**CHAPTER 9**

**BIOSENSORS AND NANOTECHNOLOGY IN MICROBIAL DETECTION**

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**ABSTRACT**

In clinical microbiology, the identification of microorganisms is important for the immediate diagnosis and treatment of infectious diseases. Traditional methods include PCR and culture-based techniques, which often involve lengthy processes, exorbitant costs, and the need for specialized laboratory facilities. Biosensors integrated with nanotechnology have become useful tools for rapid, accurate, and cost-effective microbial detection in response to these problems. An analytical instrument is known as a biosensor, which consists of a bioreceptor such as antibodies, nucleic acids, or enzymes; a transducer that could be electrochemical, optical, or piezoelectric; and a signal processor. Some of the most appealing advantages that biosensors are offering as compared to the traditional methods include real-time detection, very high specificity, and downsizing into point-of-care applications. Electrochemical and optical biosensors have demonstrated great promise among the various types of biosensors used in microbial diagnostics due to their extreme sensitivity and simplicity of integration with digital platforms. Nanotechnology improves sensitivity, specificity, and detection limits, which further improves biosensor performance. Nanomaterials, including carbon nanotubes, quantum dots, and nanoparticles, have been effectively integrated into biosensor systems to enhance signal detection and make it possible to identify microbes at extremely low concentrations. Bacterial pathogens, such as *Escherichia coli* and *Staphylococcus aureus,* and viral and fungal diseases, like SARS-CoV-2 and HIV, have been detected with very effective biosensors. Successful microbial identification is portrayed by applications in the real world related to environmental monitoring, food safety, and healthcare situations. The creation of point-of-care biosensors has transformed microbial diagnostics, allowing quick bedside testing. The integration of lab-on-a-chip and microfluidics technologies has further made them more portable and user-friendly to be used in the field for medical practitioners to carry out tests with minimal sample preparation. Despite promising encouragement, biosensor technologies face challenges in large-scale commercialization, including manufacturing costs, regulatory permissions, and standardization. Future studies will focus on the development of nanomaterial production, enhancing multiplexed detection capabilities, and the application of artificial intelligence to enhance real-time data analytics. This could potentially revolutionize microbiological diagnostics by ensuring early identification, prompt treatment, and better patient outcomes.

**Keywords**- Biosensors; Nanotechnology; Microbial Diagnostics; Point-of-Care Testing; Infectious Disease Detection

**I. INTRODUCTION**

Clinical microbiology has an exciting past, a noteworthy present, and a bright future. It has been evolving since the Dutchman Anton van Leeuwenhoek invented the microscope more than 200 years ago. The development of the light microscope was the main medical technology advancement that made it possible to directly view microorganisms. The modernization of Western medicine began with the discovery of bacterial illnesses, and clinical bacteriology later became an important part of diagnostics. Clinical microbiology today uses standard techniques including molecular biology, in vitro culture, antigen and antibody testing, and Gram stain morphology to diagnose and monitor the progression of microbial infections. The identification and characterization of pathogens for newly developing infectious illnesses has been greatly aided by clinical microbiology. The diagnosis and treatment of infectious disorders depend on the detection and identification of microorganisms, which is made possible by clinical microbiology [1]**.**

Traditional methods like Gram staining, culture, and antigen-antibody tests are still crucial in clinical practice. However, molecular biology techniques, especially nucleic acid amplification, have greatly improved pathogen detection and identification, including antimicrobial resistance. PCR is notably effective, enhancing accuracy and speed. Blood cultures remain vital for assessing disease severity. As molecular methods become more efficient and affordable, their use in clinical microbiology is likely to grow. [1, 2]. Clinical microbiologists are responsible for identifying the bacterial, viral, fungal, and parasitic agents that cause human disease, providing diagnostic and therapeutic support for clinical patient management, and preventing the spread of infectious diseases in the community and healthcare system. The number of infectious diseases that have been found has been gradually rising since the 1940s [3].

The polymerase chain reaction (PCR) was invented in the 1980s to amplify nucleic acids, enhancing clinical microbiology with quick and sensitive techniques. Reverse transcriptase (RT), discovered in 1970, led to the widely used RT–PCR for detecting RNA. Quantitative PCR (qPCR), developed in 1993, allows precise measurement of pathogens in samples. Automation of Sanger sequencing in 1987 made rapid genome sequencing of bacteria like *Haemophilus influenzae* and *Mycoplasma genitalium* possible. [1] Clinical microbiology has altered in the early 21st century as a result of new techniques for sample and culture, as well as technologies like MALDI–TOF mass spectrometry (MS), PCR, and high-throughput sequencing [4]. The advances in technology that begun in environmental microbiology have revealed a far larger microbial world than was previously believed [5].

Clinical microbiology diagnostic techniques fall into five major areas. The first is morphological observation, which can be done under a microscope or with the unaided eye. The second is pathogen antigen tests, which are widely used for outpatient testing because of their accuracy and rapidity. The third approach, which is regarded as the gold standard for culturable diseases, is the culture method. Although they are rarely employed for rapid detection, the fourth method is the use of serological methods to identify host reactions. Molecular biology techniques are included in the last category; PCR is the most popular tool for identifying viral infections. [1]

**A. Current challenges in traditional diagnostic methods**

For many years, traditional diagnostic methods and procedures have been employed to identify and diagnose a wide range of illnesses. To make a diagnosis, these techniques combine imaging tests, laboratory testing, and clinical evaluation. Traditional approaches continue to be an essential tool in the management and prevention of disease, even while new diagnostic processes and techniques are constantly being created. New and creative methods for identifying and diagnosing illnesses are referred to as emerging diagnostic techniques and procedures. These approaches are always changing and are frequently more effective, sensitive, and focused than conventional ones.

Traditional methods for diagnosing infectious diseases, like culture techniques and histopathology, have problems with low sensitivity and are slow. This has led to new molecular diagnostic methods. Polymerase Chain Reaction (PCR) is now a gold standard for detecting infections quickly and accurately. Other advanced techniques include next-generation sequencing, radiometric methods, and various serologic tests. However, implementing these technologies can be difficult in areas with limited resources, and ethical and data privacy issues need to be resolved for patient safety. [6].

The traditional way of diagnosing infectious diseases involves the doctor examining the patient, identifying a clinical syndrome, and then testing for possible pathogens until a diagnosis is reached. However, the increasing number of new pathogens makes it hard for doctors to remember all possible pathogens for each disease and order the right tests. Standardized diagnostic kits based on the syndrome can be employed to minimize delays, streamline test ordering significantly faster, and improve workflows for medical professionals. [5]

 Many immunocompromised people are at serious danger from invasive fungal infections. The need for innovative detection techniques is emphasized by the lower sensitivity of conventional diagnostic techniques like histopathology and culture. Novel molecular and serologic methods are being evaluated in clinical settings. In clinical practice, tests like the β-glucan test for invasive *Candida spp.* and the galactomannan antigen test for aspergillosis are becoming more significant. Moreover, molecular techniques such as MALDI and PCR are promising but require uniformity.

Before a new diagnostic test is used in clinical practice or included in guidelines, it must undergo a long validation process. This process checks the test's sensitivity, reproducibility, accuracy, and quantification limits. It's challenging to determine accuracy without a gold standard test, especially for fungal diagnostics. After analytical validation, clinical validation assesses the test's usefulness, which is difficult due to few fungal disease cases. The types of specimens, like blood or urine, complicate validation further. Factors such as ease of use and cost impact the adoption of new tests. Non-molecular methods are still used but are less sensitive. There is a need for faster and more accurate tests, and while new methods are emerging, inconsistencies hinder their widespread use. Standardizing these techniques is crucial for improving detection and treatment of fungal pathogens. [7]

For many years, the identification of viral infections has been delayed by the expense, effort, and expertise needed for the cell culture techniques used, as well as the slow development and typically low sensitivity of many viruses in artificial conditions. For many viruses, the clinical detection of antibodies is rather insensitive, serology is frequently ineffective in the early stages of infection, and obtaining specific antisera for the serology tests can be challenging. Therefore, the detection of some of these viruses has been enhanced by PCR technique. For a number of persistent viral illnesses, monitoring viral DNA or RNA loads has become the accepted treatment. Viral load is measured using branched chain DNA signal amplification, competitive PCR techniques, or more recently real time PCR.

A variety of biochemical characteristics, including oxygen demand, Gram staining, carbohydrate metabolism, and the presence of particular enzymes, have long been used in the phenotypic identification of bacterial isolates. However, even when automated, phenotypic identification methods—like tiny strips—are expensive and time-consuming, and that's why MALDI–TOF MS has surpassed them during the last ten years. Due to the improved workflow efficiency and related cost savings when employing this method, MALDI–TOF MS has been used by numerous big CMLs worldwide [5]

A group of simple and fast molecular devices is replacing antigen testing for quick diagnosis at the point of care. "Syndromic panel" kits with multiplex-PCR are commonly used to find various pathogens with similar symptoms. Quantitative pathogenic testing is important for monitoring infection treatment. Next-generation nucleic acid sequencing and gene editing are being used to detect specific nucleic acids of pathogens. MALDI-TOF MS is also used in laboratories for fast identification. Molecular methods help discover new pathogens like hantavirus and influenza A (H7N9) [1]

Next-generation sequencing (NGS) is one of the most impressive developments since PCR. The discovery of novel strains and genetic variants is made possible by NGS, which enables the sequencing of whole pathogen genomes in a single run. There are several benefits to this technology, especially when it comes to monitoring antibiotic-resistant strains, figuring out virulence factors, and comprehending the molecular epidemiology of outbreaks. NGS is being utilized in clinical diagnostics more and more as bioinformatics tools and data analysis capabilities become more widely available. The management of infections has been significantly accelerated by the quick identification of virulence factors and resistance genes [8].

Molecular diagnostic techniques now use advanced strategies like CRISPR-based systems along with PCR and NGS. CRISPR is known for its ability to change genes and quickly identify specific DNA or RNA sequences for diagnosing infectious diseases. However, implementing these techniques in clinical settings, especially in low- and middle-income countries, faces challenges like lack of infrastructure, skilled workers, and funding. Advancements in portable and affordable PCR and NGS technologies could help address these issues. Each diagnostic method, including PCR, NGS, LAMP, NASBA, and CRISPR, has unique strengths and weaknesses in clinical applications. [ 9, 10].

The genomes of an increasing number of microbial species/strains have been sequenced, the door has been opened wide for “omic” technologies to be used more broadly. Metagenomic next-generation sequencing (mNGS)- enabled surveillance methods offer the opportunity to improve the detection of both known and yet-to emerge pathogens. Besides genomics, the laboratory may use transcriptomics, proteomics, and metabolomics, each of which may carry potential diagnostic utility [1].

Molecular diagnostics play a crucial role in public health by supporting the fast and accurate diagnosis of contagious diseases. This approach helped control outbreaks and improved the management of significant bacterial infections. Early diagnosis of diseases like *B. pertussis,* *M. tuberculosis,* and *N. meningitidis* is essential for preventing their spread, achieved through both conventional and molecular testing. Economic factors will drive the development of cheaper, automated testing methods, with advances in automated extraction and purification systems and pipetting robots helping to reduce labour in molecular technology [2].

One of the main issues facing the food, beverage, and pharmaceutical industries is the detection of harmful bacteria. Bacterial identification is typically accomplished by detecting their distinctive metabolites or cellular reproduction cycles, which are difficult, time-consuming, less sensitive, and deceptive [11]

**B. Overview of how biosensors and nanotechnology address these challenges.**

A biosensor is an analytical instrument that quantifies alterations in biological processes and transduces them into an electrical signal. Anything that is biological, such as enzymes, tissues, microbes, cells, acids, etc., can be referred to as a biological process. The transducer will either produce current or voltage (electric form) as its output, depending on the kind of enzyme and the materials employed in the biological element. Since Clark created the first biosensor in 1962, biosensors have been intensively researched and used in a variety of contexts due to their enormous potential. Generally speaking, biosensors can be grouped according to their basic platforms, which include microbes, enzymes, protein receptors, and antibodies [12]

The field of healthcare and related services encompasses a number of unique biosensor capabilities. Biosensors are useful in a wide range of fields, including medical mycology, disease detection, retinal prostheses, contrast imaging during MRIs, heart diagnosis, health monitoring, and more. With superior social services, these extensive capabilities further elevate healthcare to a new level. [13, 14]

Applications of biosensors in a variety of industries, such as pharmaceuticals, biomedical, clinical, and healthcare, are being researched more and more. They are employed in patient monitoring, rehabilitation, disease prevention, and human health management. Without drawing a spontaneous blood sample, biosensors are able to detect bacteria, viruses, and other diseases. Decentralized healthcare is replacing laboratory-based concentrated healthcare because it enables rapid pathogen identification and disease propagation monitoring. Using optical and electrochemical transducers, biosensors transform the responses of biological materials into readable signals, facilitating rapid disease monitoring and diagnosis [15].

Biosensors based on metabolites monitor clinical parameters like blood glucose, urea, lactate, cholesterol, and uric acid, offering advantages for laboratory analysis. They have potential to detect virus outbreaks and diseases, and are particularly useful in heart diagnosis. Cardiovascular diseases are the highest cause of death globally, and biosensors using biomarkers are crucial for the diagnostic revolution. Designing sensitive and specific biosensors using surface chemistries and nonmaterials is vital for precise heart disease diagnosis [15].

Due to the growing demand for prompt and preventive diabetes diagnosis, the incidence of diabetes and the use of biosensors are driving company earnings. Biosensors are being employed extensively in diabetes spaces due to their increasing sensitivity and precision. Wearable technology lowers healthcare expenses, making portable electronic technologies essential for healthcare. New business potential may be created by the growing number of elderly individuals and the inclinations of young people for wearable technology. Biosensors aid in the detection of pathogens, dangerous substances, and diseases.

Biosensors are widely used for point-of-care monitoring in portable devices because biomolecular detection provides speed and automated analyte detection. Although there are still technical obstacles to overcome, implantable biosensors have the potential to revolutionize patient care and disease treatment. By detecting processes like anticorps, enzymes, glucose thresholds, microbial diseases, tumor growth detection, infections, and toxins, biosensor creation has advanced the medical industry.

Microbial biosensors have been integrated with modern micro/nanotechnologies to overcome traditional detection limitations. Advancements in machining tools and measuring equipment have led to improvements in microbial biosensors, such as stable transducer immobilization and high-throughput screening. Carbon nanotubes (CNTs) have been used to modify electrodes, improving sensitivity. However, CNT-based electrodes have limitations like high background current and decreased electron diffusion rate. A method combining CNT and redox osmium polymer solution was introduced for phenol detection. Deng et al. (2010) developed a novel device using a silk-derived carbon fibrous mat with metallic nanoparticles. [12]

Nanomaterials like nanowires, nanotubes, and nanoparticles raise the sensitivity of detection. Additionally, microfabrication technology enables high-throughput analyte screening. Biosensors are simple, inexpensive, and extremely sensitive analytical instruments used in the domains of food analysis, bioterrorism, environmental issues, and human health. Pathogen-specific DNA sequence detection is crucial for clinical, environmental, and food analysis because it offers reliable, fast, and accurate detection. Hazardous bacteria in food, the environment, or living things can now be detected through recent advancements in genosensor technology, including optical fiber genosensors, SPR sensors, MB sensors, QD-capture sequence, piezoelectric sensors, colorimetric sensors, electrochemical genosensors, microfluidics genosensors, sensor arrays, etc. The inclusion of nanoparticles significantly boosts the sensor's sensitivity. Still a challenge, though, is developing a device that is both portable and useful. Reliability, repeatability, robustness, and usability remain critical issues for lab-on-a-chip pathogen sensors. [11]

The development and implementation of new diagnostic methods need significant financial investment. The high cost of clinical trials, research and development, and regulatory approval may limit the development and use of innovative diagnostic techniques. Overcoming the challenges of developing and implementing novel diagnostic techniques requires interdisciplinary collaboration, innovative thinking, and a commitment to improving patient outcomes. By addressing these challenges, new diagnostic techniques can provide patients more accurate results, earlier detection, and better treatment options. [6]

The increasing prevalence of drug-resistant pathogens indicates a significant need for antimicrobial susceptibility testing platforms that are capable of providing susceptibility data within hours rather than days. Electrochemical biosensors are well suited for molecular diagnostics, that uses sequence specific hybridization of bacterial 16S rRNA for the molecular identification of bacterial pathogens [16]

Research on nanomedicine is growing as a result of the current need for faster, more accurate, and more efficient medical tools for the early diagnosis and subsequent prognosis of any disease. The multidisciplinary nature of nanomedicine has created many difficulties in the development of an ideal point-of-care biosensor, as a commercially available biosensor necessitates concepts from electronics, chemistry, physics, materials science, and molecular biology, among other point-of-care testing. Intriguing, imaginative, integrated, automated system prototypes have been made possible by emerging technologies such as microfabrication and micro/nano fluidics.[17]

**II. FUNDAMENTALS OF BIOSENSORS**

Biosensors are a rapidly developing subject with the potential to revolutionise many aspects of our lives. Their ability to detect and measure biological events in real time opens up several prospects for improving healthcare, environmental monitoring, and other fields. Biosensors are the devices that catch biological signals and transform them into observable electrical signals. It entails combining biological entities such as DNA, RNA, and proteins/enzymes with electrochemical transducers to detect and observe biological analytes such as antibody-antigen interactions. Different types of biosensors have been developed and effectively used in the environmental, biomedical, and food industries to detect and eliminate particular toxins, whether non-living or living creatures. Sensors often used nowadays include amperometric, optical, surface plasmon resonance, enzymatic, DNA, phage, and bacterial sensors. These biosensors can detect a wide range of biological analytes and have demonstrated improved responsiveness and performance in medical laboratories, food bioanalysis, and microbial detection, among other applications. Detection of the lower or upper limits of glucose in the body, microbial invasion in the body and food, heavy metals detection in soil, water, and airborne microbes, pesticides in water and soil, and various harmful chemicals produced by the body can be easily and quickly monitored with high precision using the various types of biosensors with few modifications [18].

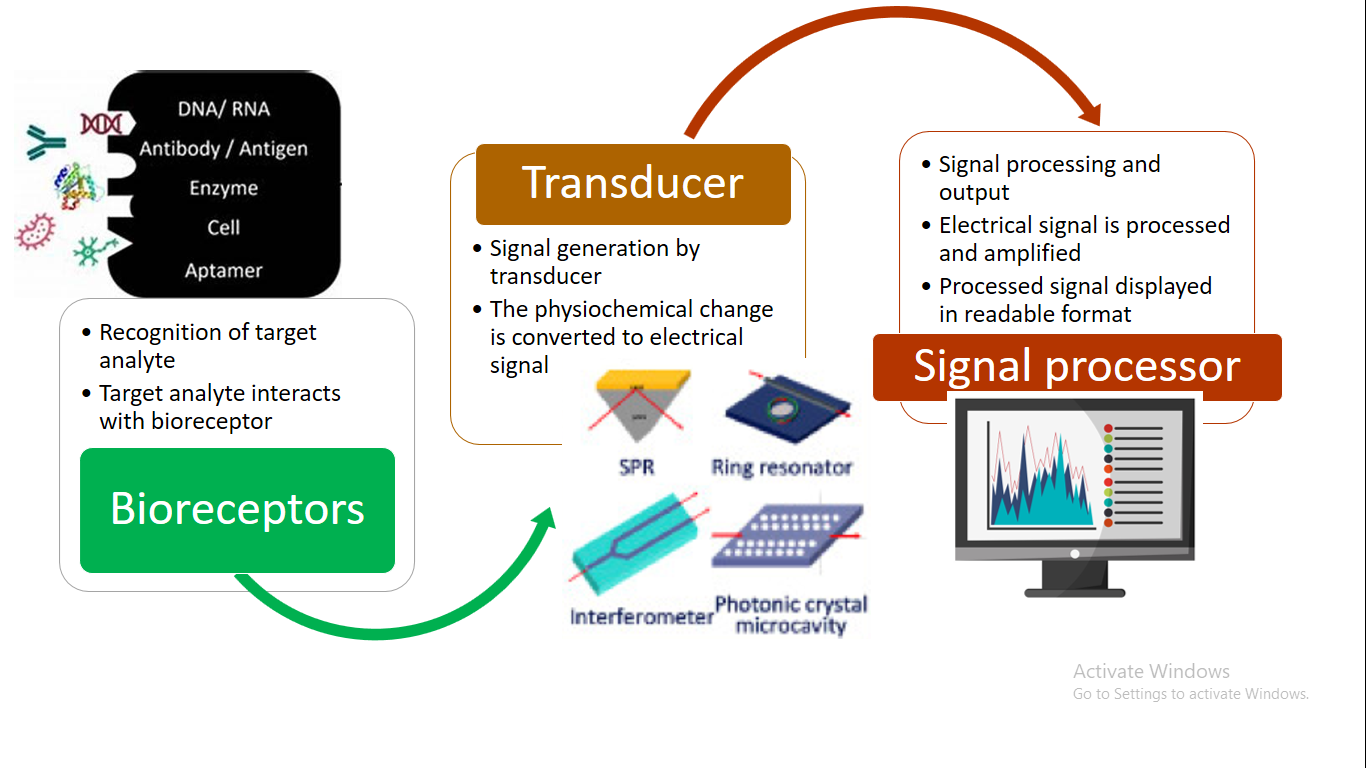
Biosensors are analytical devices that combine a biological component with a physicochemical detector to measure the presence or concentration of chemicals, biological molecules, or microorganisms. They play a crucial role in various fields including medical diagnostics, environmental monitoring, and food safety. Defined as analytical devices that combine a biological recognition element with a physical transducer, biosensors convert biological interactions into measurable signals. The International Union of Pure and Applied Chemistry (IUPAC) defines a biosensor as "a self-contained integrated device capable of providing specific quantitative or semi-quantitative analytical instrumentation using a biological recognition element (biochemical receptor) in direct spatial contact with a transducer element." Biosensors often convert physical, chemical, or biological phenomena into quantifiable signals [19]. A biosensor consists of three main components: a bioreceptor (e.g. enzymes, proteins, nucleic acids, aptamers, antibodies, organelles, microorganisms, or cell receptors) that selects the target analyte, and a transducer (e.g. optical, electrochemical, physicochemical, piezoelectric, mechanical, or thermal) that converts the biorecognition event to the target analyte [20].The concept of biosensor has evolved; for some authors it is a self-contained analytical device that responds selectively and reversibly to the concentration or activity of chemical species in biological samples. A first chemical or physical signal consecutive to molecular recognition by the bioactive layer is converted by the transducer into a second signal, generally electrical, with a transduction mode that can be electrochemical, thermal, optical, or based on mass variation. The biosensing element must be either intimately connected to or integrated within a physicochemical transducer. Numerous attempts to find a universal transducer that matches any kind of reaction have been reported [21]

**C. Biosensors: Principles and Mechanisms**

Biosensors have evolved into three generations based on the attachment of components. In the first generation (Ist gen), biosensors measure the content of analytes and products of bioreceptor reactions, producing an electric response. Leland Charles Clark Jr., the father of biosensors, first described components in 1956. In 1967, Updike and Hicks modified Clark's work, resulting in the first functional enzyme electrode-based on glucose oxidase immobilized on an oxygen sensor. In 1969, Guilbault and Montalvo demonstrated the first potentiometric enzyme electrode-based sensor for urea detection. In 1973, Guilbault and Lubrano described glucose and lactate enzyme sensors based on hydrogen peroxide detection. In 1976, Clemens et al. incorporated an electrochemical glucose biosensor in a "bedside artificial pancreas." [22]. In the third generation (IIIrd gen), the bioreceptor molecule becomes an integral part of the base sensing element, allowing for low design cost and repeated measurements. This ground breaking invention paved the way for modern biosensors, which now incorporate advanced materials such as nanostructures and artificial intelligence to enhance sensitivity and functionality. Biosensors today are capable of detecting analytes at femtomolar concentrations and offer multiplexing capabilities, allowing simultaneous detection of multiple pathogens in a single assay.

The principle underlying biosensors is the detection of specific biological interactions, such as enzyme-substrate binding or antigen-antibody recognition, and their conversion into quantifiable signals. Biosensors typically consist of three core components: a biological recognition element, a transducer, and a signal processor. A biosensor works by identifying a particular biological molecule and using a set of precise procedures to transform this contact into a measurable signal. High specificity is ensured by the bioreceptor's fundamental ability to attach to the target analyte selectively, whether it be an enzyme, antibody, nucleic acid, or entire microbial cell. The transducer then records the physicochemical change that is brought about by this binding event, such as mass fluctuation, heat production, or electron transfer. This biological contact must be transformed into a measurable physical signal which could be mechanical, thermal, optical, or electrical by the transducer.

In an electrochemical biosensor, for example, the bioreceptor starts a reaction that produces electrons, which results in a detectable change in impedance, voltage, or current. Similarly, thermal biosensors measure the amount of heat emitted during a response, optical biosensors detect changes in light, and piezoelectric biosensors detect changes in frequency brought on by changes in mass. After being generated by the transducer, the raw signal is amplified and transformed into a usable output, like a graphical display or a numerical number, by a signal processor. One useful example is the glucose biosensor, in which hydrogen peroxide (H2O2) is produced when glucose is oxidised by the enzyme glucose oxidase (GOx). After detecting this reaction, the electrode transforms it into an electrical signal proportional to the concentration of glucose, which is subsequently shown on a digital screen for easy interpretation.



**Fig: 1 Basic Working Principle of a Biosensor: Detection, Transduction, and Output**

**D.1. Parts of a Biosensor**

The structural design of a biosensor is integral to its performance. A sensor is described as a device or module that assists in detecting changes in physical quantities, such as pressure, heat, humidity, movement, force, and an electrical quantity like current, and therefore converting them into signals that can be monitored and analysed. The sensor is the heart of any measuring system. An ideal sensor should have the following characteristics: range, drift, calibration, sensitivity, selectivity, linearity, high resolution, reproducibility, repeatability, and reaction time [22]. Biological sensors detect biomolecular processes such as antibody-antigen interactions, DNA contacts, enzymatic interactions, and cellular communication. Biological sensors are also abbreviated as biosensors. The biological recognition element forms the sensor’s core, ensuring specificity in detecting microbial targets. For example, DNA probes are commonly used in microbial detection to identify specific genetic sequences unique to pathogens such as *E. coli* or *Salmonella*. Similarly, antibodies serve as bioreceptors for detecting microbial toxins or surface antigens. A biosensor combines a biological element, such as an enzyme or antibody, with an electrical component to produce a detectable signal. The electronic component detects, records, and communicates data about a physiological change or the presence of various chemical or biological substances in the environment.. A typical biosensor includes an analyte, a bioreceptor, a transducer, electronics, and a display.

The transducer plays a pivotal role in translating biological interactions into measurable signals. A transducer is an essential component of a biosensor. It turns the biorecognition event into a quantifiable (electrical) signal that corresponds to the quantity or presence of a chemical or biological target. This process of energy conversion is referred to as signalisation. Transducers provide visual or electrical signals based on the number of analyte-bioreceptor interactions. Transducers can be classified as electrochemical, optical, thermal, electronic, or gravimetric based on their functioning mechanism. Different types of transducers are employed based on the nature of the interaction. Electrochemical transducers, for instance, measure changes in current or impedance during the detection of microbial metabolites. Optical transducers, on the other hand, rely on changes in light properties, such as fluorescence or absorbance, to detect microbial activity. Piezoelectric transducers are particularly useful in detecting mass changes, as seen in applications like biofilm formation studies. Thermal transducers measure heat changes during microbial enzymatic reactions, offering insights into metabolic activity [23].

Signal processors amplify and process these transduced signals for display. Modern biosensors integrate these processors with Internet of Things (IoT) devices, enabling real-time monitoring and remote data transmission. A practical example is the integration of biosensors in food processing facilities to ensure microbial safety in packaged goods [22].

**D.2. Characteristics of a biosensor**

A biosensor's effectiveness hinges on a combination of essential characteristics that ensure its reliability, precision, and accuracy in diverse applications. These qualities are essential for highly precise analyte detection, long-term consistency in results, and precise measurement even under a variety of situations. Designing and developing sophisticated biosensors requires an understanding of these essential features such as specificity, consistency, durability, sensitivity and proportionality in order to function at its best. One of the most critical characteristics of a biosensor is its ability to specifically detect a target analyte within a complex sample. This selectivity is essential for ensuring that the sensor provides accurate results, especially when the sample contains numerous other substances that could interfere. The choice of bioreceptors, such as antibodies, plays a pivotal role in achieving this specificity. For a biosensor to function consistently over time, it must be dependable. For this consistency, reproducibility- the capacity to produce the same outcomes under the same circumstances is essential. Precision, which guarantees consistent findings in multiple tests, and accuracy, which guarantees that the results are near the genuine analyte concentration, are the keys to high repeatability. Durability is another crucial factor that determines the long-term efficacy of a biosensor. A stable biosensor can maintain its performance even when exposed to external factors such as temperature fluctuations or the degradation of the bioreceptor. This characteristic is especially important in scenarios that require prolonged incubation periods or continuous monitoring, where a sensor’s ability to withstand environmental stressors can significantly impact the accuracy and consistency of the results. Sensitivity defines the biosensor's limit of detection (LOD), which is the smallest quantity of the analyte that can be reliably identified. In medical diagnostics, for example, a highly sensitive biosensor can detect analytes at concentrations as low as ng/ml or fg/ml. This level of sensitivity is critical for applications such as early disease detection, where even trace amounts of biomarkers need to be identified accurately, which is essential for timely and accurate medical interventions. Proportionality of a biosensor ensures that it can accurately quantify the analyte across a wide spectrum of concentrations. Resolution and linear range are two essential components of proportionality. The linear range is the concentration interval throughout which the biosensor's response is exactly proportionate to the analyte concentration, whereas resolution is the smallest discernible change in analyte concentration. Both elements are necessary to guarantee that the biosensor can produce accurate and dependable readings at different target analyte concentrations [24].

**D.3. Classification of Biosensors**

Biosensor classification is a broad, multidisciplinary field. The classification of biosensors is based on a variety of characteristics, as indicated in Table 1. Bioreceptors are regarded as the primary component in biosensor fabrication. Biosensors are classified into four types based on the bioreceptor: enzymatic biosensors the most common biosensor, immunosensors which have high specificity and sensitivity and are especially useful in diagnosis, aptamer or nucleic acid-based biosensors which have high specificity for microbial strains and nucleic acid-containing analytes, and microbial or whole-cell biosensors. The second classification is based on the transducer, with sensors classified as electrochemical, electronic biosensor, thermal biosensor, optical, and mass-based or gravimetric. The electrochemical biosensor is further divided into potentiometric, amperometric, impedance, and conductometric. Another group includes bioreceptor-analyte combinations, which are restricted. Classifications are formed based on the detecting system- optical, electrical, electronic, thermal, mechanical, and magnetic and technology- nano, surface plasmon resonance (SPR), biosensors-on-chip.

**Table: 1. Classification of Biosensors**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Based on Bio receptor** | **Based on Technology** | **Based on Detection system** | **Based on Transducer** | **Electrochemical biosensors** |
| Optical biosensors | Electrochemical biosensors |
| Enzyme based biosensor | Electronic biosensors | Optical biosensors | Amperometric |
| Antibody based biosensor | Biosensors-on-chip | Electrical biosensors | Electronic biosensors | Potentiometric |
| Aptamer based biosensor | Electrometers | Thermal biosensors | Thermal biosensors | Voltammetric |
| Whole cell biosensor | Nano biosensors | Magnetic biosensors | Gravimetric biosensors | Impedometric |
| Nano biosensor | SRP biosensors | Mechanical biosensors | Acoustic biosensors | Conductometric |

**E. Types of Biosensors in Microbial Detection**

Biosensors are broadly classified based on their transduction mechanisms. Each type has distinct advantages tailored to specific applications in microbial detection. Biosensors serve an important role in microbial detection, providing extremely sensitive, specific, and quick techniques for detecting pathogens and monitoring microbial activity in a variety of domains including healthcare, food safety, and environmental monitoring.

**E.1. Electrochemical biosensors**

Electrochemical biosensors are frequently employed in microbial detection because they can monitor electrical changes such as current, voltage, or impedance caused by enzyme reactions or microbial growth. These sensors often employ biological recognition components, such as enzymes or antibodies, which catalyse processes when bound to target microorganisms. The electrical signal produced is proportional to the concentration of the pathogen, making these sensors perfect for detecting bacteria, viruses, and fungus in clinical samples, food items, and water [25]. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) Conductometric, (2) Amperometric and (3) Potentiometric [26]. An amperometric biosensor is a self-contained integrated device based on the amount of the current resulting from the oxidation, providing precise quantitative analytical information.

**E.2. Optical biosensors**

Optical biosensors detect changes in light characteristics such as absorption, fluorescence, or refractive index when a microbiological activity takes place. These biosensors are extremely sensitive and are used for pathogen detection, environmental monitoring, and diagnostics. Surface Plasmon Resonance (SPR) techniques provide real-time monitoring of microbial interactions by measuring a shift in the refractive index produced by microbial attachment, providing excellent specificity and sensitivity without the need of labelled probes [27].

**E.3. Piezoelectric biosensors**

Piezoelectric biosensors, such as Quartz Crystal Microbalance (QCM), detect changes in the resonance frequency of a piezoelectric crystal as microbial cells adsorb to its surface. This technology is very helpful for detecting bacterial adherence, biofilm development, and microbial growth, and it offers a label-free, real-time sensing tool for microbial contamination in water, food, and healthcare situations [28].

**E.4. Thermal biosensors**

Thermal biosensors detect heat changes that occur during biological processes or microbial metabolism. As microorganisms grow or metabolise substrates, they produce or consume heat, which may be monitored using thermal sensors. These sensors are commonly employed to monitor bacterial development, particularly in food safety applications where quick and reliable detection is required [27].

**E.5. Fluorescent biosensors**

Fluorescent biosensors use fluorescent markers or probes that attach selectively to microbial components, such as DNA or proteins, and produce fluorescence upon binding. This allows for high-throughput, specific pathogen identification, notably in clinical diagnostics and environmental monitoring, because the fluorescent signal is straightforward to quantify and can detect even low microbial quantities quickly [28]. Fluorescent microbial biosensors are frequently utilised in analysis processes because they may emit fluorescent light that is directly proportional to the analyte concentration at low levels. The fluorescent microbial biosensor is based on fusing an inducible promoter to a reporter gene to create a fluorescent protein that emits measurable fluorescence in a genetically engineered bacterium. Green fluorescent protein is the most often utilised component in the development of fluorescent microbial biosensors due to its stability and sensitivity. To detect arsenic, recombinant Escherichia coli cells were transformed using plasmids that contained three tandem copies of the ars promoter/operator-the gfp gene. Recombinant Escherichia coli cells doubled the signal-to-noise ratio and reduced the detection limit from 20 to 7.5 μg/L, compared to cells using single-copy plasmids. When exposed to genotoxins, the recombinant yeast Green ScreenTM produces fluorescence by producing green fluorescent proteins. Based on this process, a microfluidic device containing yeast was created for the detection of hazardous substances [29].

**E.6. Bioluminescence based microbial biosensors**

Bioluminescence based microbial biosensors have been extensively used in environmental monitoring for detection of toxicity due to its ability to closely reflect to toxicity . As a proportional response to the concentration of the analytes, the changes in the density of the bioluminescence emitted by the living cells can be measured by the bioluminescent microbial biosensor. According to the mechanism of production of bioluminescence, the method to control the expression of the lux gene can be divided into two manners: the constitutive manner and the inducible manner. In the constitutive manner, the bioluminescence caused by lux gene-coded luciferase exists constitutively as long as the organism is active. As the density of the bioluminescence can be affected by the additional compounds such as the toxicity, it can be used as a parameter to determine the additional compounds. In the inducible manner, the lux gene is fused with a promoter regulated by the concentration of the analytes. Based on this mechanism, the bioluminescence cannot be detected until the concentration of the analytes approaches a critical value. Several bioluminescent microbial biosensors have been developed in recent years. A whole-cell bioluminescent biosensor, based on genetically engineered Escherichia coli bacteria, carrying a recA::lucCDBAE promoter-reporter fusion, was developed for the detection of water toxicity. Further, Kuncova et al. constructed a biosensor for the detection of water pollutions, based on Pseudomonas putida TVAS, harboring chromosomal tod-lux CDABE fusion. By immobilizing bioluminescent bacteria, TV1061 strain, in wells of a microtiter plate, Eltzov et al. fabricated a microbial biosensor for air toxicity monitoring and achieved a good response to a low concentration of chloroform (6.65 ppb) [30].

**E.7. Mass-sensitive biosensors**

Mass-sensitive biosensors detect changes in mass on a sensor surface as a result of microbe adhesion and growth. Microcantilevers and surface acoustic wave devices can precisely measure this minute mass changes, enabling real-time detection of microbial adherence, pathogen detection, and biofilm growth. These sensors are employed in clinical diagnostics, environmental testing, and bioreactor monitoring, making them an effective and sensitive instrument for microbial identification [31]. Each of these biosensors has distinct benefits, such as fast reaction times, great sensitivity, and the capacity to do real-time, on-site monitoring, making them important tools for a wide range of applications from medical diagnostics to environmental protection and food safety.

Electrochemical Biosensors are among the most widely used due to their simplicity, sensitivity, and cost-effectiveness. They measure changes in current, voltage, or impedance resulting from biological interactions. For instance, a DNA-based electrochemical biosensor for *E. coli* detection leverages impedance changes when microbial DNA hybridizes with a complementary probe. Such sensors have been employed to detect *Salmonella* in poultry samples within two hours, significantly reducing diagnostic timelines. The electrochemical biosensors are a class of biosensors which convert biological information such as analyte concentration that is a biological recognition element (biochemical receptor) into current or voltage. Electrochemical biosensors depict propitious diagnostic technology which can detect biomarkers in body fluids such as sweat, blood, feces, or urine [32].

The efficiency of electrochemical biosensors are improved by the introduction of multienzyme system. Multienzyme biosensors are sophisticated devices that detect target analytes by sequentially activating numerous enzymes. These biosensors use enzyme cascades, in which one enzyme generates a substrate or cofactor for the next, enhancing signal production and detection accuracy. Oxidase-peroxidase biosensors are used to detect glucose and cholesterol, dehydrogenase-based biosensors for clinical diagnostics, and hydrolase-based biosensors for pesticide and toxin detection. Applications include medical diagnostics, environmental monitoring, and the food sector. Despite their benefits, problems include enzyme degradation, complicated manufacture, and possible interference from cross-reacting chemicals [33].

**F. Biosensors-on-Chip: Enhancing Point-of-Care Diagnostics**

Biosensors-on-chip (BoC) devices combine biosensors with microfluidic technology to improve sensitivity, specificity, automation, and accuracy in point-of-care (PoC) diagnostics. Recent advancements include electrochemical biosensors using nanomaterials for cancer diagnosis, optical biosensors (optofluidics) such as surface-enhanced Raman spectroscopy (SERS) for virus detection, microcantilever-based sensors for bacterial identification, magneto-immunosensors for parasitic infections, and paper-based microfluidic biosensors for antibiotic resistance detection. These advancements have greatly improved cancer diagnoses by allowing liquid biopsy-based identification of circulating tumour cells (CTCs), microRNAs, and tumour DNA alterations. Amperometric biosensors can detect infections such as Pseudomonas aeruginosa and Staphylococcus aureus at extremely low concentrations, whilst magneto-immunosensors help with malaria diagnosis. Although restricted, neurodegenerative illness diagnostics are increasing with biosensors detecting critical biomarkers like α-synuclein and amyloid-β. Electrolyte-gated organic field-effect transistors (EGOFET) achieving detection limits as low as 0.25 pM for Parkinson’s disease. Future developments in BoC technology, integrating nanotechnology, artificial intelligence, and lab-on-a-chip systems, hold promise for revolutionizing PoC diagnostics, particularly in resource-limited settings [20].

**G. Advantages of Biosensors over Traditional Methods**

Biosensors offer several advantages that make them superior to traditional microbial detection methods. Speed is a critical factor; while culturing can take 24–72 hours, biosensors can deliver results within minutes. This rapid response is particularly valuable in clinical diagnostics, where timely detection can significantly impact patient outcomes. For example, tuberculosis detection using biosensors has reduced diagnostic time from weeks to hours, enabling faster treatment initiation.

Another significant advantage is the high sensitivity and specificity of biosensors, which allow them to detect even trace levels of pathogens. CRISPR-based biosensors, for instance, can identify single-nucleotide polymorphisms in microbial DNA, providing unparalleled precision. Portability is another key feature, as many biosensors are designed as handheld devices suitable for field applications. Portable biosensors for *E. coli* detection in drinking water have been deployed in rural areas, ensuring safe water supplies without the need for complex laboratory infrastructure.

Traditional methods for microbial detection, such as culturing and microscopy, are often time-consuming, labor-intensive, and require specialized expertise. In contrast, biosensors offer real-time detection with minimal sample preparation, making them ideal for high-throughput applications. For instance, during the COVID-19 pandemic, biosensors like CRISPR-based diagnostic tools played a crucial role in quickly identifying viral RNA, ensuring timely intervention. Market studies predict rapid growth in the biosensor industry, with projections estimating a market size of $36.7 billion by 2027, driven largely by applications in disease diagnostics and food safety.

Despite their advantages, biosensors face challenges that hinder widespread adoption. High production costs, limited stability of biological components, and environmental sensitivity remain significant barriers. For instance, the shelf-life of enzymes and antibodies used in biosensors can be affected by temperature fluctuations, impacting their performance. Future advancements in biosensor technology are focused on addressing these limitations. Nanotechnology offers promising solutions by enhancing the sensitivity and stability of biosensors. Gold nanoparticles, for example, have been used to improve the detection capabilities of biosensors for pathogens like *Listeria*. CRISPR-based biosensors represent another cutting-edge innovation, enabling precise and rapid detection of microbial DNA and RNA. Additionally, integrating artificial intelligence and machine learning algorithms with biosensors can improve data analysis and pathogen prediction, further enhancing their utility.

**III. ROLE OF NANOTECHNOLOGY IN BIOSENSORS**

**H. Introduction to nanomaterials**

Nanomaterials are the material whose sizes are normally between 1 and 100 nm and exhibit unique properties due to their tiny size[34]. The size of the particle is an essential metric for the characterization of nanoparticles. Recently this become a major focus of research, with the potential to serve as a foundational technology for the future. Nanotechnology involves studying and designing materials or devices at the atomic and molecular levels. Since a nanometer is just one-billionth of a meter—containing about 10 atoms—it opens the door to precisely rearranging matter with atomic-level accuracy. The size of the particle is an essential metric for the characterization of nanoparticles. The size effect originates from quantum confinement, which alters the electronic characteristics of materials, including density of states, discrete energy bands, and adjustments at the edges of conduction and valence bands. These modifications in electronic properties result in significant changes in material behaviors compared to their bulk forms. For instance, substances that are normally opaque can appear transparent (like copper); materials that are typically inert can act as catalysts (such as platinum); substances that are generally stable can become combustible (like aluminum); solids can transform into liquids at room temperature (as seen with gold); and insulators can turn into conductors (for example, silicon)[35].The ratio of surface area to volume in a nanoparticle and also its chemical composition greatly influences its characteristics and performance[36]. These materials can be classified based on their origin as natural, incidental, bioinspired, or engineered [37]. Examples include nanoparticles, nanotubes, quantum dots, liposomes, dendrimers, and nanoshells[38]. These can be classified as zero-dimensional, for instance, quantum dots and metal nanoparticles, one-dimensional, for example, nanotubes and nanorods, two-dimensional, or bulk nanostructured[39].

Depending upon the method of synthesis and types of materials nanomaterials are again classified into organic, inorganic and carbon based. Organic nanoparticles are micelles, liposomes, dendrimers, polymeric and protein nanoparticles and nanogels. Currently liposomes are used for different therapeutic applications like cancer diagnosis and treatment, delivery of targeted drug and antimicrobial therapy. Dendrimers are widely utilized in clinical settings because of their small size (1–5 nm) and unique structure, and they serve as carriers for vaccines, genes, or medications. Polymeric nanoparticles (NPs) can be categorized as either synthetic or natural and are further divided into nanocapsules or nanospheres and can be engineered to encapsulate therapeutic agents at high concentrations, allowing for targeted release. PMMA, a widely utilized amorphous synthetic polymer, shows promise for biomedical purposes owing to its non-toxic nature, affordability, minimal inflammatory response in tissues, and ease of processing. Protein nanoparticles can be created through the self-assembly of protein polymers, which are made up of isolated proteins obtained from animal or plant sources, such as collagen, gelatin, albumin, or elastin. Abraxane and ontak are the different types of protein nanoparticles. Inorganic nanoparticles typically comprise metal and metal oxide nanoparticles, quantum dots, and silica-based nanoparticles. Carbon-based nanoparticles can be categorized into fullerenes, graphene, carbon nanotubes, carbon nanofibers, and carbon black. Nanoparticles composed solely of carbon are referred to as carbonaceous nanoparticles[40].

Nanomaterials can be created and/or produced through physical, chemical, and biological methods via two primary approaches: bottom-up and top-down, based on the initial precursors used for nanoparticle formation. Both methodologies have potential applications across various fields, including nano-molecular electronics, optoelectronics, sensors, energy storage materials, composite materials, nano-biotechnology, and nano-medicine. The top-down method involves breaking down suitable raw materials into minuscule nanoparticles through lithographic techniques such as milling, sputtering, grinding, chemical etching, and laser ablation. In contrast, the bottom-up approach employs various chemical methods (like sol-gel, laser pyrolysis, aerosol, and vapor deposition) and biological techniques (such as bacteria, fungi, yeast, and plants) to produce nanoparticles by allowing atoms to self-assemble and form new nuclei that ultimately evolve into nano-sized particles [41]. The synthesis methodologies include gas-phase methods such as inert-gas condensation, vapor synthesis, and plasma-related techniques.Nanomaterials are a class of materials that exhibit very different optical, electrical, and mechanical properties compared with the same material when it exists in a larger form. For example, semiconducting nanoparticles exhibit quantum confinement phenomena, while metallic nanoparticles exhibit plasmon resonance. Their applications extend across multiple industries, especially in the biomedical sector, where customized nanomaterials are being engineered[38]. Methods of nanomaterials' characterization include the estimation of the surface area, study of crystalline structure, electron microscopy, etc[42].

A biosensor is an analytical instrument used for detecting target molecules such as harmful biomolecules and chemical substances and these target molecules specifically bind to sensing materials such as enzymes, antibodies, or designed nucleic acid sequences. Depending on the type of sensing molecule used, biosensors can be categorized into enzyme-based, DNA-based, immunosensors, etc. Different techniques like electrochemical, fluorescence, and optical property analysis, surface plasmon resonance measurements, and surface-enhanced Raman spectroscopy (SERS), are used for analyzing the biosensing reactions between sensing molecules and target materials[43]. Over the past ten years, the design of biosensors has concentrated on miniaturizing the devices while maintaining their detection effectiveness. To accomplish this, nanoparticles (NPs) have been integrated into the design of biosensors, leading to the creation of a nanoscale platform. Indeed, NPs serve as signal transducers in various parts of the biosensor, transforming biomolecular interactions into electrical, optical, or magnetic signals. This ability within the biosensor arises from unique nanometric characteristics, including surface area, small size, affinity for specific biomolecules, catalytic properties, and autofluorescence. Similar to conventional biosensor devices, nanobiosensors consist of three primary components: a biorecognition probe, a transducer, and an amplifier. The NPs are the component of transducer section, enhancing the transduction of biochemical, electrical, magnetic, or optical signals. Additionally, the incorporation of functionalized NPs into the biorecognition component allows for straightforward and efficient signal reading[44].

Nanotechnology has significantly improved the sensitivity and selectivity of biosensors used in pathogen, toxin, and cancer biomarker detection. Nanomaterials, such as nanotubes, nanowires, and nanoparticles, with large surface areas and distinct characterizations, have complemented these biosensors greatly in performance[45]. The use of nanomaterials as signal amplifiers or generators in no-wash biosensors is extremely promising and opens a wide range of opportunities for the diagnosis of cancer[46]. The presence of nanomaterials in electrochemical sensors and biosensors significantly improved their sensitivity and selectivity to many classes of analytes: from small molecules and enzymes up to cells[47]. Moreover, gold nanoparticles, magnetic nanoparticles, and multi-layer thin film structures were used in enhancing the sensitivity of the surface plasmon resonance sensors. Methods that enhance the refractive index of analyte-nanoparticle complexes and amplify the electromagnetic field of surface plasmons result in highly sensitive optical sensors[48].

Nanomaterials have transformed the field of biosensor technology by improving sensitivity, selectivity, and detection limits. Nanostructures and nanomaterials have unique properties that make them useful for biomedical diagnostic applications. - Different types of nanostructures (0D, 1D, 2D, and 3D) have been used in biosensors to improve their sensitivity, selectivity, detection limits, and speed. These nanostructures have been integrated into various types of biosensors, including electrochemical and optical, to enhance their performance[49]. In optical biosensors, the interaction between the analyte and the receptor generates luminescence as a result of electromagnetic excitation at optical frequencies. The level of sensitivity is influenced by the detection methods employed, such as fluorescence, Raman Spectroscopy, Surface Enhanced Raman Scattering (SERS), refraction, and others. Biological components bind to nanostructures, impacting their light-emitting properties. Changes that occur from molecular binding influence the excitation of nanomaterials. This effect can be utilized to identify a specific analyte. For example, surface plasmon resonance (SPR) sensors detect variations in the refractive index of the medium at the sensing surface that occur due to the attachment of molecules. There are two categories of SPR sensors: propagating SPR (PSPR) sensors and localized SPR (LSPR) sensors. Both PSPR and LSPR sensors can detect concentrations below 1 pm. Gold nanoparticles (AuNPs), commonly known as plasmonic NPs, are utilized to enhance the sensing capabilities of LSPR sensors[50]. Commonly used nanomaterials include nanoparticles, nanowires, carbon nanotubes, and quantum dots, which possess distinct characteristics like high surface-to-volume ratios and excellent conductivity[22]. These remarkable nanomaterials, especially graphene, carbon nanotubes, zinc oxide, and gold nanoparticles, have shown outstanding results in the development of genosensors, immunosensors, and enzymatic biosensors for medical applications, facilitating the detection of various biomolecules, pathogens, and clinical biomarkers[51].Bottom of Form

Inorganic nanomaterials such as gold, platinum, and silver nanoparticles, as well as organic nanomaterials including carbon nanotubes and graphene, have been effectively utilized in enzyme-based electrochemical biosensors[52]. This noble metal nanomaterials, including gold (Au), silver (Ag), and platinum (Pt), are widely utilized in biosensor development due to their quantum mechanical properties, small size, biocompatibility, and ease of modification. Their remarkable electrochemical characteristics allow them to be seamlessly integrated into biosensor platforms, significantly improving sensor sensitivity and selectivity. For example, an electrochemical DNA biosensor was designed using Au nanoparticles of different sizes to take advantage of their superior electron transfer recovery properties. Similarly, Ag and Pt nanomaterials have been employed in electrochemical biosensor fabrication. A notable case is the development of a Pt/Ag nanowire electrode for an electrochemical biosensor, where Pt nanoparticles on Ag nanowires enhanced electrode conductivity and provided electrocatalytic activity for methanol electro-oxidation. Additionally, key characteristics of nanowires—such as length, radius, and noble metal composition—can be easily adjusted, directly influencing electrode electrical properties and optimizing sensitivity and selectivity[53].

**IV. APPLICATIONS IN MICROBIAL DETECTION**

Pathogens contribute to a significant number of deaths in both rural and urban environments. Unfortunately, the inaccurate identification of these pathogens results in inadequate management of their impacts. Furthermore, current pathogen detection methods depend on procedures that necessitate prolonged analysis times and are constrained by laboratory settings, such as microscopy and culture-based approaches, rendering them less suitable for field use. Therefore, there is a need to create highly effective pathogen detection systems that are user-friendly, cost-effective, and straightforward to implement. Traditional techniques for identifying and detecting pathogens mainly rely on (i) culture and colony enumeration methods (which involve counting bacteria); (ii) immunological approaches (that include antigen-antibody interactions); and (iii) the polymerase chain reaction (PCR) technique (which focuses on deoxyribonucleic acid (DNA) analysis). However, these methods have limitations as culture-based techniques can be expensive. Additionally, pathogens in the analyzed sample often exist in very low concentrations, which makes their detection extremely challenging. Identifying a single bacterial cell faces significant challenges, primarily due to the necessity for rapid, real-time detection and the requirement for high sensitivity in the analysis. With significant advancements made in recent years, nano biosensors have developed into practical alternatives to conventional methods employed for pathogen detection. Furthermore, enhancing pathogen detection methods could reduce the inappropriate prescribing of medications and help prevent the spread of diseases[54].

Nanotechnology has emerged as a powerful tool for microbial detection, offering rapid, sensitive, and cost-effective methods compared to traditional techniques [55,56]. Nanotechnology-based detection methods are particularly suitable for on-site and low-resource settings, providing economical access to safe food, water, and affordable diagnostics[55]. Integrating nanoparticles in microbial detection holds promise for enhancing patient outcomes and mitigating the spread of harmful bacteria in healthcare settings. Various nanoparticle-based approaches have been developed, including Surface Plasmon Resonance (SPR) sensors, Surface-Enhanced Raman Scattering (SERS) sensors, and bacteriophage-based sensors, enabling precise detection of bacteria and viruses[57]. These technologies have shown promise in detecting pathogens such as HIV, SARS coronavirus, and hepatitis viruses[56].Customizable nanoparticles have the potential to overcome the limitations of existing procedures, contributing to rapid microbiological diagnosis[58]. Gold nanoparticles have been utilized for microbial detection due to their unique optical and physicochemical properties, providing faster and more cost-effective alternatives to conventional methods[59]. Incorporating gold nanoparticles into various biosensing platforms, such as nucleic acid-based, immunosensors, enzyme, fluorescence, and bacteriophage biosensors, can improve their diagnostic capabilities[60]. Terahertz metamaterials have demonstrated potential for highly sensitive and selective microbial sensors, capable of detecting small amounts of microorganisms in both ambient and aqueous environments[61]. The combination of nanotechnology-based sensors, modern technologies, and machine learning/wireless communication represents the future trend in the diagnosis of infectious diseases[62].

Electrochemical biosensors convert biochemical interactions into electrical signals (current, potential, impedance, or resistance). They are classified into **biocatalytic** (enzyme-based) and **affinity** biosensors, which rely on selective biomolecular binding. Affinity biosensors include **immunosensors** (antibody/nanobody-based), **aptasensors** (DNA/RNA aptamers), and **genosensors** (ssDNA-based). Additionally, some biosensors for pathogen detection use peptides, phages, microRNA, antibiotics, or molecularly imprinted polymers (MIPs) as recognition elements. Various electrochemical techniques enable different signal detection mechanisms. Electrochemical biosensors that incorporate nanomaterials can be used for the detection and monitoring of pathogens. Various nanomaterials, including quantum dots, gold, silver, magnetic nanoparticles, carbon nanomaterials, metal oxides, and other two-dimensional nanomaterials, can enhance the efficacy of electrochemical biosensors used for detecting foodborne pathogen. Utilizing different nanomaterials can enhance the analytical capabilities of electrochemical sensors through signal enhancement. The incorporation of nanomaterials with the electrode expands the surface area, thereby increasing the loading capacities and the mass transport of reactants, leading to amplified signals. In addition, nanomaterials can serve as carriers for redox probes to enable sensitive detection or can enhance the kinetics of redox exchanges, significantly boosting the output. The integration of different nanomaterials into nanocomposites can address the shortcomings of conventional technologies and facilitate the development of biosensors that are rapid, highly sensitive, specific, and cost-effective[63]. Nanomaterials like nanochannels and metallic nanoparticles can be used in the development of innovative and sensitive biosensing systems for on-site detection of plant pathogens[64]. Semiconducting nanowire (NW) based field-effect transistor (FET) biosensors that are enhanced with receptor probe molecules to improve specificity are among the most advanced systems in the realm of electrochemical biological detection today. These biosensor devices have the ability to effectively identify a broad spectrum of biological, chemical, and foreign microorganisms. Moreover, NW-based sensors facilitate real-time detection and conversion of electrical output signals, all integrated within a single unit, thereby broadening the possibilities for next-generation disease diagnostics[65].

Graphene and its derivatives have attractive properties like large surface area, optical and magnetic properties, and high elasticity, making them suitable for preparing graphene-based nanocomposites for biosensor applications. Graphene-based nanocomposites have been used to detect various microorganisms including prions, viroids, viruses, bacteria, fungi, protozoa, microbial toxins, and antibiotics from microbial sources. Graphene and its derivative nanomaterials possess exceptional potential to expand the possibilities of biosensor applications in the near future. The outstanding characteristics of graphene, such as its vast surface area, superior electrical conductivity, and biocompatibility, support the development of highly sensitive and selective biosensors. These versatile materials make it possible to conduct molecular diagnostics and point-of-care testing for the detection of various diseases, while also facilitating the continual monitoring of food safety, health, and environmental threats. Various forms of graphene-based nano biosensors exist, including optical, electrical, and electrochemical biosensors. In the field of biomedical research, graphene-based nano biosensors have been extensively utilized for biomarker identification, cancer cell diagnosis, and the detection of pathogenic microorganisms. Additionally, graphene and its composite materials used in biosensing applications offer new insights for researchers, fostering the creation of advanced nanocomposites with improved detection capabilities[66].

**I. Detection of bacterial pathogens**

Nanotechnology offers promising approaches for detecting bacterial pathogens with high sensitivity and specificity. Large surface area, adjustable morphology, unique and adaptable optical properties, and outstanding mechanical, electromagnetic, and chemical properties make these nanoparticles to be used as nanoprobes[62]. The most commonly studied pathogens through nanobiosensors are E. coli, S. aureus, and S. typhimurium, with E. coli being the most prominent because of its importance as an indicator of fecal contamination and its association with various diseases[51]. Nanobiosensors using various nanomaterials such as carbon nanotubes, quantum dots, and gold nanoparticles have shown great potential for rapid and accurate bacterial detection [66, 67]. These sensors can be functionalized with aptamers or antibodies to target specific bacterial strains[68]. The integration of nano biosensors with microfluidic systems and advanced analytical techniques has further enhanced their performance, making real-time monitoring and point-of-care applications possible[67]. Immune-based sensors, Aptasensors, Bacteriophage-Based Sensors, Array-Based Sensors, and Optoelectronic Nose are the new nanotechnology-based approaches for bacterial detection[69]. The integration of molecular modelling and solid phase synthesis facilitated the effective synthesis of molecularly imprinted polymer nanoparticles (nanoMIPs) that can recognize and detect endotoxins using the highly sensitive SPR biosensor with triethylamine method[70]. In a study a polyacrylonitrile nanofiber bioreceptor, designed with bacterial antigen-specific antibodies, was developed to capture airborne bacteria. Tested with a specialized air filtration system, the antibody-coated membranes demonstrated significantly higher efficiency in capturing *E. coli* and *S. aureus* compared to unmodified nanofibers. This innovative bioreceptor could pave the way for cost-effective, rapid, and highly selective bionanosensors to detect bacterial contamination in commercial and medical environments[71]

DNAzymes have been used broadly in micro-sensor applications due to their inexpensive, stable, and advantageous properties. It can function as molecular recognition elements (RNA-cleaving DNAzyme) and reporter elements (peroxidase mimicking DNAzyme) for pathogenic bacteria detection. - DNAzyme-based biosensors have been developed that can detect pathogenic bacteria at very low concentrations (10-10 CFUs/mL) in complex matrices[72]. These biosensors primarily utilize two types of DNAzymes: hemin/G-quadruplex DNAzyme (HGD) and RNA-cleaving DNAzyme (RCD). Recent advancements include the development of a hook-like DNAzyme-activated autocatalytic biosensor, which demonstrates improved sensitivity and adaptability for detecting various pathogenic bacteria[73]. Another innovative approach involves a multi-component all-DNA biosensing system that uses a 4-way junction to transduce DNAzyme reactions into amplified signals, enabling detection of as few as 50 *E. coli* cells/mL within 85 minutes[74]. CRISPR/Cas-based nanosensors can be used to identify a variety of hazardous waterborne pathogens, such as Salmonella enteritidis, Salmonella species, and Pseudomonas species and it offers excellent specificity, outstanding sensitivity, and simplicity, rendering it suitable for practical use[75]. Surface-enhanced Raman scattering (SERS) has emerged as a powerful technique for multiplex detection of pathogens, offering rapid, sensitive, and specific identification of multiple bacterial strains simultaneously. Recent studies have demonstrated SERS-based methods for detecting foodborne pathogens at concentrations as low as 101 CFU/mL using novel covalent organic frameworks and lectin-functionalized magnetic nanoparticles[76]. In another study a novel bionanosensor utilizing surface-enhanced Raman scattering (SERS) has been developed for the rapid and sensitive detection of bacterial pathogens and this assay combines magnetic separation with SERS-active nanoparticles functionalized with strain-specific antibodies, allowing simultaneous isolation and detection of multiple bacteria. The sensor successfully identified *Escherichia coli, Salmonella typhimurium,* and methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, it demonstrated multiplex detection of all three pathogens in a single sample, confirmed through principal component analysis. The system's fast, selective, and multiplexing capabilities highlight its potential for point-of-care diagnostics and biomedical advancements[77]. Combining SERS with PCR amplification has enabled the detection of as few as 100 copies of target bacterial DNA[78]. Label-free SERS approaches have also shown promise, with one study successfully identifying 20 out of 22 common pathogenic bacterial strains using silver nanorod substrates and mathematical analysis methods[79]. These advancements in SERS technology are driving the development of point-of-use applications for rapid pathogen detection in industrial and medical settings[80], offering significant advantages over traditional time-consuming microbial culture methods.

**J. Viral diagnostics**

Nanotechnology offers promising applications for viral pathogen detection, addressing the limitations of conventional methods. Nanomaterials like gold nanoparticles, graphene, and quantum dots enable rapid, sensitive, and accurate virus detection due to their unique physical and chemical properties. These nanomaterials can be functionalized with biological ligands for selective binding to viral targets, enhancing detection specificity [81,82]. Nanotechnology-based approaches have been applied to detect various viruses, including influenza, hepatitis, HIV, and dengue[82]. Single virus tracking technology using fluorescent nanoprobes allows the monitoring of virus life cycles within living cells[83]. Biosensors incorporating nanomaterials such as graphene oxide, carbon nanotubes, and metal nanoparticles have shown potential for real-time, selective virus detection[84]. However, challenges remain, including reducing false-positive rates and improving in vivo tracking capabilities[83].

The development of various biosensors, such as affinity-based nano-biosensors, graphene affinity-based biosensors, optical nano-biosensors, surface Plasmon Resonance-based optical nano-biosensors, and electrochemical nano-biosensors, has greatly improved the rapid and sensitive detection of viruses. In addition, the incorporation of nanomaterials for signal amplification, including gold and silver nanoparticles, quantum dots, and iron oxide nanoparticles, has boosted the accuracy and sensitivity of biosensors[85]. Various metal-based nanomaterials including metal oxides and noble metals have been used for the fabrication of electrochemical and optical biosensors due to their unique properties. Metal-based nanomaterials are particularly well-suited for creating highly sensitive plasmon-enhanced biosensors, as their sharp corners and edges generate significantly amplified electric fields when exposed to a broad spectrum of excitation wavelengths. Their unique plasmonic properties have drawn substantial interest, as they can effectively influence the optical signals of nearby molecules, enhancing detection sensitivity and precision. For example, gold (Au) nanorods have been incorporated into LSPR biosensors due to their unique optical properties, particularly their enhanced near-infrared (NIR) and visible (Vis) absorption compared to Au nanoparticles. Silver (Ag) nanopillars can be used to detect the DNA of the avian influenza A (H9N2) virus. Ag nanopillars can be efficiently fabricated using a porous aluminium oxide template, and their properties can be fine-tuned by adjusting the manufacturing conditions. By optimizing these parameters, the researchers developed an effective SERS (surface-enhanced Raman scattering biosensors platform), enabling precise detection of the target viral DNA. Nanotechnology-assisted techniques like lithography, CRISPR/Cas systems, and DNA nanotechnology have been employed to develop advanced viral nucleic acid biosensors[56].

Recent advancements in nanotechnology have led to the development of biosensors for rapid and accurate detection of pathogenic RNA viruses, including SARS-CoV-2. The COVID-19 pandemic has affected millions worldwide and has weakened the global economy. While RT-PCR remains the most reliable method for virus detection, it requires time and specialized expertise, emphasizing the need for faster alternatives. The sensor-based detection methods using nanotechnology, such as gold nanoparticle (AuNP) lateral flow assays, which enable quick, accessible, and reliable testing even for asymptomatic individuals[86]. Gold nanoparticles (AuNPs) silver and nanoparticles (AgNPs) have gained significant attention due to their unique sensing properties and ability to amplify signals[87,88]. These nanoparticle-based biosensors can detect various analytes, such as nucleic acids, aptamers, and proteins in clinical samples, offering higher sensitivity and specificity compared to traditional immunological methods[88]. AuNPs, in particular, have been widely used in colorimetric detection methods for SARS-CoV-2[91]. Other nanomaterials, including graphene oxide, carbon nanotubes, and magnetic nanoparticles, have also shown promise in virus detection[84]. 2D carbon nanomaterials like graphene and graphene oxide have been extensively used to create highly sensitive and rapid biosensors for virus detection. Graphene oxide has proven effective in identifying a variety of viruses including Rotavirus, Ebola, Influenza, HIV, Hepatitis B, and Hepatitis C through fluorescent biosensing methods. Multiplexed fluorescent biosensors using graphene and other 2D carbon materials have been designed to detect multiple viruses simultaneously[89]. The integration of these nanomaterials into various biosensing platforms, such as nucleic acid-based biosensors and immunosensors, has improved their diagnostic capabilities for on-site, point-of-care pathogen detection[60]. Paper-based lateral flow biosensors (LFBs) have emerged as an efficient tool for the rapid, specific, and sensitive detection of pathogen. The LFB employs polyclonal antibodies conjugated with gold nanoparticles for signal visualization. The developed LFB is a simple, cost-effective, and robust method for the detection of Noda virus or fish nervous necrosis virus and suitable for field use in aquaculture facilities[90].

**K. Detection of fungi and other microorganisms**

Nanotechnology offers promising approaches for rapid and sensitive detection of microorganisms, including fungi. Numerous fungal pathogens can cause significant crop damage, leading to financial losses in the billions of dollars annually. Additionally, fungi can impact humans by contaminating and degrading food. Graphene-based nanobiosensors can be used to detect a variety of microorganisms including fungi. An electrochemical biosensor utilizing impedance techniques that incorporates graphene-Au nanoparticles for the detection of *Aphanomyces invadans* has been reported. As mentioned, graphene and its derivatives, including GO, reduced GO, and graphene quantum dot nanocomposites, are excellent nanomaterials suitable for identifying fungi[91]. The carbon-based nanomaterial sensor technology can detect the presence of specific fungal microorganisms in a sample in under 15 minutes. The electrical signals from the carbon nanotube sensors correlate with results from quantitative PCR, providing a comprehensive evaluation of the nanotube-based detection system. - The new technology offers advantages over conventional methods like blood culture and qPCR, including increased sensitivity, cost-effectiveness, and time efficiency[92]. The magneto-nanosensor biochip is a promising technology for the sensitive detection of fungal pathogen[93]. Biosensors offer significant advantages over conventional detection methods for airborne fungal spores, including high selectivity, real-time measurement, and potential for miniaturization and portability. Various biosensing technologies, such as optical, electrochemical, and microfluidic platforms can be used for the detection of airborne Aspergillus spores and other fungal pathogens. The integration of advanced biosensing technologies with sampling devices, microfluidics, and data analysis techniques has the potential to revolutionize the monitoring and detection of airborne fungal contamination[94].

**V. ADVANCES IN POINT-OF-CARE TESTING**

There has been significant progress in point-of-care testing, especially in lab-on-a-chip technologies, microfluidics, and portable biosensors. These developments are changing diagnostic procedures by allowing quick and precise testing at or close to the patient care location. Portable biosensors for bedside diagnostics are compact, user-friendly devices that enable rapid, accurate microbial detection at the point of care. They revolutionize diagnostics by minimizing lab dependence, accelerating treatment, and enhancing patient outcomes in clinical settings.

Quick and affordable electrochemical biosensors for bacterial detection include amperometric, potentiometric, and impedance biosensors. Impedimetric biosensors are viable options for the detection of whole bacteria due to their great sensitivity and lack of labels. Optical biosensors are quick, accurate, and reasonably priced for real-time on-site detection. Whole bacterial cells can be detected by a label-free technique called surface plasmon resonance (SPR). Thermistors and thermocouples are used in thermal biosensors, while piezoelectric biosensors use gold electrodes coated with quartz crystal. Due to their extreme sensitivity to temperature changes, these biosensors are ideal for detecting harmful microorganisms and chemicals. However, labelling of samples is required for fluorescence-based optical biosensors, which increases the cost and time [95].

Carbon nanotubes (CNTs) are being researched in polyolefin (POC) systems to analyze biological analytes for the detection of glucose in diabetes and cancer biomarkers. Between 2008 and 2011, the usage of CNTs in biosensors increased; they accounted for 20% of published healthcare industry papers and 53% of chemical publications. The analytical performance of the biosensors in terms of their repeatability and reproducibility, LOD, sensitivity, and selectivity have to be well considered. Several studies are working on POC testing based on CNT biosensors and other technologies for cancer biomarkers using devices like microfluidic and immunosensing devices for glucose detection using implantable devices [17].

The microfluidics and LOCs provide a biosensing technique in small-scale operations of the laboratories. These devices decrease the time of analysis, the cost of reagents, and chemical waste. Microfluidics refers to the behavior, control, and manipulation of fluids at the sub-millimeter scale. The design and construction of microfluidics hardware are significantly different from those of macroscale hardware, making scaling down conventional devices challenging. Microfluidics can be processed, mixed, separated, or moved using passive fluid control techniques or external actuation means like rotary drives, micropumps, or microvalves. Miniaturization of lab processes onto a single chip can increase the efficiency, portability, and reduce sample and reagent volume.

For public health and food safety, it is necessary to detect pathogenic microorganisms quickly and with high sensitivity. Some of the modern detection methods include electrochemical biosensors, qPCR, SPRi, ELISA, fluorescence assay, SERS, and CL, but they all have drawbacks in terms of the laborious extraction of DNA, expensive equipment, and so forth. Because it is sensitive, has high throughput, is relatively inexpensive, and easy to handle, microfluidic chip detection technology has gained attention. Microchip capillary electrophoresis (MCE) is an encouraging alternative technique since it can be automated and uses little sample. Therefore, sensitive and rapid methods for the identification of pathogenic microorganisms need to be developed [96].

A variety of chemical applications have been developed for microfluidic devices, including droplet-based systems with isolated reaction sites and reduced sample consumption and traditional systems using microchannels for continuous flow regimes. Digital microfluidic (DMF) devices make use of electrostatically actuated electrodes to decrease volumes. These were first introduced in the early 2000s. Micro Total Analysis Systems (μTAS) integrate laboratory procedures to analyze a single chemical and provide multiple features on a single platform. μTAS technologies are more widely applicable than LOCs, which handle only small fluid volumes [97]. Some of the advantages of these systems include higher throughput, automation, shorter analysis times, lower reagent use, and fewer sample requirements. They are ideal for point-of-care and near-patient testing as they also minimize iatrogenic blood losses, reduce sample volume, cut costs, and expedite processing times [98].

Micro-laboratory analysis systems utilize the new 3D structure chips called microfluidic paper-based chips (mPCs). They have rapidly developed due to their cost-effectiveness, portability, and thermal stability, which make them useful for detecting infections, cleaning the environment, and ensuring food safety. The preparation method consists of substrate modification, hydrophilic/hydrophobic processing, and selection of the paper substrate. These small laboratory analysis devices are known as microfluidic paper-based chips (mPCs) and use paper substrates instead of more conventional silicon, quartz, glass, and other polymers. These chips allow for self-driven flow and reaction on the chip by creating hydrophilic/hydrophobic channels and analytical units on the surface. The ultimate goal is the transfer of laboratory analyses to portable chips for home use and tailoring. Several reviews have provided comprehensive descriptions of microfluidic systems, including new developments in virus detection, fiber-optic sensor applications, and fabrication techniques. It goes without saying that as interest grew in microfluidic paper-based chips, vital reviews that highlighted fabrication techniques and commercialization problems and new report communications involving both the design and fabrication, and detection methods plus wide-ranging applications of mPADs produced pertinent references for development of paper chips [99].

Lab-on-a-chip technology is being developed to identify various microorganisms from biological samples, including HIV, malaria, TB, diarrheal illnesses, pertussis, and dengue fever. One promising use is the Disposable Enterics Card (DEC), which can identify *Salmonella, Shigella dysenteriae, Shiga Toxin-producing Escherichia coli (STEC), Campylobacter jejuni,* and *Escherichia coli* O157:H7 from feces on a single microchip. The DEC lyses bacteria, uses specific antibodies to trap them, and then uses the polymerase chain reaction (PCR) to amplify the bacteria's DNA. Amplified DNA is then detected using a laser light. However, DEC testing's adaptation to field situations is limited because it requires a compact machine for PCR reaction and product detection. It has limited utility in field settings, although it is helpful in small analytical labs or clinics. Miniaturized electrode arrays on silicon cartridges enable Abbott Diagnostics' iSTAT lab-on-a-chip device to quickly evaluate analytes from blood samples. The cartridge is filled with the sample, which is then exposed to chemical reagents and placed into a portable electromechanical readout unit. The electrode arrays utilize potentiometry, amperometry, or conductimetry to measure the concentrations of gases, blood electrolytes, among many other analytes. After use, chips are discarded [100].

A handheld proof-of-concept device such as the "Lab-On-A-Drone" device has been designed to rapidly deploy nucleic acid-based diagnostics in the field using consumer-grade quadcopter drones. The gadget utilizes a smartphone camera and an integrated image analysis app to accomplish time-resolved fluorescence detection and quantification of DNA samples, as well as isothermal PCR with a single heater. Another interesting feature is the use of 3D-printed accessories to turn drone rotors into centrifuges. Examples include the Corgenix ReEBOV Antigen Rapid Test kit, the low-cost, field-ready POC devices for Zika virus detection, and the cellphone-sized real-time PCR equipment for Ebola virus RNA detection [101].

Portable biosensors are now revolutionary bedside diagnostic tools offering rapid and accurate microbial detection directly at the point of care. The instant analysis and the real-time result required for making immediate medical decisions make portable biosensors different from the centralized laboratories used in conventional diagnostic methods. These small, user-friendly devices enable high sensitivity and specificity detection of pathogens, monitoring of biomarkers, and determination of antibiotic resistance. They are essential in emergency and critical care units because earlier diagnosis and treatment are crucial determinants of patient outcome. The portable biosensors can make diagnosis easier with low reliance on infrastructure and in low-resource and remote places, closing healthcare gaps. Modern, decentralized healthcare systems cannot function without such cutting-edge technologies as nanomaterials and microfluidics integrated into its framework.

Serious health problems can arise from communicable diseases that affect a number of systems: neurological, cardiovascular, and urinary systems. Communicable diseases such as Helicobacter pylori have a close relationship with certain chronic diseases. To enhance patient outcomes, stop the transmission of disease, and avoid blind medicine, research on infectious diseases is essential. In low-resource locations, standard laboratory techniques including sequencing, immunological assays, and nucleic acid testing are inappropriate for prompt detection. In impoverished communities, point-of-care testing, or POCT, has become a viable way to enhance health management and surveillance. Rapid diagnosis has been achieved through POCT platforms like plasmonic-based platforms, smartphone-based biosensors, lateral flow immunoassays (LFIA), and paper-based diagnostic platforms. Enhancements in POCT have been noticed in the identification of infectious diseases such as the Ebola virus, COVID-19, Zika virus, malaria, and AIDS. Different infections were detected with the help of developed Point-Of-Care Biosensors. Rapid POC tests are nucleic acid and antibody tests for COVID-19 infections. The tests of nucleic acids detect the virus in sputum or nasal secretions, while the antibody tests collect blood samples containing antibodies against the virus. The virus triggers an immune response and produces antibodies in the plasma, serum, or whole blood of the patient. Quantitative real-time polymerase chain reaction (qRT-PCR) is the gold standard for COVID-19, with higher clinical sensitivity and specificity. The POC biosensors include chip-based, paper-based, and material-based biosensors [102].

As one of the leading causes of death today, there is a need for quick point-of-care (POC) HIV infection diagnostics. Sensitive enough to identify the illness at seroconversion, anti-HIV antibodies have shown the LOC device's capability to reduce the window of detection for HIV acute phase and increase its sensitivity. As an example, Lee et al. used the RT-PCR-based chemiluminescence test to build a disposable LOC device based on polymer for the purpose of clinical POC diagnosis of HIV. Chen et al. created a self-contained, integrated, disposable, and sample-to-result polycarbonate microfluidic cassette, appropriate for HIV nucleic acid detection at POC. Using a self-digitization chip platform, Wang and Chiu et al. designed a digital format of nucleic acid sequence-based amplification that was more precise and sensitive than real-time quantitative NASBA for measuring HIV-1 RNA in plasma samples [103].

Bacterial infection, especially tuberculosis, is a serious concern to world health, for which multidrug-resistant tuberculosis (MDR-TB) frequently needs costly treatment. There is a need for immediate detection of these diseases in a rapid, sensitive, and accurate way. For molecular detection in laboratories or resource-poor areas, POC diagnostics with LOC devices is beneficial. Disposable Lab-on-a-Film devices for MDR-TB detection from sputum extracts have been prepared through research. Closed droplet-based LOC devices for mycobacteria differentiation have also been prepared using the devices that combine hybridization, the processes of post-hybridization wash, and multiplex amplification in one system. The most common bacterial infectious diseases are *Salmonella enterica*, *Staphylococcus aureus*, and *E. coli*. Several cost-effective molecular diagnostic tools have been developed during the past few years for sensitive and rapid detection. One such device includes a TEM-1 β-lactamase system containing zinc finger protein arrays specifically designed for *E. coli* O157:H7. It can detect double-stranded DNA with high specificity and sensitivity at a detection limit of as low as 1 nM target DNA. The highly integrated, self-powered LOC diagnostic equipment can directly and quantitatively detect Methicillin-resistant Staphylococcus aureus (MRSA) DNA from blood. By using isothermal recombinant polymerase amplification, this technology enables the quantitative detection of MRSA DNA in 30 minutes, which offers encouraging starting points for upcoming low-cost molecular diagnostic tests [103]. The misuse of antibiotics and lack of new antibiotics are turning drug-resistant pathogens into a global issue.

Standard microbiological diagnosis of bacterial infections, such as UTI, requires bacterial culturing, which takes from 18 hours up to 72 hours in a laboratory. Classical AST methodologies include disk diffusion and microdilution, involving primary isolation steps before the commencement of the test for 18 hours. In conclusion, what is needed, then, is the development of platforms for conducting an AST for antimicrobial drugs within hours. Electrochemical biosensors are well-suited for molecular diagnostics, and studies have reported rapid b-AST (biosensor-based Antimicrobial Susceptibility Test) that combines phenotypic assay validity with genotypic specificity using molecular probes. This method measures bacterial phenotypic response to different antibiotics, providing genotypic specificity and eliminating the need for initial pathogen isolation [16].

Urinary tract infections (UTIs) are common bacterial infections, with Enterobacteriaceae species accounting for over 80% of cases.

This gap between clinical presentation and the microbiology report leads to an empiric antibiotic prescription. Evidence-based antibiotic selection for UTIs may help stem increases in antibiotic resistance. Trimethoprim/sulfamethoxazole (TMP/SMX) was once the first-line treatment for UTI, but ciprofloxacin is recommended for complicated UTIs due to lower resistance rates. Point-of-care (POC) diagnosis of UTI can improve patient care by rapidly identifying the causative pathogen and the best treatment choice. Electrochemical biosensors are quite suitable for POC diagnostics because of quick answers, high sensitivity, and small size. The electrochemical biosensor assay would take 1 hour for the molecular identification of uropathogens and can help identify urine samples containing one or more bacterial species. Biosensor-based approach has shown phenotypic AST of common uropathies against standard bactericidal and bacteriostatic antimicrobials with a limit of detection of 103 colony-forming units (cfu)/ml [104]. Biosensors have emerged as a less time-consuming, low-cost, and highly sensitive technique for H. pylori detection. Challenges in biosensor fabrication include selecting transducers, bio-recognition elements, and immobilization. DNA biosensors are the most studied, while immunosensors, apt sensors, and microfluidic-based biosensors provide low LOD, high sensitivity, and selectivity for effective and rapid diagnosis. Despite available methods for detection and treatment, infected individuals are at higher risk for gastric cancer. A microfluidic magnetic immunosensor was designed to detect human serum IgG antibodies against H. pylori. The device was immobilized on a magnetic microsphere, which made it easier to interact with antibodies. The device was developed to evaluate antibiotic resistance caused by H. pylori strains due to single point mutations [105]. Increasing promise has been shown in several applications, including in biosensors, through microfluidic devices—fluid handling devices having a very small volume of material flowing into channels.

Some of the advantages of few requirements of samples or reagents, simple target analyte localization, reduced times for analysis and functionalization of the inner surface of the channel. Electrical and electrochemical biosensors without the use of labels have gained popularity as they do pretty well with microfabrication techniques. It has been demonstrated in studies by showing how LAMP amplification can be integrated onto lab-on-a-chip devices for the detection of Salmonella and on-chip artificial pore sensors to detect bacterial pathogens. More μD for pathogen detection, including microfluidic cell-based pathogen sensors, microfluidic antibody/aptamer sensors, and microfluidic protein/enzyme-based pathogen sensors. Recent research has developed thread-based fuel cells and 3D paper-based microfluidic electrochemical glucose biosensors to detect infections in manageable samples under research environment settings [106].

In vitro diagnostics, imaging, and treatment have all extensively used nanomaterials, including metal nanoparticles, graphene, and carbon nanotubes. They can serve as signal reporters for the sensitive and targeted detection of analytes or act as carriers to load signal markers. In addition, upon being utilized as function materials on an electrode surface, it can accelerate electron transfer. Due to their special qualities, the sensitivity, specificity, repeatability, and dependability of the detection systems could be improved through nanomaterials. Incorporation of nanomaterials into micro-biosensor systems has provided the means to develop portable, user-friendly, and reasonably priced sensors. Detecting a huge number of clinical factors such as homocysteine (HcySH), α-fetoprotein (AFP), prostate-specific antigen (PSA), and carcinoembryonic antigen (CEA) is achievable using gold-modified electrodes. Electrode-based devices have shown outstanding sensing with respect to the detection of tumor cells in human IgG antibodies, serum albumin, CEA, AFP, beta amyloid, and ApoE. Protein chip assays, biobarcode assays, and integrated biochips for the detection of therapeutically important biomarkers have been developed as a result of the interaction between GNPs and lab on a chip (LOC) platform. These devices are more sensitive and efficient than traditional ELISA tests because they can detect several analytes on a single chip without causing non-specific reactions [107].

Several technologies and gadgets have been used based on carbon nanotubes because of their sensitivity, specificity, speed, cost-effectiveness, and ease of operation. Real-time diagnosis for point-of-care analysis, portability, functionality, and dependability has all improved. There are three types of CNT biosensors, namely CNT-based LOC, CNT-based printed electrode, and CNT-based LFA. Very recently, an LFB based on MWCNT was developed using carboxylated MWCNT as a label and a streptavidin-biotinylated probe on a nitrocellulose membrane. The same biosensor presented a 40 pM detection limit and was able to detect DNA sequences quickly and sensitively. Another research developed a novel immunosensor for the electrochemical and colorimetric detection of DNA oxidative damage biomarker (8-OHdG) in urine samples using lateral flow immunostrip and CNT conductive paper [108].

Graphene, which is a honeycomb lattice of just one atom, has been interesting due to its mechanical, electrical, and thermal characteristics. Researchers have fabricated graphene materials on electrically insulating surfaces using mechanical exfoliation, chemical vapor deposition, electric arc discharge, and epitaxial growth. Graphene and its derivatives, including graphene-based printed electrodes and graphene-based LOCs, have provided a potential platform for sensitive clinical detection and POC diagnostics. In recent research, scientists have utilized microfluidic paper-based electrochemical immuno-devices and graphene-based amplification techniques to multiplex the assessment of cancer biomarkers. To identify D-amino acid (D-AAs) enantiomer-biomarkers linked to Vibrio cholera infections, Batalla et al. created an enzyme-based microfluidic chip connected to graphene electrodes. The development of a functionalized graphene-gated biochip to detect the cardiac Troponin I (cTnI) biomarker has resulted in potential applications in routine monitoring of cTnI in blood samples [108].

Several biosensors to detect MTB have been made possible by breakthroughs in nanotechnology and microfluidics. These platforms contain analytical equipment and biological sensors, that can be subdivided into either mass/piezoelectric sensor, biochemical, electrical, or optical sensors via nucleic acid hybridization, whole mycobacteria, or antibody-antigen interaction. MTB can be detected at the level of 20 CFU/mL in sputum samples with no processing time using a DMR device in just 30 minutes. The system is made up of three parts: the micro coil array, the microfluidic networks, and on-board NMR electronics. Turbid materials like blood, sputum, or urine can be treated by magnetic particles covered with antibodies uniquely targeting the biomarkers of interest. With this increase in system mass sensitivity towards avidin up to 80-fold, this technique stands poised for POC testing. Specific nucleic acids of MTB have been assayed through nanotechnology, such as probe tests relying on gold nanoparticles. These technologies, however do require PCR amplification, which introduces problems at POC. Toward a POC electrochemical biosensor devoid of PCR for the detection of MTB genomic DNA, the gold nanoparticles have been dual labeled with alkaline phosphatase and some specific DNA oligonucleotides. In clinical sputum samples, this technique demonstrated similar sensitivity and specificity as PCR and detected MTB DNA down to 1.25 ng/mL [109].

**L. Opportunities and Limitations**

POC diagnostics present numerous opportunities but also come with several limitations. Since POC testing provides results in minutes compared to traditional diagnostic methods, it facilitates timely decision-making in critical care and emergencies. Early disease detection helps prevent disease progression and its transmission to others. Portable devices are very suitable for remote areas and resource-limited settings where the central, well-equipped laboratories cannot be accessed. Rapid diagnosis allows rapid therapeutic intervention and reduces mortality. It also avoids unnecessary antibiotic use and limits the development of antibiotic resistance in pathogens. Another benefit of POC devices is that they require a minimal volume of samples, which means that patients suffer less discomfort while obtaining the samples. Most of the devices are simple to use and can be run with a short training course. The linking of POC devices with digital technology like smartphone apps ensures that real-time data is transmitted, consultation may be made via remote sites, and record keeping is made easier. POC devices can effectively diagnose all types of microbial infections like tuberculosis, malaria, HIV, and COVID-19. These have applications in food safety and environmental monitoring as well.

However, POC devices have some limitations. They may be less sensitive or specific in certain applications compared to centralized techniques like qPCR, which may lead to false positives or negatives. The limitation of having many of the devices that only detect one or a few pathogens at a time reduces their applicability in cases of multiple infections. The initial cost of all such devices may be too high, restricting their broader applications. Their performance may depend on environmental conditions, such as temperature and humidity, where they are operated. In remote locations, portable equipment may require regular calibration, maintenance, and consumable supplies, posing logistical challenges. Moreover, certain microbial infections may demand complex detection methods or larger sample sizes that are unsuitable for smaller POC platforms.

Compact biosensors are needed for high sensitivity, specificity, and integration in locations that may not have access to trained staff. Still challenging, however, is the development of a fully integrated device, particularly for sample pretreatment. The advantages of programmable fluid handling and low-test volume aside, DMF remains an attractive platform for automated proof-of-concept applications. Limitations such as nanoscale signal output integration and on-chip reagent storage are still present, though. Handheld instrument-assisted techniques are commercially available and relatively inexpensive compared to large analytical instruments.

Cost is another important factor, and for commercial use and cost reduction, stable mass production is necessary. The microfluidic platform-based biosensing devices can lower the costs of the devices because of their mass-production capabilities, low-cost material usage, and small-volume reagent requirements. The great challenge for miniaturized biosensors in POC tests lies with cost per test and consistency and reliability. So, further work should focus on the betterment of stable signal transducer components and the parallel production of microfluidic chips. Resource-limited settings also have some challenges related to commercialization, such as acceptance from customers, market adoption, and limited funding. However, future development of portable, integrated, and automated biosensing technologies could considerably streamline healthcare and better clinical outcomes for people in resource-limited settings [99].

**VI. CHALLENGES AND FUTURE PERSPECTIVES**

**M. Technical and Economic Barriers in the Commercialization of Biosensors**

Despite the great promise of biosensors, their broad commercialisation is hampered by technical and economic constraints. The technological issues largely include the reliability, sensitivity, specificity, and stability of biosensors under real-world circumstances. Many biosensors rely on biological components like enzymes, antibodies, or microbes, which can deteriorate over time, resulting in reduced effectiveness. Furthermore, the complexity of biosensor systems, such as the necessity for accurate calibration, signal amplification, and the integration of biological and electrical components, poses major challenges to production scaling [110].

On the economic front, the commercialisation of biosensors is hampered by high development and manufacturing costs, which are frequently associated with the use of expensive materials and sophisticated production procedures. Furthermore, a lack of standardisation in biosensor design and testing processes might cause confusion for clients, delaying the adoption of these technologies. Market adoption is also an issue, as potential users may be unwilling to replace old techniques with biosensors due to perceived high upfront costs or unfamiliarity with the technology. In industries such as healthcare, reimbursement and insurance coverage for biosensor-based diagnostics continue to be important difficulties, restricting their wider implementation. The expanding biosensor market offers both possibilities and challenges to start-up biosensor entrepreneurs. The primary difficulty and threat for these entrepreneurs is predicting the biosensor market and transforming promising biosensor technologies into commercialised biosensors [111].

**N. Technical Barriers**

**Sensitivity and Specificity Limitations:** Biosensors face a significant challenge in achieving high sensitivity and specificity due to the influence of environmental factors such as temperature, pH, and the presence of interfering substances. These factors can affect the accuracy of biosensors, leading to false positives or false negatives and reducing their overall performance. To overcome this, advanced materials like nanomaterials and molecularly imprinted polymers, along with improved enzymatic or antibody-based recognition elements, can be employed to enhance both sensitivity and specificity. Additionally, incorporating multi-step detection methods, such as multienzyme cascades or coupled biosensors, can further improve selectivity, enabling more accurate detection of target analytes [112]

**Stability and Shelf-life:** The stability and shelf-life of biosensors are crucial challenges, particularly due to the degradation of biological recognition elements such as enzymes, antibodies, and microorganisms over time. This degradation can compromise the reliability and accuracy of the biosensor. To address this issue, researchers are exploring strategies like enzyme immobilization on stable substrates, the use of stabilizing agents, and the development of more robust synthetic biological components. Additionally, incorporating non-biological recognition elements, such as synthetic aptamers, can enhance the shelf-life of biosensors, providing more durable and reliable sensing solutions [112]

**Manufacturing and Scalability**: Biosensors face significant challenges in scaling up from laboratory prototypes to mass production, primarily due to the precise fabrication techniques required to integrate biological recognition elements with transducers in a cost-effective manner. Reproducibility, quality control, and ensuring uniformity in sensor performance are common obstacles when transitioning to large-scale production. To overcome these challenges, advances in microfabrication and nanofabrication technologies, such as inkjet printing, roll-to-roll processing, and microfluidic chip production, can enable the mass production of high-quality biosensors. Additionally, automation and standardized manufacturing processes are essential for ensuring scalability without compromising product quality [113]

**Complexity of Calibration**: Biosensors often require complex calibration due to their varying responses under different environmental conditions or with different sample types, which can make the process time-consuming and demand specialized expertise. To address this issue, integrating self-calibrating systems or utilizing portable reference sensors could reduce the need for complex calibration, making the process more efficient. Furthermore, the development of advanced software that compensates for environmental variables can simplify the calibration procedure, making biosensors more user-friendly and accessible for a broader range of applications [114].

**Regulatory Approvals:** Biosensors, particularly those intended for medical diagnostics, must navigate stringent regulatory requirements before reaching the market. The approval process is often lengthy and costly, with standards varying significantly across different regions. To expedite commercialization, streamlining regulatory pathways and developing international standards for biosensor approval are essential. Collaboration among industry stakeholders and regulatory bodies is crucial to establish clear guidelines for biosensor testing and approval, thereby facilitating a more efficient and globally harmonized process [115]

**O. Economic Barriers**

**High Development and Manufacturing Costs**: Biosensor development and manufacturing involve significant research and development (R&D) costs, including the design, testing, and optimization of components such as biological recognition elements, transducers, and signal amplifiers. Additionally, materials with high sensitivity and specificity can be expensive, further driving up the cost of biosensors. To reduce these costs, researchers are exploring the use of more affordable materials, such as low-cost enzymes or synthetic recognition elements. Leveraging efficient production methods, including microfabrication and high-throughput screening, can also help reduce overall expenses. Strategic material selection during the design stage is crucial for managing costs and improving product reliability, manufacturability, and scalability [116].

**Lack of Standardization**: The lack of standardisation in biosensor design, testing, and application restricts their market viability. Customers may have difficulty assessing the reliability and accuracy of different biosensors in the absence of standardised methods for performance assessment and comparison. To overcome this issue, worldwide regulatory and standards organisations must establish industry-wide standards for biosensor performance, calibration, and validation. These standards have the potential to increase market acceptability, encourage wider deployment of biosensors across a variety of sectors, and minimise testing and certification costs. Clear rules can also boost customer confidence and promote the worldwide scalability of biosensor technology [117]

**Limited Market Acceptance and Awareness**: The commercialization of biosensors faces resistance from potential customers who may be unfamiliar with their benefits, particularly in industries like food safety, environmental monitoring, and clinical diagnostics. Additionally, the initial investment in biosensor technology may be perceived as too high for many businesses, especially in resource-constrained settings. To address these challenges, educating potential users about the advantages of biosensors, such as their ability to provide rapid, on-site testing with high accuracy, can increase market acceptance. Additionally, demonstrating the cost-effectiveness of biosensors through case studies and pilot programs can encourage adoption.[118]

**Competition with Conventional Methods:** Biosensors often face competition from traditional detection methods, such as culture-based microbiological testing and conventional chemical assays, which are well-established and may be perceived as more reliable or cost-effective. To overcome this challenge, manufacturers need to demonstrate the added value of biosensors, including faster results, higher sensitivity, and ease of use. Offering biosensors at competitive prices and providing clear evidence of their superior performance compared to traditional methods can help drive market adoption [119].

**P. Need for regulatory approvals.**

Microbial biosensors are analytical tools that combine microbes and transducers to identify analytes; they may find use in food, environmental, and medical monitoring [29]. These biosensors have limitations, including low sensitivity, poor selectivity, and impractical mobility, despite their potential. In order to overcome these constraints, scientists have combined microbial biosensors with micro/nanotechnologies, improving their functionality and broadening their range of uses [12]. Improved immobilization of microorganisms by nanomaterials has resulted in more dependable and selective biosensors. Furthermore, sensitivity has grown due to developments in transducer technology [29]

Using a variety of biorecognition components, including molecularly imprinted polymers, nucleic acids, and antibodies, the incorporation of nanotechnology into biosensors has substantially improved pathogen detection. Future advancements in this area might concentrate on resolving issues with integration into lab-on-a-chip systems, characterisation, quality assurance, and nanotoxicity [120]. Biosensors and nanotechnology are crucial in industries like healthcare, food safety, pharmaceuticals, and environmental monitoring due to their direct contact with biological systems and potential environmental impacts. Regulatory licenses ensure safety, effectiveness, accuracy, and dependability, necessitating governmental monitoring. In order to ensure consistent accuracy across various settings and operators, regulatory bodies evaluate biosensors and nanodevices for microbial agent detection with few false positives or negatives.

To guarantee product quality and reduce faults, biosensors and nanotechnologies must abide by international standards, Good Manufacturing Practices (GMP), and regulatory clearances. Biosensors are employed in the medical field for outbreak prevention, infection control, and diagnostics. Before being marketed or disseminated, pre-market approvals are required by organizations such as the FDA, EMA, and CDSCO. After a device is released, post-market surveillance makes sure it keeps working as planned.

Applications for nanotechnology are numerous and include environmental monitoring, pharmaceuticals, food safety, and medical diagnostics. Regulations such as the FDA's 21 CFR Part 809 or the EU's IVDR require clearance. FDA, EFSA, or FSSAI requirements must be followed by food safety equipment. The application of nanotechnology in microbiological detection also requires pharmacovigilance. EPA approval may be needed for environmental monitoring. The FDA regulates medical biosensors and nanotechnology through its Center for Devices and Radiological Health; regulatory procedures differ based on jurisdiction and application.

Nanobiosensors, especially those used for microbial detection, encounter a number of formidable obstacles in the regulatory approval process. These difficulties result from the unique characteristics of nanomaterials, the intricate ways in which they interact with biological systems, and the shortcomings of the regulatory frameworks that are already in place. Nanobiosensors frequently make it difficult to distinguish between many types of medical items, including medications, devices, and biologics. Because current frameworks might not sufficiently address the unique properties and mechanisms of action connected to nanotechnology, this ambiguity makes the regulatory road more difficult. For example, certain items have been difficult for the U.S. Food and Drug Administration (FDA) to classify, which can result in varying regulatory requirements and approval process delays.

A bright future for a number of sectors is presented by the development of nanosensors. But making sure they are developed and used responsibly necessitates paying close attention to ethical issues, environmental effect, public participation, regulatory requirements, and international cooperation. By taking proactive measures to resolve these issues, researchers and policymakers may fully utilize nanosensors while respecting moral standards and preserving the environment and society [121]. Nanomedicine, which attempts to cure diseases or repair damaged tissues, is a result of nanotechnology, which is the creation of nanoparticles with special properties. This field enhances treatment modes, detection, and diagnostic capabilities by combining biological, mechanical, and chemical properties. In the future, a single nanomedicine product might be able to target particular organs and tissues, offer photos, evaluate vital signs, diagnose in real time, and administer personalized medicines.

Under the Food, Drug, and Cosmetic Act (FDCA) and the Public Health Service Act (PHSA), the FDA regulates medications, medical devices, and treatments that come from biological sources. Items must be described in accordance with the definitions and principles set out by Congress. The FDA regulates products involving nanotechnology, and new medications and biological products must pass three stages of clinical studies before being approved. Products created to cure significant illnesses or unmet health needs may be eligible for breakthrough treatment classification. Devices can enter the market through premarket approval or premarket notification procedures, or through generic and biosimilar pathways. New innovations at the nexus of biologics, devices, medications, or all three product categories are evaluated by the Office of Combination Products (OCP). For the purpose of discovering and evaluating combination goods, the 21st Century Cures Act requires clear and uniform procedures. Nanotechnology-derived goods are frequently classified as combination products, guaranteeing both efficacy and safety [122]

To find possible risks associated with nanoparticles in products, thorough risk evaluations are necessary. Research in toxicology is essential to comprehending the consequences on health. It is important to assess exposure scenarios at each phase of the product's lifecycle. It is essential to use risk management strategies such containment measures, PPE, and engineering controls. Effective communication is also essential. For safe handling, storage, disposal, environmental impact assessment, and safety procedures, regulatory compliance with nanomaterials laws, standardization protocols, clear labeling, monitoring systems, emergency response plans, and continuous improvement are essential [123].

**Q. Emerging trends and future research directions**

Biosensor techniques will revolutionize the future of precision medicine, encompassing drugs, instruments, and diagnostics. Implantable biosensors can accelerate the development of personalized medicines by accurately monitoring drug effects on the body and diagnosing complex blood DNA mutations. Biosensor technology can be utilized in cost-effective and reversible care-point equipment to track multiple health parameters simultaneously. The integration of biosensors with the Internet of Things (IoT), Artificial Intelligence (AI), and 5G will make the healthcare industry more confident, sensitive, and customized. Biosensors provide detailed mechanical insights at the molecular level across pharmaceuticals, health, food, agriculture, environmental technology, and biotechnology processes. Consequently, the market for biosensors is thriving due to their extensive applications in healthcare and medicine. Portable biosensors enable real-time health monitoring, reducing the need for routine check-ups [101].

Microfluidic technologies have significantly advanced in recent years, especially in point-of-care (POC) testing. These technologies have enabled the fabrication of lab-on-a-chip platforms with automated analysis procedures and minimized device sizes. Furthermore, microfluidic systems support molecular motors, nanorobots, biomedical implants, biomimetics, on-chip supramolecular chemistry, and hybrid systems integrated with blood vessels. However, several hurdles remain, such as avoiding non-specific adsorption, automating and integrating technology, and developing suitable sample preparation techniques. Microfluidic devices can be integrated with POC testing systems via smartphones, but a major challenge is developing integrated circuits and sensors on a single chip. Future proof-of-concept platforms will focus on non-invasive detection of molecular markers in bodily fluids and customized devices [108].

The 'post-antibiotic' era, driven by the overuse and misuse of antimicrobials, threatens the future of medicine. Rapid POC methods can enhance the efficacy of these drugs while reducing inappropriate use. For better patient outcomes and minimizing adverse medication reactions (AMR), repeated utilization of rapid POC testing before antibiotic administration is essential. Compared to traditional diagnostics, cell phones offer advantages such as speed, affordability, and user-friendliness. Emerging technologies like advanced material manufacturing and CMOS sensors make POC diagnostics more accessible. Smartphones can be integrated into fully featured analytical platforms with external peripherals [110].

Biosensors are vital analytical tools in biomedical diagnostics, disease monitoring, and environmental analysis. Electrochemical biosensors are particularly valued for their low detection limit, high specificity, and ease of operation. Nano-electrochemical and nano-biosensors, enhanced by versatile nanostructures, significantly improve biosensor performance. Approximately 200 companies invest in biosensors and bioelectronic development, with 85–90% of existing products dedicated to glucose monitoring in diabetic patients. Challenges such as sensor stability, cost, lifetime, and accuracy can be addressed through strong collaboration between industry and academic researchers. Future wearable biosensing devices will enable non-invasive monitoring and testing for multiple analytes, facilitating sophisticated self-diagnosis. However, the use of non-invasive biosensors faces resistance from medical practitioners and biomedical societies until further validation and successful applications in human testing are achieved [111].

Electrochemical sensors, due to their low cost, portability, rapid analysis, and high detection limits, have become a substantial market. The immobilization of analytes using polymers and nanomaterials enhances the sensitivity and detection capabilities of biosensors. High-sensitivity electrochemical sensors with real-time analysis have been developed using biofabrication approaches like contact or non-contact-based patterning. However, their regenerative ability is limited for extended use. FRET, fluorescence-based, surface plasmon resonance, and bioluminescent resonance energy transfer-based transducers have shown enhanced single-analyte detection in contact-based sensing. Non-contact sensors developed using 3D bioprinting or laser direct methods show promising results but involve high costs and customization requirements. Amperometric electrochemical biosensors have been designed for disease diagnosis using bodily fluids, excelling in detecting single DNA or peptide molecules, particularly in forensic sciences and biomedicine. Although the detection limit has not yet reached the femto-level, improvements in micro- and nanoelectromechanical biosensors have yielded better results through biofabrication approaches. Despite the technological advancements of genetically encoded or synthetic fluorescent biosensors, their preparation and use require sophisticated instrumentation. Future goals include developing durable regenerative biosensors for long-term use, benefiting patients and physicians by enabling efficient diagnosis and treatment [112].

Through the integration of biosensors with the Internet of Things (IoT) and artificial intelligence (AI), the ability to monitor in real time and provide data-driven insights is revolutionizing microbial detection. IoT-enabled biosensors can wirelessly transmit microbial detection data for centralized monitoring in the cloud, improving the capacity to monitor illnesses and spot trends in outbreaks. Artificial intelligence enhances diagnostic precision by analyzing huge amounts of biosensor-generated data to identify minute trends and predict disease outbreaks. Moreover, this technological combination facilitates automated diagnosis, reducing the need for professionals in low-resource or remote-access locations. Wearable technology and portable biosensors are becoming indispensable instruments for real-time, point-of-care (POC) microbiological identification. These instruments are perfect for emergencies or in remote locations with little access to centralized labs as they provide quick results with minimal sample processing. Wearable technology, such as skin patches or smartwatches, can continuously monitor physiological variables and detect microbiological biomarkers in human fluids like perspiration or saliva. This innovation increases patient outcomes by enabling early intervention and reducing diagnostic delays and patient visits to healthcare facilities.

Nanotechnology is leading the biosensor innovation race by utilizing materials such as carbon nanotubes, graphene, gold nanoparticles, and quantum dots to improve device performance. These nanoparticles enhance the sensitivity, selectivity, and stability of biosensors, enabling the detection of minute quantities of microbial pathogens in complex biological samples. Functionalized nanostructures allow for targeted interactions with specific microbiological biomarkers, ensuring accurate diagnoses. The ability to tailor nanomaterials for specific applications is enabling superior performance in next-generation biosensors. Lab-on-a-Chip (LoC) devices are revolutionizing microbial detection by automating and condensing laboratory processes into a single chip. These devices integrate sample preparation, reagent mixing, and detection processes into small, portable platforms, drastically reducing diagnostic time and costs. Microfluidics, a key component of LoC devices, enables precise control of tiny fluid volumes, making multiplex pathogen detection possible. Advancements in LoC systems are improving access to rapid diagnoses in resource-scarce environments and enabling high-throughput analysis for large-scale testing during pandemics.

Single-analyte detection biosensors are being replaced by multiplex platforms capable of identifying numerous microbial infections simultaneously. This development is particularly important for diagnosing polymicrobial illnesses, or co-infections, which are common in clinical settings. Multi-analyte biosensors reduce the time, expense, and sample volume required for diagnoses, increasing their utility in epidemiological research and comprehensive health evaluations. Simultaneous monitoring of several biomarkers enhances diagnostic precision and provides a more comprehensive picture of patient health. Non-invasive biosensors, which rely on exhaled breath, perspiration, saliva, or tears for microbial detection, represent a major breakthrough in patient-centric diagnostics. These techniques eliminate the discomfort associated with conventional methods like blood draws or biopsies. Minimally invasive methods, requiring only a few microliters of sample, are also gaining popularity for their efficiency and simplicity. These developments enhance patient compliance and allow for regular monitoring of chronic infections or during treatment regimens.

Electrochemical biosensors are gaining popularity due to their affordability, portability, and quick response times. Recent research focuses on increasing the sensitivity and specificity of these devices using nanostructured materials such as metal-organic frameworks, conductive polymers, and nanowires. These advancements enable the detection of microbial infections at extremely low concentrations, making electrochemical biosensors highly reliable for early diagnosis. Innovations like self-powered electrochemical sensors are also being explored to make devices more sustainable and reduce operating costs.

The use of CRISPR-Cas systems in biosensors is revolutionizing the diagnostic process by offering unparalleled accuracy and specificity. CRISPR-based biosensors use guide RNAs to identify specific DNA or RNA sequences of pathogens, enabling rapid and precise identification. This technology is particularly valuable for detecting genetic variations, new infections, and antibiotic resistance genes. As a user-friendly technique capable of delivering results in minutes, CRISPR-based biosensors are highly suitable for proof-of-concept applications. The application of 3D printing in biosensor development allows for the creation of high-performance, affordable, and customizable devices. This technology facilitates the rapid prototyping of intricate geometries and integrated features, such as multi-analyte detection capabilities. 3D-printed biosensors are particularly valuable in resource-scarce or geographically isolated regions where conventional manufacturing methods may not be feasible. Customizing these devices for specific microbial detection applications increases their adaptability and usefulness.

With the increasing application of biosensors for the detection of resistant genes and infections, they are becoming pivotal in addressing the global challenge of antimicrobial resistance (AMR). Such tools ensure the quick identification of AMR markers, guiding appropriate antibiotic use and slowing resistance rates. Predictive analytics on AMR trends, derived from integrating biosensors with AI-driven platforms, may help inform public health policies and improve antimicrobial stewardship.

Biosensors are also playing a critical role in the evolution of personalized medicine by enabling patient-specific diagnostics and treatment monitoring. The integration of biosensors with genomics, proteomics, and metabolomics platforms allows for tailored healthcare solutions. Personalized biosensors can monitor disease progression, evaluate treatment efficacy, and provide real-time feedback for therapeutic adjustments, revolutionizing healthcare delivery. A key area of research is the development of regenerative biosensors with enhanced durability and reusability. These devices are designed for long-term monitoring of microbial infections, reducing the need for frequent replacements and lowering overall healthcare costs. Regenerative biosensors also reduce environmental waste, making them a more sustainable diagnostic solution.

**VII. CONCLUSION**

Nanotechnology coupled with biosensors has transformed microbial detection in unprecedented levels of sensitivity, specificity, and speed in diagnostics. Nanomaterials-based incorporation into the platform of biosensing has been very efficient for detecting microbial pathogens, antibiotic resistance genes, and biomarkers for disease diagnosis with improved precision and effectiveness. Clinical uses aside, its applications expand to veterinary medicine, environmental surveillance, food safety assessment, and bioterrorism surveillance detection. Constant progress in the use of nanotechnology-based biosensors shall change microbiological diagnosis landscapes for centuries-old practice models into an efficient, completely automated, in situ real-time systems and portable apparatus for detection purposes. Improvement in biosensors' sensitivity through engineered nanomaterials, such as carbon nanotubes and graphene, gold nanoparticles, and quantum dots, are some of the key advantages of nanotechnology in the detection of microbes.

The nanomaterials improve signal amplification, which permits the detection of microbial pathogens at ultra-low concentrations, even in complex biological samples. Using functionalized nanostructures provides selective targeted interaction between nanostructures with the microbial biomarker. Therefore, biosensors and nanotechnology ensure increased diagnostic specificity. Thus, the application of biosensors and nanotechnology has recently enabled early and precise detection of diseases. Its advantages include not only short diagnosis times but also well-timed clinical interventions. Moreover, artificial intelligence combined with machine learning algorithms is revolutionizing biosensing with modern advances. The application of AI-driven analytical tools in biosensing has enabled real-time monitoring, data analysis, and predictive modelling.

IoT-enabled biosensors can wirelessly transmit microbial detection data to centralized cloud platforms, facilitating remote diagnostics and large-scale epidemiological surveillance. Such as convolutional neural networks and recurrent neural networks, has demonstrated incredible ability in biosensor read accuracy improvements based on big datasets analysis and extraction of minor patterns with reduction in false positives and false negatives. It is, indeed, the combination of AI with biosensors which forms a revolution in the way disease diagnosis is taken and disease outbreak is monitored especially in remote areas and under resource constraint regions where a conventional laboratory diagnosis would be hard to establish. Portable and wearable biosensors have developed further, revolutionizing the field of microbial detection. The miniaturized devices can indeed provide real-time, on-the-spot pathogen identification without complex laboratory infrastructure. Wearable biosensors in the form of smart patches or wristbands can constantly monitor physiological changes and detect microbial biomarkers in body fluids, such as sweat, saliva, or tears. This is particularly important in the early detection of infectious diseases, providing the possibility of disease transmission control through timely intervention. POC biosensors are proving to be invaluable in emergency settings, outbreak-prone regions, and underdeveloped healthcare systems by providing rapid, accessible, and cost-effective diagnostic solutions. Another notable advancement is the emergence of lab-on-a-chip (LoC) technology, which integrates multiple laboratory functions into a single microfluidic platform. LoC devices integrate the entire diagnostic process, from sample preparation and reagent mixing to pathogen detection, into a compact, portable system. Microfluidic biosensors allow for multiplex detection of multiple pathogens simultaneously, reducing diagnostic time, cost, and the need for large sample volumes. LoC devices are game-changers for global health surveillance, especially during pandemics or bioterrorism threats, because they can function in low-resource settings without sophisticated laboratory equipment.

Multiplex biosensing platforms have also received much attention due to their ability to detect multiple microbial infections within a single assay. Unlike traditional single-analyte biosensors, these advanced systems provide comprehensive diagnostic insights, particularly for polymicrobial infections and co-infections commonly encountered in clinical settings. The ability to analyze multiple biomarkers at once enhances the accuracy of diagnoses, facilitates more informed treatment decisions, and contributes to personalized medicine. By leveraging multiplex biosensors, clinicians can conduct epidemiological studies more efficiently and track disease progression with greater precision. In addition to the traditional biosensing methods, non-invasive biosensors are becoming a promising alternative for microbial detection. These biosensors analyze exhaled breath, sweat, saliva, or tears as sample materials, eliminating the need for invasive procedures such as blood draws or biopsies. The adoption of non-invasive diagnostic approaches improves patient compliance, reduces discomfort, and facilitates routine monitoring of infections, particularly in chronic disease management. The rising popularity of these biosensors’ points to the growing demand for patient-friendly diagnostic solutions that do not compromise accuracy or sensitivity. Another area of research in the development of biosensors is the incorporation of electrochemical sensing mechanisms. Electrochemical biosensors have many advantages, including affordability, portability, and rapid response times.

The latest advancements in this field involve the application of nanostructured materials like metal-organic frameworks (MOFs), conductive polymers, and nanowires to further improve sensor performance. These materials enhance the electrochemical biosensor's sensitivity for the detection of microbial infections at concentrations as low as possible. Another innovation is the concept of self-powered electrochemical sensors to increase sustainability and lower operational costs. Probably the most revolutionary innovation in the recognition of microbes was the CRISPR-Cas technology-based application in biosensors. CRISPR-based biosensors utilized guide RNAs that recognized specific pathogen DNA or RNA sequences with high specificity and accuracy. This can be particularly helpful in the identification of emerging infectious diseases, genetic mutations, and antibiotic resistance genes. The simplicity, speed, and adaptability of CRISPR-based biosensors make them highly suitable for point-of-care diagnostics, paving the way for widespread adoption in clinical and research settings. Despite the tremendous progress in nanotechnology-based biosensors, several challenges remain in their commercialization and large-scale implementation. The sensitivity and specificity of colorimetric biosensors still require further refinement to ensure reliable, on-site diagnostics. Another challenge includes streamlining regulatory approval processes of nanotechnology-based diagnostic devices into mainstream healthcare. Reproducibility, stability, and affordability are further key focus points for future research. Translating laboratory innovations into commercially viable, real-world diagnostic tools shall be possible with these solutions addressed. In the near future, microbial detection continues to evolve along with the integrated applications of nanotechnology, AI, and automation. Miniaturized, smart biosensors are expected to play a pivotal role in disease surveillance, biodefense, and outbreak control. Nanorobotics, which can navigate biological systems to detect and eliminate infections at the source, hold great promise for precision medicine. Hybrid biosensors, which integrate multiple sensing modalities such as optical, electrochemical, and piezoelectric techniques, will further enhance diagnostic accuracy and efficiency.

Rapid developments in molecular biology and nanotechnology are about to usher in a new age of microbial diagnostics. The scientific community is poised to witness the general acceptance of miniaturized, automated, cost-effective, and high-performance biosensors. More nanotechnology-based assays will soon enter the market, and future microbiologists and healthcare professionals will be required to include these state-of-the-art diagnostic tools in their daily practice. Nanotechnology-enabled biosensors, which come with promise to revolutionize global healthcare, food safety, and environmental monitoring, may also bring about greater improvement in disease prevention, early intervention, and personalized medicine once continued research and innovation ensue. Thus, it can be concluded that the combination of biosensors and nanotechnology at the core of research is revolutionizing microbial detection. Such work in research and advancement in technology would continually shape diagnostics, ensuring fast, more accurate, and more accessible ways to detect pathogens. By conquering the existing issues and developing beyond the level of innovation within biosensors, science is at its best prepared to unlock all potential within nanotechnology for changing microbial diagnostics and public health.

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