al.*, 2020). It has had a significant impact on the culture-free molecular diagnosis of infectious diseases. Its high sensitivity and specificity, along with its capability to amplify nucleic acid from a small number of microorganisms in a sample by billions of times, has led to major advancements in the field (Daher *et al.*, 2016).

However, the utilization of fluorescent technologies and new rapid PCR cycle instruments in real-time PCR (rtPCR) detection has transformed the process by eliminating the requirement for complex multi-step post-PCR analysis (Navarro *et al.*, 2015; Wang *et al.*, 2023). This advancement has resulted in a reduction in hands-on time and minimized the risk of contamination caused by carryover amplification of previously generated amplicons. The methodology is thus essential for the diagnosis of fungi and viruses (Wickes & Wiederhold, 2018; Compton 2020). As a result, it is highly regarded for its sensitivity and specificity while detecting low organisms and disorders connected with pathogen microorganisms.

The nucleotide sequence composition of pathogen genomes can be accurately determined using gene-centered sequencing techniques, such as Next-Generation Sequencing (NGS) or Sanger Sequencing (Mustafa & Makhawi, 2021). Sanger sequencing is an original standard for shortening a piece of DNA, and most bacteriological researchers find it one of the most convenient tools in their molecular lab. Further, NGS technologies involve sequencing, which is used to detect emerging pathogens, strain typing, and investigate genetic variants associated with antimicrobial resistance (Satam *et al.*, 2023).

Microarray technology enables the identification of the nucleic acid sequences from multiple samples with concurrent

detection in a single experiment. Microarrays are DNA chips containing immobilized probes that hybridize the targeted sequences in the sample. According to Lantos *et al.* (2021), they signal hybridization for them to be detected, analyzed, and data used to verify and quantify the gene samples. Microarrays have not only been utilized for pathogen detection, gene profiling, and antimicrobial resistance screening but are also effective in high-volume and efficient screening, according to (Salmanton-García *et al.*, 2023; Ko *et al.*, 2023). Loop-Mediated Isothermal Amplification (LAMP) is a thermophilic isothermal nucleic acid amplification technique in which movements of a DNA strand with excellent specificity occur under constant temperature conditions. LAMP-based assays were created for the identification of multitudes of pathogens, such as bacteria, viruses, and parasites according to (Menon *et al.*, 2020; Hayden *et al.*, 2024; Lim *et al.*, 2020; Duan *et al.*, 2021). These assays are essential in decentralized diagnostics that provide testing in limited resource settings. Digital PCR (dPCR) is a quantitative PCR method that separates the samples into thousands of individual containers, each reacts differently to the nucleic acid targets, according to (Mann *et al.*, 2021; Maljkovic Berry *et al.*, 2020 Rodrigues & Nosanchuk, 2021). It enables absolute quantification of the specific nucleic acid targets. Therefore, the application of molecular diagnostic tests in the early and accurate diagnosis of infectious diseases is one of these tests' most significant features. These technologies bring high sensitivity to the detection of pathogens, precise, correct identification and characterization of antimicrobial resistance, and monitoring of infectious disease outbreaks.

Antimicrobial Resistance:

Antimicrobial resistance (AMR) is an issue that hinders people's health on a global level. As such, molecular diagnostics serve as a core technology that actively fights against AMR by making diagnostics of resistant pathogens. For example, the update

to the World Health Organization's guidelines on the detection of tuberculosis (TB) and drug-resistant TB includes three new classes of technologies (World Health Organization (WHO), 2021). The first is moderate complexity automated Nucleic acid amplification technologies (NAATs), which are recommended for detecting TB and resistance to rifampicin and isoniazid. This addresses the gap in the rapid diagnosis of isoniazid-resistant and rifampicin-susceptible TB. The second is low-complexity automated NAATs, which are recommended for detecting resistance to isoniazid and second-line anti-TB agents. This improves access to testing for fluoroquinolone resistance. The third is high-complexity reverse hybridization-based NAATs, which are recommended for detecting pyrazinamide resistance. These tests are the first molecular tests for determining resistance to this drug (World Health Organization (WHO), 2021). However, healthcare professionals should have a clear understanding of the strengths and limitations of certain technologies in order to make informed decisions in fighting antibiotic resistance and improving patient outcomes (Kotze *et al.*, 2020; Yamin *et al.*, 2023).

In addition, molecular diagnostics can lead to the identification of genes and mutations contributing to antibiotic resistance and point out the horizontal gene transfer process itself (Lantos *et al.*, 2021; Menon *et al.*, 2020). Due to the whole-genome sequencing (WGS) method, it is essential to perform the complete identification of novel resistance factors and distinct genetic ways. The idea is supposed to develop resistance to several antibiotics (Lantos *et al.*, 2021; Menon *et al.*, 2020). The extensive amount of information acquired through WGS provides numerous applications across diverse fields (Logsdon *et al.*, 2020). Molecular diagnostics allows physicians to apply the most suitable antibiotics in managing infections by discovering antimicrobial drugs. Through the use of the latest diagnostic practices, which include PCR and the microarray, medical professionals can reidentify the resistant bug

strands and select the most appropriate antimicrobials to prescribe in response (Hayden *et al.*, 2024; Lim *et al.*, 2020; Duan *et al.*, 2021). Utilizing such an approach will minimize the chances of treatment failures, the side effects will not intensify, and therapies will be personalized to the specific problem. Molecular diagnostics and their quick and accurate contribution to antimicrobial resistance surveillance initiatives not only improve antimicrobial stewardship but also make the future of the field of medicine more collaborative and sustainable. Surveillance methods adopt applications such as PCR and WGS to track antibiotic-resistant illnesses in healthcare facilities and the community.

Point-of-Care Testing (POCT):

Point-of-care testing (POCT) is undoubtedly a revolution in medicine as it involves providing fast screening and treatment directly to the location of the affected patient. One of the many benefits for patients initially diagnosed with POCT is that treatment is promptly administered. One crucial feature of POCT is that it can give diagnostic results to medical professionals within a short period (Elsheikha *et al.*, 2020; Jayamohan *et al.*, 2021). Utilizing molecular diagnostic's rapid turnaround times strategy helps accelerate diagnosis, and more accurate treatment is of the essence, according to (Akber *et al.*, 2023; Zlotnikov *et al.*, 2023). This is critical when managing infectious illnesses, where patient recovery and infection containment depend on early diagnosis and commencement of suitable treatment (Mögling *et al.*, 2020; Gopalswamy *et al.*, 2020; Tang *et al.*, 2020). The POCT devices are designed to require a minimum of setup and user training and are readily adaptable. They can be utilized in high-end clinical locations and remote settings with scarce resources. Point-of-care tests are user-friendly and highly transportable.

Consequently, rapid diagnosis and management are assured. Such a situation does not require rescheduling appointments or referring patients (Binnicker, 2020;

Sreepadmanabh *et al.*, 2020; Donnelly *et al.*, 2020). Rapid POCT diagnostics improve the detection of infectious causes, leading to faster use of appropriate treatment and better patient outcomes. Ultimately, POCT provides an opportunity to assess patient response to treatment better and more precisely and achieve optimal outcomes.

Challenges and Future Perspectives:

There could be improvements and the promotion of molecular diagnoses in the healthcare system, but despite this, they have radically changed the method for managing infectious diseases. For instance, a molecular diagnostic test's start-up cost and the necessary equipment are significant barriers, particularly in second-world countries (Tahamtan & Ardebili, 2020; Vaz *et al.*, 2020). Establishing equitable access to molecular diagnostics requires creative financing patterns in addition to technology transfer stratagems. Establishing molecular diagnostics depends on appropriate laboratory capabilities and qualified professionals. As such, healthcare institutions, particularly in developing countries, are in shortage of facilities to make high-quality molecular diagnoses. Variabilities in assay quality and lack of quality control measures can affect test accuracy and reproducibility.

Impact on Clinical Practice:

Modern molecular diagnostics enable rapid and precise identification of mutated microorganisms that, in turn, facilitate doctors to develop the best selection of treatments instantly. As such, molecular tests are a more convenient tool for the accurate and fast diagnosis of diseases as they give quick results. The speed enables patients and clinicians to initiate the proper treatment and control of the infection. According to Koehler *et al.* (2021), diagnostics are indispensable in deploying the precision medicine strategy in the battle against infectious diseases. It allows the physicians to select the most appropriate treatment strategy based on the individual patient characteristics and the virulence of the pathogens. Identifying a particular genetic

marker involved in the antimicrobial resistance or virulence mechanism is sufficient to enable Medics to pick the most appropriate antimicrobial agent, according to Koehler *et al.* (2021). This, in turn, optimizes the treatment regimen, helping patients to recover more than is expected and reducing the failure rate of treatment. Point-of-care molecular assays have added a new dimension in the practitioner's clinics by eliminating delays in clinical diagnosis experienced when prior diagnostic and interventional tools were used. Also, POCT devices perform rapid diagnoses and allow for immediate treatment initiation. Thus, incorporating the test into the workflow is very beneficial since it can decrease the time to diagnosis. Molecular diagnostics, considering the surveillance of infectious diseases and tackling any outbreak promptly, is a core task of public health.

In conclusion, the emergence of molecular diagnostics in infectious disease management has altered the diagnosis and management of these diseases. These technologies are now fast and precise, allowing pathogens to be identified that guide the targeted treatment. The developments improve the patient's response to treatment. Molecular diagnostics also assist in promoting precision medication and antimicrobial stewardship initiatives, which improve the treatment of antimicrobial resistance and the efficiency of healthcare resource administration. Also, the joint use of molecular diagnostics in clinical practice will refine infection control and outbreak control and make public health surveillance systems more effective. In the process, cooperative efforts between diversified parties bring industry professionals, academic researchers, government officials, and industry players into play, which ensures the benefits of molecular diagnostics are taken advantage of to the maximum and results in healthier lives for individuals and communities.

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