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# Nanomaterials in bacterial detection: current trends and future outlook

## ABSTRACT

*Contamination by pathogenic bacteria represents a severe risk to public health and well-being. We outlined current approaches to detecting and sensing harmful bacteria by integrating recognition elements with nanomaterials (NMs) in this study. Nanomaterials have emerged as a transformative technology for bacterial detection due to their unique physicochemical properties, including high surface area, quantum effects, and enhanced reactivity. This review highlights the current trends in the application of various nanomaterials, such as gold nanoparticles, carbon nanotubes, and quantum dots, in the detection of bacterial pathogens. These materials enable the development of selective, and rapid detection methods through mechanisms like surface plasmon resonance, electrochemical sensing, and fluorescence. Furthermore, integrating nanomaterials with microfluidic devices and biosensors is discussed, showcasing advancements in point-of-care diagnostics. Challenges such as stability, reproducibility, and potential toxicity of nanomaterials are addressed, alongside regulatory considerations. The future outlook emphasizes the potential of emerging nanomaterials, such as graphene and metal-organic frameworks, to revolutionize bacterial detection. This review aims to enhance the scalability, cost-effectiveness, and environmental sustainability of these technologies, paving the way for widespread clinical and environmental applications.*
***Keywords:*** *Contamination, nanomaterial, nanoprobes, pathogenic bacteria, sensing*

## 1. INTRODUCTION

Rapid identification of pathogenic bacteria in hospital and clinical diagnostics, environmental and water quality controls, and resource-constrained situations may all help reduce food- and water-borne outbreaks [1]. Approximately one-third of all fatalities worldwide are attributed to bacterial food poisoning, which also causes 47.8 million ailments across many states annually and expensive product recalls [2]. Most cases of bacteria originate from infection or poisoning with *Listeria monocytogenes*, *Escherichia coli* O157:H7, tuberculosis, *Bacillus cereus*, *Salmonella typhimurium*, and *Clostridium perfringens*. Medicines may be used to treat the majority of bacterial illnesses; however, a significant issue arises because certain harmful bacteria have developed resistance to one or more medicines. The World Health Organisation predicts that throughout the next one to two decades, the ability of present antibiotics to combat diseases will decline [3]. The development of bacterial biofilms on production equipment surfaces in food and medical processing environments may lead to raised expenses and risk by promoting corrosion, increasing fouling, and contaminating products [4].

The PCR and ELISA have long been the gold standards for bacterial infection diagnosis [5]. Staining a urine sample is the non-culturing strategy used to identify bacterial infections; however, this method takes more time and has lower accuracy [6]. To lessen the danger of these new hazards in food, medicine, and the environment, it is urgently necessary to create reliable, early-stage screening techniques.

Magnetic NPs, gold NPs, silver NPs, and quantum dots (QDs) are examples of nanotechnology-based methods that demonstrate selective target-binding properties. They are the best options for the biosensing and detection of bacterial illnesses because of their qualities [7]. The NMs

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used in biosensors for the detection of bacteria were reviewed in this paper. Using nanomaterials, we highlighted the key biosensing recognition components and processes of bacterial sensing.

## 2. BACTERIAL SENSING AND ITS IMPORTANCE IN VARIOUS FIELDS

Bacteria can proliferate as single cells, a process known as planktonic growth, or as cellular clumps known as biofilms [8]. Increased resistance to antimicrobials and the host defence systems are among the unique physical and chemical characteristics that the biofilm community benefits from, due to a self-produced matrix of extracellular polymeric molecules [9]. At now, there are no techniques used for the identification of biofilm, despite its clinical significance [10]. Instead, most bacterial detection methods use culture-based procedures that enable the growth of bacteria to levels that can be recorded using optical density before being analyzed on a chromogenic or selective medium. These approaches have a lengthy turnaround time and need skilled users, which raises the total cost of therapy dramatically. Therefore, techniques for the accurate, quick, as well as reliable identification of harmful bacteria continue to be difficult to come across [11].

Complying with the need for lightweight, prompt diagnosis, easily navigable equipment is being designed. The development of biosensors for uses ranging from infection detection to metabolic illnesses has been fuelled by the need for point-of-care biosensor devices for healthcare purposes to have a straightforward appearance [12, 13].

## 3. NANO-MATERIALS FOR BACTERIAL SENSING: TYPES AND PROPERTIES

Compared to normal materials, NMs have distinct mechanical, optical, magnetic, electrical, and physical properties because of their very tiny size. This is the nano biosensor's extra benefit and the source of all its improved sensing capabilities. Various NMs have been used by researchers to improve biosensing findings' accuracy, sensitivity, and shelf life [14]. A wide range of potential uses exist for biosensors in agricultural production, and environmental monitoring, including online, in-field, and real-time detection of antibiotics, microbes, toxic materials, pathogens, pesticides, proteins, odor-causing bacteria, etc. in water, air, animals, food, soil, plants, and food processing. Biosensors are combined with cutting-edge techniques in NMs, microfluidics, and molecular biology.

Recently, there has been a lot of interest in biosensors. They are thought to be potent new instruments for identifying different biomarkers in healthcare and environmental monitoring [15]. A biosensor is a tiny apparatus that converts an identification of a biological molecule—complete cell, antibody, DNA or RNA, protein, etc. into an optical signal, nanomechanical signal, piezoelectric signal, electrical signal, mass-sensitive signal, etc [16]. When compared to traditional analytical procedures, biosensors may give a quick response in an ultrasensitive biomolecule detection, a short amount of time, and the ability to be miniaturized for portable usage with little sample processing. Fig. 1 represents a schematic image of a conventional biosensor.

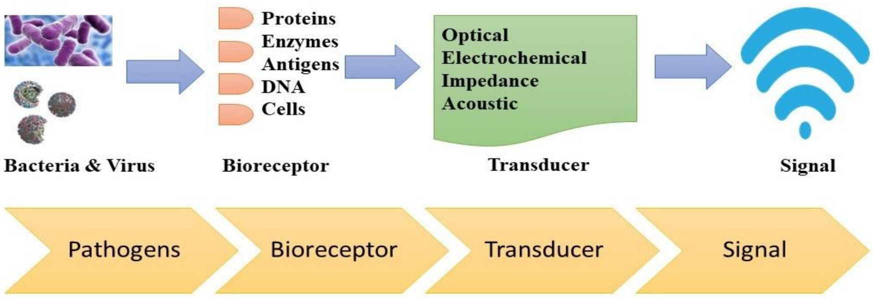


*Figure 1.Guidelines for using biosensors [17]*

Biosensors may find use in the following areas: bacterial sensing, water analysis, farming, healthcare,water treatment, and agriculture [15]. Numerous applications that operate in real-time have made use of biosensors. One possible method to address the aforementioned demands is to use biosensor-based detection techniques [19]. A transducer, which converts the binding event into a quantifiable signal, and a recognition element, which binds to target analytes, are the essential parts of a biosensor [20]. The surface plasmon resonance (SPR) that metal nanoparticles show is the basis for biosensor development being worked on by several research groups [21]. Various metal nanoparticle compositions and sizes may be engineered to scatter light at various wavelengths based on their unique surface plasmon resonance [22].

It is known that materials' magnetic, mechanical, optical, as well as electrical characteristics change as they are reduced to nanoscopic scales. Particles with diameters less than 100 nm that exist at the nanoscale level are referred to as nanoparticles. Because of their distinctive physical characteristics, including homogeneity, special optical qualities, conductivity, and a high surface area to volume ratio that provides a greater area for sensing and identification, nano-materials are of interest to scientists and biologists [23]. Particles with diameters less than 100 nm that exist at the nanoscale level are referred to as nanoparticles. Various kinds of NPs and the sensing techniques employed for bacterial sensing are shown in Fig. 2.

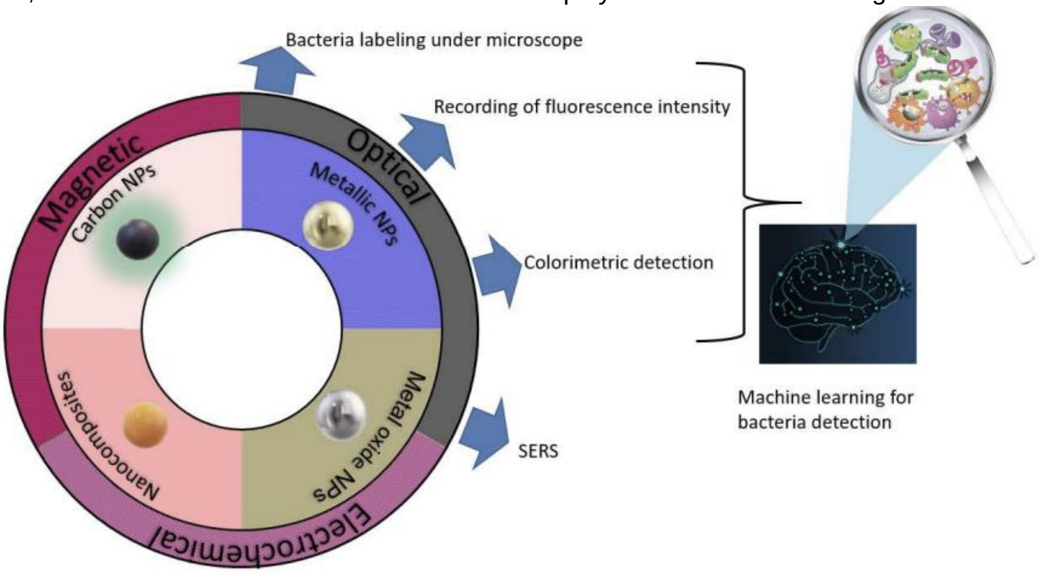


*Figure 2.Figure illustrating the many types of nanoparticles, the methods of detecting bacteria, and the critical role that machine learning may play in creating a point-of-care system for infectious illness detection [24]*

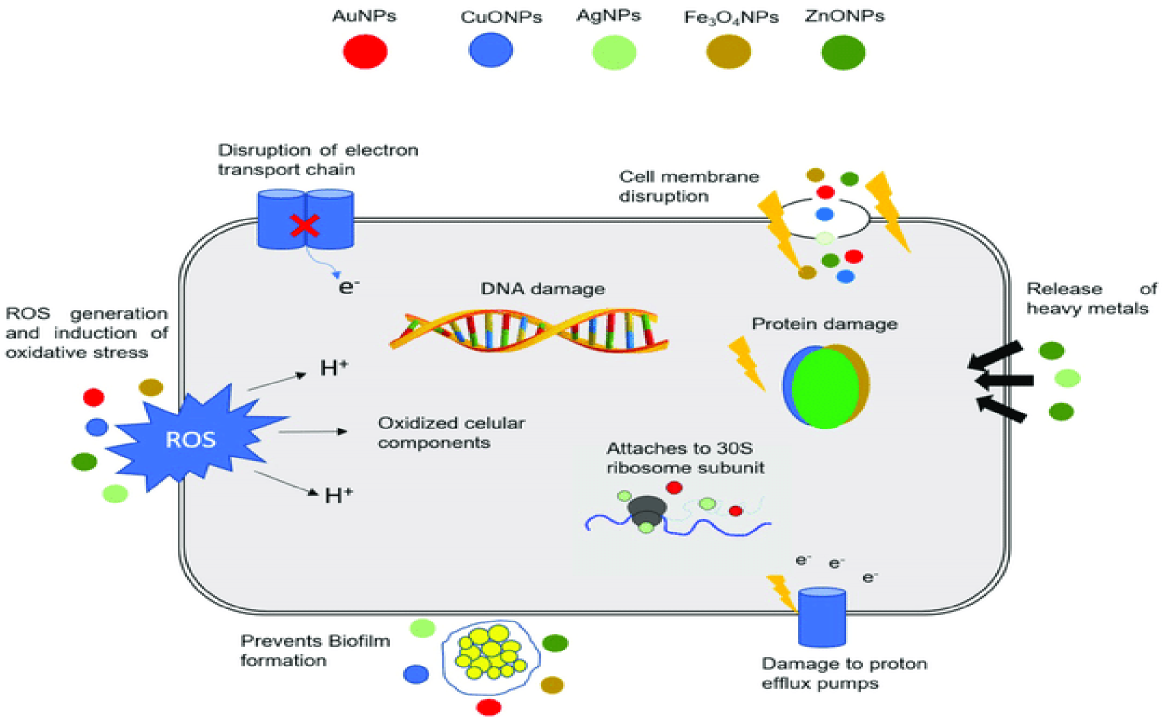


*Figure 3.Several ways that NPs work in bacterial cells [26]*

## 4. MECHANISMS OF BACTERIAL SENSING USING NANOMATERIALS

Many different mechanisms allow NMs to exert their antibacterial activity: (1) induction of intracellular effects, such as interactions with DNA and proteins; (2) reactive oxygen species (ROS) production; (3) triggering of both innate and adaptive host immune responses; and (4) direct contact with the cell wall of the bacteria; (5) reduction of the development of biofilms. They are very useful against MDR bacteria since they do not exhibit the same mechanisms of action as regular antibiotics (Figure3) [25].

*Table 1. Biosensors based on nanoparticles for identifying harmful microorganisms*

| Trans-duction | NP | Pathogen | Function of NP | Advantages | LOD | Ref. |
|---|---|---|---|---|---|---|
| | Paramagnetic Nanoparticles | *E. coli* | Biosensing system | Greater sensitivity than biosensors using a self-assembling multilayer structure | 10 CFU/mL | [31] |
| Magnetic | Magnetic Nanoparticles | *S. aureus* | Signal amplification | Assay time 15 min<br>Turbid sample measurements made without any prior preparation<br>Sample volumes (5–10 lL) | 1 * $10^3$ CFU/mL | [32] |
| Magnetic | Superparamagnetic Nanoparticles | *E.coli* nucleic acid<br>*Salmonella* antibiotic-resistant genes | Biosensing system | Assay time 3 min | 4–250 pM | [33] |
| | Superparamagnetic Nanoparticles | *E. coli*O157:H7 | Biosensing system | Long-term stability<br>Minimized noise and interferences | 1 * $10^5$CFU/mL | [34] |
| Magnetic | Gold Nanoparticles-modified screen-printed carbon electrodes | *E. coli*O157:H7 | Signal amplification | – | 6 CFU/ strip | [35] |
| | Polyaniline-coated magnetic NPs | *B. anthracis* endospores | Biosensing system | Assay time 16min | 4.2 * $10^2$ CFU/mL | [36] |
| Magnetic | SWCN (Single-walled carbon nanotubes) | *E. coli* | Biosensing system | Assay time < 20 min | – | [37] |
| | Nanogold/chitosan-MWCN (multi-walled carbon nanotubes) | *S. aureus* enterotoxin B | Biosensing system | – | 0.5 ng/mL | [38] |
| | Platinum Nanoparticles-coated gold nanoporous film | *E. coli* | Signal amplification | No sample pretreatment | 10 CFU/mL | [39] |
| | Potassium ferrocyanide encapsulated liposomes | Cholera toxin | Biosensing system | – | 1015 g/mL | [40] |
| | SWCN (Single-walled carbon nanotubes) | *S. infantis* | Biosensing system | Assay time 1 h<br>Label-free system | 100 CFU/mL | [41] |
| | Gold Nanoparticles-based graphite epoxy composite | *Salmonella sp*. ssDNA | Signal amplification | Novel material with improved properties | 9 fmol | [42] |
| | Magnetic Nanoparticles-coated carbonnanotubenanocomposite | *E. coli* | Biosensing system | – | 10 CFU/mL | [43] |
| | Gold Nanoparticles<br>Magnetic Nanoparticles | *S. enteritidis* insertion element gene | Biosensing systems | – | 1 ng/mL | [44] |
| | Gold Nanoparticles | *E. coli*<br>*S. aureus* | Signal amplification | LOD was 5500 times lower than it was in the absence of Au NP | 58.2 ± 1.37 pg/mL<br>7 * $10^5$CFU/mL | [45] |
| Electr | Gold -silver nanoparticles | *S. aureus* enterotoxin B | Biosensing system | - | 0.1 ng/mL | [46] |
| | Gold nanoparticles | *B. subtilis* genomic DNA | Biosensing system | – | 2.5 fM | [47] |
| Optical | PAMAM-NH2dendrimer | *E. coli* | Signal amplification | – | 1 * $10^4$CFU/mL | [48] |
| Optical | CdSe/ZnS core/shell dendronnanocrystals | *E. coli*O157:H7 | Biosensing system | Sensitivity greater than comparable published systems | 2.3 CFU/mL | 49] |
| | Dye-doped silica Nanoparticles | *E. coli*O157:H7 | Biosensing system | High sensitivity<br>Assay time 20 min | 1 CFU/m | [52] |
| Optical | Liposome | RNA sequences from *B. anthracis*, *C. parvum, E. coli* | Signal amplification | Universal system | 10–1000 fmoL | [51] |
| Optical | PAMAM-OH dendrimer | *P. aeruginosa* | Signal amplification | 350% increase in sen-sitivity over the absen-ce of PAMAM-OH | – | [52] |

## 5. TYPES OF NANOMATERIAL-BASED BACTERIAL SENSORS

The process of translating biological information into a digital signal by chemical or physical means underlies the idea of bacterial detection utilizing biosensors. This biological information is obtained by the interactions among recognition elements (bacteria, antibodies, enzymes, nucleic acids, and viruses) and their associated biological targets. Recognition components are usually immobilized through adsorption, cross-linking, covalent binding, trapping, or encapsulation onto a transducer surface [27].

Indirect (labeled) and direct (label-free) detection methods are the two categories into which biosensors fall. While the result of biological processes is identified through a labeled probe utilizing a sensor in indirect detection systems, physical changes caused by the interaction of the target analyte are measured directly and in real-time in direct detection systems, meaning no label is needed. Due to their simplicity of use, cheap cost, high sensitivity, quick response, high signal-to-noise ratio, adaptability in using recognition components, instrumental simplicity, and little interference with food matrices, electrochemical biosensors are among the numerous transducer types that show great promise [28]. Even at low concentrations in a turbid medium, bacterial cell may be detected with high sensitivity and selectivity thanks to electrochemical biosensors.

Zero-dimension (0D) and three-dimension (3D) carbon NMs have been modified for the creation of electrochemical biosensors [29]. When it comes to electrochemical sensing of bacterial infections, carbon NMs like graphene, carbon nanotubes (CNTs), and their derivatives provide a number of benefits, particularly with regard to their high field-effect sensitivity and mobility [30]. Table 1 provides an overview of the typical nanomaterial-based sensors and how well they work for quickly detecting water contaminants.

## 6. RECENT ADVANCES IN NANO-MATERIALS FOR BACTERIAL SENSING

NMsare being used in commercial goods at a very fast rate. By 2010, there were about four times as many commercial items on the market that claimed to have improved qualities because they used nanomaterials in 2006 [53]. The most often utilized nanomaterial in goods is silver, which is followed by materials based on carbon and metal oxides like $TiO_2$.

Recently, biosensors have been considered as appealing substitutes for the traditional pathogen detection platforms that are now in use. Biosensors are devices that are intended to identify and measure different biological substances such as certain DNA sequences, proteins, oligonucleotides, viruses, and bacteria [54]. A lot of the biosensors that have been created so far are affinity-based, which means that the target molecule is selectively bound by an immobilized capture probe. Detecting a change at a localised surface therefore becomes the difficulty of detecting a target in solution. A range of techniques, including surface plasmon resonance, evanescent wave, and amperometric measurements, may then be used to quantify this shift [55].

Surface water resources that are contaminated with water-borne pathogens pose a serious hazard to human health and life on a global scale [56]. Therefore, maintaining pathogen (bacteria and virus) control is essential. A unique, multifunctional magneto-plasmonic nanosensor (MPnS) with target antibodies (MPnS-Ab) was created by Panchal et al., [57] based on replaceable sandwich ELISA. In order to directly detect the bacterium *Escherichia coli* (*E. coli*), Nair et al., [58] created a unique silicon nanowire (SiNWs)-based sensing platform covered with reduced graphene oxide (RGO). *E. coli* showed preferential adherence to the SiNWs network throughout the study, which led to a drop in resistance and an increase in current. As a direct, quick, and accurate nanosensor for the detection of *E. coli* bacteria in aqueous solutions, the discovered technology shows great promise. Salaun et al., [59] described silicon nanowire-based biosensors for electrical detection of *Escherichia coli*. The sensors displayed great specificity, which was made possible by chemically modifying the nanowires to bind certain antibodies to the intended *E. coli* target. Silver nanoparticles (Ag-NPs) functionalized with fruit extracts from banana peel (*Musa paradisiaca*) and grape (*Vitis vinifera*) were green synthesized, as shown by Nqunqa et al., [60], and utilized as optical and electrochemical sensors for the detection of *E. coli*. The published limit of detection values for the optical and electrochemical sensors, respectively, are $1x10^2$ CFU/mL and $3.5x10^1$ CFU/mL, which are both within the range for *E. Coli* in saltwater.

## 7. CHALLENGES AND LIMITATIONS

Differentiating and identifying living from dead bacteria is a major problem when it comes to germs. Agar medium plating is capable of distinguishing between live bacteria. However, it does not include viable but uncultivable cells. Different types of optical sensors provide a number of obstacles. For example, label-based biosensors involve tagging samples with distinct fluorescent tags, which increases detection time and expense and may harm cell function. When creating biosensors to identify bacteria, the design should have high specificity, no label, viable distinguishability, affordability, small size, portability, ease of use, and minimal sample preparation processes.The biosensor designs that are now on the market can detect the sample at various concentrations ranging from fM to aM [61]. Consequently, a highly sensitive biosensing platform that falls within the pM, zM, and yM range must be able to detect the target sample at a very low concentration. One major obstacle in the development of implanted biosensors is power consumption, which may be overcome with a self-powered gadget. Optical sensors are more expensive and difficult to use in environments with limited resources since they need filters, spectrometers, microscopes, light sources, and fluorometers. For the Patho BactD, only colorimetric and fluorescence-based sensors among many optical biosensing systems are commercially available [62]. The development of interferometric, SERS, and plasmonics-based sensors is ongoing. It's crucial to note that because of the electromagnetic field's shallow penetration depth into bacteria, plasmonic sensors' commercial uses are restricted to the detection of tiny compounds, cancer cells, and chemicals. One of the key issues is that calibration which labels may help with is necessary for the estimation of the amount of bacteria that the sensor detects [63].Other problems are more innate to the newly introduced innovative technology, like poor selectivity in microbial mixtures and intricate physiological settings [64].

## 8. ENVIRONMENTAL AND SOCIETAL IMPACT

Bacteria emit tiny membranous structures known as BEVs into the extracellular milieu. From a conceptual standpoint, BEVs are conceptually comparable to EVs seen in other animals, especially eukaryotes. Experimental evidence has demonstrated that different BEV phenotypes can arise from different bacterial origins and the unique mechanism of BEV production, leading to unique biological capabilities. The source of membrane vesicles (MV) is Gram-positive bacteria. In this review, all of these vesicle subtypes that originate from bacteria are referred to as BEVs, regardless of the biogenesis mechanism [65].Microbes, such as bacteria, fungi, as well as viruses, are often found in water systems. Many of these microbes are harmful to humans. A subspecies of the bacterium *Escherichia coli* (*E. coli* O157:H7) [66], which is extensively distributed in the environment and may be very harmful to humans, is one of the most hazardous diseases [67]. The “US Environmental Protection Agency (EPA)” has advised keeping an eye on *E. coli* in freshwater as an indicator organism for water danger. Thus, one of the most important requirements for maintaining such a vital natural resource is the ongoing monitoring of water quality for different pollutants in water distribution and treatment systems. To maintain continuous control over the quality of the water and to prevent tragedies that might endanger public safety, this calls for precise and easily accessible detecting systems. via enhancing water safety, the use of fast sensors to detect contaminates in the water might improve public health via early notification of biological and chemical contamination.

Few reviews have concentrated on the rapid detection of water contaminants, despite the fact that numerous reviews have summarised the current state-of-the-art achievements of nanomaterial-based sensors for water contaminant detection, such as 2-D graphene nanosheets, 1-D nanowires, and CNTs, and 0-D nanoparticles (NPs) [68]. Early recognition is one of the most promising approaches for future water sensors and smart systems since it eliminates negative impacts on the environment and human health by allowing early notifications of water pollution or ongoing water quality monitoring.

## 9. FUTURE PROSPECTS AND EMERGING TRENDS

The development of inexpensive, eco-friendly, user-friendly, and portable sensors that need a small amount of material for analysis has gained traction in recent years. Contemporary techniques for detecting water quality in real-time, such as MEMS, optical sensors, and biosensors, are fiercely competitive with more established ways. These sensors can analyze data in real-time and have excellent selectivity, sensitivity, and rapid reaction times. They can also identify pathogens (bacteria) and minute amounts of contaminants with high confidence and little sample preparation [69]. Combining contemporary sensing methods into one system may provide better sensitivity and selectivity as well as the potential for real-time data processing.

A transducer (optical, electrochemical, thermal, etc.) to produce a signal and a biological-indicator molecule interface make up a biosensor [70]. Antibodies, tissue slices, microbial organisms, enzymes, and cell membrane receptorsthat interact with a certain parameter might all be considered biological indicators [71]. Transducers describe a change in a physical or chemical process by generating a signal (potential or current) that is proportionate to the goal [72]. Biosensors use the selective target-immobilized target indicator interaction. There are some microfluids assays also which have been developed for the same purpose of work. They have outstanding sensing properties and advances in nanomaterials especially high surface area to volume ratio that facilitated the expansion of their application to various purposes [73].

## 10. CONCLUSION

Nanomaterials have undeniably revolutionized the field of bacterial detection, offering unprecedented sensitivity, selectivity, and speed. Selecting appropriate NMs provides sensitive signal transduction pathways for the sensing of bacterial cells. The development of "next-generation" biosensors that can meet societal demands is made possible by carefully choosing the right blend of recognition components and nanomaterial transducers. The unique properties of nanomaterials, such as high surface area and enhanced reactivity, enable the development of innovative diagnostic tools that significantly improve the efficiency of pathogen detection. Current trends demonstrate the successful integration of nanomaterials with advanced technologies like microfluidic devices and biosensors, fostering the creation of portable and user-friendly point-of-care diagnostics. Despite the remarkable progress, several challenges remain. Issues related to the stability, reproducibility, and potential toxicity of nanomaterials must be thoroughly addressed to ensure safe and reliable applications.

Moreover, regulatory frameworks need to evolve in parallel with technological advancements to facilitate the commercialization and widespread adoption of nanomaterial-based detection systems. Looking ahead, emerging nanomaterials such as graphene and metal-organic frameworks hold great promise for further enhancing the capabilities of bacterial detection methods. Continued research and development efforts are essential to overcome existing limitations and to improve the scalability, cost-effectiveness, and environmental sustainability of these technologies. As these advancements come to fruition, nanomaterials are poised to play a pivotal role in safeguarding public health through more efficient and accessible bacterial detection methods, with significant implications for clinical diagnostics, environmental monitoring, and food safety.

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## IZVOD

### NANOMATERIJALI U DETEKCIJI BAKTERIJA: TRENUTNI TRENDOVI I BUDUĆI IZGLEDI


*Kontaminacija patogenim bakterijama predstavlja ozbiljan rizik po javno zdravlje i dobrobit. U ovoj studiji izneli smo trenutne pristupe otkrivanju štetnih bakterija integracijom elemenata za prepoznavanje sa nanomaterijalima (NM). Nanomaterijali su se pojavili kao transformativna tehnologija za detekciju bakterija zbog svojih jedinstvenih fizičko-hemijskih svojstava, uključujući veliku površinu, kvantne efekte i poboljšanu reaktivnost. Ovaj pregled naglašava trenutne trendove u primeni različitih nanomaterijala, kao što su nanočestice zlata, ugljenične nanocevi i kvantne tačke, u detekciji bakterijskih patogena. Ovi materijali omogućavaju razvoj selektivnih i brzih metoda detekcije kroz mehanizme kao što su površinska plazmonska rezonanca, elektrohemijski sensing i fluorescencija. Štaviše, raspravlja se o integraciji nanomaterijala sa mikrofluidnim uređajima i biosenzorima, pokazujući napredak u dijagnostici na licu mesta. Izazovi kao što su stabilnost, ponovljivost i potencijalna toksičnost nanomaterijala su adresirani, zajedno sa regulatornim razmatranjima. Izgledi za budućnost naglašavaju potencijal nanomaterijala u nastajanju, kao što su grafen i metal-organski okviri, da revolucionišu otkrivanje bakterija. Ovaj pregled ima za cilj da poboljša skalabilnost, isplativost i ekološku održivost ovih tehnologija, utirući put širokoj kliničkoj i ekološkoj primeni.*



***Ključne reči:*** *Kontaminacija, nanomaterijal, nanosonde, patogene bakterije, sensing*

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