

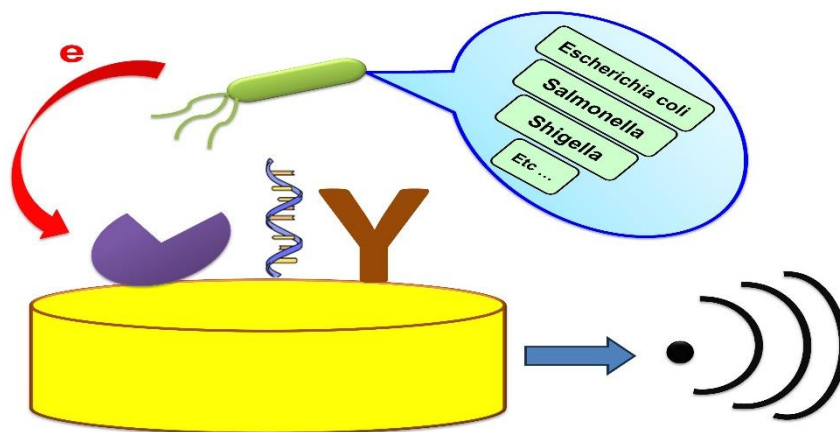
Identification of pathogenic bacteria by biosensors in water and wastewater

Sakineh Hargol Zadeh¹, Soheila Kashanian^{1,2*}, Maryam Nazari¹

¹Applied Chemistry Department, Faculty of Chemistry, Razi University, Kermanshah, Iran.

²Nanobiotechnology Department, Faculty of Innovative Science and Technology, Razi University, Kermanshah, Iran.

GRAPHICAL ABSTRACT



Article info

Article type:

Research Article

Article history:

Received 17 July 2024

Received in revised form 28 October 2024

Accepted 29 October 2024

Available online 2 December 2024

Keywords:

Escherichia coli

Water and wastewater

Biosensor

Fluorescence

Electrochemical



© The Author(s)

Publisher: Razi University

Abstract

Pathogenic microorganisms, such as *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *legionella*, *Shigella* and etc. can contaminate drinking water and lead to disease and even death. On the other hand, due to the ability of antibiotics to prevent or treat bacterial infections, they have been used as the main method of infection treatment in humans and animals for the past two decades. The irresponsible use of these antibiotics is one of the most important reasons for the emergence of microbial resistance, which has become a global issue. Therefore, timely diagnosis of these pathogens is very important. The use of specialized personnel, machines, and tools in molecular methods such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are expensive, and traditional methods such as plate culture are time-consuming. Today, a variety of biosensors are reported to identify these bacteria, which are fast, accurate, and cost-effective. In this review, we described a number of important pathogenic bacteria and biosensors made to identify these pathogens.

1. Introduction

Pathogenic bacteria cause disease, hospitalization, and death of over 15 million people worldwide each year. These pathogens directly affect human health, the safety of food and drinking water sources. So far, several pathogens have been identified, including *Escherichia coli* (*E. coli*). The Strains of Uropathogenic *Escherichia Coli* (UPEC) are causative agents in urinary tract infections (Mulvey *et al.*, 2000). *Staphylococcus aureus* with the production of several toxins are the most important causes of microbial contamination and food poisoning (Berrettoni *et al.*, 2004). According to previous reports, contamination of water sources with the pathogen *P. aeruginosa* causes hospitalization and death of thousands of people in the United States annually (Anaissie, Penzak and Dignani, 2002). *Salmonella enterica* subspecies *enterica* serovar *Typhi* (*Salmonella typhi*) causes typhoid fever and severe gastrointestinal issues in humans mainly via contaminated food and water (Mathai *et al.*, 1995; Kidgell *et al.*, 2002;

Quaresma *et al.*, 2022). *Shigella* is another important pathogenic bacteria that contaminate water and food, and the agent of Shigellosis is an acute invasive enteric infection that causes severe diarrhea and digestive diseases. Pathogenicity caused by this pathogen is a major challenge in many countries, especially developing countries. Obviously, consuming clean water is one of the ways to prevent the epidemic of this bacteria (Schnupf and Sansonetti, 2019; Bengtsson *et al.*, 2022).

Considering the infection and pathogenicity caused by these microorganisms, their timely identification is very important, but the use of specialized personnel, machines, and tools in molecular methods such as enzyme-linked immunosorbent assay (ELISA) (Rajapaksha *et al.*, 2019) and polymerase chain reaction (PCR) are expensive, and traditional methods such as plate culture are time consuming (Sieuwerts *et al.*, 2008; Valones *et al.*, 2009; Does, 2013; Rajapaksha *et al.*, 2019). In recent years, biosensors have overcome the problems and limitations of traditional methods and have attracted a great deal of

*Corresponding author Email: kashanian_s@yahoo.com

attention in medicine, food and beverage safety, and health owing to their simplicity, high speed, and being inexpensive (Amiri *et al.*, 2018). Some of these biosensors and their details are gathered in Table 1.

2. Biosensors

Biosensors are analytical devices that are used to determine the presence and concentration of a particular analyte in a biological analysis that are generally composed of several basic components required for functionality. Biosensors components include the bioreceptors, transducer, and biosensor reader (Fig. 1) which are described in the following.

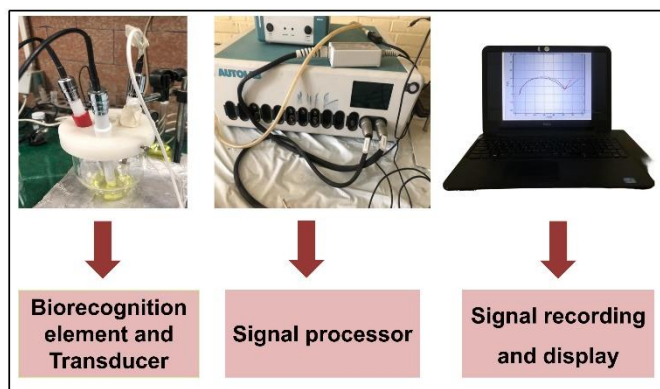


Fig. 1. A three-electrode electrochemical system.

2.1. Bioreceptors

This part performs the act of identifying a specific analyte which is one of the most important parts in the biosensor. Various biological bioreceptors have been used in the construction of biosensors such as DNA (Izadi *et al.*, 2016), RNA aptamer (Wu *et al.*, 2012; Zhang *et al.*, 2018), antibody (Yang, Li and Erf, 2004), enzyme (Kaur *et al.*, 2021; Soy, Sharma and Nigam, 2022), etc., to identify bacterial components.

2.2. Transducers

Transducers are devices that convert energy from one form to another. Biosensors cite the cooperation of receptors that identify target analytes and the transducers that convert this recognition to a detectable signal. (Kaur *et al.*, 2021).

2.3. Biosensor reader

Transducer in the biosensor is directly connected to a biosensor reader that records the results and signals from the transducer and displays them in a user-friendly way, which can be a computer.

2.4. Signal amplifier

Amplification of signals from analyte interaction with bioreceptors is directly related to improving the sensitivity of biosensors designed to identify bacteria. A signal amplification method in different categories of biosensors such as optical, electrochemical, piezoelectric, and thermal biosensors is the use of nanomaterials such as gold nanoparticles in electrochemical and piezoelectric biosensors (Roushani and Shahdost-Fard, 2015; Devi, Sasidharan and Sundramoorthy, 2018; Pohanka, 2018; Fang *et al.*, 2019; Bharti *et al.*, 2020; Yadav, Chhillar and Rana, 2020), silver nanoparticles in optical and electrochemical biosensors (Jiang *et al.*, 2012; Wang *et al.*, 2015), other metal and their oxides nanoparticles, (Muniandy *et al.*, 2019; Pangajam, Theyagarajan and Dinakaran, 2020), and magnetic nanoparticles (Pedrero, Campuzano and Pingarrón, 2012), carbon nanostructures (Ng and Liu, 2009; Jahanbakhshi and Habibi, 2016; Douaki *et al.*, 2020; Pangajam, Theyagarajan and Dinakaran, 2020; Amri, Shukla and Lee, 2021), and quantum dots (Jahanbakhshi and Habibi, 2016; Douaki *et al.*, 2020) (Li *et al.*, 2020; Pangajam, Theyagarajan and Dinakaran, 2020; Choi *et al.*, 2021) in optical and electrochemical biosensors, etc. These nanomaterials can affect the performance of biosensors by providing properties such as creating a high surface-to-volume ratio as a result of creating a broad substrate for the stabilization of other materials and by affecting the electrical or optical properties of the biosensors.

3. Construction and classification of transducers in biosensors

Biosensors are as a combination of bioreceptors and transducers based on identifiable signals are classified into four general categories, including electrochemical (Arduini *et al.*, 2019), optical (Wu *et al.*, 2012), piezoelectric, and thermal signals (Fig. 2) (Muramatsu *et al.*, 1989; Arreguin-Campos *et al.*, 2023). In these devices, the specific output can be electrical current, resistance potential, fluorescence, piezoelectric temperature changes, or mass changes, based on which biosensors convert the signals from analytes to detectable biological inputs.

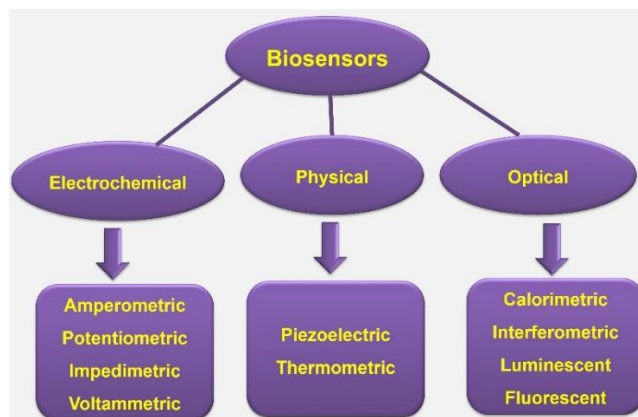


Fig. 2. Different types of biosensors.

4. Pathogenic bacteria and biosensors designed to detect these pathogens

4.1. *Escherichia coli* bacteria and its biosensors

E. coli is a commensal of humans, but also a major pathogen. Consequently, most isolates are innocuous, but some of them cause severe food-borne infections in humans. This gastrointestinal infections can be caused by the transmission of this pathogenic bacterium by contaminated vegetables, such as lettuce, spinach, contaminated water, and raw or poorly pasteurized milk. Members of some pathovars of this bacterium, such as *E. coli* O157:H7, and *E. coli* O104:H4, produce deadly toxins such as Shiga toxin that cause infection and dissemination in live organs (Pandey *et al.*, 2017; Zaraee *et al.*, 2020). Such an infection with enterohaemorrhagic *E. coli* O157:H7 can lead to dysentery and, if not diagnosed early and treated quickly, can lead to renal failure and death. Due to the severity of infection by these pathovars of *E. coli*, the diagnosis time and prevention measurements are highly important.

Zaraee *et al.* designed an optical biosensor for *E. coli* bacteria. This biosensor consists of a modified chip and an optical setup. The chip contains a Si/SiO₂ layered substrate modified with MCP-4 copolymer (copoly DMA-NAS-MAPS). *E. coli* antibody was immobilized on the MCP-4 and bovine serum albumin (BSA) was used as negative control instead of *E. coli* antibody. The prepared chip was then placed under an optical setup, and detection was performed very quickly using the Interferometric Reflectance Imaging Sensor (IRIS) sensitive detection and imaging technique, without the need of complex methods and a skilled user. The Limit of detection (LOD) in this biosensor was reported 2 CFU/mL. The specificity of this biosensor was validated by comparing the response of that to target bacteria *E. coli* and non-target bacteria such as *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*. The performance of biosensor was investigated in tap water sample (Zaraee *et al.*, 2020).

Pourmadadi *et al.* designed an aptasensor using glassy carbon electrodes (GCE) modified with graphene oxide (GO) and gold nanoparticles to detect *E. coli* lipopolysaccharides (LPS) type O55:B5 based on voltammetry method. The electrode demonstrated desirable selectivity and the LOD of this biosensor was obtained 30 fg/mL. To design this biosensor, Mg/CQD (carbon quantum dot) was used as the redox-active medium to reduce the electrochemical potential in LPS detection. The specificity of this biosensor was confirmed by comparing the signal response of the nanoprobe electrode to LPS compared to the non-target analytes of glucose, albumin, and fetal bovine serum (Pourmadadi *et al.*, 2019).

4.2. *Shigella dysenteriae* and its biosensors

Shigella is a gram-negative bacterium without spores and a major cause of dysentery. This bacterium was named after its discoverer Kiyoshi Shiga, who was a Japanese bacteriologist. There are four subtypes of this bacterial species, and all of them are capable of damaging the epithelial cell lining of the large intestine. The type of

dysenteriae can cause long and widespread epidemics, resulting infection being more severe, possessing a longer duration than other *Shigella* species, and often associated with mortality. Successful infection of this pathogen can require less than one hundred cells. The symptoms of *Shigella* are nausea, pain, and abdominal cramps. This

bacterium is similar to *E. coli* (infact a subspecies of *E. coli* possessing species status due to particular infection phenotypes) and *Salmonella* and only causes pathogenesis in humans and primates. Dairy products, and contaminated milk and water samples can be the main sources of this bacterium (Mukama et al., 2017).

Table 1. Different types of biosensors for identifying pathogenic bacteria.

Metod	Biorecognition element	Target analyte	Linear dynamic range	LOD	Reference
Electrochemical	Biorecognition element free	<i>E. coli</i>	$10^{3.2}-10^6$ CFU/mL	$10^{3.2}$ CFU/mL	(Lin et al., 2022)
Electrochemical	Carbohydrate	<i>E. coli</i>	$1.3 \times 10^1-1.3 \times 10^6$ CFU/mL	2 CFU/mL	(Hargol Zadeh, Kashanian and Nazari, 2023)
Electrochemical	Anti- <i>E. coli</i> antibody	<i>E. coli</i>	-	53 CFU	(Malhotra et al., 2022)
Electrochemical	DNA Biosensor	<i>E. coli</i>	1×10^{-10} to 1×10^{-5} μ M	1.95×10^{-15} μ M	(Yuhana Ariffin et al., 2020)
Electrochemical	Biochar-based immunosensor	<i>E. coli</i>	10^4 to 10^7 CFU/mL	4 log CFU/mL	(Sobhan et al., 2022)
Electrochemical	Antigen-antibody-based	<i>H. pylori</i>	0.1 to 12.8 ng/mL	$0.3 \mu\text{A}^{-1}$ ng /mL	(Saxena et al., 2022)
Colorimetric	Aptamer	<i>Salmonella typhimurium</i>	10 to 10^7 CFU/mL	7 CFU/mL	(Wei et al., 2022)
Colorimetric	Aptamer	<i>Salmonella typhimurium</i>	-	3.2×10^3 CFU/mL	(Li et al., 2023)
Fluorescence	Aptamer-functionalized horseradish peroxidase	<i>Shigella</i>	2.3×10^2 to 2.3×10^7 CFU/mL	32 CFU/mL	(Song et al., 2023)
Fluorescence	Aptamer-Based	<i>Shigella sonnei</i>	10^3 to 10^7 Cells/mL	10^3-10^7 Cells/mL	(Song et al., 2017)
Fluorescence	Aptamer	<i>Salmonella typhimurium</i>	30 to 3×10^4 CFU/mL	13 CFU/ mL	(Liu et al., 2022)
Fluorescence	Nanoprobe-Based FRET	<i>Shigella</i>	1.2×10^2 to 1.2×10^8 CFU/mL	30 CFU/mL	(Chen et al., 2022)
Optical	Oligonucleotide-Gold Nanoparticles	<i>Salmonella spp</i>	-	<10 CFU/mL	(Quintela et al., 2019)
Optical	Monoclonal antibodies	<i>Escherichia coli K12</i>	-	10^4 Cells/ mL	(Massad-Ivanir, Shtenberg and Segal, 2013)
Termal biosensore	Copper chip holder	<i>E. coli</i>	-	10^3 CFU/mL	(Arreguin-Campos et al., 2023)

Zarei et al. designed an aptasensor using gold nanoparticle-modified glassy carbon electrode (GCE) for *Shigella dysenteriae*. After aptamer immobilization on the surface of gold nanoparticles, residual free gold nanoparticles were blocked with 6-mercaptop-1-hexanol. Detection of *Shigella dysenteriae* was done by determining electrical surface resistance changes in different concentrations of *Shigella dysenteriae* in the presence of hexacyanoferrate as a chemical probe. The LOD of this work was reported 10 CFU/mL, and the prepared aptasensor successfully detected *Shigella dysenteriae*. Selectivity of this biosensor was examined and the aptasensor did not show a significant response against other pathogenic bacteria (Zarei, Soleimani-Zad and Ensafi, 2018).

4.3. *Staphylococcus aureus* (SEA) and its biosensors

Staphylococcus aureus is a threat to food safety and health, which can produce a variety of neurotoxins and cytotoxins (Jia et al., 2013; Rasooly et al., 2019). Currently, 24 subtypes of this bacterium have been reported, among which *Staphylococcal Enterotoxin A* (SEA) cause to water and food poisoning worldwide (Desouza et al., 2009). Consumption of fifty ounces of contaminated water or food such as meat, eggs, milk, and dairy products (per 70 kg body weight) can cause food poisoning, toxic shock, and exacerbate various diseases caused by toxin ingestion (Zhang et al., 2020). In addition to food poisoning, the species of this bacterium cause diseases such as post-surgery infections, purulent skin lesions, pneumonia, etc (Davydova et al., 2016). In 2020, Zhang et al. designed a label-free fluorescence aptasensor to identify staphylococcal Enterotoxin A, abbreviated SEA, using functionalized aptamer with silver nanoclusters. In this biosensor, DNA sequences were modified to increase the fluorescence intensity with the sequence of C6GC6. Polypyrrole nanoparticles (PPyNPs) were used to fabricate this biosensor which silver nanoclusters and DNA were adsorbed through fragment accumulation within PPyNPs; therefore, energy transfer took place directly from silver nanoclusters as donors to PPyNPs as energy receptors. The LOD in this work was reported 0.3393 ng/mL. Selectivity of the biosensor was examined with three nontarget bacteria including *S. aureus* enterotoxins such as staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C1 (SEC1), and staphylococcal enterotoxin D (SED) (Zhang et al., 2020). In 2020, Bagheri et al. also designed an iron ion fluorescence- whole cell-based aptasensor to detect the *Staphylococcus aureus* using fluorescence resonance energy transfer (FRET) between green CQDs and AuNPs. The CQDs and gold nanoparticles act as donors and energy receptors, respectively. The reported biosensor, detected *Staphylococcus aureus* up to 10 CFU/mL. Specificity of this biosensor was validated by comparing the response to the target bacteria *Staphylococcus aureus* versus non-target bacteria *E. coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. (Pebdeni, Hosseini and Ganjali, 2020).

4.4. *Pseudomonas aeruginosa* and its biosensors

Pseudomonas aeruginosa is a gram-negative environmental bacterium present in soil and water. It is also present on the surface of plants and animals (Cutelod, Seddon and Neubert, 2011). Nevertheless, *P. aeruginosa* can cause serious infections in patients with weakened immune systems, organ transplants, skin burns, and cystic fibrosis (Gao et al., 2018). In addition, *P. aeruginosa* can efficiently colonize artificial devices and is a major cause of infection in ventilator-associated pneumonia, (El Solh et al., 2008). Therefore, to ensure the importance of nutrition and protect common health, it is extremely vital to develop rapid and practical methods to detect *Pseudomonas aeruginosa* (Gao et al., 2018). Ran Gao et al. designed a fluorescence-supported fluorescence aptasensor with graphene oxide quantum dots (GOQDs) for rapid detection of *Pseudomonas aeruginosa*. In the absence of *Pseudomonas aeruginosa*, the complementary strand with the fluorescent 5-carboxyfluorescein-labeled complementary DNA (FAM-cDNA) was hybridized with the part of aptamer sequence, and the fluorescent FAM was quenched by GOQDs. By adding the target bacterium, the aptamer detected the biological element (analyte) and bound to a specific aeruginosa. As a result, it was repelled by GOQDs (desorption), so the fluorescence of FAM was restored. The LOD was 100 CFU/mL, and the detection time was 2 hours. In addition, selectivity of the aptasensor was examined and successful results toward was demonstrated compare to other pathogenic bacteria (Gao et al., 2018).

4.5. *Mycobacterium tuberculosis* and its biosensors

Mycobacterium tuberculosis (MTb) is a major cause of infection and causing chronic and acute tuberculosis that is seriously life-threatening. The cure requires long-term treatment with a low likelihood of eradicating the infection (Diouani et al., 2017; Zhang et al., 2018). Research has shown that water and wastewater contamination with *mycobacterium tuberculosis* is one of the ways of environmental transmission of this pathogen to humans. So early and rapid detection of *Mycobacterium tuberculosis* (MTb) is an important factor in the rapid diagnosis and control of tuberculosis. ESAT-6 (6-kDa early secreted antigenic target) is a 6-kDa primary antigen secreted by this bacterium in the first stage of MTB infection. Therefore, the identification of ESAT-6 is considered to be of great importance in the initial diagnosis of tuberculosis (Zhang et al., 2018).

Li et al. designed an aptasensor for ESAT-6 using a nanofiber hybrid material composed of a reduced graphene oxide metal-organic framework (rGO-MOF). This rGO-MOF was placed on a glassy carbon electrode, and toluidine blue (TB) was used as the electroactive compound. Platinum/Au core/shell (PtNPs @ AuNPs) were used to concentrate the thiolated aptamer (EBA) on the modified electrode and increase sensitivity to the ESAT-6 response. The LOD was 3.3×10^{-5} ng/mL (Li et al., 2018). In another approach, Diouani et al. designed an

unlabeled immunoelectrochemical biosensor for the diagnosis of ESAT-6. In this work, an anti-ESAT-6 monoclonal antibody was used as a bioreceptor, and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was used as a redox probe. Finally, the LOD of 7 ng/mL was obtained in this work (Djouani et al., 2017). Also, Sypabekova et al. designed another aptasensor using the gold solid support and a thiolated linker of OP (O₂) O- (CH₂CH₂O)₆, that the LOD was obtained 4.1 fM (Nikonovas et al., 2020).

4.6. *Salmonella* bacteria and its biosensors

Salmonella is a gram-negative bacterium that is an important food pathogen for humans and animals. Annually, this bacterium causes more than 1 billion infections worldwide and causes over 155,000 deaths due to the consumption of food contaminated with this pathogen (He et al., 2023). *Salmonella* is found in meat, eggs, milk, fruit juice, and poultry (Dill, Stanker and Young, 1999). Among the all serotypes of *Salmonella*, one of the important serotypes that associated with human disease is *S-Typhimurium*. The consumption of water contaminated with this bacteria can lead to immune deficiencies and other symptoms such as fever, gastrointestinal disorders, diarrhea, and even death within a short time (Park et al., 2015). Therefore, rapid and reliable diagnosis of this pathogen is very important (Lee et al., 2015). In 2016, Sheikhzadeh et al. designed an unlabeled biosensor, in which a gold disk-shaped electrode was polished with a micropad and alumina powder 0.1, 0.3, 0.05, and then the clean gold disk was modified by the copolymer to covalently stabilize the aptamer on the surface. The aptasensor identified *Salmonella typhimurium* with high specificity and a LOD in 3 CFU/mL at a short time (Sheikhzadeh et al., 2016).

4.7. Pathogenic bacteria (total bacteria) and its biosensors

Pathogenic bacteria and contamination of various environments with these bacteria are serious threats to the health of humans and living organisms. They contaminate water, food, and pharmaceutical products, leading to pathogenicity and even death in humans (Lâm et al., 2010). Therefore, it is essential to discover a fast, sensitive, and reliable method for timely diagnosis and screening. There are several methods, including colony counting as the gold standard, ELISA, and PCR that all of which are expensive and require a skilled user. In the development of suitable on-site bacterial identification methods, colorimetric methods have attracted a lot of attention due to easy operation and visual detection without the need of tools and expertise (Zhu et al., 2016; Alamer et al., 2018). Thao Nguyen Le et al. reported a calorimetric method for the identification of bacteria using chitosan-coated magnetic nanoparticles (CS-MNPs), in which the surface of the MNP was coated with chitosan to produce CS-MNP. Chitosan has a positive charge (+) and that it can electrostatically attach to a negatively charged bacterial cell membrane with a very high affinity. In this work, the 2-2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution was used as colorimetric probe. CS-MNP exhibits peroxidase-like catalytic activity by producing a dense green color in the absence of bacteria. However, due to the presence of bacteria in the environment and the electrostatic interaction of bacteria, it was attached to the chitosan surface coated on MNP, which created a barrier to ABTS access to chitosan, thus reducing peroxidase-like activity of CS-MNP. The presence of bacteria was determined by a decrease in calorimetric activity. The LOD for this sensing method was at 10⁴ CFU/mL and bacteria could be identified with the naked eye. This sensor was tested in the presence of *Staphylococcus aureus* and *E. coli* (Le, Tran and Kim, 2020).

4.8. Biofilm and its biosensors

Bacterial biofilm consists of one or more bacterial species encapsulated within self-produced extracellular polymeric substances (EPSs) that adhere to wetted surfaces (Becerro, Paredes and Arana) (Funari and Shen, 2022; Werwinski et al., 2022; Römling, 2023). Due to high antibiotic resistance, bacterial biofilm is considered to cause many diseases, especially in the clinical setting (Subramanian et al., 2020; Ameer et al., 2023). Biofilms can cause many problems including biological, environmental, infectious, biofouling, or spoilage. As a result, there is a need to monitor the formation of biofilms in different environments. In recent years, biosensors have received attention including the identification of bacteria and the supervision of biofilm formation in many environmental and medical fields (Saccomano, Jewell and Cash, 2021; Funari and Shen, 2022). Fig.3 shows the steps of biofilm formation and its cycle. Liu et al. reported an interdigital microelectrode integrated biosensor chip to monitor the formation process of *Salmonella* and *E. coli* biofilms. Using electrochemical impedance spectroscopy, they showed that changes in the impedance spectroscopy of biofilms occur with the time of cultivation and the

changes in the process of decreasing and increasing of the capacity of biofilm are proportional to the process of biofilm formation (Liu et al., 2018). Brochman et al. reported a microfluidic sensor to assess biofilm formation. In this study, they used different bacterial strains and complex biofilms. The results of electrochemical monitoring showed the high dynamics of biofilms to chemical treatment strategies (Bruchmann et al., 2015). Rakhimbekova et al. reported the possibility of using optical and fiber-based sensors to identify and analyze bacterial biofilms. In this study, measuring the refractive index with an optical backscatter reflectometer determined the initial concentration of the biofilm by the crystal violet adhesion method (Rakhimbekova et al., 2022).

Matthias Fischer et al. reported a robust fiber-optic biofilm sensor for online monitoring of large biofilms in natural aquatic environments based on natural fluorescence detection of biofilm microorganisms. The working principle of this device is based on the natural fluorescence detection of biofilm-forming microorganisms. The results of marine bacterial strains showed a linear signal response characteristic with a detection of 4 x 10³ cells/cm² (Fischer, Wahl and Friedrichs, 2012).

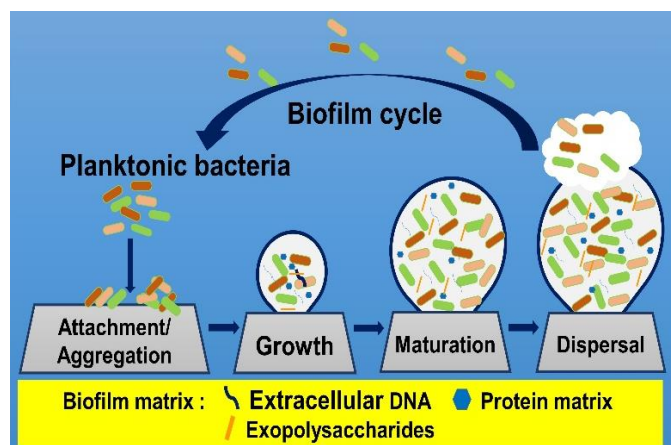


Fig. 3. The steps of biofilm formation and its cycle.

5. An overview of the advantages and disadvantages of these biosensors

Biosensors offer advantages in comparison with other bacterial detection methods, such as ELISA, PCR, or standard culture medium methods, which show that these devices have the potential to overcome the limitations of previous methods. Bacterial biosensors with high sensitivity provide the possibility of detecting very small amounts of analyte and with their high selectivity, they can identify the target analyte in complex environments. The response of these systems is fast and provides the possibility of early diagnosis. These devices have very small dimensions that make them easy to transport and they also need a small amount of sample. The performance of some of these devices may be affected by pH, temperature, or magnetic field that limited the bacterial biosensor performances. Using biosensors in some complex environments such as contaminated water is a challenge and their accuracy and sensitivity may be affected by interference. Many of these sensors are not recoverable and have a short lifespan.

6. Conclusions

In this study, some of the important pathogenic bacteria in water, their associated diseases, and their role in creating life-threatening conditions, and some fabricated biosensors for their detection quickly and accurately were investigated. These biosensors were designed with different methods such as optical, electrochemical, etc. All of these biosensors succeeded in selective identifying the target bacteria in a short time. Given the high accuracy, speed of detection, and cost-effectiveness of biosensors in identifying pathogens, seem to have a clear vision for fast detection.

Author Contributions

Sakineh Hargol Zadeh: Conceptualization, investigation, and writing the original draft.
Soheila Kashanian: Supervisor.
Maryam Nazari: Data curation and visualization.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Professor Ute Römling Professor at the Department of Microbiology, Tumor, and Cell Biology, Karolinska Institutet, Sweden for their cooperation in editing and improving the quality of the scientific content of the text.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Alamer, S. et al. (2018) 'Rapid colorimetric lactoferrin-based sandwich immunoassay on cotton swabs for the detection of foodborne pathogenic bacteria', *Talanta*, 185, pp. 275-280. doi: <https://doi.org/10.1016/j.talanta.2018.03.072>
- Ameer, S. et al. (2023) 'Electrochemical impedance spectroscopy-based sensing of biofilms: A comprehensive review', *Biosensors*, 13 (8), pp. 777. doi: <https://doi.org/10.3390/bios13080777>
- Amiri, M. et al. (2018) 'Electrochemical methodologies for the detection of pathogens', *ACS Sensors*, 3 (6), pp. 1069-1086. doi: <https://doi.org/10.3390/bios13080777>
- Amri, C., Shukla, A.K. and Lee, J.-H. (2021) 'Recent advancements in nanoparticle-based optical biosensors for circulating cancer biomarkers', *Materials*, 14 (6), p. 1339. doi: <https://doi.org/10.3390/ma14061339>
- Anaissie, E.J., Penzak, S.R. and Dignani, M.C. (2002) 'The hospital water supply as a source of nosocomial infections: a plea for action', *Archives of Internal Medicine*, 162 (13), pp. 1483-1492. doi: <https://doi.org/10.1001/archinte.162.13.1483>
- Arduini, F. et al. (2019) 'Origami multiple paper-based electrochemical biosensors for pesticide detection', *Biosensors and Bioelectronics*, 126, pp. 346-354. doi: <https://doi.org/10.1016/j.bios.2018.10.014>
- Arreguin-Campos, R. et al. (2023) 'Functionalized screen-printed electrodes for the thermal detection of Escherichia coli in dairy products', *Food Chemistry*, 404, p. 134653. doi: <https://doi.org/10.1016/j.foodchem.2022.134653>
- Becerro, S., Paredes, J. and Arana, S. (2015) 'Multiparametric biosensor for detection and monitoring of bacterial biofilm adhesion and growth' In *6th European Conference of the International Federation for Medical and Biological Engineering, MBEC, 2014. 7-11 September 2014, Dubrovnik, Croatia*: Springer, pp. 333-336.
- Bengtsson, R.J. et al. (2022) 'Pathogenomic analyses of Shigella isolates inform factors limiting shigellosis prevention and control across LMICs', *Nature Microbiology*, 7 (2), pp. 251-261. doi: <https://doi.org/10.1038/s41564-021-01054-z>
- Berrettoni, M. et al. (2004) 'Electrochemical sensor for indirect detection of bacterial population', *Sensors and Actuators B: Chemical*, 102 (2), p. 331-335. doi: <https://doi.org/10.1016/j.snb.2004.04.022>
- Bharti, A. et al. (2020) 'Electrochemical biosensor for miRNA-21 based on gold-platinum bimetallic nanoparticles coated 3-aminopropyltriethoxy silane', *Analytical Biochemistry*, 609, p. 113908. doi: <https://doi.org/10.1016/j.ab.2020.113908>
- Bruchmann, J. et al. (2015) 'Multi-channel microfluidic biosensor platform applied for online monitoring and screening of biofilm formation and activity', *PLoS one*, 10 (2), p. e0117300. doi: <https://doi.org/10.1371/journal.pone.0117300>
- Chen, M. et al. (2022) 'Upconversion fluorescence nanoprobe-based FRET for the sensitive determination of Shigella', *Biosensors*, 12 (10), p. 795. doi: <https://doi.org/10.3390/bios12100795>
- Choi, H.K. et al. (2021) 'Noble metal nanomaterial-based biosensors for electrochemical and optical detection of viruses causing respiratory illnesses'. *Frontiers in Chemistry*, 9, p. 672739. doi: <https://doi.org/10.3389/fchem.2021.672739>
- Cuttelod, A., Seddon, M. and Neubert, E. (2011) 'European red list of non-marine molluscs', Publications Office of the European Union Luxembourg. doi: <https://doi.org/10.2779/84538>
- Davydova, A. et al. (2016) 'Aptamers against pathogenic microorganisms', *Critical Reviews in Microbiology*, 42 (6), pp. 847-865. doi: <https://doi.org/10.3109/1040841X.2015.1070115>
- Desouza, I.A. et al. (2009) 'Role of sensory innervation in the rat pulmonary neutrophil recruitment induced by staphylococcal enterotoxins type A and B', *European Journal of Pharmacology*, 613 (1-3), pp. 128-134. doi: <https://doi.org/10.1016/j.ejphar.2009.04.010>
- Devi, N.R., Sasidharan, M., and Sundramoorthy, A.K. (2018) 'Gold nanoparticles-thiol-functionalized reduced graphene oxide coated electrochemical sensor system for selective detection of mercury ion', *Journal of the Electrochemical Society*, 165 (8), pp. B3046-B3053. doi: <https://doi.org/10.1149/2.0081808jes>
- Dill, K., Stanker, L.H., and Young, C.R. (1999) 'Detection of salmonella in poultry using a silicon chip-based biosensor', *Journal of Biochemical and Biophysical Methods*, 41 (1), pp. 61-67. doi: [https://doi.org/10.1016/S0165-022X\(99\)00027-5](https://doi.org/10.1016/S0165-022X(99)00027-5)
- Diouani, M.F. et al. (2017) 'Detection of ESAT-6 by a label free miniature immuno-electrochemical biosensor as a diagnostic tool for tuberculosis', *Materials Science and Engineering: C*, 74, p. 465-470. doi: <https://doi.org/10.1016/j.msec.2016.12.051>
- Garibyan, L. et al. (2013) 'Polymerase chain reaction', *The Journal of Investigative Dermatology*, 133(3), pp. 1-4. doi: <https://doi.org/10.1038/jid.2013.1>
- Douaki, A. et al. (2020) 'Flexible screen printed aptasensor for rapid detection of furaneol: A comparison of CNTs and AgNPs effect on aptasensor performance', *Nanomaterials*, 10 (6), p. 1167. doi: <https://doi.org/10.3390/nano10061167>
- El Solh, A.A. et al. (2008) 'Persistent infection with Pseudomonas aeruginosa in ventilator-associated pneumonia', *American Journal of Respiratory and Critical Care Medicine*, 178 (5), pp. 513-519. doi: <https://doi.org/10.1164/rccm.200802-239OC>
- Fang, L. et al. (2019) 'Copper nanoparticles/graphene modified green rusts for debromination of tetrabromobisphenol A: Enhanced galvanic effect, electron transfer and adsorption', *Science of the Total Environment*, 683, pp. 275-283. doi: <https://doi.org/10.1016/j.scitotenv.2019.05.273>
- Fischer, M., Wahl, M. and Friedrichs, G. (2012) 'Design and field application of a UV-LED based optical fiber biofilm sensor', *Biosensors and Bioelectronics*, 33 (1), pp. 172-178. doi: <https://doi.org/10.1016/j.bios.2011.12.048>
- Funari, R., and Shen, A.Q. (2022) 'Detection and characterization of bacterial biofilms and biofilm-based sensors', *ACS Sensors*, 7 (2), pp. 347-357. doi: <https://doi.org/10.1021/acssensors.1c02722>
- Gao, R. et al. (2018) 'Graphene oxide quantum dots assisted construction of fluorescent aptasensor for rapid detection of Pseudomonas aeruginosa in food samples'. *Journal of Agricultural and Food Chemistry*, 66 (41), pp. 10898-10905. doi: <https://doi.org/10.1021/acs.jafc.8b02164>
- Hargol Zadeh, S., Kashanian, S., and Nazari, M. (2023) 'A Label-free carbohydrate-based electrochemical sensor to detect escherichia coli pathogenic bacteria using D-mannose on a glassy carbon electrode', *Biosensors*, 13 (6), p. 619. doi: <https://doi.org/10.3390/bios13060619>
- He, Y. et al. (2023) 'Epidemiology of foodborne diseases caused by Salmonella in Zhejiang Province, China, between 2010 and 2021', *Frontiers in Public Health*, 11, p. 1127925. doi: <https://doi.org/10.3389/fpubh.2023.1127925>
- Izadi, Z. et al. (2016) 'Fabrication of an electrochemical DNA-based biosensor for Bacillus cereus detection in milk and infant formula', *Biosensors and Bioelectronics*, 80, pp. 582-589. doi: <https://doi.org/10.1016/j.bios.2016.02.032>
- Jahanbakhshi, M., and Habibi, B. (2016) 'A novel and facile synthesis of carbon quantum dots via saep hydrothermal treatment as the silver nanoparticles support: Application to electroanalytical determination of H₂O₂ in fetal bovine serum', *Biosensors and Bioelectronics*, 81, pp. 143-150. doi: <https://doi.org/10.1016/j.bios.2016.02.064>
- Jia, G. et al. (2013) 'Tetraether biomarker records from a loess-paleosol sequence in the western Chinese Loess Plateau', *Frontiers in Microbiology*, 4, p. 51234. doi: <https://doi.org/10.3389/fmicb.2013.00199>
- Jiang, P. et al. (2012) 'Water-soluble Ag₂S quantum dots for near-infrared fluorescence imaging in vivo', *Biomaterials*, 33 (20), pp. 5130-5135. doi: <https://doi.org/10.1016/j.biomaterials.2012.03.059>

- Kaur, K. et al. (2021) 'Quantitative E. coli enzyme detection in reporter hydrogel-coated paper using a smartphone camera', *Biosensors*, 11 (1), p. 25. doi: <https://doi.org/10.3390/bios11010025>
- Kidgell, C. et al. (2002) 'Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old', *Infection, Genetics and Evolution*, 2 (1), pp. 39-45. doi: [https://doi.org/10.1016/S1567-1348\(02\)00089-8](https://doi.org/10.1016/S1567-1348(02)00089-8)
- Lâm, T.-T. et al. (2010) 'Phagolysosomal integrity is generally maintained after Staphylococcus aureus invasion of nonprofessional phagocytes but is modulated by strain 6850', *Infection and Immunity*, 78 (8), pp. 3392-3403. doi: <https://doi.org/10.1128/iai.00012-10>
- Le, T.N., Tran, T.D., and Kim, M.I. (2020) 'A convenient colorimetric bacteria detection method utilizing chitosan-coated magnetic nanoparticles', *Nanomaterials*, 10 (1), p. 92. doi: <https://doi.org/10.3390/nano10010092>
- Lee, K.-M. et al. (2015) 'Review of Salmonella detection and identification methods: Aspects of rapid emergency response and food safety', *Food Control*, 47, pp. 264-276. doi: <https://doi.org/10.1016/j.foodcont.2014.07.011>
- Li, C. et al. (2020) 'Biosensors based on advanced sulfur-containing nanomaterials', *Sensors*, 20 (12), p. 3488. doi: <https://doi.org/10.1016/j.foodcont.2014.07.011>
- Li, J. et al. (2023) 'A Simple colorimetric Au-on-Au tip sensor with a new functional nucleic acid probe for food-borne pathogen Salmonella typhimurium', *Angewandte Chemie*, 135 (20), pp. e202300828. doi: <https://doi.org/10.1002/ange.202300828>
- Li, L. et al. (2018) 'Aptamer based voltammetric biosensor for Mycobacterium tuberculosis antigen ESAT-6 using a nanohybrid material composed of reduced graphene oxide and a metal-organic framework', *Microchimica Acta*, 185, pp. 1-9. doi: <https://doi.org/10.1007/s00604-018-2884-5>
- Lin, Y.-K. et al. (2022) 'A new biorecognition-element-free ID μ E sensor for the identification and quantification of E. coli', *Biosensors*, 12 (8), p. 561. doi: <https://doi.org/10.3390/bios12080561>
- Liu, L. et al. (2018) 'Monitoring of bacteria biofilms forming process by in-situ impedimetric biosensor chip', *Biosensors and Bioelectronics*, 112, pp. 86-92. doi: <https://doi.org/10.1016/j.bios.2018.04.019>
- Liu, X. et al. (2022) 'Aptamer-Based fluorescence detection and selective disinfection of Salmonella Typhimurium by using hollow carbon nitride nanosphere', *Biosensors*, 12 (4), p. 228. doi: <https://doi.org/10.3390/bios12040228>
- Malhotra, S. et al. (2022) 'A low-cost, 3D-printed biosensor for rapid detection of Escherichia coli', *Sensors*, 22 (6), p. 2382. doi: <https://doi.org/10.3390/s22062382>
- Massad-Ivanir, N., Shtenberg, G., and Segal, E. (2013) 'Optical detection of E. coli bacteria by mesoporous silicon biosensors', *Journal of Visualized Experiments*, (81), p. 50805. doi: <https://doi.org/10.3791/50805>
- Mathai, E. et al. (1995) 'Significance of Salmonella typhi bacteriuria', *Journal of Clinical Microbiology*, 33 (7), pp. 1791-1792. doi: <https://doi.org/10.1128/jcm.33.7.1791-1792.1995>
- Mukama, O. et al. (2017) 'An update on aptamer-based multiplex system approaches for the detection of common foodborne pathogens', *Food Analytical Methods*, 10, pp. 2549-2565. doi: <https://doi.org/10.1007/s12161-017-0814-5>
- Mulvey, M.A. et al. (2000) 'Bad bugs and beleaguered bladders: interplay between uropathogenic Escherichia coli and innate host defenses', *Proceedings of the National Academy of Sciences*, 97 (16), pp. 8829-8835. doi: <https://doi.org/10.1073/pnas.97.16.882>
- Muniandy, S. et al. (2019) 'A reduced graphene oxide-titanium dioxide nanocomposite based electrochemical aptasensor for rapid and sensitive detection of Salmonella enterica', *Bioelectrochemistry*, 127, pp. 136-144. doi: <https://doi.org/10.1016/j.bioelechem.2019.02.005>
- Muramatsu, H. et al. (1989) 'Piezoelectric crystal biosensor system for detection of Escherichia coli', *Analytical Letters*, 22 (9), pp. 2155-2166. doi: <https://doi.org/10.1080/00032718908051244>
- Ng, M.-Y., and Liu, W.-C. (2009) 'Fluorescence enhancements of fiber-optic biosensor with metallic nanoparticles', *Optics Express*, 17 (7), pp. 5867-5878. doi: <https://doi.org/10.1364/OE.17.005867>
- Nikonovas, T. et al. (2020) 'Near-complete loss of fire-resistant primary tropical forest cover in Sumatra and Kalimantan', *Communications Earth & Environment*, 1 (1), p. 65. doi: <https://doi.org/10.1038/s43247-020-00069-4>
- Pandey, A. et al. (2017) 'Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic E. coli O157: H7', *Biosensors and Bioelectronics*, 91, pp. 225-231. doi: <https://doi.org/10.1016/j.bios.2016.12.041>
- Pangajam, A., Theyagarajan, K., and Dinakaran, K. (2020) 'Highly sensitive electrochemical detection of E. coli O157: H7 using conductive carbon dot/ZnO nanorod/PANI composite electrode', *Sensing and Bio-Sensing Research*, 29, p. 100317. doi: <https://doi.org/10.1016/j.sbsr.2019.100317>
- Park, J.Y. et al. (2015) 'Colorimetric detection system for Salmonella typhimurium based on peroxidase-like activity of magnetic nanoparticles with DNA aptamers', *Journal of Nanomaterials*, 2015 (1), p. 527126. doi: <https://doi.org/10.1155/2015/527126>
- Pebdeni, A.B., Hosseini, M., and Ganjali, M.R. (2020) 'Fluorescent turn-on aptasensor of Staphylococcus aureus based on the FRET between green carbon quantum dot and gold nanoparticle', *Food Analytical Methods*, 13 (11), pp. 2070-2079. doi: <https://doi.org/10.1007/s12161-020-01821-4>
- Pedrero, M., Campuzano, S., and Pingarrón, J.M. (2012) 'Magnetic beads-based electrochemical sensors applied to the detection and quantification of bioterrorism/biohazard agents', *Electroanalysis*, 24 (3), pp. 470-482. doi: <https://doi.org/10.1002/elan.201100528>
- Pohanka, M. (2018) 'Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications', *Materials*, 11 (3), p. 448. doi: <https://doi.org/10.3390/ma11030448>
- Pourmadadi, M. et al. (2019) 'A glassy carbon electrode modified with reduced graphene oxide and gold nanoparticles for electrochemical aptasensing of lipopolysaccharides from Escherichia coli bacteria', *Microchimica Acta*, 186, pp. 1-8. doi: <https://doi.org/10.1007/s00604-019-3957-9>
- Quaresma, A.J.P.G. et al. (2022) 'Molecular epidemiology of sporadic and outbreak-related Salmonella Typhi isolates in the Brazilian north region: a retrospective analysis from 1995 to 2013', *Infectious Disease Reports*, 14 (4), pp. 569-573. doi: <https://doi.org/10.3390/idr14040060>
- Quintela, I.A. et al. (2019) 'Simultaneous colorimetric detection of a variety of Salmonella spp. in food and environmental samples by optical biosensing using oligonucleotide-gold nanoparticles', *Frontiers in microbiology*, 10, p. 1138. doi: <https://doi.org/10.3389/fmicb.2019.01138>
- Rajapaksha, P. et al. (2019) 'A review of methods for the detection of pathogenic microorganisms', *Analyst*, 144 (2), pp. 396-411. doi: <https://doi.org/10.1039/C8AN01488D>
- Rakhimbekova, A. et al. (2022) 'Biofilm detection by a fiber-tip ball resonator optical fiber sensor', *Biosensors*, 12 (7), pp. 481. doi: <https://doi.org/10.3390/bios12070481>
- Rasooly, R. et al. (2019) 'T cell receptor V β 9 in method for Rapidly quantifying active staphylococcal enterotoxin type-A without live animals', *Toxins*, 11 (7), p. 399. doi: <https://doi.org/10.3390/toxins11070399>
- Römling, U. (2023) 'Is biofilm formation intrinsic to the origin of life?', *Environmental Microbiology*, 25(1), pp. 26-39. doi: <https://doi.org/10.1111/1462-2920.16179>
- Roushani, M., and Shahdost-Fard, F. (2015) 'Fabrication of an ultrasensitive ibuprofen nanoaptasensor based on covalent attachment of aptamer to electrochemically deposited gold-nanoparticles on glassy carbon electrode', *Talanta*, 144, pp. 510-516. doi: <https://doi.org/10.1016/j.talanta.2015.06.052>
- Saccomano, S.C., Jewell, M.P., and Cash, K.J. (2021) 'A review of chemosensors and biosensors for monitoring biofilm dynamics', *Sensors and Actuators Reports*, 3, pp. 100043. doi: <https://doi.org/10.1016/j.snr.2021.100043>
- Saxena, K. et al. (2022) 'Electrochemical immunosensor for detection of h. Pylori secretory protein vacA on g-c3n4/zno nanocomposite-modified au electrode', *ACS Omega*, 7 (36), pp. 32292-32301. doi: <https://doi.org/10.1021/acsomega.2c03627>
- Schnupf, P., and Sansonetti, P.J. (2019) 'Shigella pathogenesis: new insights through advanced methodologies', *Bacteria and Intracellularly*, pp. 15-39. doi: <https://doi.org/10.1128/9781683670261.ch2>

- Sheikhzadeh, E. et al. (2016) 'Label-free impedimetric biosensor for Salmonella Typhimurium detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer', *Biosensors and Bioelectronics*, 80, pp. 194-200. doi: <https://doi.org/10.1016/j.bios.2016.01.057>
- Sieuwerts, S. et al. (2008) 'A simple and fast method for determining colony forming units', *Letters in Applied Microbiology*, 47 (4), pp. 275-278. doi: <https://doi.org/10.1111/j.1472-765X.2008.02417.x>
- Sobhan, A. et al. (2022) 'A novel activated biochar-based immunosensor for rapid detection of E. coli O157: H7'. *Biosensors*, 12 (10), p. 908. doi: <https://doi.org/10.3390/bios12100908>
- Song, M.-S. et al. (2017) 'Detecting and discriminating Shigella sonnei using an aptamer-based fluorescent biosensor platform', *Molecules*, 22 (5), p. 825. doi: <https://doi.org/10.3390/molecules22050825>
- Song, Y. et al. (2023) 'A novel nanopatform based on biofunctionalized MNPs@ UCNPs for sensitive and rapid detection of Shigella'. *Chemosensors*, 11 (5), p. 309. doi: <https://doi.org/10.3390/chemosensors11050309>
- Soy, S., Sharma, S.R., and Nigam, V.K. (2022) 'Bio-fabrication of thermozyne-based nano-biosensors: their components and present scenario', *Journal of Materials Science: Materials in Electronics*, 33 (8), pp. 5523-5533. doi: <https://doi.org/10.1007/s10854-022-07741-9>
- Subramanian, S. et al. (2020) 'Microsystems for biofilm characterization and sensing—A review', *Biofilm*, 2, p. 100015. doi: <https://doi.org/10.1016/j.biofilm.2019.100015>
- The, H.C. et al. (2016) 'The genomic signatures of Shigella evolution, adaptation and geographical spread', *Nature Reviews Microbiology*, 14 (4), pp. 235-250. doi: <https://doi.org/10.1038/nrmicro.2016.10>
- Valones, M.A.A. et al. (2009) 'Principles and applications of polymerase chain reaction in medical diagnostic fields: a review', *Brazilian Journal of Microbiology*, 40, pp. 1-11. doi: <https://doi.org/10.1590/S1517-83822009000100001>
- Wang, G. et al. (2015) 'A glassy carbon electrode modified with graphene quantum dots and silver nanoparticles for simultaneous determination of guanine and adenine', *Microchimica Acta*, 182, pp. 315-322. doi: <https://doi.org/10.1007/s00604-014-1335-1>
- Wei, S. et al. (2022) 'On-site colorimetric detection of Salmonella typhimurium', *npj Science of Food*, 6 (1), p. 48. doi: <https://doi.org/10.1038/s41538-022-00164-0>
- Werwinski, S. et al. (2022) 'Monitoring aerobic marine bacterial biofilms on gold electrode surfaces and the influence of nitric oxide attachment control', *Analytical Chemistry*, 94 (36), pp. 12323-12332. doi: <https://doi.org/10.1021/acs.analchem.2c00934>
- Wu, W. et al. (2012) 'An aptamer-based biosensor for colorimetric detection of Escherichia coli O157: H7', *PLOS one*, 7 (11), p. e48999. doi: <https://doi.org/10.1371/journal.pone.0048999>
- Yadav, N., Chhillar, A.K., and Rana, J.S. (2020) 'Detection of pathogenic bacteria with special emphasis to biosensors integrated with AuNPs', *Sensors International*, 1, p. 100028. doi: <https://doi.org/10.1016/j.sintl.2020.100028>
- Yang, L., Li, Y., and Erf, G.F. (2004) 'Interdigitated array microelectrode-based electrochemical impedance immunosensor for detection of Escherichia coli O157: H7', *Analytical Chemistry*, 76 (4), pp. 1107-1113. doi: <https://doi.org/10.1021/ac0352575>
- Yuhana Ariffin, E. et al. (2020) 'A highly sensitive impedimetric DNA biosensor based on hollow silica microspheres for label-free determination of E. coli', *Sensors*, 20 (5), p. 1279. doi: <https://doi.org/10.3390/s20051279>
- Zaraee, N. et al. (2020) 'Highly sensitive and label-free digital detection of whole cell E. coli with Interferometric Reflectance Imaging', *Biosensors and Bioelectronics*, 162, p. 112258. doi: <https://doi.org/10.1016/j.bios.2020.112258>
- Zarei, S.S., Soleimani-Zad, S., and Ensafi, A.A. (2018) 'An impedimetric aptasensor for Shigella dysenteriae using a gold nanoparticle-modified glassy carbon electrode', *Microchimica Acta*, 185, pp. 1-9. doi: <https://doi.org/10.1007/s00604-018-3075-0>
- Zhang, X. et al. (2020) 'A label-free fluorescent aptasensor for detection of staphylococcal enterotoxin A based on aptamer-functionalized silver nanoclusters', *Polymers*, 12 (1), p. 152. doi: <https://doi.org/10.3390/polym12010152>
- Zhu, L. et al. (2016) 'Development of a double-antibody sandwich ELISA for rapid detection of Bacillus Cereus in food', *Scientific Reports*, 6 (1), p. 16092. doi: <https://doi.org/10.1038/srep16092>