

## Biological, Pathological, Molecular Diagnosis of Microbes and their Role in Pharmaceutical Field as Biological Target

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DOI: [10.36348/sb.2021.v07i07.002](https://doi.org/10.36348/sb.2021.v07i07.002)

| Received: 11.05.2021 | Accepted: 17.06.2021 | Published: 06.07.2021

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### Abstract

Different infections in blood leads to infectious diseases that are caused by viruses, bacteria and parasites. Microorganisms are used in the production of antibiotics, vaccines, steroids, etc. The advantages and limitation of molecular techniques including real-time polymerase chain reaction, genome sequencing, molecular typing, microarrays, PCR and multiplexing required advancements at molecular level. Nucleic acid-based tests are used in diagnosing infectious diseases for isolating nucleic acids from through gel electrophoresis, and nucleic acid hybridizationsn techniques to analyze DNA or RNA. Polymerase chain reaction (PCR) is an in situ DNA replication process that allows for the exponential amplification of target DNA in the presence of synthetic oligonucleotide primers. The advances in chemistry that made real-time PCR possible were also significant, and modifications of these chemical reactions continue today. In situ hybridization has been introduced into the clinical microbiology laboratory and should prove to be a useful technology for the rapid characterization of bacteria and fungi in positive blood culture samples. Multilocus sequence typing (MLST) is an unambiguous, portable and nucleotide-based technique for typing bacteria using the sequences of internal fragments of (usually) seven house-keeping genes. Microorganisms are used in the production of antibiotics, vaccines, steroids.

**Keywords:** Molecular diagnosis, microbes, PPCRR, bacteria, parasites, pharmaceutical sciences.

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### 1. INTRODUCTION

There are different pathogens that cause variety of diseases in blood stream due to their strong interaction with parameters in blood [1, 2]. Microorganisms such as pathogenic fungi, parasites, bacterial toxins, viruses are the most leading for searing of infectious diseases in human. It ultimately leads to affect the large populations all around the world. There is need to designed strategies to control the infections caused by pathogens at cellular level in order to inhibit their action the initial level [3-5].

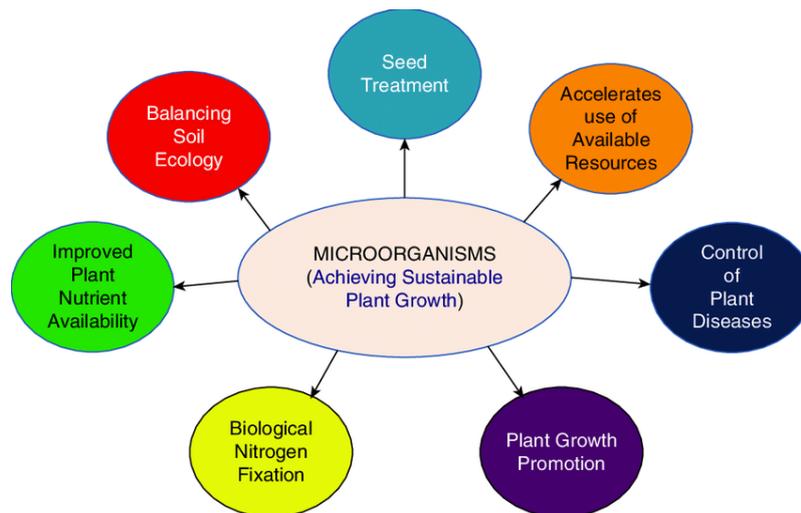
There are many techniques used for diagnosis of microbes at molecular level [6]. The advantages and limitation of molecular techniques including real-time polymerase chain reaction, partial or whole genome sequencing, molecular typing, microarrays, broad-range

PCR and multiplexing have made revolution in the fields of biomedical sciences. PCR is used to amplify the specific segment of the DNA that can possible through the advanced primers. Electrophoresis separates the different bands of the DNA on the basis of their size. Genome sequencing is set to sequencing the particular sequence of indicial or organism [7, 8].

Besides PCR and genome sequencing there are many other techniques or methods used for diagnosis of infectious diseases [9]. Nucleic acid-based tests used in diagnosing infectious diseases use standard methods for isolating nucleic acids from organisms and clinical material and restriction endonuclease enzymes, gel electrophoresis, and nucleic acid hybridization techniques to analyze DNA or RNA. Each method has its own advantages and limitations to diagnose the specific disease. While these advances aim to improve

laboratory performance and efficiency and the quality

of patient care, they are not without drawbacks [10].



**Fig-1: Shows the potential of microbial functions st cellular level**

### 2.1 Diagnosis of Microbes through Molecular Approach

There are many techniques that are used for diagnoses of infectious disease caused by microbes. It is necessary to identify the specific disease either caused by, bacterial infection, viral and parasitic attack on the specific cell of human body. The most important techniques are PCR,

### 2.2. PCR and its Types, Role in Molecular Diagnosis

PCR is the most important situ DNA replication process in which specific primers are potentially used for the amplification of the particular target of the DNA[11]. This can be carried out in the presence of enzyme such as thermostable DNA polymerase. Buffers are also prepared to control the reactions. It only carried out in small tube with reaction mixture under thermocyclers. This reaction dependent on the temperature on each specific step and excess of temperature leading to misleading the accuracy of results and hence poor quantification [12].

RT-PCR also knows as reverse transcription-polymerase chain reaction that is used to detect the mRNA both at cellular and molecular level under laboratory conditions [13]. RT-PCR is relatively most advanced techniques as compared to the other traditional techniques in detection of mRNA using the small amount of sample while on the other hand; other molecular techniques are not significant as due to large amount of samples for detection. There are various methods to validate the PCR generated amplicons, as they serve to differentiate the target amplification from non-specific amplification. Real-time PCR automates this otherwise laborious process by quantitating reaction products for each sample in every cycle [14].

### 2.3. Role of genome Sequencing

Each organism has specific sequence of nitrogen bases found in DNA. Sometime, there is breakage of in any DNA strands that leads t sere mutations. Detection of mutations or any defect helpful to diagnosis the specific disease. Sometime, mutations leads to defects in DNA that might be harmful for the survival of organisms. Molecular biology helpful in detecting of any disease thus assisting in diagnosis of pathogenic diseases [15, 16].

DNA sequencing is important to different the organisms from each other on the basis of specific nucleotides in order to determine their position in right direction. DNA sequencing means determining the order of nucleotides in a DNA molecule for which This technique can be used to study the structure of gene, detect mutations, and compare genetic relatedness and to design oligonucleotide primers. DNA replication, transcription and translation are the most important events for survival of organisms. DNA sequencing is sued for detection of particular sequencing in each process occurs in the particular cell [16, 17].

### 2.4. Role of In situ hybridization

This technique based on molecular biology approach in order to diagnose the variety of organism. Through this technology, bacteria as well as fungi easily to characterize in the blood. In situ hybridization is used to reveal the location of specific nucleic acid sequences on chromosomes or in tissues, a crucial step for understanding the organization, regulation, and function of genes [17].

There are many advantages of using of in situ hybridization as compared to the traditional techniques that required much time and costly and hence not affordable for cellular detection of particular sequences of DNA or RNA in relation to the chromosomal sites, the major advantage of in situ hybridization is that it

enables researchers to determine how the distribution of specific nucleic acids is related to protein products of the target gene and their relation with cellular structures using immunohistochemistry. This technology is specially employed in order to localized the specific sequences to the chromosomal sites. Hence, it helpful for detection of cellular damage of any tissue or replication of microbes in the specific cell [18-20].

### 2.5. Role of Multilocus sequence typing

There are many other techniques used for detection of nucleotides sequences in the particular cell. It also helpful for diagonals of microbial infection either due to bacteria and viruses. One of such techniques in the molecular biology is the Multilocus Sequence Typing (MLST) that is the most unambiguous, portable and nucleotide-based technique for typing bacteria using the sequences of internal fragments of (usually) seven house-keeping genes. In MLST, different sequences within a bacteria species are assigned as distinct alleles for each house-keeping gene and the alleles at each end of the seven loci define the allelic profile or sequence type for each isolate [21-23].

There are lots of advantages of using of MLLST as compared to the traditional molecular biology technique that are not reliable due to equipment of large quantity of samples. This technique is used for the detection of specific bacterial sequences in appropriate manner. The great advantage of MLST over MLEE and over molecular typing methods that rely on the comparisons of DNA fragment sizes is the unambiguity and portability of sequence data, which allow results from different laboratories to be compared without exchanging strains. Through this technique, different strains of bacteria can be diagnosed easily with low cost and high accuracy rate. Sometimes, human error occurs due to pathogen strains that disrupt the quality of results [24-27].

### 2.6. Role of microbrews in Clinical/Medical Diagnosis

Since the microbes are causing different disease and resisted increasing day by day as they can attack on the human cells and damage them. In this regard, lots of antimicrobial drugs have been discovered in order to control the growth of may bacterial specific and ultimately connected the number of infectious diseases [28-30]. Probiotics also helpful in digestion as major event of some useful bacteria. Another important role in pharmaceuticals is the use of microbes for medically important studies, such as Bacteriorhodopsin, a protein from the plasma membrane of *Halobacterium salinarum*. Matrix-Assisted Laser Desorption/Ionization Time-of- Flight mass spectroscopy (MALDI-TOF MS) is used for detection of bacterial pathogens such as lactic acid bacteria in fermented food, bacteria involved in spoilage of milk and pork, bacteria isolated from milk of dairy cows, and pathogens contaminating

powdered infant formula-food [30-35]. This technology potentially differentiated the bacteria against the other strains by marking the laser flow at the specific direction. For example, *Shigella* is often confused with *E. coli*, and different *Streptococcus* species are often indistinguishable through MALDI-TOF [36, 37].

### CONCLUSION

There are different methods or techniques used for detection of microbes; each method has its own limitation and efficiency the specific diseases. Variety for primers also used to diagnose them at advanced level. PCR, electrophoresis, MLST, hybridization techniques and other nucleic acids detection techniques are used. Sometime, these techniques identify the particular at high cost. This has reflected in the relatively very low available literature on the application of molecular techniques to detect or type foodborne pathogens isolated from ducks.

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