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REVIEW

Biosensing technology for detection and assessment of pathogenic microorganisms

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ABSTRACT

At present, the prevalence of infectious diseases is rising annually, making it an important risk factor for human health that should not be neglected. Consequently, infection control and prevention have become even more important. The key to determining and designing the most effective anti-infectious medication depends upon the immediate and accurate identification of the causative agent. The standard techniques used for routine infection screening and surveillance tests are shifting toward biosensors. Furthermore, biosensors are projected to be employed for microbiological detection to satisfy the higher accuracy required for clinical diagnosis. This is because of their compact size, real-time monitoring and ability to analyze large sample numbers with less sophistication and manpower requirement, which have allowed them to develop quickly with extensive uses. Biosensors have multiple applications in food safety, environmental surveillance, drug sensing and national security because they offer several advantages such as quick response, outstanding sensitivity, remarkable selectivity, high degree of accuracy and precision, ease of use and affordable price. This review highlights the performance aspects of recently developed biosensors for the detection of infectious bacteria and viruses in biological and environmental samples and emphasizes the significance of nanotechnology in signal amplification for enhanced biosensor performance and dependability.

PLAIN LANGUAGE SUMMARY

Biosensors are special tools used to identify bacteria and viruses in environmental and clinical samples. They work quickly and are simple to operate, so can be used at home or in small clinics. One of the best things about biosensors is that they are very sensitive, which means they can find even small numbers of infectious bacteria or viruses before disease symptoms appear. Early identification of such infectious agents can stop the spread of the illness. Biosensors are becoming compact, less costly and simpler to use. This makes them a useful tool for communities that do not have straightforward access to healthcare. In short, biosensors are very useful because they allow people to find infections rapidly so that treatment can be started sooner.

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1. Introduction

The increased demand brought on by population expansion and industrial development has made water shortages a significant problem during the past few decades [1]. Due to the scarcity of freshwater bodies, people all around the world are forced to rely on alternative water sources generated by applying various water purification techniques such as desalination, industrial water treatment, etc. to fulfill the need for freshwater for consumption as well as other applications. However, it poses a risk of water-mediated transmission of infectious agents [2]. Apart from the increase in population, factors such as habitat invasion, overseas travel and commercialization are also other factors resulting in the transmission of novel infections that may present a risk to overall population health [3]. Along with microorganisms and other organic matter, heavy metals and synthetic chemicals have increased in the environment as major pollutants [4].

The increase in pollution necessitates ongoing research and development in the field of detection and identification of pathogens from environmental and clinical samples [5]. There are multiple types of pathogenic microbes found in environmental and clinical samples; however, bacteria predominate in mass and diversity [6]. Some of the potential pathogenic bacteria include species of *Pseudomonas, Mycobacterium, Escherichia, Corynebacterium, Salmonella, Vibrio, Yersinia, Legionella, Klebsiella, Bacillus, Clostridium, Listeria, Enterococcus, Heliobacter, Campylobacter, Neoehrlichia, Alloscardovia, Waddlia, Parachlamydia, Actinobaculum, Simkania, Aerococcus* and *Bartonella* [7]. They have led to the development of severe clinical manifestations such as diphtheria, diarrhea, meningitis, pneumonia,

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liver abscess, typhoid fever, cholera, gastrointestinal syndromes, urinary tract infections, nosocomial infections, anthrax, botulism, bacteremia, listeriosis, leptospirosis, infective endocarditis, neoehrlichiosis and miscarriages. They are also sometimes associated with onchocerciasis and lymphatic filariasis [8,9]. Table 1 represents the summary of various pathogenic bacterial species found in environmental and clinical samples with their associated diseases.

The environment has also been found to contain a variety of verified enteric viruses, such as enteroviruses, caliciviruses, hepatitis A and adenoviruses. Viruses found in water bodies are one of the major concerns for public health and the environment [23]. The viruses found in water bodies exhibit common traits and can cause a wide range of illnesses, including acute as well as, chronic cases of hepatitis, conjunctivitis, gastroenteritis, respiratory disorders and infections of the brain and nervous system. Enteric viruses, including coxsackie, echo and polioviruses, can persist in freshwater environments for extended periods of time. Their average rates of inactivation range from 0.174 log10 d^{-1} in groundwater to 0.576 log10 d^{-1} in surface water obtained from taps [24].

Along with enteric viruses, a variety of non enteric viruses which include west Nile, dengue virus (DENV), coronaviruses and influenza are also reported in the environment and are a topic of concern for the community [25]. A list of potential pathogenic viruses found in the environment and clinical samples has been summarized in Table 2.

Disease-causing microorganisms are crucial to food safety, environmental health and human health. As a result, it is critical to accurately, swiftly and readily identify these microbes. With all of these characteristics, biosensors provide a special method of microbe detection. They are already in use in related industries and are developing as a more ergonomic and flexible technology [37].

Mass screening, along with appropriate isolation and treatment is the most efficient approach to identifying these infectious disorders. Compared with traditional detection techniques, the installation of biological sensors in the field of detection may achieve significantly higher levels of testing, proper desolation and analysis [38].

When compared with traditional approaches, the development of biosensors showed various advantages because they are portable, quick and simple to use with minimum handling or manual error. Sensors that employ biological reactions as an identifying factor are known as biosensors. They consist of the transducer and the biorecognition element as their two primary parts [39]. The biorecognition element can be microorganisms, proteins, antibodies, nucleic acids or whole cells; on the other hand, the transducer can be electrical, electrochemical, optical, thermal, acoustic or piezoelectric. A biochemical reaction induced by an interaction between the analyte and bioreceptor is converted into the desired signal by the transducer component of the biosensors [40].

Research on the development of biosensors for pathogen detection has been in progress in recent times. Biosensors designed for the detection of pathogenic microbes are capable of giving real-time analysis and monitoring of microbial contamination. Early detection and notification of infectious disease-causing pathogens in the environment can shield the population against potential hazards in the near future and help prevent the infection from becoming epidemic. However, present biosensor research for pathogen detection is still in its nascent stage, indicating the need for more study in this area [41]. The fact that the environment consists of a complex matrix makes it crucial to take into account the precision of the biosensors toward one specific or class of pathogen detection in the environment and clinical samples [42]. As a result, optimizing the fabrication parameters to improve the biosensor response is significant. The incorporation of nanomaterials in the biosensor design increases the sensitivity of the biosensors, making them more accurate and precise in the analysis of real environmental samples. The affinity and biocompatibility of nanomaterials for biological receptors must be taken into account while introducing them into the biosensor's fabrication. In certain cases, functionalized/capped nanomaterials are more suitable for biosensor design as they provide additional functional groups for efficient and successful immobilization of the bio element on the surface of nanomaterials [43]. Such nanomaterial-bioreceptor conjugates can be more effectively coated on the surface of electrodes or other materials to create a biosensing platform that can easily interact with the analyte, resulting in a change in the physiochemical property of the reaction medium, that can serve as a sensory cue. The effectiveness of the produced physiochemical changes significantly depends upon the electrical, magnetic, optical and electronic characteristics of the nanomaterials [44]. More sensitive biosensors have been made possible as a result of the use of nanomaterials in the biological sensing industry. Utilizing these unique features has made nanomaterial-based biosensors competitive [45].

2. Biosensors for the detection of pathogens

Professor Clark first described an enzyme-based biosensor in the Annals of the New York Academy of Sciences in 1962. The glucose oxidase (GOx) in this biosensor

Table 1. List of potential pathogenic bacteria found in environment and clinical samples.

Class	Genera	Pathogenic species	Virulence factor	Disease-associated	Ref.
Actinobacteria	*Corynebacterium*	*C. diphtheria* and *C. jeikeium*	Adherence, toxins formation	Diphtheria and nosocomial infections	[10]
Gammaproteobacteria	*Escherichia*	Enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, enteroaggregative, diffusely adherent, uropathogenic *E. coli*	Adherence ability, toxin, protease, iron uptake, invasion, transportation system	Diarrhea, urinary tract infections, bacteremia, sepsis, meningitis, pelvic infection and abdominal infection	[11]
	Klebsiella	*K. pneumoniae, K. oxytoca*	Adherence, capsule formation	Pneumoniae, liver abscess	[12]
	Legionella	*L. pneumophila*	Adherence, endotoxins, enzymes, iron uptake	Legionnaires' disease, nonpneumonic Pontiac fever	[13]
	Pseudomonas	*Pseudomonas aeruginosa*	Adherence, antiphagocytosis, iron uptake	septic shock due to infectious wound and eye infection. Infection of the lung leading to cystic fibrosis.	[14]
	Salmonella	*S. typhimurium, S. typhi, S. dublin, S. enteritidis, S. virchow, S. hadar, S. anatum, S. bongori*	Surface attachment, immune evasion, magnesium uptake	Gastroenteritis, typhoid fever	[15]
	Vibrio	*V. cholerae*	Adherence, regulation, secretion system, toxin	Cholera	[16]
	Yersinia	*Y. pestis, Y. enterocolitica, Y. pseudotuberculosis*	Adherence, capsule formation, Invasion, Uptake of iron, plasminogen activation	Plague, gastroenteritis, mesenteric adenitis and lymphadenitis	[17]
Epsilonproteo - bacteria	*Arcobacter*	*A. butzleri, A. cryaerophilus, A. skirrowii*	Adherence, invasion, cytotoxicity	Gastroenteritis, Bacteremia	[18]
Firmicutes	*Bacillus*	*B. anthracis, B. cereus*	Adherence, anti phagocytosis	Anthrax, diarrhea	[19]
Clostridia	*Clostridium*	*C. tetani, C. perfringens, C. difficile, C. botulinum*	Formation of biofilm, attachment, capsule and flagella formation	Tetanus, gastrointestinal infection and botulism	[20]
Actinomycetia	*Mycobacterium*	*M. tuberculosis, M. leprae*	Macrophage invasion, utilization of iron, multiple drug resistance	Tuberculosis, leprosy	[21]
Spirochaetia	*Treponema*	*T. pallidium, T. pertenue, T. endemicum*	Attachment, invasion	Syphilis, yaws, bejel	[22]

Table 2. List of potential pathogenic viruses found in environment and clinical samples.

Family	Genus	Reported disease	Transmission route	Ref.
Flaviviridae	*Hepacivirus*	*Hepatitis C*	Bloodborne	[26]
Herpesviridae	*Varicellovirus*	Chickenpox, varicella zoster infection, encephalitis, post chickenpox syndrome	Respiratory system	[27]
Coronaviridae	*Betacoronavirus*	Novel coronavirus	Respiratory	[28]
Matonaviridae	*Rubivirus*	*Rubella*	Respiratory	[29]
Togaviridae	*Alphavirus*	Encephalitis, chikungunya	Vector-borne	[30]
Picornaviridae	*Hepatovirus*	*Hepatitis A*	Enteric	[31]
Hepeviridae	*Orthohepevirus*	*Hepatitis E*	Enteric	[32]
Caliciviridae	*Norovirus*	*Norovirus*	Enteric	[33]
Hepadnaviridae	*Orthohepadnavirus*	*Hepatitis B*	Bloodborne	[34]
Retroviridae	*Lentivirus*	*HIV*	Bloodborne, semen, breast milk, vaginal fluid	[35]
Filoviridae	*Ebolavirus*	*Reston, Sudan, Tai Forest, Zaire ebolavirus*	Blood and body fluids	[36]

was coated on the oxygen electrode's surface using a dialysis membrane; hence, the relationship between the drop in oxygen and the glucose concentration was established [46].

The International Union of Pure and Applied Chemistry (IUPAC) defines a biosensor as an autonomous, integrated device that can directly integrate a transducer and a biometric element for quantitative or semi-quantitative target analysis. According to this definition, the basic idea behind biosensors is to record quantifiable signals (such as electrical, optical, acoustic, temperature, etc.) produced due to physical or chemical responses generated as a result of interaction between targets and biosensitive elements, such as phages, proteins, cells, tissues, enzymes, lectin, aptamers, DNA and antibodies captured and amplified by the transducer, such as optical fibers, field-effect transistors, surface plasmon resonance (SPR), thermistors and microelectrodes, etc. Figure 1

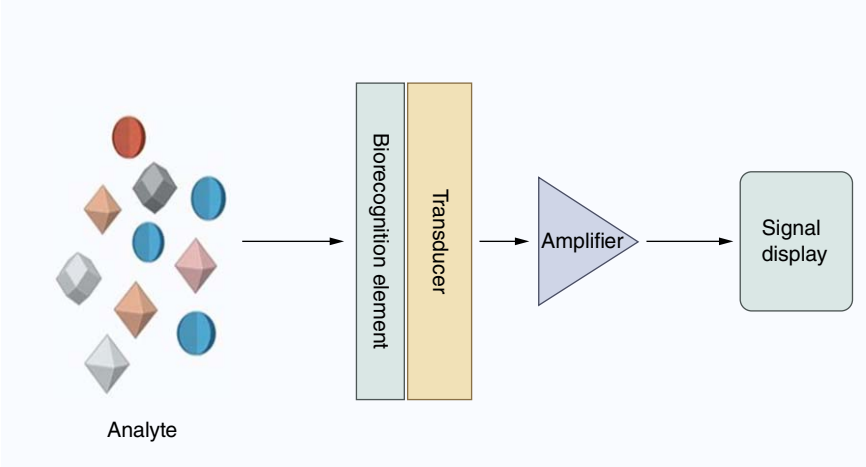

Figure 1. The working principle of biosensor describing the interaction of various components is schematically represented.

shows the diagrammatic representation of a typical biosensor working flow.

Numerous biosensors available are classified both based on the bio element used as well as, based on the transducer component employed. Based on the transducer element, biosensors can be amperometric, potentiometric, impedimetric, voltammetric, piezoelectrical, thermometric or optical. On the other hand, immunosensors, whole cell biosensors, aptamer-based, nucleic acid-based, biomolecule-based and biomimetic biosensors are categories of biosensors based on their bio-element [46,47]. Figure 2 shows the diagrammatic representation of a typical biosensor working mechanism for microbial detection.

2.1. Biosensors designed for the detection & identification of pathogenic bacteria

Several therapy and detection strategies have been developed and implemented to increase the effectiveness of diagnosis. Although immunology-based techniques, cell culture, quantitative polymerase chain reaction (qPCR) and colony counting methods are reliable and precise, they are often costly and time consuming. Additionally, a lab setup is necessary to carry out the detection.

To overcome the limitations of conventional methods available for the detection and monitoring of pathogenic bacteria in clinical and environmental samples, biosensors have been proposed as a feasible alternative. Biosensors offer economical, handy, precise, and point-care devices for environmental assessment and monitoring. Biosensors also offer advantages like a lower detection limit, high sensitivity and selectivity, less involvement of hazardous chemicals and reliable output [48]. In subsequent paragraphs, some of the biosensors designed for the detection of bacteria in recent times have been discussed.

2.1.1. Detection via immunosensor

E. coli is a well-known opportunistic pathogenic bacterial species that can cause waterborne illnesses. It has been widely utilized as a definitive index of coliforms in water bodies. The conventional procedure for the identification of *E. coli* requires a duration of 2–3 days. Thus, in the past few years, researchers have developed biosensors for *E. coli* serotype detection. Most of the research group has developed immunosensors by modifying screen-printed carbon electrodes. Lin and the group developed an immunosensing disposable strip for rapid detection of *E. coli* O157:H7. The designed screen-printed carbon electrode immunosensor exhibited high specificity toward *E. coli* O157:H7 with a detection limit of 6 CFU per strip and a detection range from 10^2 to 10^7 CFU/ml. Their assay technique was based on an indirect sandwich enzyme-linked immunoassay in which *E. coli* O157:H7-specific antigens first and second (conjugated with horseradish peroxidase) and intact *E. coli* cells were used. Ferrocenedicarboxylic acid and hydrogen peroxide were used as substrates for horseradish peroxidase. The response of the sensor was enhanced 13-fold by modifying screen-printed carbon electrodes with gold nanoparticles (AuNPs) [49]. In another attempt, Huang developed an electrochemical immunosensor for quantification of *E. coli* O157:H7 in a label freeway. They developed a gold nanoparticle-modified screen-printed carbon electrode immobilized with an *E. coli* O157:H7-specific antibody. The resultant immunosensor worked on the principle of antigen-antibody reaction on the working electrode. The response was recorded in the form of cyclic voltammetry (CV) and electrochemical resistance. The detection range was found to

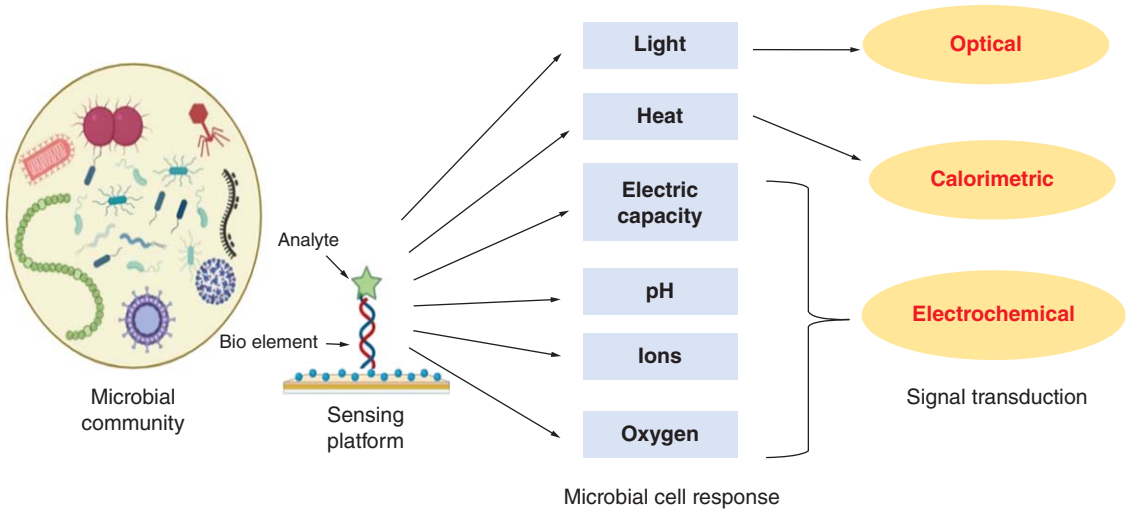

Figure 2. Mechanism of microbial biosensing: Interactions between the microbial cells and the sensor's detection components resulting in microbial cell response and detection.

be 1.19 × 10^3 to 1.19 × 10^9 CFU/ml with a lower detection limit of 5.94 × 10^2 CFU/ml [50]. In the same year, Mo and colleagues also reported a polyaniline and reduced graphene oxide-neutral red-gold nanoparticle @Platinum composite modified screen-printed carbon electrode for quantitative analysis of *E. coli* O157:H7. Their electrochemical immunosensor gave a linear response in the range of 8.9 × 10^3 to 8.9 × 10^9 CFU/ml with a limit of detection of 2.84 × 10^9 CFU/ml [51]. Recently, Vu and the group have reported a label-free electrochemical biosensor for rapid detection of bacterial pathogens. Their electrochemical biosensor comprises an anti-*E. coli* O 157 antibody immobilized via N-hydroxy succinimide cross-linking on a gold nanoparticle-coated screen-printed carbon electrode. The biosensor response was recorded with the help of a cyclic voltammeter. This label-free approach exhibited a detection range of 10 to 10^6 CFU/ml and a limit of detection of 15 CFU/ml [52]. In addition, Savas and the group focused on developing an antibody sensor that could detect *V. cholerae* using a conventional immunoassay. To do that, spherically synthesized AuNPs of different sizes were produced in-house. The Turkevich method also referred to as the citrate reduction method, was used to create colloidal AuNPs. The boiling gold (III) chloride solution was mixed with a decreasing quantity of sodium citrate to create AuNPs of various sizes. These AuNPs were then coupled with a secondary antibody-horseradish peroxidase enzyme complex, and their potential impact on the lowest detection limit of *V. cholerae* was examined. With high sensitivity, low limit of detection (1 CFU/ml), and specificity, the developed AuNPs-immunosensor allowed the quantification of *V. cholerae* in a wide concentration range. Thus, a novel electrochemical immunoassay was created for the detection of *V. cholerae* in drinking water in less than 5 min [53]. All these examples show the potential of biosensing platforms for monitoring pathogenic agents with the help of point-of-care devices in a selective, sensitive and economical manner.

2.1.2. Detection via aptamer-based biosensor

An aptasensor for the identification of *E. coli* O157:H7 and *Salmonella typhimurium* was also developed by Fang based on the transient wave dual color fluorescence technique. A fiber nano probe coated with a silica fiber optic nanoporous layer containing aptasensors Cy3- apt-e and Cy 5.5- apt- s employing the tube surface carving method was developed and placed in a microfluidic cell. This fiber nanopore was connected to a photodetector and optical controller through a single multimode fiber optic coupler. The bacterial sample buffer and washed water were pumped over the surface of the fiber nanoprobe using a peristaltic pump during the course of detection. When excitation light of 520 and 635 nm was alternatively introduced into the fiber nanoprobe, the excitation of the two aptasensors was recorded by the photodetector. In the presence of a varying concentration of pathogenic bacteria, the fluorescent intensity recorded by the photodetector decreases due to the specific interaction of the bacteria with the corresponding aptasensor on the nanoprobe surface when excited with a laser of wavelength 520 and 635 nm, respectively. Due to the nano-size effect of free aptasensor and bounded aptasensor, the detection of *Salmonella typhimurium* and *E. coli* O157:H7 was conducted using the fabricated biosensor. Target molecules may be identified with ease using a fluorescent-labeled aptasensor in conjunction with a nanoporous layer and a fiber nanotube acting as a

transducer. The quantification was completed in less than 35 min, and the limit of detection was found to be 340 and 180 CFU/ml for *S. typhimurium* and *E. coli* O157:H7, respectively [54].

Song developed a quadruplex DNA aptamer with peroxidase-like activity to design an aptasensor for the detection of *H. pylori*. The sensor was fabricated by immobilizing G- quadruplex DNAzyme and magnesium-dependent DNAzyme substrate on a gold electrode. The sensor works on linear isothermal amplification reaction, and the response was recorded with the help of differential pulse voltammetry. *H. pylori*, 17 nucleotide target DNA sequence was the analyte to be detected by the fabricated sensor. The assay was simple, selective and sensitive toward *H. pylori* detection. The sensor response was linear in the range of 2.1–67.2 pg with a limit of detection of 1.3 pg [55].

A triple-helix molecular switch was employed in another study by Cai and the group to create a biosensor with electrochemical activity for the identification of *Staphylococcus aureus*. A broad linear response in the range of 60–6 $\times$ 10^7 CFU/ml and a detection limit of 8 CFU/ml were determined for *S. aureus* by the fabricated aptasensor [56]. In addition, Hou and colleagues concentrated on photoelectrochemical biosensors as a recently developed detection method with cost–effectiveness, low noise, ease of use, high sensitivity and accuracy in comparison to conventional methods to detect *V. parahaemolyticus*. This photoelectrochemical aptasensor was constructed employing a layer-by-layer self-assembly method on the surface of a glass working electrode. The glass electrode surface was coated with bismuth nanoparticles and silver nanoparticles; the functionalized layer of silver nanoparticles was then used for immobilization of *V. parahaemolyticus* specific aptamer. The detection was monitored by recording the decrease in photocurrent due to a specific reaction between *V. parahaemolyticus* and the aptamer. The sensor was successfully tested for quantification of *V. parahaemolyticus* in the seafood with no interference by bacteria belonging to *Staphylococcus, Salmonella or Bacillus* species at the concentration of 3.2 $\times$ 10^5 CFU/ml. This device measured a LOD of 40 CFU/ml with a linear range from 3.2 $\times$ 10^2 to 3.2 $\times$ 10^8 CFU/ml with good precision and high stability [57].

2.1.3. Detection via fluorescent biosensor

To determine *S. aureus*, a dual-mode nano-biosensor with ratiometric fluorescence and colorimetry has been developed by Gao and colleagues. The carbon dots (BCDs) and manganese dioxide nanosheets (MnO_2 NSs) generated procedures were simple, effective, and labor-saving. The fluorescence quenching and oxidation mediated by MnO_2 NSs enhanced detection signals during analysis. The dual-mode determination had a low limit of detection of 9 CFU/ml (ratiometric fluorescence) and 22 CFU/ml (colorimetry), with a wide linear range of 37 $\sim$ 3.7 $\times$ 10^6 CFU/ml [58].

Sheini also created a fluorescent biosensor for the identification of four bacterial species, namely *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *E. coli*. They developed a paper-based microfluidic device (PAD) for the identification of bacteria. The biosensor was designed by immobilizing nanoclusters made of gold and copper on rectangular paper of dimension 1.5 $\times$ 1.0 cm. The paper device was divided into hydrophilic and hydrophobic zones by the ink-printed method. The hydrophilic zones were loaded with synthesized six different nanoclusters (OVA-coated gold nanoclusters, pepsin-coated gold nanoclusters, trypsin-coated gold nanoclusters, GSH-coated-copper nanoclusters, pepsin-coated copper nanoclusters and trypsin-coated copper nanoclusters). The analyte present in human serum was tested by injecting it into a clean glass sheet, followed by pasting the fabricated PAD on it. After 15 s of incubation, the PAD was placed in a fluorimeter. The analyte (bacteria) in the serum sample interacts with the nanocluster, and when irradiated with a wavelength of 365 nm in the fluorimeter under dark conditions, the change in the fluorescence intensity was recorded as a measure of analyte detection. The sensor was able to detect low concentrations of bacteria in the sample with detection limits of 43.0, 63.5, 26.0, and 47.0 CFU/ml for *S. aureus*, *S. pyrogens, E. coli* and *P. aeruginosa*, respectively [59].

Some more biosensors developed for bacterial detection and identification have been listed in Table 3.

2.2. Biosensors developed for the detection of pathogenic viruses

Viral diseases pose a serious threat to the population and safety. Inadequate identifying instruments are the cause of their high prevalence. Consequently, there is a notable need for the quick, precise, and selective identification of viruses. Numerous biosensors have been created and tested for their ability to detect viruses in environmental samples to prevent their harmful impact on the human population. Real-time molecular target identification is made possible by nanotechnology. Human excrement is a major source of waterborne viruses because an infected individual can shed anywhere from 10^5 to 10^{12} virus particles per gram. The real-time detection of viruses is one of the main advantages and contributions that biosensors could offer. This makes early detection of potentially fatal viruses in the environment, and thus their spread and impact can be controlled [75]. In subsequent

Table 3. Biosensors for identification of pathogenic bacteria.

Targeted organism	Biorecognition element	Biosensor	Detection time (minutes)	LOD (CFU/ml)	Linear range (CFU/ml)	Ref.
Shigella sonnei	DNA Aptamer	Dual-mode immunochromatographic test strip (DITS) biosensor	10	1×10^4	–	[60]
Vibrio parahaemolyticus	DNA2 Probe	Electrochemiluminescent	–	2.11	$7.04–1 \times 10^7$	[61]
Clostridium perfringens	Monoclonal antibody	Electrochemical immunosensor	–	–	–	[62]
Mycobacterium tuberculosis	DNA aptamer	Electrochemical	–	–	–	[63]
Streptococcus pneumoniae	Oligonucleotide probe	Electrochemical	–	10^2	$10^1–10^8$	[64]
Salmonella Typhimurium	Anti-Salmonella polyclonal antibodies	Electrochemical	120	10	$10–10^6$	[65]
Escherichia coli O157	Anti-*Escherichia coli* O157 antibody	Electrochemical	30	15	$10–10^6$	[66]
Salmonella enterica	Polyclonal anti-Salmonella antibodies	Electrochemical immunosensor	125	10	$10–10^5$	[67]
Aeromonas hydrophila	DNAzyme	Fluorescence	10	36	$0–10^3$	[68]
Staphylococcus aureus	Vancomycin	Electrochemical impedance spectroscopy	–	<39	–	[69]
F-pili containing *E. coli*	M13 phage	Chemiresistor	–	45	$10^2–10^7$	[70]
Haemophilus influenzae	Single strand probe DNA	Electrochemical	–	10^{-10}	10^{-10}	[71]
Escherichia coli O157: H7	Functional DNA aptamer	Electrochemical	60	19	$10–10^{-6}$	[72]
Salmonella typhimurium	Nanozyme	Optical	50	100	$10^4–10^6$	[73]
Salmonella enteritis	Molecularly imprinted polymers	Electrochemical	20	100	$3 \times 10^2–3 \times 10^7$	[74]

paragraphs, some of the biosensors designed for the detection of viruses in recent times have been discussed.

2.2.1. Detection via lateral flow biosensor

Shi and group designed a lateral flow biosensor based on AuNPs that was integrated with reverse transcription loop-mediated isothermal amplification. They designed a primer that was made from different genotypes of the hepatitis C virus, namely 1b, 3b, 2a, 3a and 6a. The device was utilized for testing the hepatitis C virus in clinical and environmental samples. The detection and identification process took approximately 40 min without any need for extra instruments, and the limit of detection value comes out to be 20 copies per serum sample. The sensor developed was precise and stable [76].

2.2.2. Detection via optical biosensor

With an estimated 2 billion cases worldwide, the hepatitis B virus (HBV) ranks among the most common viral illnesses in Asian nations. Though vaccination is available for its prevention, approximately 25 million people are still affected, and nearly one million deaths are recorded due to hepatitis B virus infection. ELISA is the most common technique for its detection, but due to its low sensitivity, its effectiveness is unsatisfactory. As a result, quantitative PCR is a benchmark for HBV detection. PCR techniques need sophisticated lab setup along with professional, skilled personnel. As a result, efforts have been made to create a biosensor that can detect HBV at the point of care. Chang et al. reported a "light-sensitive sensor" for the detection of HBV. In their approach, they have prepared helical gold nanorods functionalized as optical probes. HBV DNA strands isolated from the sample were converted to hybridization chain products that exhibit quadruplex DNAzyme structure when treated with hemin and potassium ions. This prepared quadruplex DNAzyme, when incubated with the helical gold nanorods, results in an etching of the helical gold nanorods and a decrease in scattering intensity that can be captured by dark field microscopy. Helical gold nanorods were reported to the excellent biosensor probe with a limit of detection as 30.15 fM. Their approach opened an avenue for sensitive detection of viral DNA detection [77].

2.2.3. Detection via colorimetric biosensor

Duyen developed a colorimetric biosensor for the identification of DENV that was based on the precise binding of dengue's genomic RNA to a DNA-conjugated gold nanoparticle, which forms a DNA–RNA duplex structure and causes the gold nanoparticle to aggregate, giving the ruby red color to turn blue under acidic condition. A change in the color is a visual indication of the presence of the DENV, and the same can be quantified by recording the absorbance of the developed blue color at 620 nm. The detection limit was measured as $\sim$1 pg/μl. The time taken to complete the reaction process along with the extraction of genomic RNA was 1 h [78].

2.2.4. Detection via electrochemical biosensor

Uygun and Tasoglu developed a biosensor system that was a peptide-based antimicrobial biosensor system utilizing electrochemical impedance spectroscopy for the identification of gp 120, a viral envelope protein of HIV. The biosensor uses silver nanoparticles and a gold-coated carbon electrode with MXene to detect the HIV envelope protein gp120. On the biosensor surface, the presence and dispersion of MXene and silver nanoparticles were verified by scanning electron microscope. To guarantee dependable and stable functioning, the antimicrobial peptide was applied to the electrode surface in a way that reduced the breakdown of the biorecognition receptor. For the detection of gp120, the sensor showed a linearity range of 10–4000 pg ml^{-1}, showing good repeatability in actual samples. Additionally, it was determined that the limits of quantification and detection were, respectively, 0.14 pg and 0.05 pg ml^{-1} [79].

Viral flu is easily confused with common flu during the majority of flu pandemic cases globally. In 1918, approximately 50–100 million deaths were reported due to the outbreak of H1N1 flu; a similar case was also reported in 2009, killing 4 lakh people. Earlier detection of the flu causative agent can be very helpful in controlling such outbreaks. RT-PCR and virus culture are conventional detection methods for such viruses. However, these methods are influenced by the external environment, are time-consuming, and cannot be miniaturized therefore sensitive, precise, accurate and dependable alternative techniques for viral detection are in need of time. Recently, Yang et al. developed an electrochemical sensor for the identification of influenza viruses. A single-stranded DNA (ssDNA) probe was designed and synthesized that has an H1N1 target sequence reorganization element as well as a primer sequence that can initiate rolling circle amplification. When this ssDNA probe was incubated with a specific target H1N1 RNA, probe cleavage was initiated, followed by loop-based amplification to reduce multiple biotin-labeled ssDNA that can be detected by the streptavidin present on the working surface of a modified gold-coated electrode. The sensor response is measured with the help of a cyclic voltameter and by electrochemical impedance spectroscopy. The developed biosensor can serve as an economical and reliable tool for clinical application, and the strategy of biosensor design can be implemented for the detection of other viruses in different types of samples [80]. With a high global prevalence, severe symptoms, and concerning mortality rates, DENV is the most common arbovirus in the world. Do Couto and colleagues created an electrochemical biosystem for the detection of DENV genotypes 1 and 2, utilizing anti-DENV antibodies, cadmium telluride quantum dots and cysteine, in response to the limitations of existing diagnostic techniques. Atomic force microscopy (AFM), fourier transform infrared spectroscopy (FTIR), SPR, CV, and electrochemical impedance spectroscopy were used to analyze the performance of immunosensor. There were observable angular and topographic variations in the AFM and SPR data, which supported the biomolecular identification. The study examined varying concentrations of DENV-1 and DENV-2 (0.05 $\times$ 106 to 2.0 $\times$ 106 PFU ml-1), yielding maximum anodic shifts of 263.67% $\pm$ 12.54 for DENV-1 and 63.36% $\pm$ 3.68 for DENV-2. The response of the detection methods to the rise in virus concentration was linear. Across a wide concentration range, excellent linear correlations were established, with R^2 values of 0.95391 for DENV-1 and 0.97773 for DENV-2. High repeatability was observed in the analysis of the data. The quantification limits were found to be 1.49 $\times$ 10-6 PFU ml-1 and 0.06 $\times$ 106 PFU ml^{-1}, whereas the detection limits were 0.34 $\times$ 106 PFU ml^{-1} and 0.02 $\times$ 106 PFU lL^{-1} [81].

2.2.5. Detection via aptamer-based biosensor

In addition, Khan and colleagues created an aptamer-based biosensor that can quickly and accurately identify viruses, opening up new possibilities for COVID-19 detection on a broader scale. They described the use of molecular docking and bioinformatics tools in conjunction with an aptamer-based electrochemical and computational technique to characterize the interaction between a 60-base single-stranded (ss) DNA aptamer and the viral S protein. The DNA aptamer was attached to the surface of an electrode modified with AuNPs to produce the biosensor. Methylene blue was utilized as a redox indicator. In the presence of S-protein, conformational changes in the aptamer's structure were obtained that increased the methylene blue redox signal. This signal had a linear range of 10 pM–6 nM and a differential pulse voltammetry limit of detection of 91.1 pM [82]. The Zika virus (ZV) can cause hemorrhagic fever, which can be fatal. A quick diagnostic method is needed to identify ZV infection. To address this issue, Jang and colleagues created a quick electrical biosensor using the alternating current electrothermal flow (ACEF) method and a DNA aptamer mounted on an interdigitated gold micro-gap electrode. To lower production costs for the creation of biosensors, the truncated ZV aptamer (T-ZV apt) was created, which demonstrated binding affinity comparable to the original ZV aptamer. This biosensor which used the pulse-voltammetry was made of an immobilized T-ZV apt on an interdigitated micro-gap electrode. Target virus envelope protein found in the serum binds to the aptamer, and detection can be achieved within 10 min. When the serum's Zika envelope

concentration increases, the biosensor waveform grows linearly, with a detection limit of 90.1 pM [83].

Different types of biosensors have been developed to identify the viral pathogens, which are summarized in Table 4.

2.3. Effect of nanomaterials on the electrochemical performance for sensing the microbial existence

Several nanomaterials, including metal oxides, metal sulfides, graphene nanocomposites, AuNPs, silver nanoparticles (AgNPs) and quantum dots, have been used in biosensor fabrication. They have characteristics related to optics, electrical, thermal and catalytic. Nanomaterials offer many advantages, such as a high surface-to-volume ratio, small size and unique optical and electronic properties; this makes them a powerful tool for improving biosensor performance in detecting target analytes. When nanoengineered materials are incorporated into biosensors, they greatly influence their performance metrics, including sensitivity, specificity, response time and overall cost. Nanomaterials can enhance the selectivity of sensors, enabling better differentiation of various microorganisms, and can also differentiate viable and nonviable cells. Additionally, they can boost the stability and durability of electrochemical sensors, resulting in more reliable and enduring performance for microbial detection [96].

To facilitate the electrochemical biosensor for *E. coli* detection, Zheng and colleagues created a bifunctional nanomaterial (Pt-Ni@erGO) made of platinum (Pt)-nickel (Ni) nanoparticles and epoxy-rich graphene oxide (erGO). Sharp edges and a large specific surface area of erGO are advantageous for inserting or cutting through cell membranes and loading high-density Pt-Ni nanoparticles while maintaining catalytic activity. Pt-Ni@erGO has strong antibody-like activity, both of which are favorable to increasing sensitivity. This led to the successful detection of *E. coli*, achieving a linear range of 1.5×10^2–1.5×10^7 CFU/ml with a sensitivity of 38 CFU/ml [97].

In another study, using a two-step spin coating method, Guinlet and colleagues introduced an electrochemical immuno-biosensor that included non cytotoxic silica nanoparticles (NPs). This novel technique, which uses CV measurements, enables the continuous and non-saturated detection of *E. coli* bacteria in about 5 min and can reach up to 10^3 CFU/ml in 30 min, in contrast to biosensors that have a special antibody-based layer without NPs [98].

Lai and colleagues have reported a sensitive and disposable screen-printed carbon electrode-based electrochemical biosensor strip for rapid and accurate identification of the Japanese encephalitis virus. Japanese encephalitis virus antibodies were immobilized on the electrode surface by amide bond formation between the amino group on the carbon nanomaterial and the carboxy group of antibodies. In 10 min, the electrochemical biosensor strip demonstrated a linear detection range of 1–20 ng/ml^{-1} and a low detection limit of 0.36 ng/ml^{-1} [99].

Karakus and the group devised a colorimetric technique for detection of the SARS-CoV-2 antigen using a gold nanomaterial-based biosensor. Irreversible aggregation of AuNPs in the presence of SARS-CoV-2 spike antigen resulted in a change in the color from red to purple due to antigen-antibody interaction that can be measured spectrophotometrically with a detection limit of 48 ng/ml. The same was electrochemically detected using the developed probe on a commercial screen-printed gold electrode. Electrochemical analysis showed a linear response to the SARS-CoV-2 spike antigen with a detection range of 1 pg/ml to 10 ng/ml. SARS-CoV-2 spike antigen could be detected with excellent specificity using this approach [100].

Thus, the abovementioned work indicates that nanomaterials greatly improve the electrochemical performance of sensors designed to detect microorganisms. Their small size and special characteristics enhance the sensors' sensitivity and efficiency, enabling them to detect low concentrations of microbes. Incorporating nanomaterials not only enhances the accuracy but also the reliability of biosensors for microbial detection.

3. Challenges in the development of biosensors

From technological barriers to practical issues, the development of biological sensors for pathogen detection poses a multitude of challenges. The following are some key points for addressing these difficulties:

3.1. Sensitivity & selectivity

In complicated matrices such as clinical samples and environmental samples, biosensors need to have a high sensitivity to identify pathogens at low concentrations. Attaining selectivity is of equal importance in distinguishing intended pathogens from non-pathogenic species or environmental pollutants [101]. Complex matrices can also introduce background noise and interfere with signal transduction pathways, resulting in false positives or negatives. Examples of these include biological fluids and environmental samples. Because of the significant background response in clinical samples, it is very difficult to identify bioanalytes such as specific proteins, cytokines or abnormal cells. Utilization of various nanomaterials while constructing a biosensor can lead to signal amplification

Table 4. Biosensors for identification of pathogenic virus.

Targeted organism	Biorecognition element	Biosensor	Detection time (minutes)	LOD (ng/ml)	Linear range (ng/ml)	Ref.
Nipah virus	DNAzyme	Fluorescent	20	10	–	[84]
Human monkeypox virus	Heparan sulfate receptor	Impedimetric	–	2.08	2.0–50	[85]
H1N1 virus	Single-stranded DNA probe	Electrochemical	–	6.7×10^{-8}	3×10^{-8}–3×10^{-3}	[86]
Hepatitis B virus DNA	Hepatitis B virus DNA oligonucleotides	Electrochemical	0.417	10^{-7}	5×10^{-4}–5×10^{7}	[87]
Hepatitis A virus	Molecularly imprinted polymers	Fluorescence	15	3×10^{-3}	2×10^{-2}	[88]
Dengue virus	CRISPR RNA & Cpf1	Electrochemical	30	10^{-5}	–	[89]
HIV	Spherical nucleic acid and CRISPR/ Cas12a	Electrochemiluminescence	120	0.00003	–	[90]
Human papilloma virus subtype	CRISPR/Cas12a	Electrochemiluminescence	70	0.00048	–	[91]
Influenza A H1N1 virus	Monoclonal anti-FluA antibodies	SERS-based biosensor	30	50	–	[92]
Hepatitis B surface antigen	Anti-hepatitis B antibody	Electrochemical	8.33	0.018	0.1–250	[93]
Singapore grouper iridovirus	Q2 and Q3 aptamers	Lateral flow biosensor	<90	5×10^{4}	–	[94]
HIV	Anti-gp120	Electrochemical	–	0.066	1–10	[95]

as nanomaterials exhibit excellent electrical and optical properties, leading to enhancement in the sensitivity of the biosensor. Nanomaterials also serve as excellent matrices for the immobilization of bio elements with increased loading capacity. When it comes to stability, cost, sensitivity and reaction time, nanoscale catalysts are the emerging stars, yet their specificity still has to be further enhanced [102]. Effective CRISPR/Cas network-driven autocatalysis amplification can be utilized as another technical advancement in the field of biosensor design for the detection of cellular DNA and RNA [103].

3.2. Extensive versatility

Since Multiplexing systems offer a thorough mapping of disease traits and accurate results, multiplexing assays are essential to clinical practice toward precision diagnostics. Multiple analyte detection in one test decreases variances between single plex assays it saves time and sample volume and provides maximum information regarding the sample during analysis. Sensitivity, cross-reaction and restricted signal readouts are issues with the multiplex capability. According to Shen, most of the electrochemical biosensors can analyze only three analytes in a particular sample due to signal overlap. On the other hand, Optical biosensors come up with upgraded systems because they can integrate with many optical tags, including SPR, surface enhanced Raman scattering (SERS) and upconverting nanoparticles thus optical biosensors offer a better option for diverse analyses [104].

3.3. Real-time monitoring

To continuously protect the status of health, effective healthcare depends on technology that monitors physiological variables in real time, same applies to environmental assessment and monitoring. While attaining *in situ* real-time monitoring is challenging thus modern biosensing systems need to be powered with multiplexing capabilities, quick response, low sample size with high sensitivity. Developments in implanted chips and wearable electronics have made it possible to monitor a variety of health issues continuously. These developments have greatly heightened interest in the application of wearable technology and sensors [105]. Analyte reversible signals can be obtained from a continuously operating real-time biosensing device even in situations where the analyte concentration fluctuates. Consequently, it can distinguish signals in a complicated sample environment among the substance that is being studied. Without requiring more operational processes, it can immediately supply the analyte's real-time signal in the interim. A current obstacle in the use of biosensors for constant molecular monitoring is the conversion of particular analyte information into continuously detectable signal outputs *in vivo* without drifting background signals [106].

3.4. The ecosystem's sustainability

Due to the widespread connectivity and modern expectations for quick, dependable, and accessible information about our health status, economical safer, and portable monitoring devices are in demand which can be easily fulfilled by the disposable sensors [107]. Concurrently, such disposable sensors are one-time use solutions therefore contamination-free for the user. Though disposable biosensors accelerate and simplify our lives, they also

pose a threat to the environment. To make biological sensing devices sophisticated and reliable in terms of data gathered, the addition of electronic component is needed. To safeguard the hazardous impact of used biosensor elements its biodegradability aspect has to be kept into consideration. Advancements in sustainable sensing platforms in recent times target economical environmentally benign, smart and user-friendly devices. Advanced biosensing systems include wearable biosensors, biochips, smartphone-based biosensors, recyclable biosensors, IOT-based biosensors, etc [108]. Paper-based analytical devices have emerged as semi-quantitative analyzing devices with scalable, economical and point-to-care tools [109]. The development of sophisticated and intelligent biological sensing machinery with the required analytical performance that is also sustainable to the ecosystem requires careful thought because it is difficult to create an environmentally friendly sensing system with all the desired qualities [110].

4. Conclusion

In this paper, we have discussed various biosensors developed for the identification of pathogenic microorganisms. The review initially explains the prevalence of infectious bacteria and viruses and the risks associated with them. Furthermore, biosensors as a promising tool, have been described for immediate and accurate pathogen identification. Environmentally safe and biocompatible sensors can detect and identify various bacteria and viruses in environmental and biological samples, as reported by researchers. The several biorecognition element-based biosensors that have been reported for the detection of microbes include aptamers, nucleic acids, enzymes and antibodies. Aptamers show potential as an alternative to antibodies because of their ease of synthesis, high stability, ease of modification, tissue penetration, low cost and low toxicity. Biosensors can be designed for point-care analyses as well as, monitoring of infection at its various stages. Biosensors and nanobiosensors are potent measurement tools that simplify, speed up, and effectively identify significant clinical bacteria and viruses.

5. Future perspective

Future applications face various significant obstacles that must be overcome, including the need to address the possibility of detecting low concentrations of pathogens and the requirement for bacterial strain-specific bioreceptors. One significant challenge arises when the complex environmental matrix produces significant interferences when target analytes are detected by a developed biosensor. Future developments in biosensors should prioritize the development of innovative sensing components, smart sensing materials, effective signal transduction and onsite detection of a broad variety of environmental contaminants to address the problems listed above. By improving signal to noise ratio of the sensor, one can strengthen the signal transduction process of the developed biosensor, thus improving overall performance. Nanotechnology advancements are/can aid in the development of biosensor technology. Continuous monitoring will be improved by the development of wearable and less invasive implantable biosensors that offer comfort to the user. The biocompatibility aspect of biosensors designed for infectious agent determination should be given prime importance. Comprehensive and accurate health monitoring will be made possible by noninvasive methods, multianalyte detection and nanoscale biosensors.

Article highlights

- Biosensors offer rapid pathogen detection often in minutes allowing for timely medical attention.
- Biosensors can detect pathogens at much lower concentrations than traditional methods.
- Biosensors are increasingly being used for continuous monitoring of pathogens, providing real-time data for better management of disease outbreaks and environmental contamination.
- In this review various biosensors developed for the detection of bacteria and viruses are discussed along with challenges in their development and future prospects.

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