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Bioreceptor modified electrochemical biosensors for the detection of life threating pathogenic bacteria: a review

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The lack of reliable and efficient techniques for early monitoring to stop long-term effects on human health is an increasing problem as the pathogenesis effect of infectious bacteria is growing continuously. Therefore, developing an effective early detection technique coupled with efficient and continuous monitoring of pathogenic bacteria is increasingly becoming a global public health prime target. Electrochemical biosensors are among the strategies that can be utilized for accomplishing that goal with promising potential. In recent years, identifying target biological analytes by interacting with bioreceptors modified electrodes is among the most commonly used detection techniques in electrochemical biosensing strategies. The commonly employed bioreceptors are nucleic acid molecules (DNA or RNA), proteins, antibodies, enzymes, organisms, tissues, and biomimetic components such as molecularly imprinted polymers. Despite the advancement in electrochemical biosensing, developing a reliable and effective biosensor for detecting pathogenic bacteria is still in the infancy stage with so much room for growth. A major milestone in addressing some of the issues and improving the detection pathway is the investigation of specific bacterial detection techniques. The present study covers the fundamental concepts of electrochemical biosensors, human PB illnesses, and the latest electrochemical biosensors based on bioreceptor elements that are designed to detect specific pathogenic bacteria. This study aims to assist researchers with the most up-to-date research work in the field of bio-electrochemical pathogenic bacteria detection and monitoring.

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1. Introduction

Pathogenic bacteria (PB) can cause life-threatening infections and are one of the main causes of mortality worldwide because PB poses an urgent threat to global health. Due to the shield created by PB, essential antibiotics are insufficient to fight against PB,[1] thus, antibiotics are becoming ineffective against PB.[2] Paralysis, tetanus, fever, cramping in the stomach, respiratory and urinary tract infections, cancer, diarrhea, cholera, and fever are the deadliest illnesses and diseases caused by PB.[3] The World Health Organization (WHO) reported that antibiotic resistance poses a serious worldwide problem with far-reaching implications for public health and the economy.[4] Intestinal infectious disorders are caused by bacteria that are associated with water, food, and physiological fluids. These bacteria also propagate infectious diseases among humans and animals.[5] The primary cause of foodborne illness outbreaks is undercooked or processed ready-to-eat (RTE) meat, dairy products, fruit, and vegetables.[6–8] The primary reservoirs for many foodborne diseases include the environment (soil and water), foods produced from animals (meat, milk, and eggs), and agricultural goods (fruit and vegetables).[5,9] The most common foods that can harbor bacteria such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter*, *Listeria*, *Shigella*, or *Escherichia coli* O157:H7 are fruit, vegetables, fish, meat, eggs, and dairy products. Due to the severe pandemic brought on by the life-threatening infection of PB, preventive measures should be taken.[10] Nonetheless, the development of diagnosis and prevention tools and routes can address the intense outbreak of PBs.

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Electrochemical biosensors have garnered increasing attention from researchers in recent years because of their extremely sensitive and selective method of detecting PB. The primary function of a biorecognition element on a biosensor is to give target analyte specificity. The bioreceptors are immobilized on a sensor surface to capture the target analyte. Antibodies, enzymes, cells, aptamers, DNAs, biomimetics, and phages are the most frequent types of bioreceptors or biorecognition elements, also known as molecular probes. Bioreceptor-modified electrochemical biosensors are a potent tool for the identification of pathogenic bacteria, providing quick, accurate, and targeted diagnostics in a range of applications. Table 1 summarizes the advantages of electrochemical biosensing over other methods of detecting PBs is presented below.

In recent years, electrochemical biosensors, especially portable electrochemical biosensors, have been the preferred bioanalytical method for detecting PBs. Numerous biosensors were previously reported such as mass-based (piezoelectric[21] and surface acoustic[22]), optical (light scattering,[22] fiber optics,[23] and SPR[24]), and electrochemical (amperometry,[25] potentiometric,[26] and impedance[27]). However, they all face the challenge of identifying pathogens in real samples (such as food) at low analyte concentrations with excellent sensitivity and selectivity. Nonspecific adsorption of biomolecules in the sample (either from the matrix or microbes) can severely impede the biosensor surface.[28] This increases background "noise", reduces the selectivity of the biosensor, and weakens the signal. Recently, several studies have been performed on novel and portable biosensors that can outperform conventional detection technologies. More research is needed on biosensors that can quantitatively identify and screen infections in clinical, environmental, and dietary samples.[29] Electrochemical platforms are the commonly used biosensor due to their high analyte selectivity and ability to perform multiplex analyses. Furthermore, they can achieve high analytical precession even in complex food matrices with varying densities, compositions, and pH levels. As a diagnostic tool for clinical applications, electrochemical-based biosensors have shown considerable promise. However, they still face some obstacles that must be overcome. Among these challenges, preserving the sensor's stability and repeatability in intricate real matrices, generating a low limit of detection (LOD), and preventing non-specific adsorption of interfering species are the three main obstacles faced in the development of electrochemical biosensing platforms.[30] Pathogens are electrochemically detected using a working electrode that has been modified with recognition components (such as an antibody, aptamer, or DNA probe) to guarantee measurements with high selectivity, sensitivity, and specificity. Employing a working electrode modified with recognition elements (*e.g.*, an antibody, aptamer, or DNA probe) allows for the electrochemical detection of pathogens while maintaining measurement selectivity, sensitivity, and specificity. For this reason, the most widely used biosensors are electrochemical-based platforms.

Incorporating biorecognition elements into the electrode surface of the electrochemical biosensing to selectively detect and identify biologically significant microorganisms such as pathogens can significantly enhance the accuracy and specificity of biosensing recognition platforms. Therefore, bioreceptors incorporated transducers-based bio-electrochemical sensors increase the overall platform viability for the diagnostic and detecting applications. This study covers the fundamentals of electrochemical biosensors, human PBs illnesses, and the most recent advancements in bioreceptor element-based electrochemical biosensors for the detection of specific pathogenic bacteria. To the best of our knowledge, there is no published survey that comprehensively review electrochemical biosensors based on bioreceptors surface modification for the detection of pathogenic bacteria with a great length of details on the commonly used biorecognition elements and a large selection of life threating pathogenic bacteria along with the most frequent diseases caused by these infectious microorganisms.

Table 1 Advantages of electrochemical biosensor over other methods for PBs detection[a]

Specification	Electrochemical method	Other methods
Sensitivity	Electrochemical biosensors are highly sensitive to specific analytes for the lowest limit of detection.[11]	Most of the conventional methods are low sensitive, *e.g.*, ELISA requires long incubation time (24–48 hours) with low sensitivity ($\geq 10^5$ CFU mL^{-1}) yield[12]
Selectivity and viability	Electrochemical biosensors are highly selective, bind and detect specific analyte, discriminate between live and dead bacteria.[13,14]	All methods are less selective, PCR techniques cannot distinguish between nonviable and viable bacteria cells due to false positive cross-amplification and false negative DNA polymerase inhibition.[15]
Stability	Retained their efficiency for a long time, it shows high efficiency.[16]	Insufficient stability, low efficiency of signal amplification.[17]
Linearity and limit of detection	Wide dynamic linear range of biosensor response and lowest limit of detection.[18]	Lack of linear dynamic range and poor limit of detection.[17]
Detection method	This method is rapid, real-time with portable device.[16]	Most of the conventional methods are time consuming, and long working protocol requires heavy machinery.[19,20]

[a] Abbreviations: ELISA: enzyme-linked immunoassay, CFU: colony forming unit, PCR: polymerase chain reaction.

This work can serve as a reference for researchers with interest in developing reliable and highly sensitive electrochemical based biosensors to selectively detect deadly pathogens for point of care applications in the health care sector.

2. Principle of an electrochemical biosensor

A combined receptor-transducer device that employs a biological recognition element for selected quantitative or semi-quantitative analytical data is called an electrochemical biosensor.[31] In 1962, Clark and Lyons introduced the term "biosensor". Their outstanding achievements has made them the original founders of biosensing concept.[31] To measure the amount of dissolved oxygen in blood using the amperometry technique, they developed an oxygen electrode and a glucose oxidase enzyme.[32] The electrochemical biosensors are the most extensively used biosensing platform and are successfully commercialized to monitor glucose for diabetic patients. For biosensing detection, pathogenic bacteria are collected from host body, cultured, and screened. The desired biochemical conversion is performed to bring detectable condition. Thereafter, pathogenic, or nonpathogenic bacteria can be detected using a group of bioreceptors (enzyme, antibody, cell, bacteriophage, nucleic acid) fabricated electrode.[33,34] Both live and dead bacteria can be detected by electrochemical biosensors. The interaction of bioreceptors with bacterial cellular components-which can be found in both living and dead bacteria is frequently the basis for detection mechanisms.[14,35] Additionally, the bacteriophage base biosensor cannot detect dead bacteria.[36] The receptor–analyte interaction is measured using electrochemical techniques such as amperometry, potentiometry, impedimetric, and conductometry. The most used signal measurement techniques are cyclic voltammetry (CV), differential pulse voltammetry (DPV), stripping voltammetry, alternating current voltammetry (ACV), polarography, square wave voltammetry (SWV), and linear sweep voltammetry (LSV). Different electrochemical signal measurement techniques are suitable for various biosensing applications, and each method has its own merits. The type of analyte, the desired level of sensitivity and specificity, and the practical considerations all play a role in the measurement systems selection. These approaches can be classified into many types based on which characteristics of the electrode are regulated and which are measured. For incidence, potentiometry is used to quantify the difference in electrode potentials, amperometry analyses electric current, and coulometry records charge passed during a given time period.[37] Technological developments in these areas keep improving the functionality and performance of electrochemical biosensors across a range of applications.

Recent wearable electrochemical sensors have attracted a great deal of attention due to their wide range of applications in the human body. These electrochemical devices are used numerous forms such as contact lenses, Google glass, skin-patch, mouth gourds, smartwatches, underwear, and wristbands.[38] Additionally, electrochemical biosensor has been trailed for bacteria detection in the human body. Mannoor *et al.*, developed a wireless and wearable biosensor based on graphene-electrode-silk hybrid structure for *Staphylococcus aureus* bacteria detection on tooth enamel.[39] The developed sensor displayed a high sensitivity and with detection limits down to a single bacterium. Another flexible, wearable, wireless and battery-free DNA hydrogel-based biosensor was developed by Xiong and his colleagues. The introduced biosensor can identify wound infections caused by pathogenic bacteria (such as *Staphylococcus aureus*.) prior to any evident signs of wound infection. The fully integrated wound infections sensor integrates biologically responsive DNA hydrogel and Near-field communication (NFC) module to support a smartphone-based readout for updating the wound infection condition.[40] Based on the previously reported finding that in infected wounds, the pH becomes alkaline which have been linked predominantly to

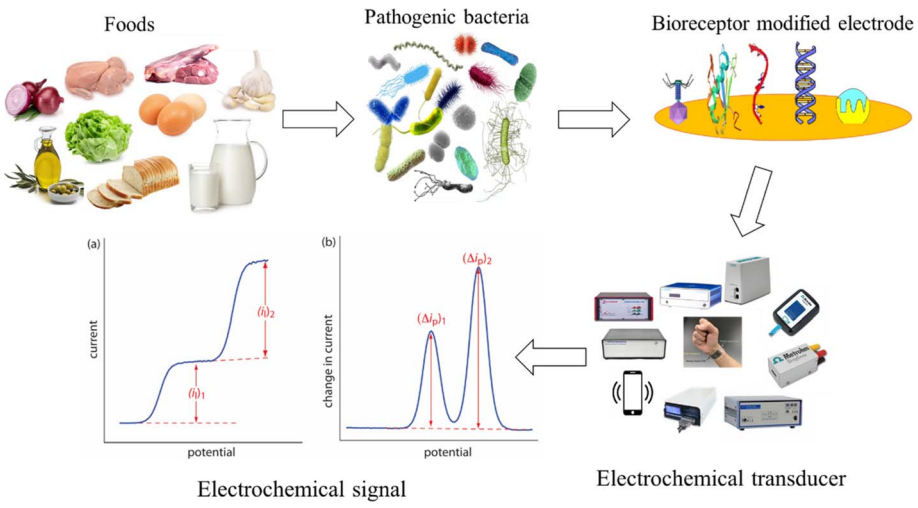

Fig. 1 Schematic diagram showing the typical electrochemical biosensing process for the detection of pathogenic bacteria. It consists of PB, bioreceptor modified electrode, electrochemical workstation measuring the receptor–analyte interaction signal.

the presence of bacteria.[41] Manjakkal *et al.* introduced graphite-polyurethane thick electrode for potentiometric electrochemical pH-based wearable sensor for pH level detection, thus determining the wound healing stage.[42]

Overall, graphical representation of pathogenic bacteria detection strategy is represented in Fig. 1. The three main parts of a biosensor are (i) a bioreceptor, also known as a biorecognition element, which identifies the desired analyte; (ii) a transducer that changes biological (electrochemical) impulses into electrical signals, (iii) a signal processing system presents these electrical signals in a recognizable format. Furthermore, biosensors are classified according to the type of transducer used, which includes electrochemical, calorimetric (thermometric), mass (piezoelectric or surface acoustic wave devices), and optical.[43] Electrochemical biosensors are becoming increasingly popular for applications in biotechnology, food safety, environmental monitoring, and clinical diagnostics due to their rapid reaction, ease of use, and low cost.

2.1. Bioreceptors

A molecule known as a bioreceptor binds to analytes through biological processes. Bioreceptors are categorized into affinity (such as nucleic acid and antibodies) and catalytic (such as enzymes, cells, and tissues) types. Biosensors are further classed according to the type of bioreceptor they use enzymes, cells, antibodies, or nucleic acid based. Immobilization techniques that can be utilized to attach bioreceptors on the surface of sensors include membrane and matrix entrapment, covalent and ionic binding, physical adsorption, and intermolecular crosslinking.[44] The physical adsorption of bioreceptors is mediated by Van der Waals forces, hydrophobic interactions, hydrogen bonds, and ionic forces. Covalent bonding between bioreceptors and sensor surfaces involves functional groups such as amino, sulfhydryl, hydroxyl, phenolic, and thiol groups, among others. It is worth noting that covalent binding is commonly utilized for enzyme immobilization. Polymeric materials such as polyacrylamide, cellulose acetate, starch, alginate, pectate, polyvinyl alcohol, polyvinyl chloride, polycarbonate, and silica gel are employed to entrap matrices or pathogens. Glutaraldehyde, hexamethylene diisocyanate, 1,5-difluoro 2,4-dinitrobenzene, and bisdiazobenzidine-2,2′-disulphonic acid are examples of bi- or multi-functional cross-linking chemicals used in intermolecular cross-linking.[45] Furthermore, successful immobilization techniques include covalent attachment to a functionalized substrate, affinity immobilization (attachment of biotinylated probes to streptavidin-coated surfaces), and self-assembling (chemisorption of thiol-modified probes onto gold surfaces). A short summary of bioreceptor bonding on transducer surface is presented in Fig. 2. Bioreceptor can be also classified into two main categories: catalytic biochemical receptors such as enzyme, cells, and DNA zymes; and affinity biochemical receptors such as antibody, aptamer, DNA/RNA oligonucleotides, engineered and protein.

2.1.1 Enzymes. Proteins called enzymes function as biological catalysts in organisms to quicken chemical reactions. Because they are composed of polypeptide chains of amino acids, the three-dimensional structure of the enzyme is determined by the arrangement of these amino acids.[47] Enzymes were the first molecular recognition components to be incorporated in biosensors.[48] Enzyme-based biosensors use a stable source of enzyme material (primarily through bio-renewable sources) and have a high possibility of modifying the catalytic properties or substrate specificity of the enzymes by means of genetic engineering. Finally, catalytic amplification of the biosensor response can be achieved by the modulation of the enzyme activity with respect to the target analyte.[46] The capacity to speed biochemical activities.[49]

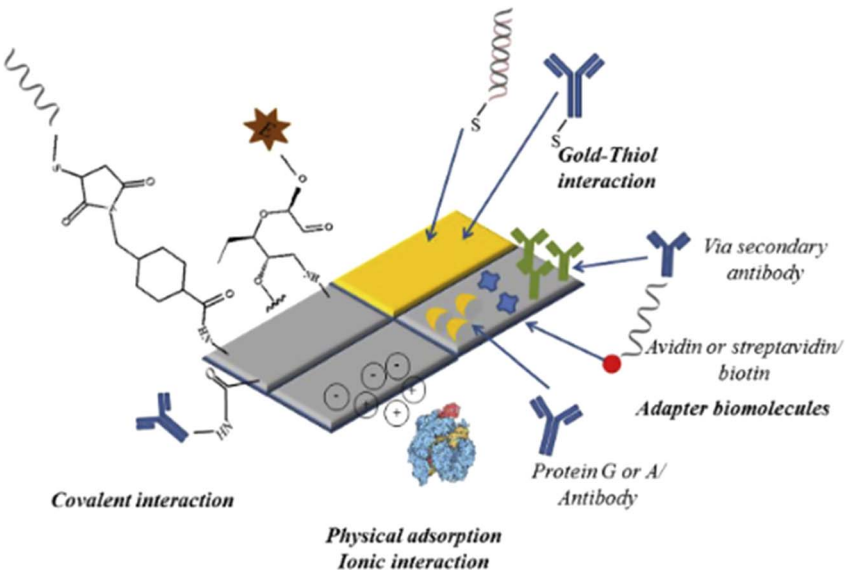

Fig. 2 Schematic diagram illustrating examples of matrix-analyte bonding formation techniques. The figure was adopted from ref. 46 with permissions, copyright @ Elsevier.

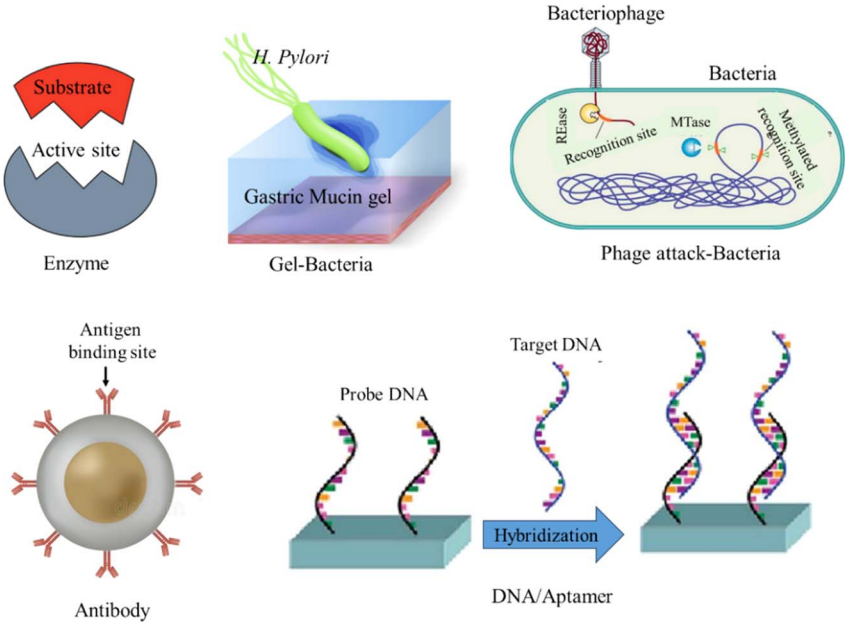

Fig. 3 Bioreceptor–analyte interaction for electrochemical biosensor detection. Figure was created by authors based on ideas by ref. 51–53.

To improve detection stability and repeatability, enzymes can be immobilized on the transducer matrix surface. Therefore, the choice of inert, stable, and resistant support material is essential for improving enzyme activity.[50] The enzymes can be immobilized on support material *via* adsorption, covalent bonding, crosslinking, encapsulation, and entrapment as illustrated in Fig. 3. Literature revealed that enzyme activity varies depending on the type of bonding with the surface of the sensor. Peroxidases and oxidoreductases have received a great deal of attention within the biosensor scientific community because they are the most stable enzymes for catalyzing oxide reduction reaction.[54] *Listeria monocytogenes*,[55] *Escherichia coli*[56], and *campylobacter jejuni*[57] pathogenic bacteria detection was reported by enzymes modified electrochemical biosensor. Even though enzyme application takes up a lot of space on the catalytic biosensor, the enzyme quickly loses its ability to function after two to four weeks after application.[58]

2.1.2 Cells. A wide range of applications, including the detection of diverse analytes from the environment, food, clinical settings, and other sources, can be fulfilled by creating biosensors through live cell-based assays.[59] It has previously been documented that several cells, including bacteria, yeast, and higher eukaryotic cells like vertebrata or mammals, were used as bioreceptors within bio-electrochemical sensors. Moreover, earlier research revealed that mammalian tissue slices or cells might be successfully employed as biorecognition components in studies utilizing biosensors.[36] The use of living cells as identification elements can result in low detection limits and functional stability due to their great sensitivity. Banerjee *et al.*,[60] investigated a cell-based sensing mechanism based on collagen-encapsulated mammalian cells for rapid detection of pathogenic bacteria and toxins. Zhao *et al.*,[61] discussed the synthesis of silica nanocomposite doped with liposome to be exploited as an artificial cell-based biosensor. The synthesized artificial cell can bind and detect hemolysin and Listeriolysin O, secreted by pathogen L. monocytogenes bacterium. One of the disadvantages of the proposed cell-based sensor is that the presence of undesired enzymes can cause a complicated response and result in a lack of selectivity.[62]

2.1.3 Antibodies. Antibodies (Ab) or immunoglobulins (Ig) are large Y-shaped glycoproteins that have a high specificity for recognizing antigens, which are substances that might trigger an immune response.[63] Each antigen contains discrete areas called epitopes to which the antibody can bind. While monoclonal antibodies are selective for only one epitope within an antigen, polyclonal antibodies can bind many epitopes inside an antigen. To analyze surface plasmon resonance (SPR) immunosensors using optical transduction, biosensor chips were coated with various secreted antibodies, including monoclonal, polyclonal, and recombinant.[64] To facilitate the conjugation of the transducer and the antibody, the transducer surface had to be modified by adding functional groups such as carboxyl, amino, aldehyde, or sulfhydryl groups using a polymer or monomer. The antigen-specific antibody binding acts as a lock and key mechanism, and it is very simple in SPR, thus making it more accurate and faster than traditional assay.[65] The results, however, were highly variable because of the limits of the antibody-decorated immunosensors with irreversibility, binding affinity, temperature, and the pH level of the reaction.[66]

2.1.4 Bacteriophages. Viruses known as bacteriophages, which range in size from 20 to 200 nm, are extracted from host cell lysis and can be employed to decorate sensitive and specific electrochemical biosensors. While most phages have a net negative charge due to their positively charged tail fibers and negatively charged capsid (head), researchers have suggested several methods to immobilize phages on electrochemical biosensor platforms, including covalent and ionic techniques. The dipole attraction force mechanism was preserved in ionic

bonding to immobilize the phage on the electrode surface.[65] Furthermore, the presence of a particular receptor on their tail protein makes the lysis bacteriophage more selective to the host strain. In addition, bacteriophages have distinct morphologies, binding affinities, and variable temperature, pH, and ionic strength compared to immunosensors, which makes them uniquely distinctive.[67] Phage function as a biorecognition component for pathogen exposure has been declared by researchers for a variety of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus anthracis spores*.[68]

2.1.5 Aptamers and nucleic acids. Small single-stranded RNA or DNA oligonucleotides, known as aptamers, typically have a length of 20 to 60 nucleotides and are highly selective and affinity-bound to target molecules. Presently produced aptamers have a broad spectrum of binding sites, ranging from simple inorganic molecules to whole cells and huge protein complexes. The aptamers are the nucleotide counterparts of antibodies; yet the synthesis of aptamers is significantly less costly and complex than that of antibodies.[69,70] Furthermore, aptamers are not poisonous or immunogenic.[69] These nucleic acid segments were bioreceptor components with broad use for transducer manufacturing. Phosphate and sugar (deoxyribose) groups alternate to form nucleic acid strands. One of the nitrogen bases; adenine (A), cytosine (C), guanine (G), or thymine (T) is held by each sugar group. As in the cases of adenine:thymine (A:T) and cytosine:guanine (C:G), one strand nitrogen base is joined to another strand by hydrogen bonds.[71] Signal amplification strategies were also studied by attaching reporter probe with suitable condition. The transducer surface is properly functionalized by a monomer, polymer, or composite to immobilize the nucleic acid receptor.[11] The ssDNA immobilized biosensor has a broad range of applications in both experimental and real sample medium. Literature from various studies reporting on nucleic acid-based electrochemical biosensors was discussed in this study.

3. Pathogenic bacteria respective diseases

PBs cause a great deal of human disorders by interfering with normal bodily functions. The intestinal mucosa serves as the body's largest interface for the colonization of both pathogenic and nonpathogenic bacterial species in most cases. Some of which combat the pathogenesis infections by fighting against infectious bacteria.[72] Beneficial bacteria may prevent the cluster of pathogenic bacteria by forming colonization and invasion. Nevertheless, pathogenesis begins with the transmission of the bacterial infectious agent to the host. Then bacteria remain at the site for colonization in the host system. The sickness is finally caused by the host immune system. The most common pathogenic bacteria and the resulting infectious diseases in humans are listed in Table 2. Hazardous infections can be

Table 2 The most common pathogenic bacteria along with the resulting infectious diseases in humans

Pathogenic bacteria	Human diseases	Pathogenic bacteria	Human diseases
(1) *Salmonella* spp.	Diarrhea, fever, abdominal cramps[73]	(14) *Pseudomonas aeruginosa*	Urinary tract, respiratory, wound infections[74]
(2) *Escherichia* spp.	Urinary tract infection, kidney failure[75]	(15) *Klebsiella pneumoniae*	Pneumonia, urinary tract infection, bloodstream infections[76,77]
(3) *Vibrio* spp.	Cholera, diarrhea, and dehydration[78]	(16) *Listeria monocytogenes*	Fever, muscle aches, meningitis[79]
(4) *Shigella* spp.	Dysentery, diarrhea, fever, stomach cramps[80]	(17) *Yersinia pestis*	Bubonic, septicemic, and pneumonic plague[81]
(5) *Bacillus* spp.	Anthrax (skin, lungs, gastrointestinal tract)[82]	(18) *Enterococcus faecalis*	Urinary tract infections, endocarditis (inflammation of heart lining)[83]
(6) *Clostridium* spp.	Tetanus, botulism, paralysis, colitis[84]	(19) *Francisella tularensis*	Tularensis illness[85,86]
(7) *Neisseria* spp.	Gonorrhea, meningitis[87]	(20) *Haemophilus influenzae*	Respiratory tract infection (pneumonia, sinusitis, and ear infections)[88]
(8) *Mycobacterium* spp.	Tuberculosis, leprosy, skin, peripheral nerves, and mucosa of respiratory tract[89]	(21) *Corynebacterium diphtheriae*	Diphtheria (pharyngitis, fever, swelling of the neck)[90]
(9) *Staphylococcus* spp.	Skin, respiratory, bloodstream, bone and joint, heart infection[91]	(22) *Bordetella parapertussis*	Pertussis, whooping cough[92]
(10) *Streptococcus* spp.	Pneumonia, skin infection[93]	(23) *Borrelia burgdorferi*	Lyme diseases (joint, heart, nervous infections)[94]
(11) *Brucella* spp.	Brucellosis, zoonotic diseases (fever, and joint pain, infections), flu-like infections[91]	(24) *Chlamydia trachomatis*	Lung carcinoma, trachoma, detrimental effects on female reproductive health[95]
(12) *Legionella* spp.	Legionnaires (pneumonia, Pontiac fever)[96]	(25) *Campylobacter* spp.	Campylobacteriosis (diarrhea, cramping, abdominal pain, and fever)[97]
(13) *Helicobacter pylori*	Gastritis, peptic ulcers, stomach cancer[98]	(26) *Serratia marcescens*	Urinary tract infections, ocular lens infections[99]

caused by a variety of bacteria, viruses, and fungi, particularly in environments that support their growth and survival. Pneumonia and diarrhea together are the third cause of death among children under 5 years of age, accounting for 2 million deaths per year.[100] Food and waterborne PB can cause acute or chronic infections in most individuals. A list of 26 human microbial species studied in electrochemical biosensor detection review on 13 PBs are summarized in Table 2. Here, the focus is on literature studies and reporting on electrode fabrication material, testing performance parameters, and concluding remarks on discussing the PBs.

4. Electrochemical biosensors for pathogenic bacteria detection

The electrode is a critical component of an electrochemical sensor that serves as a substrate for attaching the bioreceptor and target analyte while also converting the biological signal into an analogue electric signal. The performance of electrochemical sensors is affected differentially by various electrode materials. When developing high-performance electrochemical sensing platforms that use different analytical techniques to identify target molecules, electrode material is a crucial component in determining the sensitivity of electrochemical sensors.[101] That is the sensitivity of electrochemical sensors can be maximized for a variety of applications by carefully choosing the electrode materials.

4.1. *Salmonella* spp.

One of the main culprits behind bacterial foodborne illnesses worldwide is *Salmonella* spp., which is a Gram-negative bacterium. Many studies reported on the detection of *Salmonilla typhi* by ssDNA modified electrochemical biosensor. Bacchu *et al.* fabricated a highly sensitive gold nanoparticles (AuNPs) and polycysteine (P-Cys) modified screen-printed electrode (SPE), SPE/P-Cys@AuNPs, electrochemical biosensor for the detection of *Salmonella typhi* in anthraquinone-2-sulfonic acid monohydrate sodium salt (AQMS) and characterized by Differential pulse voltammetry (DPV).[102] Target DNA was detected by the developed electrochemical biosensor with a detection range of 1×10^{-6} to 1×10^{-22} mol L^{-1} and a limit of detection (LOD) of 6.8×10^{-25} mol L^{-1}. The modified biosensor has excellent discrimination ability and is reusable up to 6 to 7 times. The developed sensor displayed an excellent detection performance in real samples such as blood, poultry feces, egg, and milk. The detection summary is represented in Fig. 4. Recently all reported electrochemical biosensors research work for *Salmonilla typhi* is shown in Table 3. Nanoporous gold decorated glassy carbon electrode (GCE) aptasensor was also utilized for *Salmonella typhi* detection.

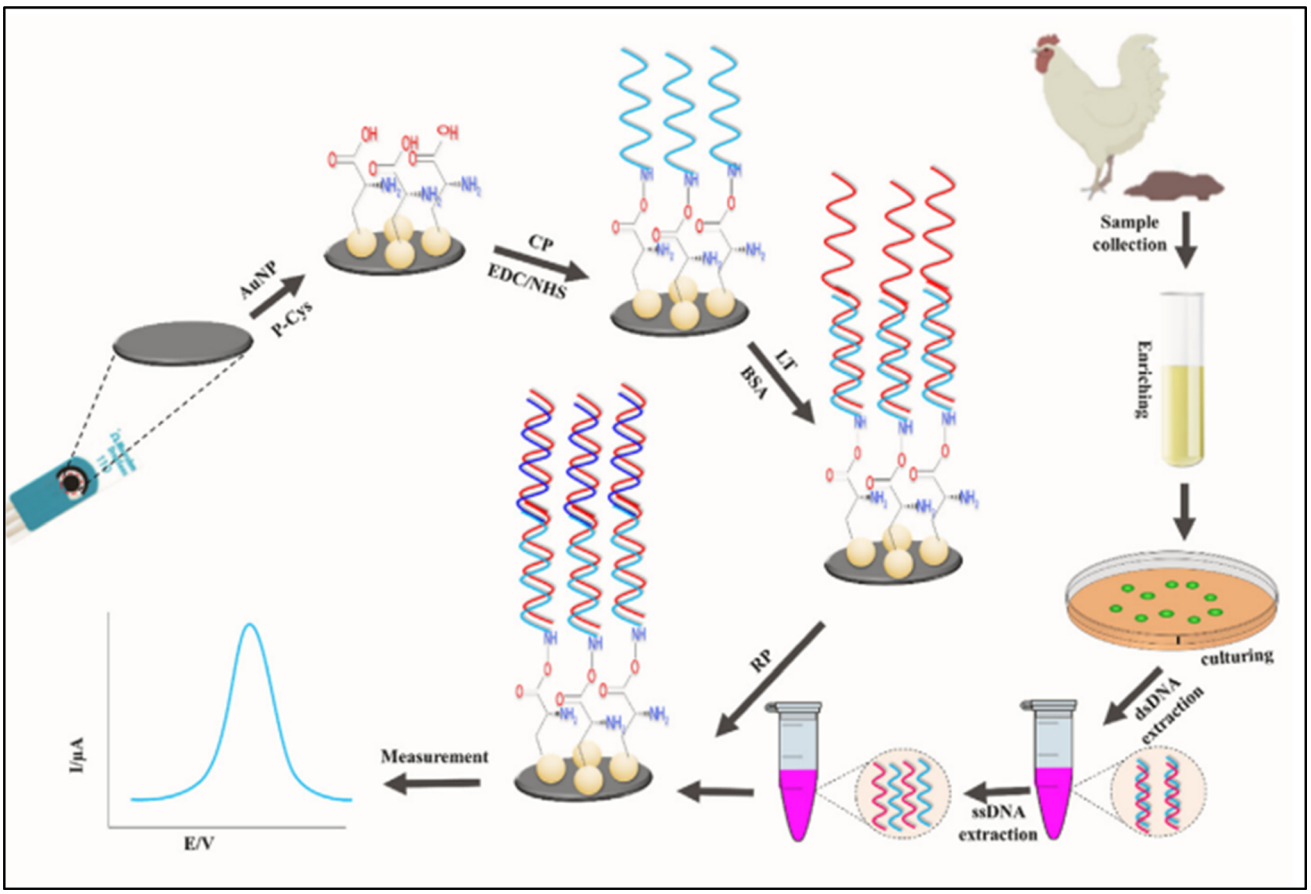

Fig. 4 Schematic illustration for the fabrication of DNA-biosensor and the detection of *Salmonilla Typhi* from real samples. The figure was adapted from ref. 102 and reproduced with permission, copyright @ Elsevier.

Table 3 Examples of the most recent reported electrochemical biosensors for the detection of pathogenic *Salmonella* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
ITO/GO-CHI	NH_2-ssDNA	*Salmonella typhi*	DNA biosensor	DPV	5.0×10^{-8} to 1.0×10^{-14} mol L^{-1}	1.0×10^{-14} mol L^{-1}	PBS	N/A	103
SPE/MPTS-AuNPs	SH-ssDNA	*Salmonella typhi*	DNA biosensor	DPV	5.0×10^{-8} to 1.0×10^{-10} mol L^{-1}	5.0×10^{-11} mol L^{-1}	PBS	Serum	104
GCE/GO/GNPs	SH-ssDNA	*Salmonella*	DNA biosensor	EIS	2.4–2.4 $\times 10^3$ CFU mL^{-1}	3.0 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Pork	105
ITO/GNAs	NH_2-ssDNA	*Salmonella typhi*	DNA biosensor	EIS	2.4×10^{-14} to 4.0×10^{-18} mol L^{-1}	4.0×10^{-18} mol L^{-1}	$[Fe(CN)_6]^{3-/4-}$	Urine and blood	106
Au/Cys/Glu	HO-ssDNA	*Salmonella typhi*	DNA biosensor	EIS	N/A	1.91 mg mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	107
GCE/Au/NPG	SH-ssDNA	*Salmonella typhi*	Aptasensor	EIS	6.5×10^2 to 6.5×10^8 CFU mL^{-1}	1 CFU mL^{-1}	PBS	Egg	108
SPE/P-Cys@AuNPs	HN_2-ssDNA	*Salmonella typhi*	DNA biosensor	DPV	1×10^{-6} to 1×10^{-10}; 1×10^{-10} to 1×10^{-22} mol L^{-1}	6.8×10^{-23} mol L^{-1}	AQMS	Blood, poultry feces, egg, milk	102

[a] ITO: indium tin oxide, CHI: chitosan, GO: graphene oxide, MPTS: organosilane 3-mercaptopropyltrimethoxy silane, GNPs: gold nanoparticles, CFU: colony forming unit, GNAs: gold nanoaggregates, Glu: glutaraldehydes, NPG: nanoporous gold; AQMS: anthraquinone-2-sulfonic acid monohydrate sodium salt, N/A: not/absent.

Electrochemical impedance spectroscopy (EIS) was applied to characterize and evaluate the results of the electrochemical detection.[108] It was concluded that the developed sensor performed well in real samples with the ability to detect and distinguish between live and dead *Salmonella* bacterial cells in egg samples. The main distinguishing mechanism between live and dead bacteria was based on the value of the charge transfer resistance (R_{ct}); it increases after binding with live bacteria and significantly decreases in the presence of dead bacterial cells. Additionally, in another research gold nanoaggregates modified surface was prepared to covalently bond with $5'NH_2$ modified ssDNA probes.[106] The target DNA was hybridized at 35 °C for 60 seconds. The binding efficiency was analyzed by standard EIS techniques in [5 mM $K_3Fe(CN)_6$] in 0.1 mol L^{-1} KCl solution. For the detection of *Salmonilla typhi*, DNA probe modified electrode displayed the most superior performance among the reported electrochemical biosensors.

4.2. *Escherichia* spp.

Among the family of *Escherichia* species, *E. coli* bacterial cells are frequently found in the digestive tracts of healthy individuals; yet a small number of clones are accountable for severe diarrhea and infections beyond the intestines. *Escherichia* spp. was detected by biosensors modified with different bioreceptors such as antibody, DNA and bacteriophage. M. Barreiros *et. al.* reported on the detection of *E. coli* O157:H7 using Anti-*E. Coli* onto an epoxy silane modified indium tin oxide (ITO) electrode (Barreiross dos Santos *et al.*, 2015).[109] In this study, EIS technique was applied with a linear range of 10 to 10^6 CFU mL^{-1} and limit of detection of 1 CFU mL^{-1}. The developed biosensor was found to be highly selective in 1 : 500 *Salmonella typhimurium*/*E. coli* O157:H7 medium. Many of these impedimetric immunosensors displayed high sensitivity and selectivity. Other antibody modified immunosensors were developed and reported for the detection of *Escherichia* spp., and they are listed in Table 4. Furthermore, Xu *et.al.* reported on NH_2-ssDNA modified ssDNA/GO/CS/GCE electrode for the electrochemical detection of *E. coli* O157:H7 using EIS and DPV. In this study, the developed sensor showed an excellent performance on the detection of *E. coli* O157:H7.[118] A T_4 phagosensor was decorated on microelectrochemical sensor with a 3-mercaptoreopionic acid modified gold electrode.[122] Utilizing DPV, the modified sensor showed a limit of detection of 14 ± 5 CFU mL^{-1} and a wide dynamic range of 1.9 × 10^1 to 1.9 × 10^8 CFU mL^{-1}. This phagosensor was successfully able to selectively distinguish between viable and dead bacteria cells. The schematic representation of the AuE/Cys/PDCIT/T_4 phage electrode fabrication aided with applied chemistry is shown in Fig. 5.

Furthermore, T4 bacteriophage modified electrochemical biosensor was developed for rapid detection of live pathogenic bacteria in urine medium with GOx/HRP-$Cu_3(PO_4)$/AuNPs/Thi composite modified electrode. This modified electrode showed a linear range of 15–1.5 × 10^8 CFU mL^{-1} with limit of detection of 1 CFU mL^{-1} in clinical settings.[67] It was concluded that bacteriophage based electrochemical biosensor detection strategy is simple, quick, highly selective, and sensitive.

Table 4 Examples of reported electrochemical biosensors for the detection of pathogenic *Escherichia* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
AuE/Ag@BSA/EDC	Anti-*E. coli*	*E. coli* O157:H7	Immunosensor	DPV	3.0×10^2 to 3.0×10^8 CFU mL^{-1}	N/A	$[Fe(CN)_6]^{3-/4-}$	N/A	110
PGE/PPy/AuNP/MWCNT @Chi	Anti-*E. coli*	*E. coli* O157:H7	Immunosensor	Amperometry	3×10^1 to 3×10^7 CFU mL^{-1}	30 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Food	111
SPE/PDA@GOx-MBs/AuNPs/GOx	Anti-*E. coli*	*E. coli* O157:H7	Immunosensor	Amperometry	10^2 to 10^6 CFU mL^{-1}	10^2 CFU mL^{-1}	Glucose	Beef	112
ITO/Epoxysilane	Anti-*E. coli*	*E. coli* O157:H7	Immunosensor	EIS	10 to 10^6 CFU mL^{-1}	1 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	113
SPCE/CDs/ZnO nanorod/PANI	pDNA	*E. coli* O157:H7	Aptasensor	DPV	1.3×10^{-18} to 10×10^{-12} mol L^{-1}	1.3×10^{-18} mol L^{-1}	$[Fe(CN)_6]^{3-/4-}$	Water	113
AuE/MCE/pDNA/GNSs	pDNA	*E. coli* O157:H7	Genosensor	SWVs	7.3 to 1.0×10^{-17} μmol L^{-1}	1.0×10^{-17} μmol L^{-1}	$(Fe(CN)_6^{3-/4-}$–KCl)	Water	114
GCE/CDs-Fe_3O_4	DNA	*E. coli* O157:H7	DNA biosensor	DPV	10 to 10^8 CFU mL^{-1}	6.88 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Milk, water	115
Au IDE/APTES	COOH-ssDNA	*E. coli* O157:H7	DNA biosensor	Amperometry	1×10^{-16} to 10×10^{-6} mol L^{-1}	0.8 fmol L^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	116
Co-aliginic acid	NH_2-ssDNA	*E. coli*	DNA biosensor	DPV	1.0×10^2 to 2.0×10^3 cells per mL	50 cells mL^{-1}	PBS	Water	117
GCE/GO/CS/EDC/NHS	NH_2-ssDNA	*E. coli* O157:H7	DNA biosensor	EIS	1.0×10^{-14} to 1.0×10^{-8} mol L^{-1}	3.584×10^{-15} mol L^{-1}	$[Fe(CN)_6]^{4-/3-}$	N/A	118
PGE/MWCNT/ssDNA	NH_2-ssDNA	*E. coli*	DNA biosensor	DPV	NA	17 nmol L^{-1}	Guanine	PCR sample	119
AuE/ECA I or ECA II or ECA–MCH	SH-ECA	*E. coli* OMPs	Aptasensor	FIS	1×10^{-7} to 2×10^{-6} mol L^{-1}	NA	$[Fe(CN)_6]^{3-/4-}$	Water	120
GCE/PEI-CNT	T_2-phage	*Escherichia coli B*	Phagosensor	EIS	10^3 to 10^7 CFU mL^{-1}	10^2 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	65
SPE/CNFs	T_4-phage	*Escherichia coli*	Phagosensor	EIS	10^2 to 10^6 CFU mL^{-1}	36 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Apple juice	121
AuE/3-MPA	T_4-phage	*Escherichia coli B*	Phagosensor	DPV	19–1.9×10^9 CFU mL^{-1}	14 ± 5 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	122
SPE/EDC	T_4-phage	*E. coli* K12 cells	Phagosensor	EIS	10^2 to 10^8 CFU mL^{-1}	10^3 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Milk	123
AuE/Cys/PDCIT	T_4-phage	*Escherichia coli*	Phagosensor	EIS	10^2 to 10^7 CFU mL^{-1}	8.0×10^2 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	124
GOx/HRP-$Cu_3(PO_4)$/AuNPs/Thi	T_4-phage	*Escherichia coli*	Phagosensor	EIS	1.5×10^1–1.5×10^8 CFU mL^{-1}	1 CFU mL^{-1}	PBS + $[Fe(CN)_6]^{3-/4-}$	Urine	67
PtE/β-gal	T_7	*Escherichia coli*	Phagosensor	DPV	N/A	10^2 CFU mL^{-1}	PAPG	Water	125

[a] EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; BSA: bovine serum albumin, NHS: *N*-hydroxysuccinimide, MWCNT: multiwall carbon nanotube, PGE: pencil graphite electrode; PPy: polypyrrole; GOx:glucose oxidase; PDA: polydopamine; SPCE: screen printed carbon electrode, CDs: carbon dots, PANI: polyaniline, MCE: mercaptoethanol, GNS: graphene nano sheets; IDE: Interdigitated electrode; APTES: (3-Aminopropyl) triethoxysilane; CS: chitosan, PCR: olymerase chain reaction, MCH: 6-mercapto-1-hexanol, DNR: daunomycin; PEI: polyethylenenimine; CNT: carbon nanotube; CNFs: carbon nanofibers; AuE: gold electrode; EIS: electrochemical impedance spectroscopy; DPV: differential pulse voltammetry; 3-MPA: 3-mercaptoreopionic acid; EI: electrostatic interaction; CL: covalent linkage; Cys: cysteamine; PDCIT: 1,4-dithiocyanate; GOx: glucose oxidase; HRP: horseradish peroxide; AuNPs: gold nanoparticles; Thi: thionine; AMP: Amperometric; AP: alkaline phosphatase; β-gal: β-galactosidase; PAPG: 4-aminophenyl-β-galactopyranoside.

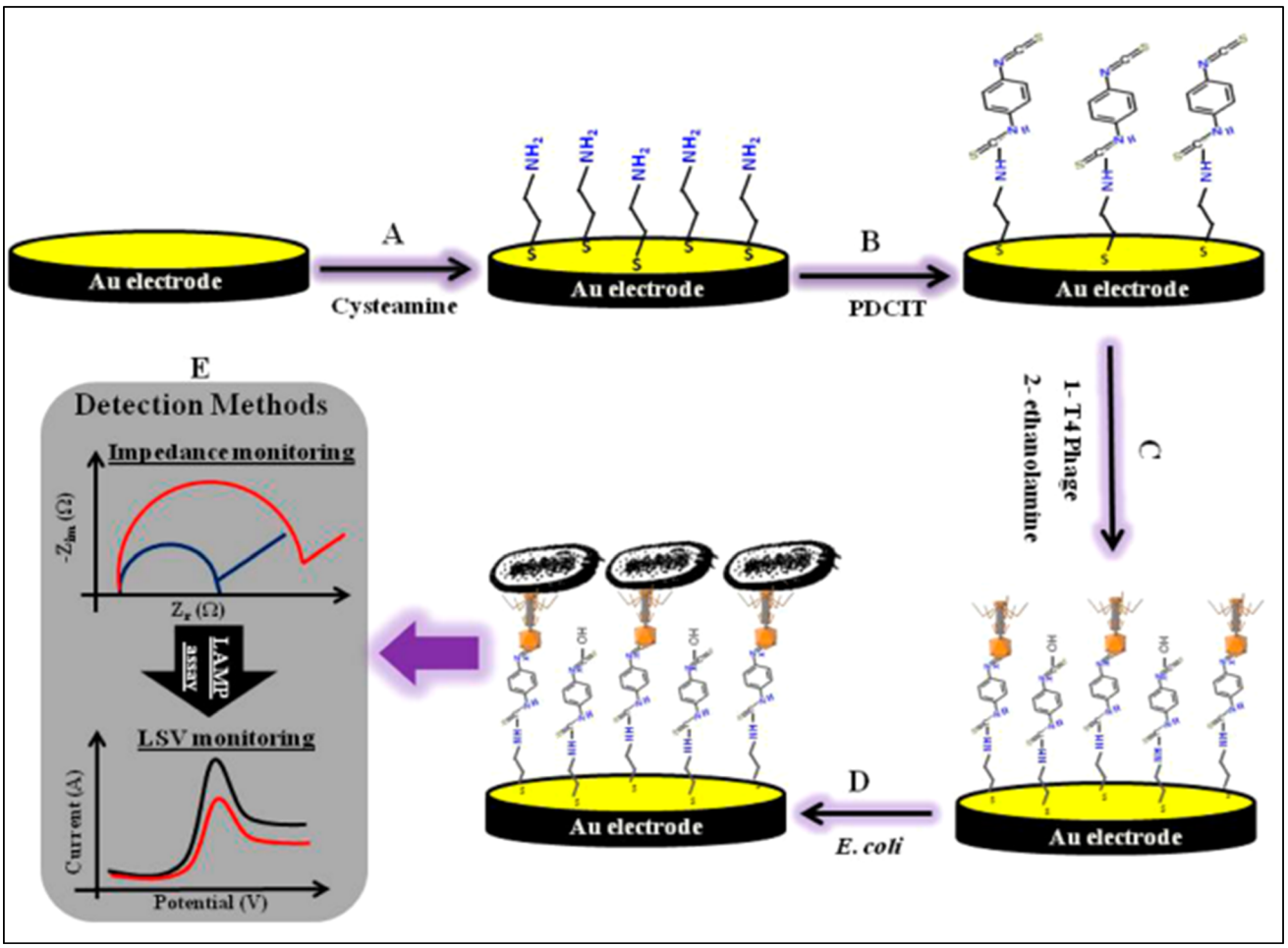

Fig. 5 Schematic illustration showing the fabrication of AuE/Cys/PDCIT/T_4 phage electrode. The figure was adapted from ref. 122 reproduced with permission, copyright @ Elsevier.

4.3. *Vibrio* spp.

Most diseases in humans connected to the natural microbiota of aquatic environments and seafood are caused by Gram-negative bacteria called *Vibrio* spp. *Vibrio cholerae* are among the most common species *of Vibrio* spp. Ali *et al.* designed and fabricated gold nanotube and 3-aminopropyltriethoxysilane/*N*-hydroxysuccinimide modified glassy carbon electrode (GCE/AuNC/APTES/NHS) for DNA modified biosensor for the detection of pathogenic *Vibrio cholera* in real sample.[126] In this work, stepwise GCE modification was performed with analytical confirmation and characterization with SEM and FTIR. The developed biosensor had a dynamic linear range of 10^{-8} to 10^{-14} mol L^{-1} to detect the target DNA. The established electrochemical biosensor was highly sensitive as shown in results summarized in Table 5. Poly (propylene imine) dendrimer (PPI) and gold nanoparticles (AuNP) composite modified electrode was chosen as a platform for antibody immobilization in the development of immunosensor.[133] Anti-cholera toxin antibody was attached to the developed PPI-AuNP composite for final electrode fabrication. The fabricated electrode was able to detect vibrio cholera toxin with a dynamic range of 10^{-7} g mL^{-1} to 10^{-12} g mL^{-1} with two different limits of detection 7.2×10^{-13} and 4.2×10^{-13} g mL^{-1}. It is worth mentioning that the LOD is the lowest reported limit of detection for the detection of vibrio cholera. In another research work, a label-free immunosensor was developed by antibody binding with amino eater composite on cerium oxide nanowire. The graphical representation of this immunosensor is shown in Fig. 6. In immunosensor assay, label free immunosensor was developed for the detection of *Vibrio cholerae O.* The immobilization of anti-*V. cholerae* O1 onto CeO_2 nanowire-deposited sensor was performed *via* an amino ester. The electrochemical response of an immunosensor modified electrode with an immobilized *Vibrio cholerae O* was measured in $[Fe(CN)_6]^{3-/4-}$ using standard EIS method. In the author's opinion, immunity receptor modified electrochemical biosensor detection mechanism have occupied great attention in research field except DNA probe modified detection strategy.

4.4. *Shigella* spp. and *Bacillus* spp.

Shigella spp. are Gram negative bacteria that are most known for their intestinal infection (shigellosis). Ali *et al.* demonstrated a label free electrochemical detection of *Shigella flexneri* in real food samples by applying a series of chemical

Table 5 Examples of reported electrochemical biosensors for the detection of pathogenic *Vibrio* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
GCE/AuNC/APTES/NHS	HN_2-ssDNA	*Vibrio cholerae*	DNA biosensor	DPV	10^{-8} to 10^{-14} 10^{-14} to 10^{-27} mol L^{-1}	7.41×10^{-30} mol L^{-1}	AQMS	Poultry feces	126
ITO/ZrO_2	ssDNA	*Vibrio cholerae*	DNA biosensor	DPV	10^{-10} to 10^{17} mol L^{-1}	10^{-17} mol L^{-1}	Methylene blue	N/A	127
AuE	SH-ssDNA	*Vibrio cholerae*	DNA biosensor	CV	500–100 ng μL^{-1}	100 ng μL^{-1}	Methylene blue	N/A	128
Carbon electrode	npcRNA	*Vibrio cholera*	DNA biosensor	SWV	NA	6×10^{-17} mol L^{-1}	Acetate buffer	PCR sample	129
SPE/AuNPs/PSA	ncDNA	*Vibrio cholera*	DNA biosensor	DPV	10^{-8} to 10^{-17} mol L^{-1}	10^{-21} mol L^{-1}	AQMS	N/A	130
ME/CeO_2@APTES/NHS/EDC	Anti-*V. cholerae* O1	*Vibrio cholerae* O1	Immunosensor	EIS	1.0×10^2 to 1.0×10^4 CFU mL^{-1}	1.0×10^2 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	131
GCE/CNF	Anti-*V. cholera toxin*	*Vibrio cholerae toxin*	Immunosensor	EIS	1.3×10^{-13} to 4.56×10^{-5} g mL^{-1}	1.2×10^{-13} g mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Water	132
GCE/AuNPs-PPI	Anti-cholera toxin	*Cholera Toxin*	Immunosensor	SWV, EIS	10^{-7} to 10^{-12} g mL^{-1}	7.2×10^{-13} (SWV), 4.2×10^{-13} (EIS) g mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	133
ITO/ZnO	Anti-cholera toxin B	*Vibrio cholerae toxin B*	Immunosensor	DPV	12.5–500 g mL^{-1}	0.16 g mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Selective medium	134
ITO/PANnf	Anti-cholera toxin B	*Vibrio cholerae toxin B*	Immunosensor	DPV	6.25×10^{-9} to 5.00×10^{-7} g mL^{-1}	22×10^{-11} g mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Selective medium	135
GCE/CNT/PPy-NTA	Anti-CT	Anti-cholera toxin antibody	Immunosensor	EIS	10^{-13} to 10^{-5} g mL^{-1}	$\sim 10^{-13}$ g mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	136

[a] npcRNA: non-protein coding RNA; PSA: polystyrene-*co*-acrylic acid; ncDNA: non complementary DNA; ME: microelectrode; PPI: poly(propylene imine) dendrimer, PANnf: polyacrylonitrile nanofiber, Anti-CT: anti cholera toxin, PPy-NTA: polypyrrole-nitrilotriacetic acid.

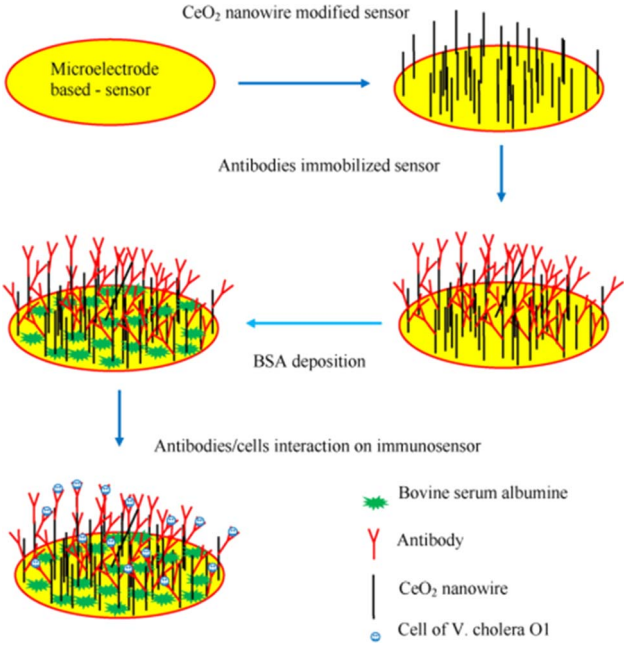

Fig. 6 Schematic illustration of the immunosensor fabrication for the detection of *V. cholera* O1 detection. The figure was adapted from ref. 133 and reproduced with permission, copyright @ Elsevier.

modification to establish a highly efficient immobilization of ssDNA capture probe.[137] For this purpose, poly-melamine, poly-glutamic acid, and disuccinimidyl suberate functionalized indium tin oxide electrode was prepared to immobilize NH_2-ssDNA. Then, using one or two base pair mismatches, a reporter probe, a modified electrode was bound with the linear target. Lastly, the DPV response was measured in anthraquinone-2-sulfonic acid monohydrate sodium salt (AQMS) acting as a redox probe mediator. They concluded that the proposed biosensor could serve as a model methodology for the detection of other pathogens. The ITO/P-Mel/PGA/DSS electrode preparation of the above-mentioned sensor is illustrated in Fig. 7. *Shigella dysenteriae* was detected *via* thiolated aptamer modified electrodeposited GCE.[138] The assay has a linear dynamic range that extends from 10^1 to 10^6 CFU mL^{-1} and a limit of detection of 10° CFU mL^{-1}. The amino functionalized ssDNA, and aptamer-based sensor have extensive applications for the detection of *Shigella* spp. compared with other reported research work.

Bacillus subtilis are among the bacterial family of Bacillus spp., Gram positive bacterial species famous for causing food-borne illness. It was previously reported that SH-ssDNA modified gold electrode was used to detect Bacillus subtilis bacteria utilizing conventional DPV technique.[139] The developed sensor successfully detected the target pathogenic species (*Bacillus subtilis*) with a linear range of 0.1–20 fmol L^{-1} and a limit of detection down to 0.08 fmol L^{-1}. They concluded that the results were in strong agreement with that of quantitative polymerase chain reaction (QPCR) detecting system. Table 6 summaries the reported electrochemical biosensors for the detection of *Shigella* spp. and *Bacillus* spp. In another detection

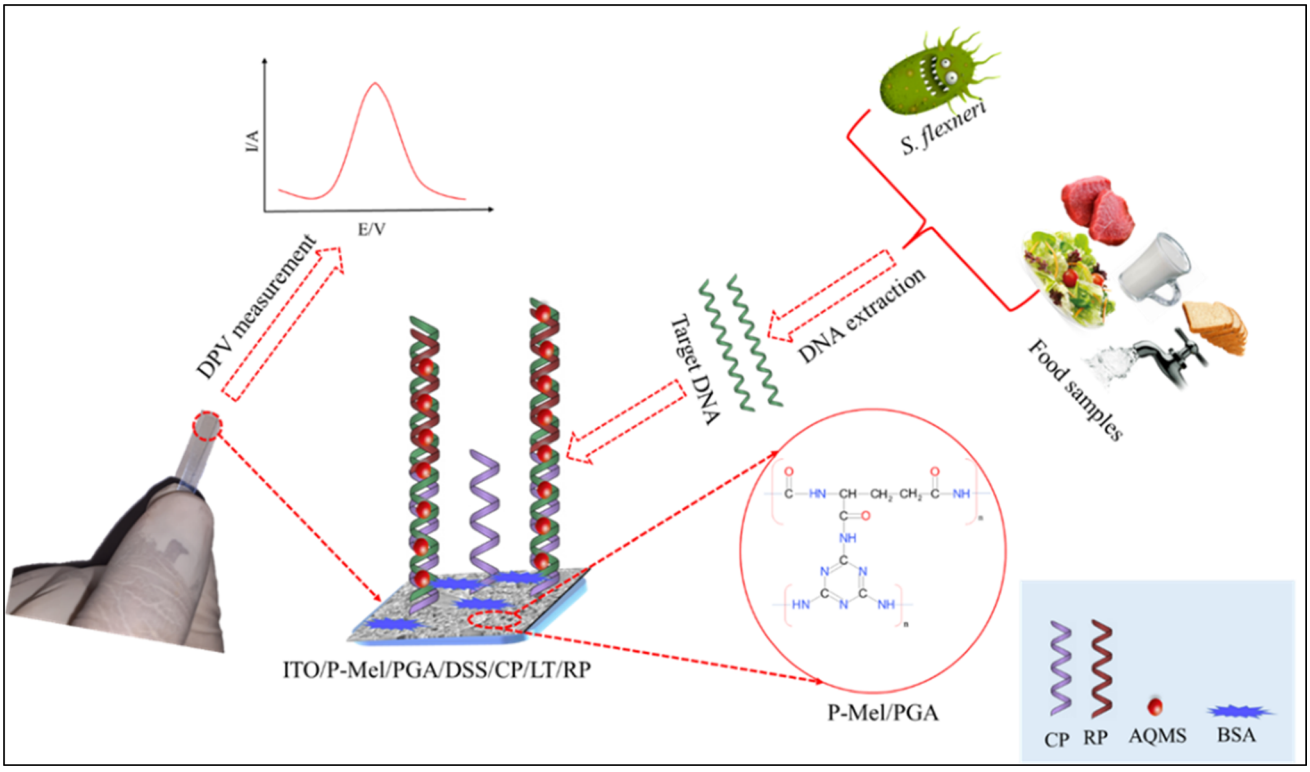

Fig. 7 Schematic diagram illustrating the fabrication process of the ITO/P-Mel/PGA/DSS modified electrode for the detection *Shigella flexneri*. The figure was adapted from ref. 137 and reproduced with permission, copyright @ Elsevier.

Table 6 Examples of reported electrochemical biosensors for the detection of pathogenic *Shigella* spp. and *Bacillus* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
***Shigella* spp.**									
ITO/P-Mel/PGA/DSS	NH_2-ssDNA	*Shigella flexneri*	DNA biosensor	DPV	1×10^{-21} to 1×10^{-6} CFU mL^{-1} 80 to 8×10^{10} cells per mL	10 cells per ml	AQMS	Food	137
GCE/AuNPs	Aptamer	*Shigella dysenteriae*	Aptasensor	EIS	10^1 to 10^6 CFU mL^{-1}	1.0 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Mik, water	138
SPE/HOOCA	Aminated-DNA aptamer	*S. typhimurium*	Aptasensor	EIS	10^1 to 10^8 CFU mL^{-1}	6 CFU mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Apple juice	140
SPE/MWCNT/SA	Anti-*S. flexneri*	*Shigella flexneri*	Immunosensor	CV	10^4 to 10^{10} CFU mL^{-1}	3.1×10^3 CFU mL^{-1}	HAc–NaAc buffer	N/A	141
SPE/MWCNT/CHI	Anti-*S. flexneri*	*Shigella flexneri*	Immunosensor	CV	104 to 10^{10} CFU mL^{-1}	2.3×10^3 CFU mL^{-1}	HAc–NaAc buffer	N/A	142
Carbon electrode	npcRNA	*Shigella flexneri*	DNA biosensor	SWV	N/A	6×10^{-17} mol L^{-1}	Acetate buffer	PCR product	129
***Bacillus* spp.**									
GE	SH-ssDNA	*Bacillus subtilis*	DNA biosensor	DPV	0.1 fmol L^{-1} to 20 fmol L^{-1}	0.08 fmol L^{-1}	N.BstNBI and NEBuffer 3	PCR product	139
PGE/GNPs-SH	SH-ssDNA	*Bacillus cereus*	DNA biosensor	EIS	0.02–10 nmol L^{-1} 0.01–10 μmol L^{-1}	100 CFU mL^{-1} 9.4×10^{-12} mol L^{-1}	$[Fe(CN)_6]^{4-/3-}$	Food	143
AuE/SWCNTs/1-PBSE	Anti-*B. subtilis*	*Bacillus subtilis*	Immunosensor	Potentiostatic	10^2 to 10^{10} CFU mL^{-1}	10^2 CFU mL^{-1}	PBS	Dust	144
AuE/Peptide/MCH	Peptide	*Bacillus licheniformis*	Peptide biosensor	DPV	0.5–100 μg mL^{-1}	0.16 μg mL^{-1}	PBS	Serum, saliva	145
GCE/Chit/GNPs	Anti-*Bacillus cereus*	*Bacillus cereus*	Immunosensor	Amperometric	5.0×10^1 to 5.0×10^4 CFU mL^{-1}	10.0 CFU mL^{-1}	MYP medium	Milk	146
SPCE/immuno-c/sNP	Anti-*Bacillus cereus*	*Bacillus cereus*	Immunosensor	CV	N/A	40 CFU mL^{-1}	HCl solution	Food, water	147

[a] P-Mel: poly-melamine, PGA: poly-glutamic acid, DSS: disuccinimidyl suberate, HOOCA: 4-amino benzoic acid tetrafluoroborate, SA: sodium aliginate, GE: gold electrode; GNPs: gold nanoparticles, MCH: mecatohexanol; GNPs: gold nanoparticles; PBSE: pyrenebutanoic acid succinimidyl ester.

strategy, protein-based *Bacillus licheniformis* detection strategy was developed by H. Wu *et al.*[145] In this assay, the biosensor was constructed using a D-amine acid containing substrate peptide *via* self-assembly of cysteine residual at the C-terminal. A biotin modifier was labelled at the N-terminal of the substrate peptide. This enabled the sensitive electrochemical detection of the intact substrate peptide using a streptavidin-conjugated alkaline phosphatase, which catalyzes the conversion of electrochemically inactive 1-naphthyl phosphate into electrochemically active phenol. Under optimized conditions, the protease can be determined in concentration range from 0.5 to 100 mg mL^{-1} with a detection limit to 0.16 mg mL^{-1}. From Table 6 and the performance parameter analysis, it is evident that DNA probe and peptide based electrochemical biosensor reported the best result for the detection of *Bacillus* spp.

4.5. *Clostridium* spp. and *Neisseria* spp.

Clostridium spp. are Gram positive bacteria, cause bacterial infection diseases such as botulism and tetanus. Qian *et al.*,[148] developed a nanocomposite modified ssDNA/CeO_2/CHIT/GCE biosensor for the detection of *Clostridium perfringens* extracted from dairy products. With favorable selectivity, they were able to achieve a linear dynamic range of 1.0×10^{-14} to 1.0×10^{-7} mol L^{-1} and a limit of detection of 7.06×10^{-15} mol L^{-1}. *Neisseria* spp. are Gram negative bacterial microorganisms that are incriminated for causing human diseases such as gonorrhea, leprosy, and *Mycobacterium leprae*. In another study, antitoxin B single domain antibody receptor was modified to decorate polyurethane (PU) nanospiked gold electrode-based label-free electrochemical immunosensor for *Clostridium difficile* toxin B detection.[149] This electrochemical immunosensor can detect within a concentration range of 1–130 pg mL^{-1} and a limit of detection of 0.5 pg mL^{-1}. Therefore, it can be concluded that immunosensor reported the lowest detection capability amongs different bioreceptor modified electrochemical biosensors.

Singh *et al.* developed DNA-biosensor for the detection of a sexually transmitted disease, gonorrhoeae, caused by *Neisseria gonorrhoeae*, pathogenic bacteria.[150] In this study, thiolated capture probe was immobilized on gold electrode and 6-Mercapto-1-hexanol (MCH) was used to block nonspecific agents to facilitate oligos "stand". A complete graphical representation of the electrode preparation and DPV analysis in methylene blue (MB) medium is shown in Fig. 8. The most updated reported research is summarized in Table 7. 5′ Aminated capture probes and super sandwiched detector probes were utilized for the detection of *Neisseria gonorrhoeae.* The capture probe modified surface hybridize with gonorrheal DNAs and after sandwich type detector probe amplified the detection signal. Further, the biosensing assay displayed a wide linear range of 100 aM to 100 nM (10^9 orders of magnitude) with an excellent sensitivity of 22.6 kΩ·(log[concentration])$^{-1}$. This type of strategic detection occupies a prizeworthy position in the research community.

4.6. *Mycobacterium* spp.

Mycobacterium spp. are Gram positive bacteria causing several infectious human diseases such as Tuberculosis, leprosy, Buruli ulcer and tuberculous mycobacterium. Among Mycobacterium spp., *Mycobacterium tuberculosis*-based DNA detection

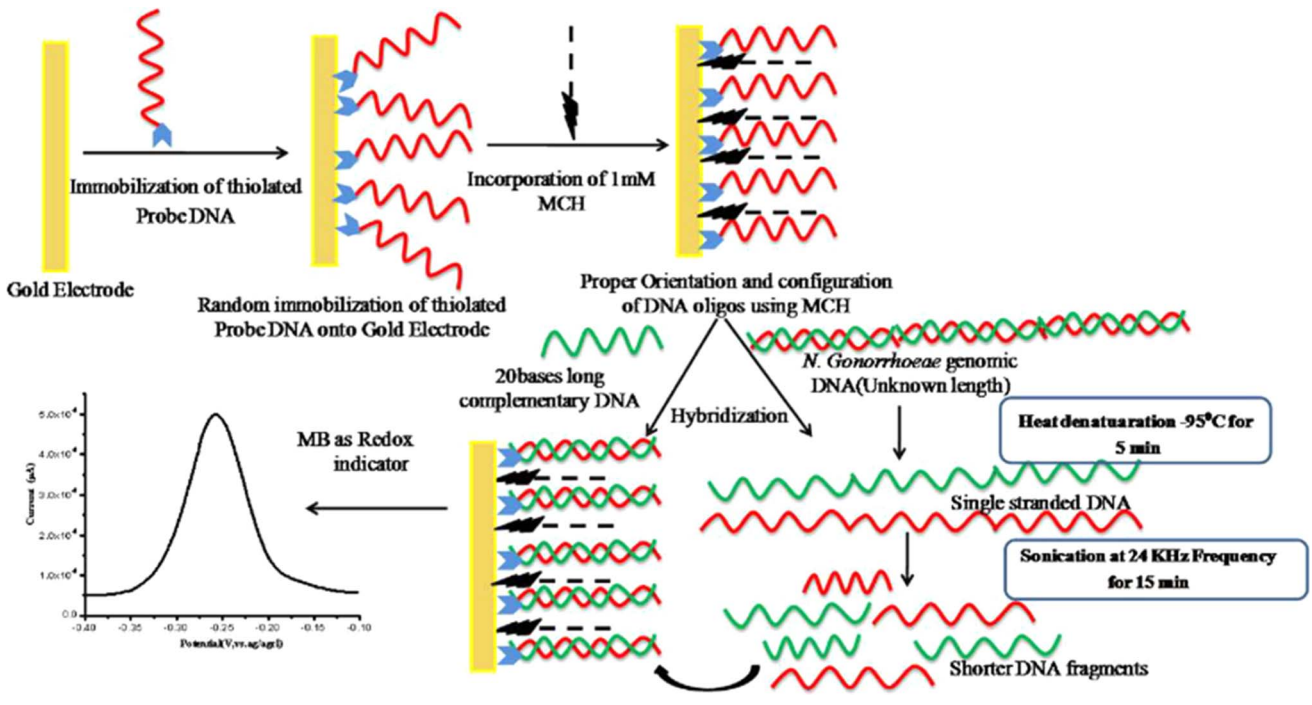

Fig. 8 Schematic diagram showing the fabrication process of the ssDNA-Au electrode for the detection of *Neisseria gonorrhoeae*. The figure was adapted from ref. 150 and reproduced with permission, copyright @ Elsevier.

Table 7 Examples of reported electrochemical biosensors for the detection of pathogenic *Clostridium* spp. and *Neisseria* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
***Clostridium* spp.**									
GCE/CHIT/CeO_2	ssDNA	*Clostridium perfringens*	DNA biosensor	EIS	10^{-14} to 10^{-7} mol L^{-1}	7.06×10^{-15} mol L^{-1}	$[Fe(CN)_6]^{3-/4-}$	Milk	148
GCE/MWCNT/AuNP	ssDNA	*Clostridium tetani*	DNA biosensor	DPV	NA	1.0×10^{-16} mol L^{-1}	MB	N/A	151
AuE/PU	Anti-C. Difficile toxin B	*Clostridium difficile*	Immunosensor	DPV	1–130 pg mL^{-1}	0.5 pg mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Stool	149
SPE/AuNPs	SA aptamer	*Clostridium perfringens*	Aptasensor	DPV	10^{-12} to 10^{-6} mol L^{-1}	10^{-12} mol L^{-1}	PBS	N/A	152
***Neisseria* spp.**									
C5@paper/cMWCNT	NH_2-ssDNA	*Neisseria gonorrhoeae*	DNA biosensor	EIS	(5 zmol–5 pmol)	(45 aM)	PBS	Selective medium	153
SPAuE	SH-ssDNA	*Neisseria gonorrhoeae*	DNA biosensor	DPV	1×10^{-15} to 1×10^{-22} mol L^{-1}	NA	PBS	N/A	154
AuE	SH-ssDNA	*Neisseria gonorrhoeae*	DNA biosensor	DPV	1.0×10^{-6}–0.5×10^{-18} mol L^{-1}	1.0×10^{-18} mol L^{-1}	PBS + MB	Selective medium	150
Glass subs/Cr/AuNPs	SH-ssDNA	*Neisseria gonorrhoeae*	DNA biosensor	CV	10–60 ng μl^{-1}	NA	PBS + MB	N/A	155
Si/Pt/ZNF	SH-ssDNA	*Neisseria meningitidis*	DNA biosensor	DPV, EIS	5–240 ng μl^{-1}	5 ng μl^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	156

[a] cMWCNT: carboxylated MWCNT; ZNF: zinc oxide nanoflower; PU: polyurethane, SA: streptavidin.

mechanism was reported based on gold nanotube array (AuNTsA) electrode platform with a 1.5 μm in length and 200 nm in diameter.[157] For the biosensor fabrication, AuNTsA was vertically aliened on Au thick film during nanotubes synthesis process and a DNA probe was immobilized using Tris–EDTA for 12 hours. Next, different complementary DNA was immobilized on captured DNA probe in an incubated environment at 37 °C for 45 minutes. The schematic representation for the biosensor decoration is presented in Fig. 9. Recently, another research work was carried out for the early diagnosis of tuberculosis caused by ESAT-6 antibody.[158] A Ni-rGO-PANI composite film modified electrode was fabricated

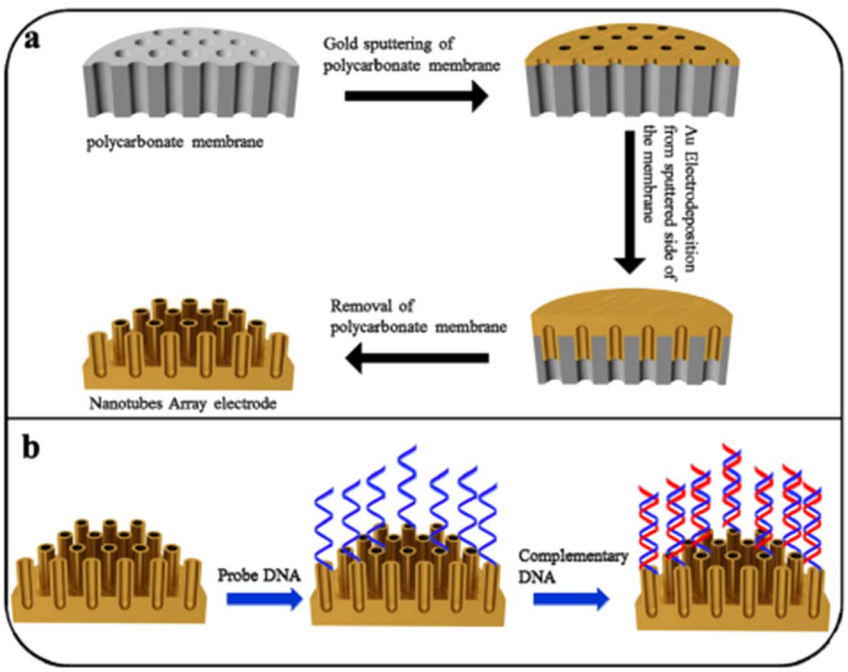

Fig. 9 Graphical representation of (a) AuNTsA synthesis; (b) decoration of DNA based biosensor on AuNTsA electrodes. The figure was adapted from ref. 157 and reproduced with permission, copyright @ Elsevier.

Table 8 Examples the previously reported electrochemical biosensors for the detection of pathogenic *Mycobacterium* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
FTO/APTES/PDITC	NH_3-ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	CV	1–100 nmol L^{-1}	1.5 ng μL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Sputum, urine	159
SPAuE	SH-ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	DPV	0.5–10 nmol L^{-1}	1.90 nmol L^{-1}	$[Fe(CN)_6]^{3-/4-}$	Sputum	160
AuNTsA	SH-ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	DPV	0.01 ng μL^{-1} to 100 ng μL^{-1}	0.05 ng μL^{-1}	$[Fe(CN)_6]^{3-/4-}$	N/A	157
GCE/AuNPs-rGO	SH-ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	DPV	1.0×10^{-15} to 1.0×10^{-9} mol L^{-1}	NA	PBS	Selective medium	161
ITO/APTES/AuNPs	SH-ssDNA	*Mycobacterium* sp. genomic DNA.	DNA biosensor	DPV	1.25 to 50 ng mL^{-1}	1.25 ng mL^{-1}	Tris–HCl	Sputum	162
SPCE/MPA-Fe_3O_4/NCC/CTAB/EDC/NHS	ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	DPV	1.0×10^{-6} to 1.0×10^{-12} mol L^{-1}	7.96×10^{-13} mol L^{-1}	$Ru(bpy)_3^{2+}$	Selective medium	163
SPCE/PANI/GP/EDC-NHS	ssDNA	*Mycobacterium tuberculosis*	DNA biosensor	DPV	10^{-6} to 10^{-9} mol L^{-1}	7.853×10^{-7} mol L^{-1}	PBS + MB	N/A	164
PANI-rGO-Ni-DSP	Anti-ESAT-6	ESAT-6	Immunosensor	CV	1–100 ng mL^{-1}	1.042 ng mL^{-1}	PBS	Blood	158
SPGE/GP/PANI/EDC-NHS	Anti-CFP10-ESAT6	CFP10-ESAT6	Immunosensor	DPV	10–500 ng mL^{-1}	1.5 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Sputum	165
SPGE/GP/PANI	Anti-CFP10	CFP10 antigen	Immunosensor	DPV	20–100 ng mL^{-1}	15 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Sputum	166
SPGE/PANI/GP	CapAptamer	CFP10-ESAT6	Aptasensor	DPV	5–500 ng mL^{-1}	1.5 ng mL^{-1}	PBS	Sputum	167

[a] FTO: tin-doped fluorine, PDITC: phenylene diisothiocyanate, MPA: mercaptopropionic acid, NCC/CTAB: nanocellulose crystalline functionalized cetyltrimethyl ammonium bromide, SPGE: screen-printed gold electrode.

Table 9 Examples of reported electrochemical biosensors for the detection of pathogenic *Staphylococcus* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
GCE@AuNP	IgY	*Staphylococcus aureus*	Immunosensor	EIS	10 to 10^7 CFU mL^{-1}	3.3 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Milk, blood	168
AuE/SAMs	Anti-*S. aureus*	*Staphylococcus aureus*	Immunosensor	EIS	10^1 to 10^7 CFU mL^{-1}	10 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	N/A	170
TTF-MPA-AuE	RbIgG	*Staphylococcus aureus*	Immunosensor	Amperometric	2.2×10^5 to 9.2×10^5 CFU mL^{-1}	1.6×10^5 CFU mL^{-1}	NaAc/NaCl buffer	N/A	171
PtE/GA-PEI	Anti-*S. aureus*	*Staphylococcus aureus*	Immunosensor	Amperometric	10^1 to 10^8 CFU mL^{-1}	10 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Milk, cheese meat	172
GCE/CNFs/CNPs/AuNPs	SH-anti-*S. aureus*	*Staphylococcus aureus*	Aptasensor	EIS	1.2×10^1 to 1.2×10^8 CFU mL^{-1}	1 CFU mL^{-1}	$Fe(CN)_6^{3-/4-}$	Serum	173
MBs	Anti-*S. aureus*	*Staphylococcus aureus*	Aptasensor	DPV	$10^{-1} \times 10^6$ CFU mL^{-1}	1.0 CFU mL^{-1}	Buffer C	Water	174
GCE/MOFs/AuNPs	SH-DNA	*Staphylococcus aureus*	Aptasensor	DPV	7–7 $\times 10^6$ CFU mL^{-1}	1.9 and 5.2 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Urine	175
AuE	DNA nanoflowers	*Staphylococcus aureus*	Aptasensor	DPV	60–6 $\times 10^7$ CFU mL^{-1}	9.0 CFU mL^{-1}	MB	Water, honey	176
GCE/AuNP-ssDNA-rGO	ssDNA	*Staphylococcus aureus*	Aptasensor	EIS	10 to 10^6 CFU mL^{-1}	10 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Food	177
SPGE	Aptamer	*Staphylococcus aureus*	Aptasensor	CV	N/A	39 and 414 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Water	178
GCE/BMZIF-derived NPCs	dsDNA	*Staphylococcus aureus*	DNA biosensor	DPV	N/A	3.7 fmol L^{-1} and 1.6 fmol L^{-1}	PBS	Water	169
SPGE-cys-GGP	GGP	*Staphylococcus aureus*	Biosensor	CV	3×10^2 and 3×10^8 CFU mL^{-1}	10^2 CFU mL^{-1}	$[Fe(CN)_6]^{4-/3-}$	Food	179
BC/c-MWCNTs-PEI	Phage	*Staphylococcus aureus*	Phagosensor	DPV	3×10^0 to 3×10^7 CFU mL^{-1}	3 CFU mL^{-1}	PBS	Milk, food	14
SPE/CNT-f-PEI	Phage	*Staphylococcus aureus*	Phagosensor	EIS	10^2 to 10^7 10^2 to 10^5 CFU mL^{-1}	1.23×10^2 1.29×10^2 CFU mL^{-1}	$Fe(CN)_6^{3-/4-}$	Blood plasma	180
SPE-COOH	Phage	*Staphylococcus aureus*	Phagosensor	EIS	2.0–2.0 $\times 10^6$ CFU mL^{-1}	N/A	$Fe(CN)_6^{3-/4-}$	Apple juice, water	181

[a] IgY: anti-protein antibody, SAM: self-assembled molecular monolayer, TTF: tetrathiafulvalene, MB: magnetic bead, BMZIF: Co–Zn bimetallic ZIF, GGP: guinea grass leaves.

for the immobilization of anti-ESAT-6 antibody. They reported a linear range of 1–100 ng mL^{-1} and a detection limit of 1.042 ng mL^{-1}. Table 8 summarizes the previously reported electrochemical biosensors for the detection of *Mycobacterium* spp. A sensitive electrochemical DNA biosensor based on functionalized iron oxide with mercaptopropionic acid (MPA-Fe_3O_4) nanoparticle for the detection of *Mycobacterium tuberculosis* was reported.[163] A DNA probe was immobilized on MPA-Fe_3O_4/NCC/CTAB electrode and sequentially bond with the target DNA and signal amplification, ruthenium bipyridyl $Ru(bpy)_3^{2+}$. The sensing mechanism offered a wide detection range of 1.0×10^{-6} to 1.0×10^{-12} M and limit of detection of 7.96×10^{-13} M.[163] Therefore, it can be concluded that with some limitations DNA biosensor, immunosensor, and aptasensor bioreceptor modified biosensor showed good result for the detection of *Mycobacterium* spp.

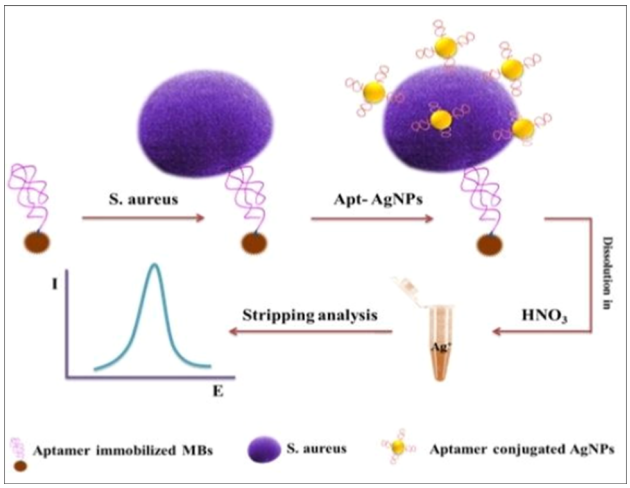

Fig. 10 Aptamer-based electrochemical determination of *S. aureus*. The figure was adapted from ref. 173 and reproduced with permission, copyright @ Elsevier.

4.7. *Staphylococcus* spp.

Gram-positive *Staphylococcus* spp. species can cause suppuration and other infectious illnesses in both people and animals. In 2020, Roushani *et al.*[168] described the use of chicken IgY antibody as an immunosensing agent that was covalently bound to AuNPs as a biosensor based on AuNPs modified GCE for the detection of *Staphylococcus aureus*. The antibody was bonded with G-producing Staphylococcus and the interference from EIS study was reported for the detection of *Staphylococcus aureus*.

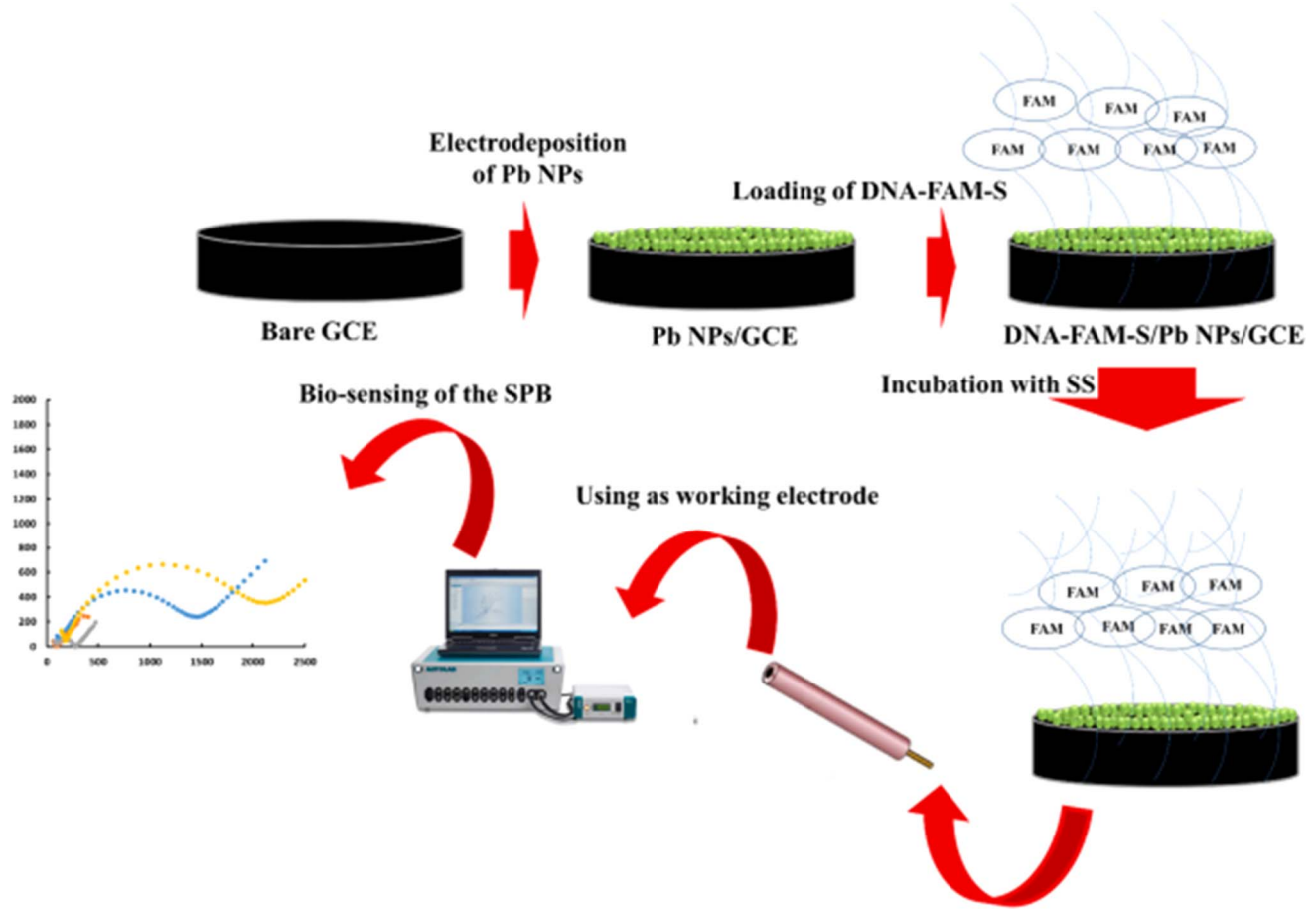

Fig. 11 Schematic diagram showing the preparation procedure for AuE/AuNPs/CysA/pDNA electrode and the detection of SPB. The figure was adapted from ref. 182 [open access].

Table 10 Examples of previously reported electrochemical biosensors for the detection of pathogenic *Streptococcus* spp. *and Legionella* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
***Streptococcus* spp.**									
AuE	DNA tetrahedron	*Streptococcus pneumonia lysate*	Immunosensor	SWV	5–100 ng mL^{-1}	0.093 ng mL^{-1}	PBS	Axilla, nasal cavity, mouth	184
SPGE/GA	Anti-*Streptococcus pyogenes*	Anti-*Streptococcus pyogenes*	Immunosensor	EIS	4.2×10^2 to 4.2×10^6 CFU mL^{-1}	9.3 CFU mL^{-1}	$Fe(CN)_6^{3-/4-}$	N/A	185
GCE/Pb NPs	DNA-FAM-S	*Streptococcus Pneumoniae*	DNA biosensor	EIS	0.0031 ng mL^{-1}- 0.4 ng mL^{-1}	0.0022 ng mL^{-1}	$Fe(CN)_6^{3-/4-}$	N/A	182
***Legionella* spp.**									
AuE/6-MHA	Anti-*L. pneumophila*	Lp02-GFP-*L. pneumophila*	Immunosensor	EIS	N/A	2.0×10^2 cells per mL	PBS	N/A	186
SPE/TMB	LP3IIG2 anti-*Legionella*	*Legionella pneumophila*	Immunosensor	Chronoamperometry	10^1 and 10^4 CFU mL^{-1}	4 CFU mL^{-1}	PBS	N/A	187
SPGE/16-AHT	Anti-*Legionella*	*Legionella pneumophila*	Immunosensor	SWV	10 to 10^8 CFU mL^{-1}	10 CFU mL^{-1}	$Fe(CN)_6^{3-/4-}$	Artificially contaminated samples	188
PtE/GA	ssDNA	*Legionella pneumophila*	DNA biosensor	DPV	10^{-13} to 10^{-6} mol L^{-1}	3.1×10^{-13} mol L^{-1}	$Fe(CN)_6^{3-/4-}$	N/A	189
AuE/AuNPs/Cys a	ssDNA	*Legionella pneumophila*	DNA biosensor	SWV	1 μmol L^{-1} to 1 zmol L^{-1}	1 zepto-molar	Tris–HCl	N/A	183

[a] 6-MHA: 6-mercaptohexanoic acid, TMB: 3,3′,5,5′-tetramethylbenzidine, 16-AHT: 16-amino-1-hexadecanethiol.

This biosensor reported by this work displayed a wide linear dynamic range from 10 to 10^7 CFU mL^{-1} and a limit of detection of 3.3 CFU mL^{-1} with a 3.0% of relative standard deviation (RSD). This sensor successfully identified the pathogenic species in both milk and human blood serum. In another study, nuc and mecA genes of methicillin-resistant *Staphylococcus aureus* (MRSA) was detected for the quantification of *Staphylococcus aureus*.[169] For this purpose, methylene blue (MB) and epirubicin (EP) were encapsulated in UiO-66-NH_2 and locked by hybrid double-stranded DNA. Based on an electroactive dye release approach, the target DNA hybridizes with the displacement DNA (DEP and DMB) from metal–organic frameworks (MOF). The detection performance was significantly enhanced by Co–Zn bimetallic zeolitic imidazolate framework-derived *N*-doped porous carbon (BMZIF) nanocomposite modified electrode compared with intrinsic electrode. Table 9 presents examples of the previously reported electrochemical biosensors for the detection of *Staphylococcus* spp.

Additionally, a sensitive and specific *Staphylococcus aureus* detection system was developed using a gold nanoparticle/carbon nanoparticle/cellulose nanofiber nanocomposite (AuNPs/CNPs/CNFs) synthesized on the surface of GCE. This combination worked as a sensing element, immobilizing a specific *S. aureus* aptamer. With a LOD of 1 CFU mL^{-1}, the fabricated aptasensor demonstrated a broad linear dynamic range (1.2×10^1 to 1.2×10^8) CFU mL^{-1} and was able to precisely identify and quantify *Staphylococcus aureus* in human blood serum, a clinical sample with a complicated matrix. The published research on an electrochemical biosensor for the detection of pathogenic *Staphylococcus* species showed good performance, with a few limitations.[173] The working procedures are summarized and presented in Fig. 10.

4.8. *Streptococcus* spp. *and Legionella* spp.

A Gram-positive bacterium called Streptococcus spp. is linked to bacterial intra-mammary infections in bovine that cause mastitis. Recently, Yaghoobi *et al.*[182] demonstrated a research work on the detection and quantification of *Streptococcus Pneumoniae* bacteria (SPB) with lead nanoparticles (Pb NPs) and DNA-FAM-S modified GCE. After binding with the complementary targeted DNA, the electrode performance was measured by standard EIS technique. The systematic representation is shown in Fig. 11. They concluded that the produced biosensor's selectivity is on par with that of the standard NanoDrop technique. In a different study, an electrochemical immunosensor was created by anchoring DNA tetrahedrons (DNA TH) with hollow structures to gold electrodes. This allowed for the quick detection of pneumococcal surface protein A (PspA) peptide and *Streptococcus pneumoniae* lysate from both synthetic and real human samples. Furthermore, with a LOD of 0.093 CFU mL^{-1}, the developed DNA-TH-based immunosensor demonstrates strong sensing efficacy against *Streptococcus pneumoniae* lysate in a therapeutically relevant linear range from 5 to 100 CFU mL^{-1}. This leads to the conclusion that Streptococcus spp. can be effectively detected in immunological and biosensors using electrochemical sensing technology.

Literature revealed that *Legionella pneumophila* was detected by DNA based bioassay of square wave voltammetry technique.[183] In this study, mip gen of *Legionella pneumophila* was detected by AuE/AuNPs/Cys A/pDNA biosensor. A linear dynamic calibration line with a range of 1 μmol L^{-1} to 1 zmol L^{-1} and low limit of detection were reported. These results are among the highest for the detection of *Legionella pneumophila*. Table 10 shows some examples of the previously reported electrochemical biosensors for the detection of *Streptococcus* spp. and *Legionella* spp. Additionally, complementary DNA was hybridized to provide a DNA probe-based detection technique.[189] For the quantification of the ss 21mer DNA sequence, the developed biosensor showed a wide linear range over seven orders of magnitude with an ultrasensitive detection limit of 3.1×10^{-13} M. It also selectively distinguished the complementary sequence from target sequences that had single base mismatches (MM1) and triple base mismatches (MM3) of

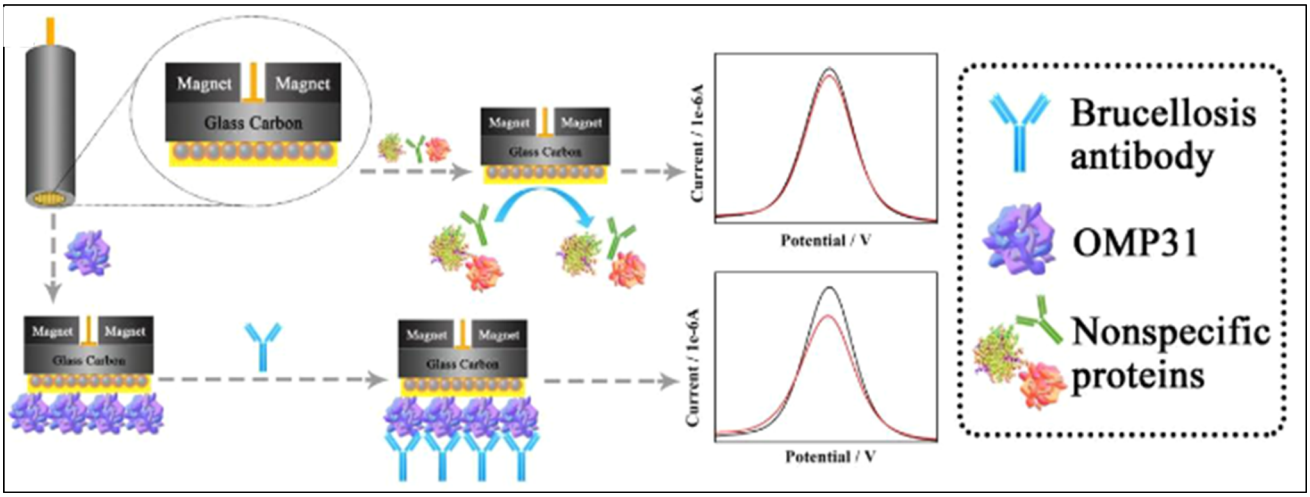

Fig. 12 Schematic diagram explanting the fabrication of Fe_3O_4@Au@PEG@HA NPs electrochemical immunosensor. The figure was adapted from ref. 190 and reproduced with permission, copyright @ Elsevier.

Table 11 Examples of reported electrochemical biosensors for the detection of pathogenic *Brucella* spp.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
Fe_3O_4@SiO_2@p(PEG-MA-GMA)	Aptamer	*Brucella melitensis*	Aptasensor	EIS	10^2 to 10^7 CFU mL^{-1}	N/A	PBS	Milk, milk products	192
AuE/nAu	p-ssDNA	*Brucella genome*	DNA biosensor	DPV	10 zmol dm^{-3} to 10 pmol dm^{-3}	1.71 zmol dm^{-3}	MB	N/A	191
AuE	ssDNA	Cas12/crRNA/target DNA	DNA biosensor	SWV	4.5×10^8 to 4.5×10^1 copies per reaction	2 copies per reaction	MB	Blood, milk	193
AuE/nPd	p-ssDNA	t-ssDNA	Genosensor	DPV	1.0×10^{-12} to 1.0×10^{-19} mol dm^{-3}	2.7×10^{-20} mol dm^{-3}	$Fe(CN)_6^{3-/4-}$	Serum	194
(GO–NW@Au-NF)FTO/Au-NF@NW-GO	OMP31 antigen	Br-antibody	Immunosensor	DPV	N/A	9.3 fg mL^{-1}	PBS	Serum	190
GCE/Fe_3O_4@Au@PEG@HA NPs	OMP31 antigen	Brucellosis antibody	Immunosensor	DPV	10^{-15} g mL^{-1} to 10^{-11} g mL^{-1}	0.36 fg mL^{-1}	PBS	Serum	190
GCE/AuNPs/GSH/HA	OMP31 antigen	Antibody	Immunosensor	DPV	2.08×10^{-15} to 1.04 $\times 10^{-12}$ g mL^{-1}	0.50 fg mL^{-1}	$Fe(CN)_6^{3-/4-}$	Serum	195
SPCE/GNP	Anti-*Brucella melitensis*	*Brucella melitensis*	Immunosensor	EIS	4×10^4 to 4×10^6 CFU mL^{-1}	1×10^4 CFU mL^{-1}	$Fe(CN)_6^{3-/4-}$	Milk	196

[a] PEG-MA: poly(ethyleneglycol)-methacrylate, GMA: glycidylmethacrylate, PEG: polyethylene glycol, HA: hyaluronic acid, GSH: glutathione.

different strains of *Legionella* spp. In contrast, other immunosensor showed unsatisfactory results.[186]

4.9. *Brucella* spp.

The Gram-negative *bacterium Brucella* spp. is the primary cause of the infectious, communicative, and contagious disease brucellosis, which mostly affects cattle, bison, and pigs. The development of novel composite materials and effective detection approach could address these issues. Fe_3O_4@Au nanocomposite has been widely used to fabricate electrochemical biosensor. A poly(ethylene glycol) (PEG) and hyaluronic acid (HA) modified Fe_3O_4@Au NPs electrochemical immunosensor was proposed by Lv *et al.* to detect disease markers that are specific to brucellosis antibodies.[190] The graphical explanation of the devolped sensor is shown in Fig. 12. It's interesting to note that this immunosensor can test in 100% serum without biological interference and has a linear response range of 10^{-15} to 10^{-11} g mL^{-1} towards brucellosis antibodies. Rahi *et al.*[191] created a three-dimensional nanostructure with gold nanoribbons encased in gold nanoblooms using the sonoelectrodeposition technique. The suggested nanostructure was employed as a transducer to create a genosensor and to immobilize a probe unique to Brucella. The zepto-molar electrochemical detection of Brucella in blood samples from brucellosis patients was carried out using this technology. Table 11 summarizes examples of previously reported electrochemical biosensors for the detection of *Brucella* spp. Additionally, a probe specific to Brucella was immobilized by fabricating 6-Mercapto-1-hexanol (MCH) on the nPd electrode surface.[194] The created genosensor was assessed for the purpose of testing the bacteria in human and cultured samples both with and without PCR. With a sensitivity of 0.02 μA dm^3 mol^{-1}, a linear concentration range of 1.0×10^{-12} to 1.0×10^{-19} mol dm^{-3}, and a detection limit of 2.7×10^{-20} mol dm^{-3}, the genosensor was able to identify the complimentary sequence. This sensor exhibited the lowest limit of detection among the surveyed literature.

4.10. Helicobacter pylori

Gram-negative *Helicobacter pylori* bacteria are well known for damaging the stomach lining and negatively impacting human health. Recently, Jaradat *et al.* developed HopQ (protein) biomarker grafted screen-printed carbon electrodes with MWCNT-COOH decorated with gold nanoparticles (AuNP), SPCE/MWCNT/AuNP, immunosensor for the detection of *Helicobacter pylori* (*H. pylori*) pathogenic bacterium.[197] It is important to emphasize that the linearity was discovered to be within the range of 10 pg mL^{-1} to 100 ng mL^{-1}. The platform showed limit of detection (LOD) and limit of quantification (LOQ) of 2.0 pg mL^{-1} and 8.6 pg mL^{-1}, respectively. Another study found that the AuNPs-based electrochemical biosensor may be used to detect *H. pylori* bacterium iDNA sequences by introducing initiator DNA (iDNA) triggered hybridization chain reaction (HCR).[198] Gel electrophoresis image confirmed that the HCR occurs with the free DNA at the DNA-modified AuNPs. Authors highlighted that HCR converts

Table 12 Examples of some previously reported electrochemical biosensors for the detection of pathogenic *Helicobacter pylori*.[a]

Electrode	Bioreceptor	Analyte	Biosensor type	Electrochemical method	Linear range	Limit of detection	Redox probe	Real sample	Ref.
AuE	SH-ssDNA	*Helicobacter pylori*	DNA biosensor	DPV	20.0–410.0 nmol L^{-1}	7.2 nmol L^{-1}	Chlorogenic acid	N/A	199
SPE@rGO/Au	MIP-CagA	*Helicobacter pylori*	Biosensor	DPV	0.05–50 ng mL^{-1}	0.05 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Blood	200
SPE@SiO_2	MIP-VacA	*Helicobacter pylori*	Biosensor	DPV, EIS	0.01–100 ng mL^{-1}	0.01 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Serum	201
GCE/Ti_3C_2Tx/AuNPs	SH-cpDNA	*Helicobacter pylori*	DNA biosensor	DPV	10^{-11} to 10^{-14} mol L^{-1}	1.6×10^{-16} mol L^{-1}	PBS	Milk, serum	202
AuE	SH-ssDNA	*Helicobacter pylori*	DNA biosensor	DPV	0.3–240 nmol L^{-1}	0.15 nmol L^{-1}	β-cyclodextrin	N/A	203
GCE/GO/AuNP	ssDNA	*Helicobacter pylori*	DNA biosensor	DPV	60.0–600.0 p mol L^{-1}	27.0 p mol L^{-1}	Oracet blue	SDS, ethanol, glucose, CTAB	204
AuE	SH-cpDNA	*Helicobacter pylori*	DNA biosensor	DPV	0.01 fmol L^{-1} to 0.5 fmol L^{-1}, 1 fmol L^{-1} to 100 fmol L^{-1}	0.68 amol L^{-1}	$[Ru(NH_3)5L]^{2+}$	N/A	198
AuE/PEDOT/rGO/Pdnano	BabA antibody	*Helicobacter pylori*	Immunosensor	CV	0.2–20 ng mL^{-1}	0.2 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Stool	205
AuET/ZnO/g-C_3N_4	VacA antigen	*Helicobacter pylori*	Immunosensor	DPV	0.1–12.8 ng mL^{-1}	0.1 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Serum	200
AuE/red-GOx/PEDOT/Pt_{nano}	CagA antibody	*Helicobacter pylori*	Immunosensor	DPV	0.1 ng mL^{-1} to 30 ng mL^{-1}	0.1 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Serum	206
SPAuE@ZnO-T	CagA antigen	*Helicobacter pylori*	Immunosensor	CV, SWV	0.2 ng mL^{-1} to 50 ng mL^{-1}	0.2 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Serum	207
AuE/Pin5COOH/c-MWCNT/TiO_2NPs	Cag aantibody	*Helicobacter pylori*	Immunosensor	SWV	0.1–8.0 ng mL^{-1}	0.1 ng mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Stool	208
SPCE/MWCNT/AuNPs	HopQ antibody	*Helicobacter pylori*	Immunosensor	SWV	10 pg mL^{-1} to 100 ng mL^{-1}	2.0 pg mL^{-1}	$[Fe(CN)_6]^{3-/4-}$	Artificial saliva	197

[a] PEDOT: poly-3,4 ethylene dioxythiophene, g-C_3N_4: graphitic carbon nitrite, Pin5COOH: polyindole carboxylic acid, ZnO-T: zinc oxide tetrapods.

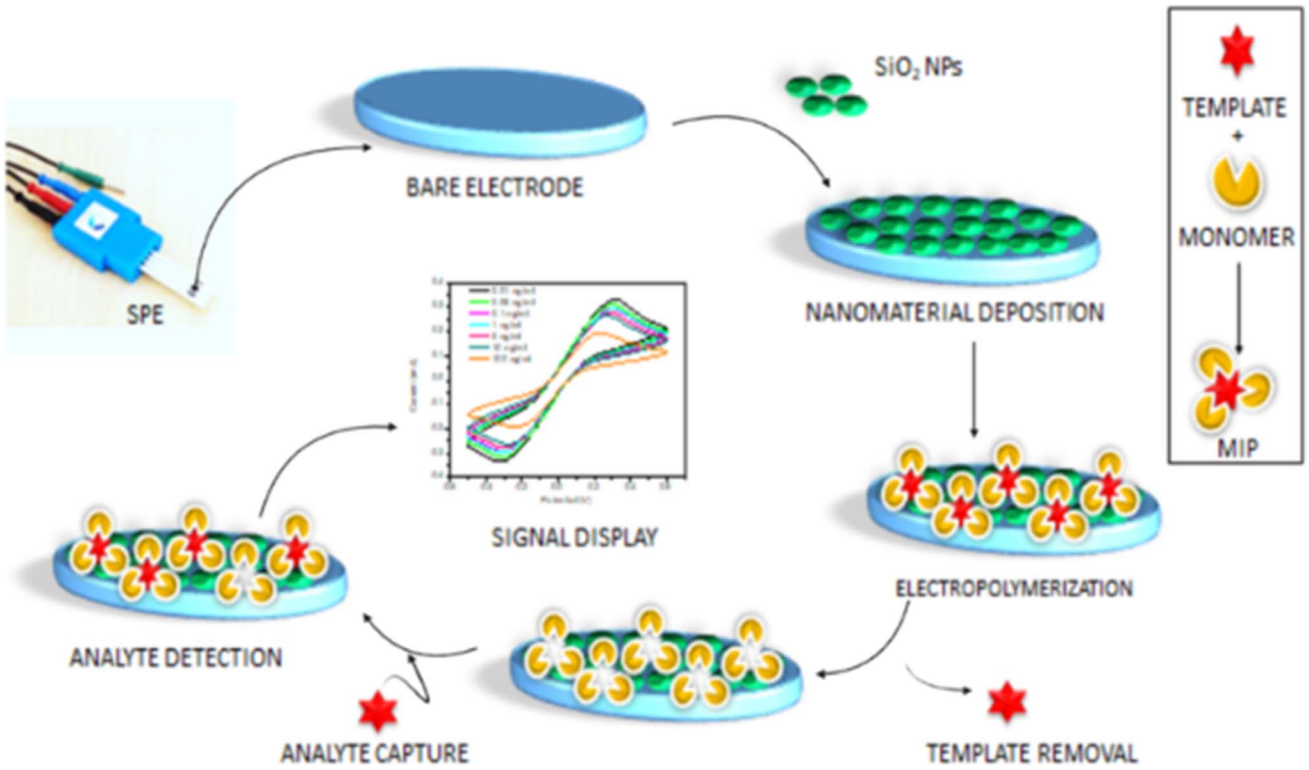

Fig. 13 Molecularly imprinted polymer (MIP) based biosensor. The figure was adapted from ref. 201 and reproduced with permission, copyright @ Elsevier.

the iDNA to long dsDNA concatemer. Furthermore, it was observed that electrochemical active molecule $[Ru(NH_3)5L]^{2+}$ intercalated into dsDNA. Table 12 sums up examples of some previously reported electrochemical biosensors for the detection of *Helicobacter pylori*. Furthermore, it was revealed that an electrochemical biosensor based on SiO_2 nanoparticle decorated molecularly imprinted polymer (MIP) was created to detect *Helicobacter pylori* on a screen-printed electrode (SPE), which is thought to function as a receptor by using template VacA antigen.[201] Schematic illustration showing the fabrication process of the devoloped biosensor is presented in Fig. 13. The developed VacA-MIP/SiO_2 @SPE sensor shows excellent sensitivity (0.304 mA ng ml^{-1}) and a very low detection limit (0.01 ng mL^{-1}) in a linear range of 0.01–100 ng mL^{-1} under optimal testing conditions. Consequently, the effective detection approach was occupied by MIP electrochemical detection techniques, DNA biosensors, and immunosensors with no restrictions.

5. Conclusions

This review of the literature concentrates on the bioreceptor theme for electrochemical biosensor modification, which offers a wide range of applications as a platform for pathogen detection. The performance metrics and a summary of the electrode construction techniques for identifying pathogenic bacteria are provided. Additionally, new developments on various bioreceptors, detection techniques, assay plans, redox probes, and material kinds are summarized and tallied. Thus, combining electrochemical-based biosensors with electrode materials and bioreceptors makes it easier to determine the optimal approach for identifying specific infections. This work also includes a comprehensive analysis of various electrochemical biosensor production methods for the detection of pathogenic bacteria and the process by which they are modified. All bioreceptors have a specific role in PB detection, however the detection limit of DNA and antibody-modified electrochemical biosensors is lower than that of other bioreceptors. Furthermore, phagosensors, or phage base sensors, are more straightforward than others. Thus, this review focuses on biosensing techniques that have led to notable advancements in the survivability, response time, selectivity, and sensitivity of bacterial detection. To prevent negative effects as early as possible, a great deal of research has recently been conducted with the goal of sensitively detecting infectious microorganisms. Additionally, combining the bioreceptor immobilized electrochemical biosensor into a single, reliable, and integrated platform for the management of healthcare is becoming a global aim for the scientific community. For scientists conducting research on electrochemically based bioreceptor modified sensors, this review can therefore be used as a starting point and a reference.

6. Present challenges and future perspectives

Water, food, hospitals, and human fluids can harbor or be the source of a variety of pathogenic bacteria that can cause

potentially fatal illnesses and become resistant to antibiotics. Due to the drawbacks of bioreceptors, such as limited stability for antibodies, difficulties binding to DNA targets for nucleic acids. That decreased sensitivity to nuclease for aptamers, electrochemical biosensors have several limitations when it comes to practical applications. However, the combination of clustered regularly interspaced short palindromic repeats (CRISPR) associated technology[209] and electrochemical DNA sensors can improve the sensitivity and precision. One of the major challenges in electrochemical biosensing of pathogenic bacteria in real samples is the need for multiple sample preparation stages. Furthermore, despite the potential for extreme sensitivity and robustness, selective biorecognition element-based bioanalytical techniques necessitate the addition of reagents to the sample and laborious sample preparation procedures, which lengthen the time-to-results (TTR). Additionally, furthermore, a wide range of molecules, including proteins, lipids, nucleic acids, and other cellular debris, are commonly present in bacterial samples and can obstruct the response of biosensors. It can be challenging to preserve the viability of bacteria during sample preparation, particularly when live bacteria is required for the analytical phases of the detection process. The wide fluctuation in bacterial concentrations in samples makes it difficult to consistently and reliably detect bacteria. False detection or a drop in signal to noise ratio may arise due to environmental conditions or contamination from other microorganisms such as intercellular bacterial leakage. Literature revealed that electrochemical biosensing still suffer from low yield during simultaneous detection. Furthermore, it is difficult to design compact, portable electronics without sacrificing effectiveness. For practical applications, biosensors must be integrated with electronic systems for data processing and wireless communication; however, this requires sophisticated engineering solutions.

Future research could benefit from adding nanomaterials to electrochemically based biosensors to increase their performance even further. The design of biosensors has been significantly impacted by the swift advancement of nanomaterials research. Moreover, a range of organic groups, including conductive polymers, thiols, and silanes, can be functionalized on the surfaces to effectively immobilize bioreceptors.[210] The development of point-of-need diagnostic instruments that incorporate nanomaterials, microfluidics, and electrochemical biosensors can benefit from cooperation between several disciplines, including nanotechnology, food science, material science, electrochemistry, microbiology, and system design and integration. At every stage of the process, including production, packaging, distribution, storage, and consumption, these devices will offer food safety evaluations and food screening capabilities. Academic and industrial researchers are collaborating to collectively commercialize a few fully integrated biosensing detection technologies. However, further research is required to precisely identify infectious bacteria.[211] Electrochemical biosensors can be used as a wearable detection tool in the near future,[16] collecting data wirelessly near the field to quickly identify pathogenic bacteria that pose a threat to human life. Such wearable and wireless integrated systems should be also commercially attractive. For the PBs detection, further bacterial culture protocol improvements is currently being aggressively sought. Achieving efficient bacterial disruption might require a combination of chemical (such as detergents), mechanical (*e.g.*, bead-beating), and enzymatic (for incident lysozyme) lysis techniques. Immuno-magnetic beads or selective growth media is often employed to extract the desired bacterial population from the sample. Removal of cellular debris through filtration and centrifugation after lysis is part sample preparation procedure. Setting up and following standardized protocols for the collection, storage, and preparation of bacterial samples are recommended steps to ensure high yield and reproducibility. By implementing efficient and reliable strategies to tackle the above challenges, electrochemical biosensors can greatly enhance the accuracy and dependability of bacterial sample analysis.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Author contributions

Md. Abdul Khaleque: conceptualized the main idea, structured, and wrote the original manuscript; S. I. Hossain: contributed to the writing and revision of the manuscript; Md. Romzan Ali: contributed to the writing and revision of the manuscript; Hala S. Abuelmakarem: wrote sections of the manuscript; Mohamed Aly Saad Aly: updated the main concepts and ideas, wrote, reviewed, and edited the manuscript, supervised and evaluated the overall work; Muhammad Shamim Al Mamun: designed, wrote, reviewed and edited sections of the manuscript; Md. Zaved Hossain Khan: contributed to the main concept, supervised and evaluated the overall concepts.

Conflicts of interest

The authors declare that there is no conflict of interest.

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