Nanonbiosensors; Rapid detection of *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp. infections

Abstract

Zoonotic diseases, which are infectious diseases transmitted from animals to humans, represent a significant global health concern. Despite efforts to eradicate or control these diseases, healthcare systems continue to face a substantial burden due to their re-emergence. Early and accurate detection of bacterial pathogens is crucial to prevent the potential health consequences associated with zoonotic infections. However, conventional diagnostic methods such as Polymerase Chain Reaction (PCR), culture-based techniques, and immunological assays have limitations, including costliness, labor-intensiveness, and lengthy turnaround times for results. There is an increasing interest in developing faster, more accurate, and cost-effective diagnostic methods to address these challenges. Nanobiosensors are emerging as promising tools for rapidly detecting infectious disease agents. These devices utilize biological recognition elements to detect specific pathogens and have the potential to revolutionize diagnostic practices. Additionally, incorporating nanotechnology, particularly Nano Particles (NPs), has been shown to enhance the performance of biosensors by improving their specificity and sensitivity. This review explores the application of biosensors and nanobiosensors to rapidly detect *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp. Infections. These innovative technologies offer several advantages over traditional diagnostic methods, including reduced cost, simplified workflows, and faster results. Nanobiosensors can detect the presence of bacterial pathogens in various sample types, including environmental samples, animal specimens, and clinical samples, making them versatile tools for disease surveillance and control. Moreover, nanobiosensors have shown promise in enhancing the sensitivity and specificity of detection assays, enabling the early identification of *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp, even at low concentrations. By leveraging advancements in nanotechnology, researchers can further improve the performance and reliability of biosensors for zoonotic disease diagnosis. Overall, integrating biosensors and nanotechnology holds great potential for enhancing the detection and characterization of *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp. These innovative diagnostic tools can revolutionize disease surveillance efforts, mitigate the spread of zoonotic diseases, and ultimately improve public health outcomes on a global scale.

Keywords: Nanobiosensors, *Salmonella*, *Clostridium*, rapid detection, nanomaterials

1. Context

Due to nanotechnology development, several new biosensors have been developed and specialized over the past few years, and many improvements have been made regarding medical sciences (1-3). At present, nanotechnology is one of the most promising topics in science, and it is being used to make biosensors that address a range of applications in medicine, drug delivery, biology, the environment, and food safety (4, 5). However, it has become one of the most critical objectives for biosensors to detect pathogens, as the health of the human population is currently affected by viral and bacterial diseases (6, 7). Several molecular techniques detect viruses and bacteria, including reverse transcription polymerase chain reaction (RT-PCR), still considered the gold standard. Several classical methods for detecting pathogens include isolation, culture, and biochemical analysis.

Furthermore, serological tests such as Enzyme-Linked Immunosorbent Assays (ELISAs) detect antibodies and immunoglobulins necessary for identification (8). The problem with some techniques is that they are complex and take a long time to achieve results. The application of nanotechnology has emerged as a suitable and easy way to detect pathogens in a faster and more efficient manner. Using NPs for various pathogenic purposes contributes to developing new devices and technologies for disease prevention. Considering zoonosis as an existing issue, the study does not only examine human diseases but also those affecting animals. It has been estimated that almost 60% of all infections identified in humans result from zoonoses. Animals and humans can contract zoonoses due to various microorganisms, including parasites, viruses, fungi, and bacteria. Although zoonoses are more commonly transmitted from animals to humans, they significantly impact public health. It is important to note that they can also pose economic costs to the livestock and poultry industries (9).

Meanwhile, Deoxyribose Nucleic Acid (DNA) biosensors and sequence-specific DNA detectors are increasingly being used for clinical studies by the international scientific community. Moreover, DNA-based piezoelectric biosensors have been used to identify specific gene sequences and to detect DNA damage. Nanobiosensors and biosensors are utilized to detect viral and bacterial clinical pathogens. Devices are fast, practical (enable Point-Of-Care (POC) testing through smartphone-based nanobiosensors), and innovative technologies that provide an alternative solution to the disadvantages presented by standard detection methods.

2. Evidence Acquisition

It has been possible to use technologies to study viruses that affect humans, such as Human Immunodeficiency Virus (HIV), Ebola virus, and recently the newly discovered Sever Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), as well as bacteria, such as *Salmonella spp* and *Escherichia coli* (*E. coli*) (10, 11). A biosensor is an analytical tool comprising a biomolecule as a sensing element and a segment that transforms a recognition event into visible or measurable information. The advantages of biosensors over conventional methods include that they provide an easy, sensitive, and fast method of detecting pathogens for effective treatment (7).

Biosensors utilizing micro- and nanotechnology may help perform complex molecular diagnostic tests for various infectious diseases. Nanobiotechnological methodologies, including real-time diagnosis, high-throughput screening, small sample volumes, and low detection limits, permit several advantages to biosensors. The study aimed to present the outcome of new nano biosensor-based diagnostic techniques to help determine the most common zoonoses of immense importance in modern medicine and veterinary medicine, such as *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp.

3. Biosensors and nanobiosensors

Biological sensors are measurement systems that combine physicochemical detectors and biological components for analyte detection. It depends on the purpose and design of the biosensor to detect analytes. A typical household device, such as a smartphone, can be used as a biosensor by adding simple accessories, as published in a paper by Soni et al., where they proposed a smartphone-based biosensor to measure urea in saliva without requiring invasive tools (12, 13). As a result, initial detection is quick and low-cost. Proteins, nucleic acids, and cells that are associated with diseases are commonly detected by biosensors. Organelles, enzymes, nucleic acids, microorganisms, and antibodies detect biomolecules.

The researchers must also determine the required functionality based on the device's intended use. It is, therefore, fundamental to conduct multidisciplinary studies before selecting the suitable material, transducer, and biological element for constructing a biosensor. A wide range of other clinical diagnostic applications can be performed with biosensors. Furthermore, biosensors can detect bacteria and viruses in water and food, which are possible sources of disease. The study by Zhao et al. developed a low-cost, portable, chemo-resistive biosensor that can detect *E.coli* in real time using AuNPs, monolayer graphene, and streptavidin-antibody system (14). Chemiresistive biosensor captures bacteria on their surface and detects them via electric readouts.

4. Principle of nanobiosensors

Traditional biosensors have been combined with nanotechnology, gaining popularity in nanobiosensors (15). It is possible to detect biological molecules at the nanoscale using nanobiosensors, which combine a biological recognition element with a transduction unit. A nanobiosensor consists of a transducer and a receptor of physicochemical components (16). The basis of biosensors is the recognition of molecules. Bacteria are only detected by biological receptors when a specific molecular recognition exists between the receptor and the bacteria. A lock-and-key model can be used to describe the interaction between antibodies and antigens in molecular recognition. A bioreceptor is a part of the sensor that interacts with the target. On the surface of the transducer, bio-receptors are fixed so that they can bind to target entities (DNA, enzymes, cells, antibodies, and aptamers) regardless of storage conditions (17).

Multiple methods of immobilizing the biological recognition element include cross-linking, adsorption, microencapsulation, entrapment, and covalent bonding. An essential challenge in nanobiosensor preparation is immobilizing nano-components. Receptors can be replaced by biologically originated molecules, including synthetic catalysts, engineered artificial proteins, recombinant antibodies, imprinted polymers, and ligands. The receptors' performance determines a biosensor's sensitivity and selectivity (18). It is possible to detect molecular recognition effects (changes in heat, light, mass, electroactivity, and pH) through transducers (thermistors, electrodes, piezoelectric devices, semiconductor pH electrodes, and photon counters). As an interface, the receptor converts measurable signals into energy. Nanobiosensors are characterized by transducers modified with NPs for rapid detection.

The presence and quantity of analytes can be detected more efficiently and accurately with nanobiosensors than simple biosensors. Further, a detector is equipped with an electronic component for amplifying and analyzing the transducer's electrical signals and a microprocessor for measuring them. Digital signals are converted to analog signals using filters and amplifiers. In addition to concentration units, the data can be displayed as a graphic, image, tabular numeric, and display. Nanobiosensors have been developed on-chip or at the point of care using smartphones to detect analytes. Using nanobiosensors' characteristics can indirectly enhance their performance (19).

Nanobiosensors are selectivity, reproducibility, linearity, and stability. The selectivity of a sensor refers to the capacity of the sensor to identify a particular analyte in many possibilities. The sensitivity of nanobiosensors determines their detection limits, which are influenced by their robustness (20). A nanobiosensors reproducibility correlates with its reliability when repeated accurately and precisely. It is a simple and effective method for determining linearity and accuracy using linear dynamic ranges or working ranges directly related to the signals they control. Sensor stability allows the quantification and detection of analytes under various measurement disturbance conditions while preserving accuracy and precision.

5. Types of nanobiosensors

A biosensor is classified according to the way it converts signals into optical, electrochemical, or piezoelectric signals. An optical biosensor analyzes data by measuring photons using a transducing element, such as an optical fiber. It is possible to use different optical sensing mechanisms to detect analytes on this type of biosensor, including absorption, fluorescence, colorimetry, or luminescence (21). A piezoelectric biosensor has a low noise level and is immune to electromagnetic interference, making it a superior biosensor to electrochemical ones. Vidal et al. have developed an innovative chromatic biosensor for fast bacterial detection, which involves non-woven fiber composites of polyvinyl butyrate-polydiacetylene. The device shows promising potential as an indicator of *S.aureus, E. coli,* and *Micrococcus luteus* infections (22). According to another study by Jeong et al., fluorescent supramolecular biosensors were constructed to detect bacteria. It is possible to detect *E.coli* by selectively producing fluorescence when pathogens bind to the supramolecular state due to conformational changes (23).

According to Ahmadi et al., viruses can be detected by optical biosensors, where the surface of a microsphere optical resonator shifts resonance to longer wavelengths when viral particles attach to its surface (24). Moreover, Surface Plasmon Resonance (SPR) is a highly effective optical immunoassay technique. Metallic thin films are deposited on dielectric waveguides, and p-polarized light is reflected along the plane of incidence to induce this type of resonance. A SPR-enhanced ellipsometry technique, sometimes called Total Internal Reflection Ellipsometry (TIRE), utilizes the perpendicular reflection properties of s-polarization (25, 26). In addition to simultaneously detecting multiple biomolecules, label-based or label-free SPR-based biosensors can monitor chemical and biological interactions of Ribose Nucleic Acid (RNA), ligands, DNA, and cofactors.

The biosensors are also suitable for clinical applications since they can quantify low molecular weight analytes, provide rapid detection, are low cost, and are specific, reproducible, and reliable. Electrochemical biosensors have been extensively used in the detection of pathogens. Using electrodes, nanobiosensors measure the electrical signals generated from specific unions or catalytic reactions with the analyte. In the previous experiment, electrons are captured by redox reactions between analytes and bio-elements (27). In addition, different readouts like potentiometry, conductometry, and amperometry are used to determine the analysis of the desired element.

Various biosensors have been improved through the use of bio- and nanomaterials. In addition to piezoelectric biosensors, there are also mechanical biosensors. Materials with piezoelectricity can produce voltage when mechanically stressed. An electric field causes crystals in biosensors to vibrate. Several materials have resonance frequencies that are characteristic of interactions with other molecules. Typically, mechanical biosensors link the change in resonant frequency to the mass of molecules adsorbing or desorbing from crystal surfaces. Vibrations provide information about the phenomena being measured (Table 1).

Table 1. Different types of nanobiosensors. This table highlights the variety of nanobiosensors used for bacterial detection, illustrating their principles, nanomaterials, target bacteria, detection methods, sensitivity, advantages, and challenges.

Type of Nanobiosensor	**Principle of Detection**	**Nanomaterial Used**	**Target Bacteria**	**Detection Method**	**Sensitivity**	**Advantages**	**Challenges**
Optical Nanobiosensor	Fluorescence, Surface Plasmon Resonance (SPR)	Quantum Dots, Gold Nanoparticles	*E. coli*, *Salmonella*	Fluorescence Spectroscopy, SPR	High (e.g., 10^2-10^3 CFU/mL)	High sensitivity, real-time detection	Complex sample preparation
Electrochemical Nanobiosensor	Conductivity, Impedance, Potentiometry	Carbon Nanotubes, Graphene	*Staphylococcus aureus*, *Pseudomonas aeruginosa*	Amperometry, Potentiometry	Very High (e.g., 10^1-10^2 CFU/mL)	High sensitivity, cost-effective	Interference from non-target species
Magnetic Nanobiosensor	Magnetic Relaxation, Magneto-Optical Detection	Magnetic Nanoparticles	*E. coli*, *Listeria monocytogenes*	Magnetic Resonance Imaging (MRI)	Moderate (e.g., 10^3-10^4 CFU/mL)	Rapid detection, easy separation	Lower sensitivity compared to other types
Piezoelectric Nanobiosensor	Mass Change Detection	Zinc Oxide Nanowires	*Salmonella*, *E. coli*	Quartz Crystal Microbalance (QCM)	High (e.g., 10^2-10^3 CFU/mL)	Label-free detection, real-time monitoring	Environmental stability issues

Colorimetric Nanobiosensor	Color Change Detection	Gold Nanoparticles, Silver Nanoparticles	*Vibrio cholerae*, *E. coli*	Visual Inspection, UV-Vis Spectroscopy	Moderate (e.g., 10^3-10^4 CFU/mL)	Simple, quick, and user-friendly	Lower sensitivity and specificity

6. Bacterial pathogen detection

Most bacterial infections in the human body are caused by Gram-negative microorganisms, which pose a particular challenge to the health of humanity worldwide today. The prevalence of multidrug resistance variants has been attributed to their indiscriminate exposure to antibiotics administered through water, food, or even through improper use of drugs on the part of patients (28). Due to the previously mentioned medical concern, different nanomaterials and biorecognition elements have been applied to develop biosensors for detecting antibiotics and bacteria (29). It is common for bacteria, such as *Salmonella typhi, Shigella spp*, and *Clostridium perfringens (C. perfringens),* to cause diseases in humans, plants, and animals (30). The bacteria that cause *S.aureus* infections are known to be extremely dangerous, as they can rapidly cause fatal diseases and are often resistant to multiple types of antibacterial agents.

Since conventional methods require at least three to five days for results, and other nucleic acid-based methods require a trained and expensive laboratory staff, it is necessary to develop new strategies for easier and faster detection (31). A biosensor developed by Suaifan et al. can detect *S. aureus* within a few minutes. The sensing tool consists of two magnetic nanobeads placed in the middle of a specific peptide substrate to measure the proteolytic activity of pathogen proteases. As a result of dissociation, the magnetic nanobeads, and the peptide moieties change color (32). Furthermore, Ahari et al. developed a potentiometric nanobiosensor capable of detecting bacteria by detecting an exotoxin they released. Usually, the method is used to detect contaminated food, although it may also be used to detect diseases clinically (33).

The software converts biological signals into information using biosensors, which utilize biological recognition and digital signals. In biosensors, substances present in living and non-living systems are detected using their characteristics, such as magnetics, optical, electrochemistry, chemicals, vibrations, and electricity. In most cases, the device comprises a transducer and biorecognition sensor. A transducer can measure an electronic signal generated by the interaction between the analyte and the bioreceptor. Various methods are used to immobilize biorecognition elements, including covalent interaction, adsorption, and encapsulation. Various biorecognition units, or receptors, can be found in cells, such as glycopeptides, lipids, lipoproteins, carbohydrates, receptor proteins, and glycoproteins. They play an essential role in infection by adhering to cell surfaces and noncellular substrates, evading immune system response, and enhancing nutrient absorption. The receptors have one significant characteristic in common besides their extracellular exposure.

The biosensors are assembled using them as biorecognition components. The detection limits of biosensors are improved by using nanomaterials. Several factors contribute, such as high electronic conductivity, large surfaces, and properties of plasmonic technology, such as the ability to store light in confined spaces. A nanomaterial can also transmit optical or mechanical signals, which makes it a potential biosensor. A nanobiosensor is a material with a diameter of less than 100 nanometers (34). To operate, they require optics, mechanics, and spectroscopy. Because nanobiosensors have smaller detection surfaces, they require less analyte to produce meaningful results. Higher-density arrays are more effective for small spaces because they allow more analytes to be detected in a single test (35). Using nanosensors, which eliminate some of the conventional processes associated with sample processing, will further simplify and reduce the expense of pathogen detection tests. A nanobiosensor uses biomimetic materials that mimic biological processes by combining enzymes, nucleic acids, antibodies, cells, substrates, antigens, and bacteria.

6.1 Detection of *Brucella spp*

As one of the most significant bacterial zoonotic diseases affecting humans and animals, Brucellosis (Malta fever) continues to pose serious health problems worldwide, particularly in the developing world (36). The disease has excellent significance on livestock from both human health and economic perspectives. Several Brucella species are believed to be involved in the development of brucellosis. Four of them are thought to be the main causative agents of human infections, including *Brucella suis (B. suis), Brucella abortus (B. abortus), Brucella canis (B. canis)*, and *Brucella melitensis (B. melitensis)* (37).

In addition to Rose Bengal plates, complement fixation, serum agglutination, and PCR tests, brucellosis can be diagnosed using several other methods. The disadvantages of diagnostic techniques include that they are less sensitive, specific, and reliable than older techniques, they might be time-consuming and labor-intensive in certain instances (38), and they require the services of experienced individuals to perform the test and interpret the results. By the way, simple methods that can detect Brucella cells directly at a high level of sensitivity seem promising.

6.2 Detection of *C. botulinum*

There is a widely distributed Gram-positive, anaerobic, rod-shaped bacillus, *C. botulinum*, in soils worldwide. The botulinum bacterium produces a potent toxin (botulinum toxin) that causes muscle flaccidity and paralysis, known as botulism disease (39). According to their antigenic reactivity, Botulinum Neurotoxins (BoNTs) are divided into seven classes, with BoNTs A, B, and E causing botulism in humans.

As a result, characterization of the BoNTs is essential for diagnosing infections caused by *C. botulinum* (40). Several methods can determine neurotoxins, including mouse bioassays, ELISAs, and PCRs, but each has limitations. Therefore, a sensitive, quick, and simple test for detecting botulinum toxin in time is crucial for public health and patient treatment.

In general, biosensors are helpful for quickly detecting biological toxins, especially BoNT (41). The work of Wang and coworkers was based on the Forster Resonance Energy Transfer (FRET) method of conducting a biosensor in aqueous media that can detect biologically active BoNT/E light chains and holotoxin within three hours using semiconductor nanocrystals (QDs) and dark quencher-labeled peptide probes (42). As a result of biologically active BoNT/E molecules cleaving the designed peptide probes when present in solution, QD photoluminescence intensities are changed due to the FRET phenomenon and allow BoNT/E to be indicated and quantified (42).

6.3. Detection of *Salmonella* spp

Salmonella is a foodborne bacterium that causes infections in humans and animals (such as poultry and livestock) (43). *Salmonella* genetic strains have been successfully identified with electrochemical antibodies, antimicrobial peptides, bacteriophages, and DNA probes combined with optical and mass-sensitive transduction techniques (44). Sun and coworkers coated blue silica- and magnetic-NPs with specific antibodies (IgG molecules) against *Salmonella pullorum* and *Salmonella gallinarum* to obtain functionalized IgG- Blue- SiNPs and IgG- MNPs as immunosensor probes for rapid detection of *Salmonella* serotypes in an optical sandwich immunoassay (45). All experiment steps were performed in less than 60 minutes, shorter than the time needed for the conventional PCR method.

6.4. Detection of *E. coli*

This Gram-negative bacterium belongs to the family Enterobacteriaceae. It involves various diseases and syndromes in humans and farm animals (e.g., cattle, pigs, sheep, goats, and poultry) (46). As a result, the animal industries suffer health risks and substantial economic losses. The bacterium is identified using optical, electrochemical, and mass-sensitive biosensors in combination with bacteriophages, antibodies, DNA probes, and aptamers. In a study by Le et al., chitosan-coated iron oxide Magnetic Nano Particles (CS-MNPs) were employed to detect *E.coli* and *S.aureus* bacteria within 10 minutes (47). Because iron oxide magnetic NPs close on bacterial cells once they attach, a reduced colorimetric response has been expected following the bacterial attachment. When the reaction was monitored by spectrophotometry and the naked eye, the detection limits were 10^2 and 10^4 CFU/ml, respectively (Figure 1) (Table 2).

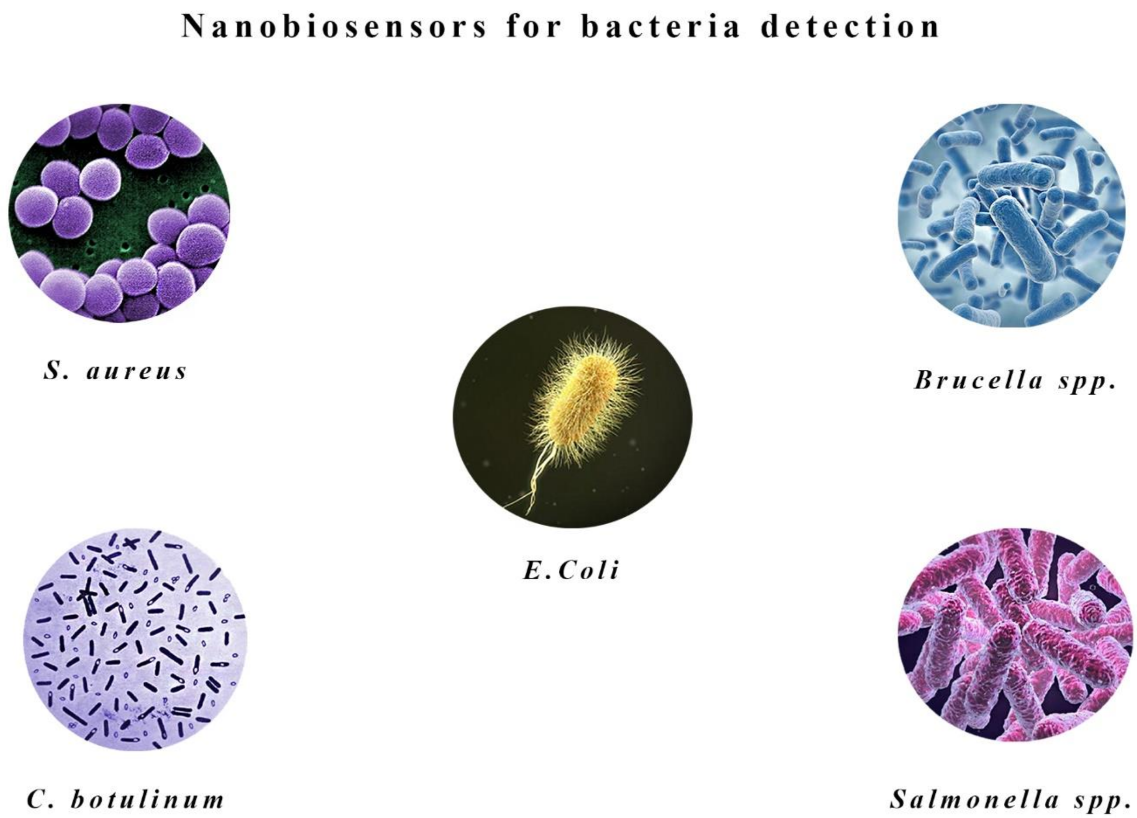

Figure 1: Advances in Nanobiosensors for Bacterial Detection.

Nanobiosensors have been developed to rapidly detect pathogenic bacteria, including *S. aureus*, *Brucella* spp., *C. botulinum*, *Salmonella* spp., and *E. coli*. These innovative biosensors offer sensitive and rapid detection methods and show potential for applications in food safety, clinical diagnostics, and environmental monitoring.

Table 2. Current status of nanobiosensors for detecting zoonotic bacterial infections.

Author	Year	Methods	Results

Peyman Ghafouri et al	2023	Nanobiosensors (NanoBioSS) are analytical devices with a biological sensor and a physicochemical converter. As an essential function of NanoBioSS, it generates a digital electrical signal directly proportional to the sum of one or several molecules being analyzed	The sensitivity and versatility of nanobiosensors make them useful in a wide range of fields, including clinical, environmental detection, and food safety
Luis Castillo-Henríquez et al,	2020	novel electrochemical-based-DNA biosensor through enzyme-amplified detection to improve the sensitivity and selectivity of the device for the pathogen	There is no vaccine or pharmacological treatment for many viruses and bacteria, and the development of a POC device for the rapid diagnosis of diseases such as COVID-19, biosensors and nanobiosensors are powerful measurement devices that can make the detection process of important clinical bacteria and virus to be easy, quick, and effective
Azam Ahangari et al.,	2022	introduced a simple and rapid cost□ effective colorimetric assay by employing chitosan□coated iron oxide magnetic nanoparticles (CS□MNPs) for the detection of both bacterial cells	The potential features of biosensors make them promising devices to introduce novel detection methods with enhanced capabilities to be replaced with conventional techniques, particularly electrochemical and optical□ based biosensors, which seem more attractive than the other types in terms of their unique properties. Optical

Anurag Jyoti et al,	2016	specific and sensitive methods for pathogen detection. Polymerase chain reaction (PCR) and real-time polymerase chain reaction (RTi-PCR) detect specific segments of the pathogen genome in less time. However, such methods require different temperature profiles and skilled personnel, thus limiting field operation. Identification of nucleic acids in clinics is limited due to complex matrices and poor availability of target nucleic acids.	Nanosensors are miniaturized devices developed by integrating various components. They include biological probes, signal transducers, and enhancers and are suitable for field use.
Ananya S. Agnihotri et al	2022	Using polymerase chain reaction (PCR) as a DNA amplification tool has paved the way for developing various methods that depend upon PCR to determine numerous harmful bacteria.	Biosensors have recently turned out to be an outstanding platform for the detection of pathogenic bacteria

7. Challenges and future perspectives

Despite their considerable potential, several challenges prevent nanobiosensors from becoming widely adopted for bacterial detection (48). Standardizing fabrication methods must be standardized so that scaleability and reproducibility can be assured, sensor performance can be optimized to achieve higher specificity and sensitivity, and biosensors' effectiveness can be validated in complex samples. It is also necessary to address issues associated with biosensors' cost-effectiveness, shelf-life, and stability related to food safety and healthcare applications.

The revolutionary potential of nanotechnology can be seen in a variety of fields. Using nanomaterials in food pathogen detection can enhance existing methods and provide novel analytical tools (49). The development of pathogen nanosensors and assays has grown in popularity recently, but many are still in the early stages. However, nanotechnology has contributed to improvement in varying degrees. Despite technological advances, others have modest enhancements, especially in whole-cell detection, because there are fewer access points and a more significant reaction center structure. A more sensitive detection system increases matrix interference, compromising certain bacteria's sensitivity and specificity. As a result of the challenge, adequate sample preparation is further highlighted.

A limited number of studies evaluate the performance of samples in natural food systems or contexts where competing organisms are present, as well as studies that examine sample preparation techniques. Due to the multidisciplinary nature of nanotechnology, there needs to be more in this area. Engineers, chemists, and material scientists have primarily investigated pathogen nanosensors and assays due to the need for more resources to evaluate and validate large-scale methods. Nanotechnology will continue to play a significant role as issues are resolved in rapid detection. Detection methods in the future will be highly sensitive and specific, highly throughput-efficient, robust, and quantitative. Nanomaterials and nanofabrication possess several advantages that make them excellent tools for addressing a wide range of problems associated with the efficient use of nanotechnology in detecting and controlling foodborne pathogens. Another study investigates black peel pomegranate extract's antioxidant and anticancer properties (50). It explores its potential as a dual reducing and stabilizing agent in biosynthesizing silver NPs, expecting enhanced biological activity.

The future of nanobiosensors in bacterial detection holds promising advancements that extend far beyond current capabilities. Emerging applications include smart packaging that detects bacterial presence and responds by neutralizing pathogens or extending shelf life through controlled release of preservatives. Innovations in wearable sensors for food handlers could also provide real-time contamination alerts, ensuring safer food handling practices. Among the various types of nanobiosensors, those based on carbon nanotubes, gold nanoparticles, and quantum dots are particularly noteworthy. Carbon nanotube-based sensors offer exceptional sensitivity and rapid response times due to their high surface area and electrical conductivity. Gold nanoparticle-based sensors excel in their ability to enhance signal detection through localized surface plasmon resonance. Quantum dot-based sensors stand out for their high brightness and photostability, which enable highly sensitive and multiplexed detection. These cutting-edge nanobiosensors are poised to transform bacterial detection, ensuring safer food production and consumption while paving the way for innovative, responsive packaging solutions.

The advent of nanobiosensors represents a significant leap forward in microbiological diagnostics, particularly in rapidly detecting pathogenic bacteria such as Salmonella, Clostridium, Escherichia coli, and Brucella spp. These pathogens are responsible for numerous infectious diseases in humans and animals, necessitating prompt and accurate detection methods to mitigate their impact. This discussion explores the mechanisms, advantages, challenges, and future perspectives of using nanobiosensors to detect these pathogens. Nanobiosensors utilize nanomaterials to enhance the sensitivity and specificity of detection systems. These sensors typically combine biological recognition elements, such as antibodies, nucleic acids, or enzymes, with nanomaterials like gold nanoparticles, carbon nanotubes, or quantum dots. The nanomaterials facilitate signal transduction, often by amplifying the detection signal or enabling real-time monitoring. For instance, a common approach in detecting *Salmonella* involves using gold nanoparticles conjugated with antibodies specific to Salmonella antigens. When *Salmonella* bacteria are present in a sample, they bind to the antibodies, causing the gold nanoparticles to aggregate. This aggregation can be detected through changes in the optical properties of the nanoparticles, providing a rapid and sensitive detection method.

Similarly, nanobiosensors for *Clostridium*, particularly *Clostridium difficile*, often employ nucleic acid-based detection. DNA or RNA sequences specific to Clostridium toxins can be immobilized on nanostructures. The hybridization of these sequences with target nucleic acids from the pathogen can be detected using fluorescent nanomaterials, providing a precise measure of pathogen presence.

The integration of nanomaterials significantly reduces the time required for detection. Traditional culture methods can take days, whereas nanobiosensors can provide results within minutes to hours. Nanomaterials enhance biosensors' sensitivity, allowing for the detection of low concentrations of pathogens. Additionally, the specificity of biological recognition elements ensures that the sensors can accurately identify specific bacterial species. Many nanobiosensors are designed to be portable and user-friendly, making them suitable for point-of-care diagnostics. This is particularly beneficial in resource-limited settings where access to laboratory facilities is restricted. Nanobiosensors can provide real-time data, enabling continuous monitoring of samples. This capability is crucial for timely decision-making in clinical and environmental contexts. Despite their numerous advantages, nanobiosensors face challenges that must be addressed for widespread adoption. Producing nanomaterials and integrating them into functional biosensors can be costly. Developing cost-effective manufacturing processes is essential for large-scale deployment. Environmental conditions can affect the stability of biological recognition elements and nanomaterials. Ensuring the long-term stability and shelf-life of nanobiosensors is critical for practical applications (Figure 2).

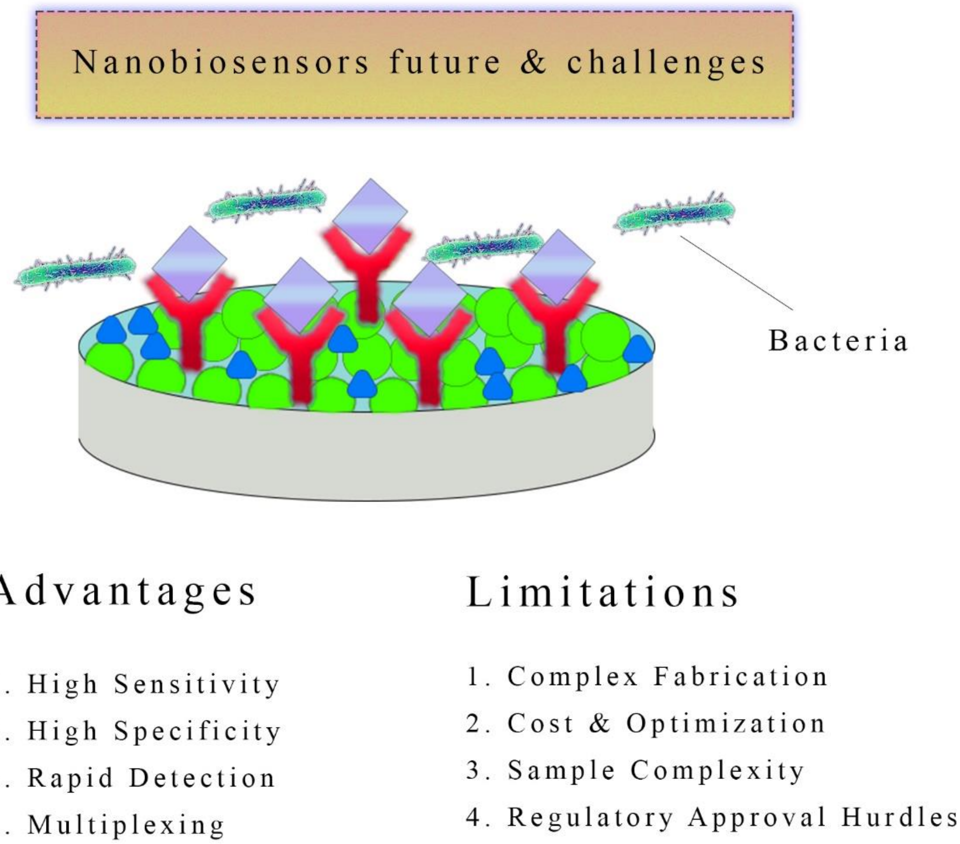

Figure 2: Advantages and Limitations of Nanobiosensors for Bacterial Detection.

Nanobiosensors, with their high sensitivity, specificity, rapid detection, and multiplexing capabilities, hold great promise as tools for bacterial detection. While they do face challenges such as complex fabrication processes, cost and optimization issues, sample complexity, and regulatory approval hurdles, these are not insurmountable. With the right approach, these limitations can be overcome, paving the way for their widespread adoption and practical use in bacterial detection applications.

Conclusion

Nanobiosensors present a transformative approach to rapidly detecting pathogenic bacteria, including Salmonella, Clostridium, Escherichia coli, and Brucella spp. Their integration of nanomaterials with biological recognition elements allows for unprecedented sensitivity, specificity, and speed in diagnostics. These advantages make them highly valuable for point-of-care testing, offering significant benefits in clinical, environmental, and food safety applications. However, cost, stability, and regulatory hurdles must be addressed to realize their full potential. Continued advancements in nanotechnology and biochemistry and strategic efforts to standardize and scale production will be crucial in overcoming these obstacles. Overall, the future of nanobiosensors looks promising, with the potential to significantly enhance our ability to detect and respond to bacterial infections rapidly and accurately.

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The datasets of the current study are available from the corresponding author upon reasonable request.

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Author contribution

Conceptualization: [Nima Komeili], …; Writing - original draft preparation: [Soheila Chaleshgari, Zahra Mostofi Fakhrani, Shahla Salimpour Kavasebi, Nima Komeili]; Writing - review and editing: [Soheila Chaleshgari, Zahra Mostofi Fakhrani, Shahla Salimpour Kavasebi, Nima Komeili], …; Funding acquisition: [Self-funding], …; Supervision: [Nima Komeili]. All authors checked and approved the final version of the manuscript for publication in the present journal.

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