Review Article

Nanonbiosensors; Rapid Detection of *Salmonella*, *Clostridium*, *Escherichia*, and *Brucella* spp. Infections

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ABSTRACT

Zoonotic diseases, defined as those that are infectious and transmitted from animals to humans, constitute a substantial global health concern. Despite concerted efforts to eradicate or control these diseases, healthcare systems continue to face a substantial burden due to their re-emergence. The early and accurate detection of bacterial pathogens is of crucial importance in order 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, labour-intensiveness, and lengthy turnaround times for results. There is an increasing interest in the development of faster, more accurate, and cost-effective diagnostic methods to address these challenges. The utilization of nanobiosensors has emerged as a promising tool for the rapid detection of infectious disease agents. The utilization of biological recognition elements by these devices enables the detection of specific pathogens, with the potential to effect a paradigm shift in diagnostic practices. Furthermore, the incorporation of nanotechnology, particularly nanomaterials, has been demonstrated 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. The capacity of nanobiosensors to discern the presence of bacterial pathogens in a variety of sample types, encompassing environmental samples, animal specimens and clinical samples, renders them a versatile instrument for the implementation of disease surveillance and control measures. Furthermore, nanobiosensors have demonstrated considerable potential in enhancing the sensitivity and specificity of detection assays, thereby facilitating 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. The integration of biosensors and nanotechnology has been demonstrated to hold significant potential for enhancing the detection and characterisation of Salmonella, Clostridium, Escherichia, and Brucella spp. The implementation of these innovative diagnostic tools has the potential to transform 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

The development of nanotechnology has led to the creation of numerous biosensors in recent years, with significant advancements being made in the field of medical sciences (1-3). Presently, nanotechnology is regarded as one of the most promising areas of research in science, with its applications encompassing a wide range of disciplines, including medicine, drug delivery, biology, the environment, and food safety (4, 5). The development of biosensors represents a significant advancement in this field, with the potential to address a diverse array of challenges and opportunities. 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). A number of molecular techniques are available for the detection of viruses and bacteria, including reverse transcription polymerase chain reaction (RT-PCR), which is widely regarded as the gold standard. A number of classical methods for detecting pathogens have been developed, including isolation, culture, and biochemical analysis. Furthermore, serological tests such as Enzyme-Linked Immunosorbent Assays (ELISAs) detect antibodies and immunoglobulins necessary for identification (8). A salient issue with certain techniques is their intricate nature and the significant time investment required to achieve tangible outcomes. The application of nanotechnology has emerged as a suitable and efficient means of detecting pathogens. The utilization of NPs for various pathogenic purposes has been demonstrated to contribute to the development of new devices and technologies for the prevention of disease. In consideration of zoonoses as a prevailing concern, the study encompasses not only the examination of human diseases but also those that affect animals. Estimates suggest that approximately 60% of all infections identified in humans are attributable to zoonoses. It is important to note that both animals and humans are susceptible to zoonoses, which are diseases transmitted from animals to humans. These zoonoses are caused by 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). Concurrently, Deoxyribose Nucleic Acid (DNA) biosensors and sequence-specific DNA detectors are being utilised with increasing frequency for clinical studies by the international scientific community. Furthermore, DNA-based piezoelectric biosensors have been utilised for the identification of specific gene sequences and the detection of DNA damage. The utilization of nanobiosensors and biosensors is pivotal in the detection of viral and bacterial clinical pathogens. Devices are characterised by their expeditiousness, pragmatism (facilitating Point-Of-Care (POC) testing through smartphone-based nanobiosensors) and innovation, thus providing an alternative solution to the disadvantages presented by standard detection methods.

2. Evidence Acquisition

Technological advances have facilitated the study of viruses that affect humans, including the Human Immunodeficiency Virus (HIV), the Ebola virus, and most recently, the recently discovered Severe 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 defined as an analytical tool comprising a biomolecule as a sensing element and a segment that transforms a recognition event into visible or measurable information. The utilization of biosensors has been demonstrated to offer several advantages over conventional methods, including the capacity to facilitate rapid, sensitive, and straightforward detection of pathogens, thereby ensuring effective treatment (7). The utilization of biosensors that are underpinned by micro- and nanotechnology has the potential to facilitate the execution of sophisticated molecular diagnostic tests for a range of infectious diseases. The employment of nanobiotechnological methodologies, encompassing real-time diagnosis, high-throughput screening, utilization of small sample volumes, and low detection limits, facilitates numerous advantages in biosensors. The objective of the study was to present the findings of novel nano biosensor-based diagnostic techniques, with a view to determining the most prevalent zoonoses of significant importance in modern medicine and veterinary medicine, including 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. The efficacy of biosensors in detecting analytes is contingent upon the purpose and design of the biosensor in question. As demonstrated in the research by Soni et al., it is possible to utilise a standard household device, such as a smartphone, as a biosensor by the addition of simple accessories (12, 13). This development proposes a novel method for the measurement of urea in saliva without the necessity for invasive tools. Consequently, the initial detection process is both rapid and cost-effective. The detection of proteins, nucleic acids and cells associated with diseases is a common application of biosensors. The detection of biomolecules is facilitated by various components, including organelles, enzymes, nucleic acids, microorganisms, and antibodies. Furthermore, the researchers must determine the required functionality based on the device's intended use. Consequently, it is imperative to undertake multidisciplinary studies prior to selecting the appropriate material, transducer, and biological element for constructing a biosensor. A plethora of additional clinical diagnostic applications can be performed with biosensors. Additionally, biosensors have the capacity to detect bacteria and viruses in water and food, which are potential 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 a streptavidin-antibody system (14). A chemiresistive biosensor is a device that captures bacteria onto its surface, whereupon an electric readout is used to detect them.

4. Principle of Nanobiosensors

The integration of traditional biosensors with nanotechnology has led to the emergence of nanobiosensors, which have gained significant popularity in recent years (15). The prospect of detecting biological molecules at the nanoscale is rendered feasible by nanobiosensors, which integrate a biological recognition element with a transduction unit. A nanobiosensor is constituted of a transducer and a receptor, which are comprised of physicochemical components (16). The underlying principle of biosensors is the recognition of molecules. The presence of bacteria is only detected by biological receptors in the context of a specific molecular recognition between the receptor and the bacteria. The interaction between antibodies and antigens in molecular recognition can be modelled using a lock-and-key paradigm. A bioreceptor constitutes a component of the sensor that interacts with the target. Bio-receptors are affixed to the surface of the transducer, enabling them to bind to target entities (e.g. DNA, enzymes, cells, antibodies and aptamers), irrespective of storage conditions (17). A variety of methodologies can be employed for the immobilization of the biological recognition element, including cross-linking, adsorption, microencapsulation, entrapment, and covalent bonding. A pivotal challenge in the field of nanobiosensor preparation pertains to the immobilisation of nano-components. Receptors can be replaced by biologically originated molecules, including synthetic catalysts, engineered artificial proteins, recombinant antibodies, imprinted polymers, and ligands. The performance of the receptors is a determining factor in the sensitivity and selectivity of the biosensor (18). The prospect of detecting molecular recognition effects (i.e. changes in heat, light, mass, electroactivity, and pH) is made possible through the utilization of transducers (namely thermistors, electrodes, piezoelectric devices, semiconductor pH electrodes, and photon counters). The receptor serves as an interface, the function of which is to convert measurable signals into energy. The term "nanobiosensors" is defined by transducers that have been modified with NPs for the purpose of facilitating rapid detection. The presence and quantity of analytes can be detected with greater efficiency and accuracy by nanobiosensors than by simple biosensors. Furthermore, a detector is equipped with an electronic component for amplifying and analysing the transducer's electrical signals, as well as a microprocessor for measuring them. The conversion of digital signals to analog signals is achieved through the utilization of filters and amplifiers. In addition to concentration units, the data can be presented in various formats, including graphics, images, tabular numeric data, and displays. The development of nanobiosensors has been undertaken both on-chip and at the point of care, with the utilization of smartphones for the detection of analytes. The employment of nanobiosensors' characteristics has the capacity to enhance their performance indirectly (19). The properties of nanobiosensors include selectivity, reproducibility, linearity and stability. The selectivity of a sensor is defined as the capacity of the sensor to identify a particular analyte in a multitude of possibilities. The sensitivity of nanobiosensors is a pivotal factor in determining their detection limits, which are, in turn, influenced by their robustness (20). The reproducibility of nanobiosensors has been demonstrated to correlate with their reliability when subjected to repeated testing, both accurately and precisely. This method is both simple and effective in determining linearity and accuracy. It does so by using linear dynamic ranges or working ranges, which are directly related to the signals they control. The capacity for sensors to maintain stability enables the quantification and detection of analytes in the presence of various measurement disturbance conditions, while ensuring the preservation of accuracy and precision.

5. Types of Nanobiosensors

The classification of biosensors is determined by the manner in which they convert signals into optical, electrochemical, or piezoelectric signals. An optical biosensor is a device that analyses data by measuring photons using a transducing element, such as an optical fibre. The utilization of disparate optical sensing mechanisms is a viable approach for the detection of analytes on this particular biosensor. These mechanisms encompass absorption, fluorescence, colourimetry, and luminescence (21). A piezoelectric biosensor has been shown to have a low noise level and is immune to electromagnetic interference (EM), thus rendering it a superior biosensor to electrochemical ones. Vidal et al. have developed an innovative chromatic biosensor for the rapid detection of bacteria, which involves non-woven fibre composites of polyvinyl butyrate-polydiacetylene. The device has been shown to have promising potential as an indicator of S.aureus, E. coli, and Micrococcus luteus infections (22). As stated in the study by Jeong et al., fluorescent supramolecular biosensors were constructed for the purpose of detecting bacteria. The selective production of fluorescence when pathogens bind to the supramolecular state due to conformational changes (23) renders E. coli detection a possibility. As posited by 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). Furthermore, Surface Plasmon Resonance (SPR) has been demonstrated to be a highly effective optical immunoassay technique. The process entails the deposition of metallic thin films on dielectric waveguides, with p-polarized light being reflected along the plane of incidence to induce this

particular form of resonance. A SPR-enhanced ellipsometry technique, otherwise referred to as Total Internal Reflection Ellipsometry (TIRE), employs the perpendicular reflection properties of s-polarisation (25, 26). In addition to the simultaneous detection of multiple biomolecules, label-based or label-free SPR-based biosensors have the capacity to monitor chemical and biological interactions involving ribose nucleic acid (RNA), ligands, deoxyribonucleic acid (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 utilised in the detection of pathogens. Electrode-based nanobiosensors are capable of measuring the electrical signals generated from specific unions or catalytic reactions with the analyte. In the preceding experiment, electrons were captured by redox reactions between analytes and bio-elements (27). Furthermore, a range of analytical techniques, including potentiometry, conductometry and amperometry, are employed to facilitate the analysis of the target element. The utilization of bio- and nanomaterials has led to significant advancements in the field of biosensors. In addition to piezoelectric biosensors, mechanical biosensors are also employed. Materials exhibiting piezoelectric properties are capable of generating an electrical potential when subject to mechanical stress. The application of an electric field has been demonstrated to induce vibrations in the crystals that constitute biosensors. It is noteworthy that a number of materials possess resonance frequencies that are indicative of interactions with other molecules. In the case of mechanical biosensors, the change in resonant frequency is typically linked to the mass of molecules adsorbing or desorbing from crystal surfaces. As demonstrated in Table 1, vibrations are a key component in the analysis of phenomena, providing a comprehensive and informative basis for the measurement process.

6. Bacterial Pathogen Detection

The majority of bacterial infections in the human body are caused by Gram-negative microorganisms, which pose a particular challenge to global health. 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). In light of the aforementioned medical concern, various nanomaterials and biorecognition elements have been employed in the development of biosensors for the detection of antibiotics and bacteria (29). It is well-documented that bacteria such as Salmonella typhi, Shigella spp., and Clostridium perfringens (C. perfringens) are known 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. Conventional methods necessitate a period of three to five days for the attainment of results, whilst other nucleic acid-based methods require the expertise of a trained and costly laboratory staff (31). The development of new strategies for the more efficient and expeditious detection of nucleic acids is therefore essential. Suaifan et al. have developed a biosensor capable of detecting S. aureus within a few minutes. The sensing tool is composed of two magnetic nanobeads positioned at the centre of a specific peptide substrate, with the purpose of measuring the proteolytic activity of pathogen proteases. It is evident that the process of dissociation results in a colour change in the magnetic nanobeads and the peptide moieties (32). Furthermore, Ahari et al. developed a potentiometric nanobiosensor capable of detecting bacteria by detecting an exotoxin they released. Typically, the method is employed for the detection of contaminated foodstuffs; however, it may also be utilised for clinical detection of diseases (33). The software in question is capable of converting biological signals into a format that can be processed using digital signals, and this is achieved by the utilisation of biosensors, which employ biological recognition mechanisms. In the domain of biosensors, the detection of substances present in living and non-living systems is facilitated by the utilisation of their distinctive characteristics, including magnetics, optics, electrochemistry, chemicals, vibrations, and electricity. In the majority of cases, the device consists of a transducer and a biorecognition sensor. A transducer is capable of measuring an electronic signal generated by the interaction between the analyte and the bioreceptor. A variety of methodologies are employed for the immobilization of biorecognition elements, encompassing covalent interaction, adsorption, and encapsulation. A variety of biorecognition units, otherwise known as receptors, can be found in cells. These include glycopeptides, lipids, lipoproteins, carbohydrates, receptor proteins and glycoproteins. These bacteria play an essential role in infection by adhering to cell surfaces and noncellular substrates, evading immune system response, and enhancing nutrient absorption. The receptors exhibit one significant commonality, in addition to their extracellular exposure. The biosensors are assembled using them as biorecognition components. The detection limits of biosensors are enhanced by the utilisation of nanomaterials. The phenomenon is attributed to a number of factors, including high electronic conductivity, substantial surface areas, and the unique properties of plasmonic technology, such as the capacity to accumulate light within confined spaces. Furthermore, the transmission of optical or mechanical signals by a nanomaterial renders it a potential biosensor. A nanobiosensor is defined as a material with a diameter of less than 100 nanometers (34). In order to function, these devices necessitate the utilisation of optics, mechanics, and spectroscopy. The reduced detection surface area of nanobiosensors necessitates a lower quantity of analyte for the generation of significant results. Higher-density arrays have been demonstrated to be more efficacious in constrained spaces, as they facilitate the

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

detection of a greater number of analytes in a single test (35). The employment of nanosensors, which serve to eliminate several of the conventional processes associated with sample processing, will result in further simplification and reduction of the expense of pathogen detection tests. A nanobiosensor is an instrument that uses biomimetic materials to mimic biological processes. Biomimetic materials combine enzymes, nucleic acids, antibodies, cells, substrates, antigens and bacteria.

6.1 Detection of *Brucella spp*

Brucellosis (Malta fever) is regarded as one of the most significant bacterial zoonotic diseases affecting humans and animals. It continues to pose serious health problems worldwide, particularly in the developing world (36). The disease is of significant concern from both human health and economic perspectives. It is hypothesised that several Brucella species are involved in the development of brucellosis. It is hypothesised that four of these bacteria are the primary causative agents of human infections, namely 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 reduced sensitivity, specificity and reliability in comparison to older techniques, the potential for time-consuming and labour-intensive procedures in certain instances (38), and the necessity of experienced individuals to perform the test and interpret the results. It is noteworthy that straightforward methodologies capable of detecting Brucella cells with a high degree of sensitivity appear to hold considerable promise.

6.2 Detection of *C. botulinum*

C. botulinum, a Gram-positive, anaerobic, rod-shaped bacillus, is distributed widely 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. Consequently, the characterisation of the BoNTs is imperative for the diagnosis of infections caused by C. botulinum (40). A number of methodologies are available for the determination of neurotoxins, including mouse bioassays, enzyme-linked immunosorbent assays (ELISAs), and polymerase chain reaction (PCR) tests. However, it should be noted that each of these approaches has inherent limitations. Consequently, the development of a sensitive, rapid and straightforward test for the timely detection of botulinum toxin is imperative for public health and the effective treatment of patients. It is widely acknowledged that biosensors are effective tools for the rapid detection of biological toxins, with botulinum neurotoxins (BoNTs) being a prime example of this application (41). The research conducted by Wang and colleagues was founded upon the Forster Resonance Energy Transfer (FRET) methodology for the implementation of a biosensor within an aqueous medium. This biosensor has the capacity to detect biologically active BoNT/E light chains and holotoxin within a timeframe of three hours by utilising semiconductor nanocrystals (QDs)

and dark quencher-labelled peptide probes (42). It has been established that the presence of biologically active BoNT/E molecules in solution results in the cleavage of the designed peptide probes. This, in turn, leads to alterations in the QD photoluminescence intensities due to the FRET phenomenon. Consequently, this process enables the indication and quantification of BoNT/E (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). The successful identification of Salmonella genetic strains has been achieved through the utilisation of electrochemical antibodies, antimicrobial peptides, bacteriophages, and DNA probes in conjunction with optical and mass-sensitive transduction techniques (44). Sun and colleagues 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). It was demonstrated that all experiment steps could be performed within a time frame of 60 minutes or less. This is in contrast to the time required for the conventional PCR method, which is known to take longer.

6.4. Detection of *E. coli*

This Gram-negative bacterium belongs to the family Enterobacteriaceae. The condition is characterised by the manifestation of diverse diseases and syndromes in both humans and farm animals, including but not limited to cattle, pigs, sheep, goats, and poultry. Consequently, the animal industries face significant challenges, including 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., the use of chitosan-coated iron oxide magnetic nanoparticles (CS-MNPs) was investigated for the purpose of detecting E. coli and S. aureus bacteria. The study concluded that detection was possible within 10 minutes (47). It has been hypothesised that the attachment of iron oxide magnetic NPs to bacterial cells will result in a reduced colourimetric response. When the reaction was monitored by spectrophotometry and visual inspection, the detection limits were 102 and 104 colony-forming units (CFU) per millilitre, respectively (Figure 1 and Table 2).

7. Challenges and Future Perspectives

Notwithstanding their considerable potential, several challenges must be overcome if nanobiosensors are to become widely adopted for bacterial detection (48). Standardising fabrication methods is imperative to ensure scaleability and reproducibility, optimise biosensor performance to achieve higher specificity and sensitivity, and validate biosensors' effectiveness in complex samples. In addition, it is imperative to address the issues associated with biosensors' cost-effectiveness, shelf-life, and stability in relation to food safety and healthcare applications. The revolutionary potential of nanotechnology can be observed in a variety of fields. The utilisation of nanomaterials in the domain of food pathogen detection has the potential to augment prevailing methodologies and furnish novel analytical instruments (49). The development of pathogen nanosensors and assays has grown in popularity recently, but many are still in the early stages. Nevertheless, nanotechnology has contributed to improvements in varying degrees. Despite the technological advances that have been made, others have made modest enhancements, especially in whole-cell detection, because there are fewer access points and a more significant reaction centre structure. The implementation of a more sensitive detection system has been demonstrated to result in an increase in matrix interference, thereby compromising the sensitivity and specificity of certain bacteria. It is evident that the aforementioned challenge has served to underscore the importance of adequate sample preparation. A limited number of studies have evaluated the performance of samples in natural food systems or contexts where competing organisms are present, as well as studies that have examined sample preparation techniques. The multidisciplinary nature of nanotechnology necessitates further research in this area. In the field of bioscience, engineers, chemists and material scientists have focused their research efforts on the investigation of pathogen nanosensors and assays. This increased focus is primarily driven by the necessity to evaluate and validate large-scale methods, which require greater resources. The role of nanotechnology is set to remain significant as issues are resolved through rapid detection. In the future, detection methods will be characterised by heightened sensitivity and specificity, enhanced throughput efficiency, robustness, and quantitative capabilities. The employment of nanomaterials and nanofabrication in addressing challenges related to the effective utilisation of nanotechnology in the detection and management of foodborne pathogens is predicated on the multifarious advantages these materials possess. Another study investigates the antioxidant and anticancer properties of black peel pomegranate extract (50). The present study explores the potential of the substance as a dual reducing and stabilising agent in the biosynthesising of silver NPs, with the expectation of 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 the presence of bacteria and responds by neutralising pathogens or extending shelf life through controlled release of preservatives. Innovations in wearable sensors for food handlers have the potential to provide real-time contamination alerts, thereby ensuring safer food handling practices. In the realm of nanobiosensors, those based on carbon nanotubes, gold nanoparticles, and quantum dots merit particular attention due to their distinctive characteristics and potential applications. Carbon nanotube-based sensors boast unparalleled sensitivity and rapid response times, a consequence of their high surface area and electrical

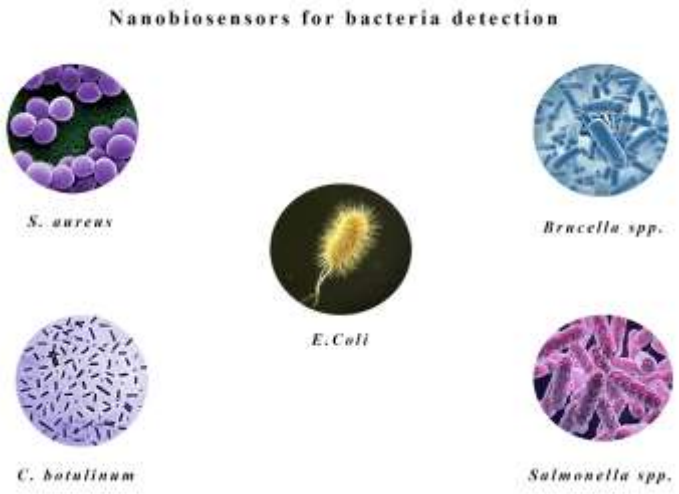

Figure 1. Advances in Nanobiosensors for Bacterial Detection. The development of nanobiosensors has enabled the rapid detection of pathogenic bacteria, including S. aureus, Brucella spp., C. botulinum, Salmonella spp., and E. coli. These biosensors represent a significant innovation in the field, offering highly sensitive and rapid detection methods with considerable potential for application in areas such as 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

conductivity. Gold nanoparticle-based sensors demonstrate a high level of proficiency in their capacity to enhance signal detection through the process of localized surface plasmon resonance. Quantum dot-based sensors are distinguished by their high brightness and photostability, which facilitate highly sensitive and multiplexed detection. These nanobiosensors represent a paradigm shift in the field, with the potential to transform bacterial detection, ensuring safer food production and consumption. Furthermore, they lay the foundation for innovative, responsive packaging solutions. The advent of nanobiosensors signifies a substantial advancement in the domain of microbiological diagnostics, with particular relevance to the expeditious detection of pathogenic bacteria such as Salmonella, Clostridium, Escherichia coli, and Brucella spp. These pathogens are responsible for a multitude of infectious diseases in humans and animals, underscoring the imperative for expeditious and precise detection methodologies to mitigate their impact. This discussion explores the mechanisms, advantages, challenges, and future perspectives of using nanobiosensors to detect these pathogens. Nanobiosensors employ nanomaterials to enhance the sensitivity and specificity of detection systems. These sensors characteristically amalgamate biological recognition elements, such as antibodies, nucleic acids, or enzymes, with nanomaterials including gold nanoparticles, carbon nanotubes, or quantum dots. The nanomaterials under discussion facilitate signal transduction, often by amplifying the detection signal or enabling real-time monitoring. A common approach to the detection of Salmonella, for instance, involves the use of gold nanoparticles that have been conjugated with antibodies that are specific to Salmonella antigens. In the presence of Salmonella bacteria in a sample, a binding process occurs between the bacteria and the antibodies. This binding results in the gold nanoparticles aggregating. The aggregation of these particles can be detected through changes in the optical properties of the nanoparticles, providing a rapid and sensitive detection method. In a similar manner, nanobiosensors for Clostridium, most notably Clostridium difficile, frequently utilise nucleic acid-based detection methodologies. Furthermore, it has been demonstrated that DNA or RNA sequences specific to Clostridium toxins can be immobilized on nanostructures. The hybridisation of these sequences with target nucleic acids from the pathogen can be detected using fluorescent nanomaterials, thereby providing a precise measurement of the pathogen's presence. The integration of nanomaterials has been demonstrated to significantly reduce the time required for detection. Conventional cultural methods can require several days, whereas nanobiosensors can yield results within minutes to hours. The incorporation of nanomaterials into biosensors has been demonstrated to enhance their sensitivity, thereby facilitating the detection of low concentrations of pathogens. Furthermore, the specificity of biological recognition elements guarantees the sensors' capacity to accurately identify specific bacterial species. A significant proportion of nanobiosensors are designed to be portable and user-friendly, rendering them suitable for point-of-care diagnostics. This is of particular benefit in settings where resources are limited and access to laboratory facilities is restricted. Nanobiosensors have the capacity to provide real-time data, thereby facilitating continuous monitoring of samples. This capacity is of pivotal importance for the timely formulation of decisions in both clinical and environmental contexts. Notwithstanding the numerous advantages inherent in nanobiosensors, challenges must be addressed for widespread adoption to be realised. The generation of nanomaterials and their subsequent integration into functional biosensors can incur substantial financial costs. The development of cost-effective manufacturing processes is imperative for large-scale deployment. It is evident that environmental conditions have the capacity to exert an influence on the stability of biological recognition elements and nanomaterials. Ensuring the long-term stability and shelf-life of nanobiosensors is critical for their practical applications (Figure 2).

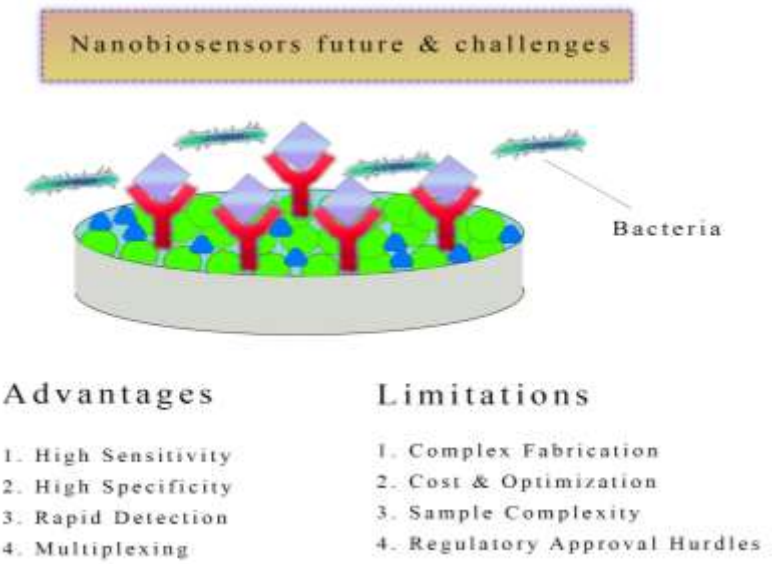

Figure 2: Advantages and Limitations of Nanobiosensors for Bacterial Detection.
Nanobiosensors offer advantages such as high sensitivity, specificity, rapid detection, and multiplexing capabilities, making them promising tools for bacterial detection. However, limitations including complex fabrication processes, cost and optimization challenges, sample complexity, and regulatory approval hurdles must be addressed for their widespread adoption and practical use in bacterial detection applications.

8. Conclusion

Nanobiosensors represent a transformative approach to the rapid detection of pathogenic bacteria, including Salmonella, Clostridium, Escherichia coli, and Brucella spp. The integration of nanomaterials with biological recognition elements allows for unprecedented sensitivity, specificity, and speed in diagnostics. These advantages render them highly valuable for point-of-care testing, offering significant benefits in clinical, environmental, and food safety applications. Nevertheless, in order to achieve their full potential, issues such as cost, stability, and regulatory challenges must be addressed. Continued advancements in nanotechnology and biochemistry, in conjunction with strategic efforts to standardise and scale up production, will be pivotal in overcoming these obstacles. The future of nanobiosensors appears to be a promising field, with the potential to enhance the rapid and accurate detection and response to bacterial infections.

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Authors' Contribution

Conceptualization: N. K.
Writing - original draft preparation: S. C, Z. MF, S. SK, N. K.
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Supervision: N. K.

Ethics

It is hereby confirmed that the relevant instructions for authors, the Ethics in Publishing policy, and the Conflicts of Interest disclosure have been reviewed and adhered to.

Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

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

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