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Rapid and accurate detection of foodborne pathogens: impedimetric immunosensor at the forefront

Abstract

Microorganisms can be categorized into five categories: bacteria, algae, fungi, viruses, and protozoa which can cause microbiological hazards, contaminating food during production, manufacture, transportation, and storage. Biosensor technology is one of the most reliable and effective analytical technique for determining a biological system's sensitivity and specificity at a very low scale. So many techniques exist for identifying and detecting food-borne illness causing bacteria. But biosensor technology is used to detect and monitor with accuracy, thus increasing its interest globally with time. Biosensors have been used for a long time to illustrate the process of regulating data in the pharmaceutical, food manufacturing, and processing industries. An immunosensor is a type of biosensor that uses the molecular recognition specificity of antigens to form a stable complex of an antibody. It is divided into two subcategories- labeled and label-free. A labeled immunosensor is primarily used to sense the immune reaction and generate signals that allow versatile detection of complex, and detect the microorganisms like *Listeria monocytogenes*, *Salmonella spp*., and *Escherichia coli.* Label-free immunosensors detect physical changes during the immune complex formation and have been explored because of their potential as a specific detection technique which can reduce the time and cost of analysis. It has been developed using several detection methods like optical changes for *Salmonella sp*., electrochemical changes for *Listeria monocytogenes* and *Escherichia coli*, and *Hepatitis B*. An impedimetric immunosensor for pathogens has been created utilizing a biosensor, electrochemical impedance spectroscopy, antibodies, affinity proteins, affimers, and other binding proteins like bio-receptors, which exhibit good selectivity. This review discusses the techniques used by immunosensors to recognize microorganisms that might cause food poisoning. These techniques consider the electrodes and base-layer components, the makeup and characteristics of distinct bacterial species, as well as the interactions between antigens and antibodies that are utilized to detect bacteria.

Keywords: biosensors, impedimetric immunosensor, electrochemical impedance spectroscopy, bacteria, antibody

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Abbreviations: EIS, electrochemical impedance spectroscopy; ECDC, European center of disease prevention and control; EFSA, European food safety authority; AuNP, gold nanoparticle; Ag-Ab, antigen-antibody; SPR, surface plasma resonance; Rs, resistance; Zw, warberge constant; Cdl, electrode surface; Ret, electron surface resistance; ELISA, enzyme-linked immunosorbent assay; F(ab')2, antigen binding fragment; QDs, quantum dots; AgNPs, silver nanoparticles; Ceo2 NPs, cerium oxide nanoparticle; CuNPs, copper based metal nanoparticle; STEC, shiga toxin-producing E.coli; IDAMs, gold integrated array microelectrode; MACA, mercaptoacetic acid; NHS, N- hydroxy succinimide; EDC, N- ethyl-N- dimethylamino propyl carbodiimide; MSNTs, magnetic silica nanotubes; TSSST-1, toxic shocking syndrome; HUS, hemolytic uremia syndrome; SPCE, screen printed carbon electrode.

Introduction

Every year, thousands of people are infected due to foodborne diseases. In the USA, a report showed roughly 9 million individuals approx. experience illness each year; among them, 56,000 are hospitalized, and 1,300 people have died.[1] Additionally, the European Center for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) in their 2020 report presented that a total number of 186,000 instances of food infection have been confirmed, with 17,000 hospitalizations and 330 deaths due to foodborne pathogenic illness.[2] In addition, it has been noted that most of these food-borne infections are brought on by the toxic effects of Shiga toxin-producing *Escherichia coli, salmonella, Listeria monocytogenes, Campylobacter spp.,* etc. bacteria.

To detect different types of pathogens rapidly, biosensor technology is needed. Biosensing is an interdisciplinary branch of foodomics that uses a biosensor, a tiny gadget that receives information from the chemical reaction and converts these signals to an electronic or any other processable information.[1–3] Biosensors consist of four components, these are Analyte, Bioreceptor, Transducer, and Electronic System.

Analyte: It can be a biomolecules that are obligatory to detect[4] microorganisms.

Ex: Glucose is an analyte for Glucose Biosensors.

Bioreceptor: Bioreceptors, can be defined as the molecules which are specific to the analyte employed.

A signal that can be triggered during biorecognition, results from the interaction between the bioreceptor and the analyte.[5]

Ex: Signal may be in the guise of temperature, pH, light, etc.

Transducer: Transducer is an important portion in which sensing or signal converts one form of energy to another form, it converts biorecognition to a detectable signal. The produced signal and analyte-bioreceptor interaction have a correlative relation.[6]

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Ex: Transducer mainly produced optical and electrical signals (Figure 1).

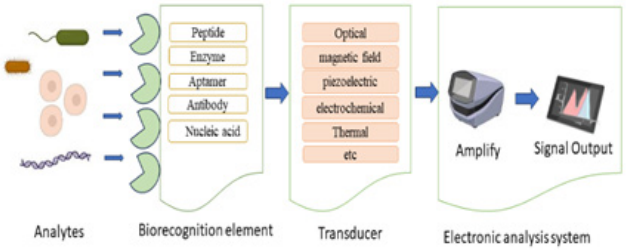

Figure 1 Parts of biosensors for assessing signal output.

Electronic System: In this electronic system, multistage amplification takes place. It comprises of signal conditioning circuit, processor, and display unit. It shows the result in a human comprehendible manner.[4,6]

Biosensors classification

In this interdisciplinary field, the classification of biosensors is based on receptors, and transducers that are used. Bioreceptors are specific to analytes for producing a signal in a transducer, for measuring and sending it to the display unit after amplification, and then converting it, understandably. Various benchmarks are involved in this classification of biosensors. Figure 2 provides a graphical representation of this categorization. The biosensor may be categorized according to the type of transducer and the bioreceptor where the following are bioreceptors- Ab-Ag, nucleic acid, DNA, enzymes, etc.[5] It can also be an electrode, piezo-electric device, pH electrode, semiconductor, etc. Electrochemical sensor techniques are the most common type of transducer. Transducers are impedimetric, amperometric, and potentiometric. In this study, we primarily concentrated on the impedimetric immunosensor technology, in which conductometric sensors are employed to calculate the propensity of the medium or electrolyte solution to let the current pass through the electrode (working) and reference/counter electrode. It is applied to examine the changes of capacitance.[6,7] Immunosensor is also a type of biosensor, and the working principle is established on the specificity of molecular recognition between antigen-antibodies, to form a stable quantifiable complex. This type of immunosensor is further classified as labeled (indirect) and label-free (direct) methods to detect food-borne pathogenic bacteria.[8]

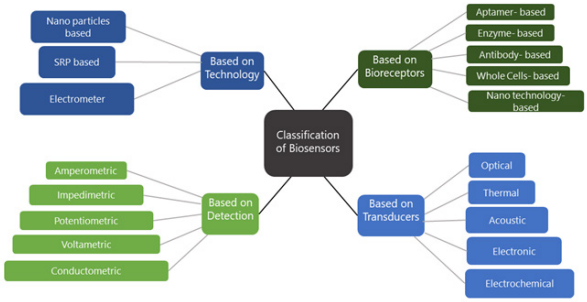

Figure 2 Classification of biosensors.

Nowadays, research, and development in biosensing technology are very popular, and the advancement in nanoscience opened a new system in the biological science and engineering field. Impedimetric sensing gives simultaneous detection of different biospecies by using electrochemical impedance, and affinity-based measurement.[3] This review intends to report and give a detailed idea about the construction, and working strategy of impedimetric immunosensors. Its role in detecting different food-borne pathogenic bacteria, and their viability, source of infection, mode of transmission, incubation period, and symptoms, detection of these bacteria.[8]

Immunosensor

As a tool for clinical diagnosis, food safety control, etc., immunosensors, i.e. a type of affinity-based solid-state biosensor, have grown its popularity. It can easily detect immunoreaction between an antibody and its target antigen that results in the formation of a stable immune complex. Here the antibody acts as a capturing agent and then generates a measurably strong signal by the transducer. [9] The main distinction between immunosensor and immunoassay is that, an immunoassay is based on the concept of antigen-antibody interaction while the immunosensor is based on the signal development detecting the immunocomplexes. Some chemical means are used to quantify the antigen-antibody complex like ELISA.[10,11] Labeled immunosensors can also be divided into competitive and non-competitive immunosensor formats.

Immunosensors can be categorized according to how they transmit information that include electrochemical immunosensors (amperometric, potentiometric, impedance, and conductometric), optical immunosensors (luminescence, fluorescence, refractive index), and piezoelectric immunosensorss.[2,12,13]

Types of immunosensor

Different types of immunosensors are described are the following:

A. Direct Immunosensor: A direct immunosensor can also be called a label-free immunosensor, which is worthy of detecting chemical and physical changes generated from the interaction between the antigen-antibody complex. Direct immunosensors do not require any types of labels and be employed for real-time analysis.[14] However, it has a substantial impact on non-specific adsorption. No signal is generated without an antigen-antibody response. However, a weak signal is always perceived due to the antigen's propensity for non-specific binding to the surface.[15,16]

B. Indirect Immunosensor: An indirect immunosensor can also be called a labeled immunosensor, these are generally based on the principle of generating a signal from one/more labels, allowing highly delicate detection. In this type of immunosensor, measurements of enzymes like catalase, glucose oxidase,[17] and alkaline phosphatase[18] Prussian blue, ferrocene are utilized as a mediators. Several nanoparticles, including gold nanoparticles (AuNPs), are employed for signal amplification (Figure 3).[13]

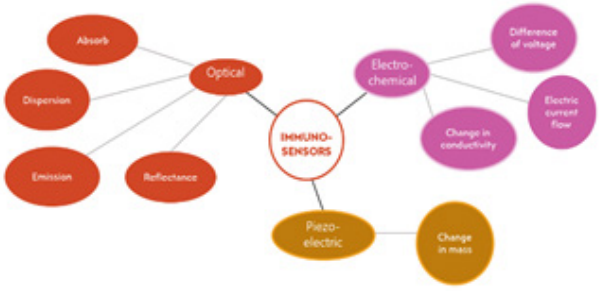

Figure 3 Types of immunosensors.

Labeled or Indirect immunosensors can also be classified into two categories described below:

A. Competitive immunosensor: Small antigens with a single epitope are detected using a competitive immunosensor. In the case of the sample analytes and labeled analytes, both have

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competed to access very few limited numbers of antibody binding sites. This limited antibody availability is known as "limited reagent assay".[13,19,21] This immunoassay is carried out in two ways:

i. Homogenous approach: Here, labeled unbound antigens are measured and there is no need for a separation procedure.[20]

ii. Heterogenous approach: Here, labeled bound antigens are measured, and a need for a separation procedure. In the washing step, unbound antigens should be removed for further procedure.[13,21]

B. Non-competitive immunosensor: Non-competitive immunosensor also called "sandwich" immunoassay is applied to large antigens along with one or more epitopes. In this process, large amounts of two types of antibodies: primary and secondary are employed and then antigens act as sandwiches in between these two antibodies.[13] Antibodies are immobilized on a solid surface to capture the primary antibody, and the antigen also called the sample analyte is sandwiched between primary and secondary antibody. Finally, a tagged secondary antibody attaches to the antigen-antibody complex to generate a quantifiable signal (Figure 4).[20]

C. Electrochemical immunosensor: The antibody is immobilized on the electrode surface which results in antigen-antibody binding reaction on the electrode generating measurable voltage. It can be subdivided into four types[22] as described below:

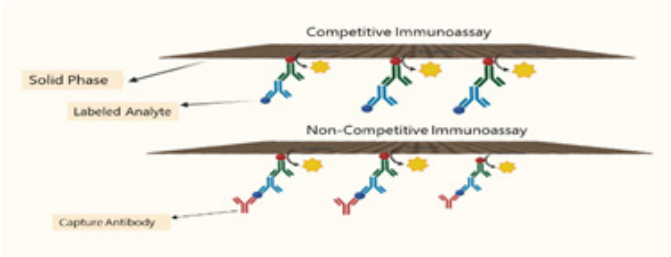

Figure 4 Categories of indirect immunosensors.

Amperometric immunosensor: This type of immunosensor is also known as voltammetric immunosensor. Here, it measures the current at constant potential on both the working and reference electrodes. The current or voltage is acquired by electrochemically oxidizing a sample or an electroactive species.[23] Amperometric immunosensors cannot be used in immune-sensing applications because most antigens and antibodies do not have any electroactivity. This is the major disadvantage of this process.[5,19] However, in the case of biosensors like glucose biosensors, they show better sensitivity than potentiometric biosensors, because their electrodes are modified with electrochemical anodization and electrochemical deposition.[24] The amperometric biosensor is used to detect specific enzymes. For instance, oxidoreductase catalyzes the biochemical reaction on the transducer, which develops into a color shift, caused by electricity flowing over an electrode surface, and the current flow is inversely related to the analyte concentration.[25] This relationship may be regulated and calculated by Faraday's Law:

$$I = n.F.A.J \quad (1)$$

In this equation, I= (Current), n = (numbers of electron transport to electrode), and F = (Faraday constant), A = (electrode area), and J = (Flux coefficient). Thus, amperometric immunosensor may have application as biosensor[26] detecting the concentration of glucose in microbial cultures, animals, and lipids like cholesterol.[27]

Impedimetric immunosensor: Impedimetric immunosensor is used to measure medium tendency or electrolyte solution that allows the passing of electrical current through the working electrode and reference electrode.[8] In case of impedance or impedimetric process, alternating current circuit is used, with an influence of inductive and capacitive effects.[28-30] A direct current circuit or flow only detects a value of resistance but an alternative current is employed to determine changes in capacitive value in the electrode by impedimetric immunosensing.[31]

Potentiometric immunosensor: Potentiometric immunosensor is based on the measurement of electron gathering or voltage in the working electrode compared to the reference electrode without significant flow of charge across them.[8] The potential difference changes because of the formation of an immune complex due to antigen-antibody interaction. Some label-free immunosensors are included in this group. Potentiometric immunosensor is used for their simplicity of operation allowing miniaturization on solid-state sensors.[5,21]

Conductometric immunosensor: These types of immunosensors are based on the relationship between biorecognition event and conductance. At the time of antigen-antibody reaction, an antigen as a biorecognition element reacts with a solution of conductivity, changes the ionic concentration of some species that alters the current flow. Supporting electrolytes change conductivity since antibodies are labeled with an enzyme, which is further conjugated with antigen in a solution of sample.[13] Then the signal is measured by an ohmmeter after completion of the reaction. This type of immunosensor is used for large-scale manufacturing industry and miniaturization even without reference electrodes.[32]

Optical immunosensor: The principles of absorbance measurement, fluorescence emission, reflectance, and NIR (near infrared) comprise the foundation of an optical immunosensor.[33] In the case of optical immunosensors, label-free method of transduction is mostly used, known as "Surface Plasmon Resonance" (SPR). The working principle of this type of immunosensor use light and the changes in refractive index[34] using biological samples. The main disadvantage of this type of immunosensor is that interference of color analytes samples like blood, urine, etc. hinders desired result. Optical Immunosensing devices are more expensive than any other immuno-sensing process.[29,35,36] Other types of immunosensor like Piezoelectric and Calorimetric immunosensors are also used for biosensing techniques (Figure 5).[37]

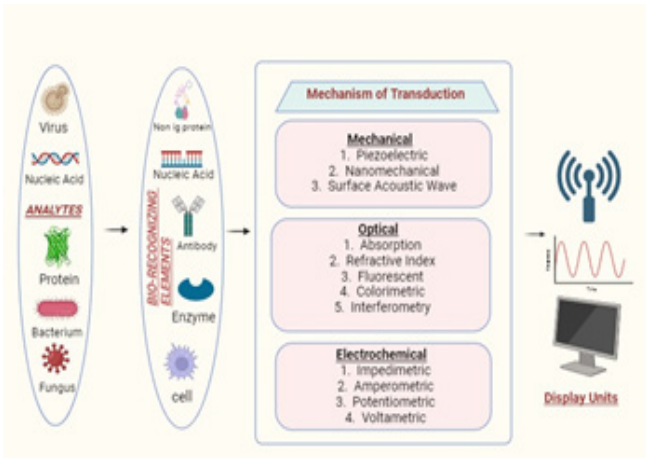

Figure 5 Types of immunosensors based on different biorecognition elements and transducers.

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In this review, the main concern is the impedimetric immunosensor, and its mechanism, working principle, and detection of food-borne pathogens through it. So, here is a brief description about this type of immunosensor.

Impedimetric immunosensor

Various categories of immunosensors are described above, to keep antibodies firmly attached to an electrode surface. Here impedance is a composite resistance that comes across when a current passes through the circuit, which consists of capacitors, resistors, or any other combination of these.[32] Molecules recognize and immobilize specific antigens causing a change between interfacial charge, thickness, resistance, mass, and capacitance in the surface of the immunosensors.[21] The electrochemical cell surface's impedance is based on spectroscopic impedance. It traverses a full alternating circuit (AC), and the space between the current and voltage phase-angle.[29]

Technique of impedance principle

Electrochemical impedance may be denoted as (z) and it is represented as an extent of (v [t]/I[t]) incremental voltage changes consequent to changes in the flow of current.[38]

$$Z = \frac{V\left(t\right)}{I\left(t\right)} + \frac{1}{Y} + \frac{V0\,sin\left(2\Pi ft\right)}{I\theta\,sin\left(2\Pi ft + \Pi\right)} \quad (2)$$

Here, V0 = Best voltage

φ= stage movement between

Current-time and voltage-time limits

I0 = Current

Y= Conductance

f= recurrence

t=period

Considering, the impact of various factors which is represented through the modulus |Z| and stage movement | φ | through ZR which is the real part, and ZI which is the nonexistence of impedance leads to impedance wobble.[38,39]

Working procedure

In the impedance technique, an equivalent circuit of an electrode undergoes a heterogeneous electron transfer. This electron transfer is followed by Randel's equivalent circuit model. This equivalent circuit is useful for impedance spectra interpretation.[38,40] At first, biological recognition elements that are highly specific to the analyst come close to each other.

i. The transducer detects and sends the signal toward the amplifier from the biological target to the mechanical signal due to the occurrence of antigen-antibody reactions.
ii. That mechanical or electrical signals are amplified.
iii. The amplified signals after processing are sent to a display unit.
iv. Then the values are displayed on the monitor.

What Is antibody?

An antibody can be defined as a substance that is naturally produced in the human body by plasma cells as an adaptive immune response for protection against pathogens. It can be categorized into 5 different categories: IgG, IgD, IgM, IgA, and IgE according, to different classes of their glycoproteins, also known as immunoglobulins. Antibodies are an essential revolutionary substance in the field of medicine. So, immunosensor technology is based on antibodies just like the ELISA (Enzyme-Linked Immunosorbent Assay) test by using antigen-antibody interaction processing.[41,42]

So, now the main concern is antigen-antibody interaction, the core concept of immunosensor technology sectors.

The antigen-antibody interaction

There are different classes of antibodies based on the presence of glycoprotein chains. Among 5 different types, IgG is the most abundant and popular class and is often used in immune-sensing techniques. In IgG structure, there are two chains: the light chain and another type is a heavy chain, these are divided into two classes depending on the order of their amino acids: variable {V} and constant {C}.[21] There are three fixed domains in the heavy chain, these are CH1, CH2, and CH3, and a single variable domain, which is VH. The light chain includes a single constant domain which is C1 and a single variable domain which is V1. An antibody is divided into two categories or fragments, one antigen-binding fragment, known as [F(ab')2], and a nonantigen-binding [Fe] fragment.[43] The antigens and antibodies are specified to each other (like a lock and key model) and it may be abbreviated by Ag-Ab type reaction. The first stage of this reaction is the Ag-Ab complex formation. Three factors interfere with this complex formation – affinity of the antibody, intermolecular forces, and closeness of antigen-antibody.

An affinity of binding an antigen and antibody may be interpreted by following equation[21]:

$$K = \frac{\left[Ab - Ag\right]}{\left[Ab\right]\left[Ag\right]} \quad (3)$$

Here, K= equilibrium constant

Ab-Ag= immunocomplex formed between specific Ab-Ag (K value range: 106-1012 Lmol-1)

The specificity of antigen towards antibody on its binding site depends on amino acid sequences. Within the V_H and V_L domain, three subregions are known as hypervariable regions.[21,43]

Spectroscopy of electrochemical impedance and data presentation

EIS or a spectroscopy of electrochemical impedance is based on application of low voltage amplitude sine-wave towards an electrical system having a broad frequency range. The ratio between the current and applied voltage is called an impedance, which indicates the opposition of electron flow in an AC circuit.[25] The antigen-antibody interaction in an immunosensor causes modification in the electrical transducer field because of the variations in the resistance of the transfer electron t and also in the capacitance within the surface of the working electrode.[30]

$$Z\left(jw\right) = \frac{V\left(jw\right)}{I(\left(jw\right)} \quad (4)$$

Here, Z= impedance, I= current, V= voltage, and w= frequency.[28,30]

If the phase angle between intensity and voltage is zero then the resistance and impedance remain the same but, in most cases, since capacitive effects have a knock-on effect, the phase angle between these two is different from zero.

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Impedance data presentation follows two paths or ways – Nyquist plots and Bode plots. Nyquist plots interpreted the relation of the imaginary and real impedance of a broad spectrum of frequencies. Bode plots interpreted the logarithm of phase shift and absolute impedance vs logarithm of frequency (excitation).[8] At a high range of frequencies, a signal is influenced and it is also controlled by the kinetic process. Before, the redox reaction the electron mediator changes the direction of charges at the surface of the electrode which causes delays in the transfer of charges in the electrode and this process is marked as the resistance (RS) frequencies (medium). Less resistance in the system is observed and it is specific for double-layer capacitance (Cdl).[42] As a result of it, there is a change in the system due to capacitance. In low frequencies, the charge is transferred by resistance since the opposition can get electron mediators because of the components of the surface.[44] Low frequencies are observed in Warburg impedance which can be plotted as the linear tail on the end of the arc of Nyquist.[28,45,46]

Uses of nanoparticles in immunosensors as electroactive labels

There are so many nanoparticles available in the market, that are used as labels in immune-sensing. Some of the nanoparticles used frequently are Gold Nanoparticles (AuNPs), Quantum Dots (QDs), Silver Nanoparticles (AgNPs), cerium oxide nanoparticles (CeO2 NPs), Copper-based metal nanoparticles (CuNPs), and so on.

Here, a brief description of using Gold Nanoparticles in immune-sensing technology is given because of their high popularity rather than any other nanoparticles.

Gold nanoparticles (AuNPs) are often used just for their simple synthesis, electrochemical properties, optical properties, narrow in size, and bi-conjugated alternatives.[47] One of the methods from the most significant study presented by Limoges' group, employs AuNPs as labels in the immunoassay of immunoglobulin G, or IgG, for their detection at extremely low g/mL levels.[48] According to reports, Salmonella typhimurium may be detected using a magneto immunoassay that employs magnetic particles and AuNPs and is connected to antibodies that can detect as little as 7 cells/mL.[49] Having a high extinction coefficient, AuNPs are emerging from the intrinsic plasmonic properties.[50] Their optical characteristics rely on the separation distance between the particles as well as on aggregation, which results in a dramatic shift in the extinction range and changes the color of suspensions.[51] AuNPs provide a suitable microenvironment because of the immobilization of biomolecules and help to facilitate transfers of electrons between the surface of the electrode and the immobilized biomolecule.[52] It has an intensive use for the establishment of an immunosensor with high and accurate analytical performance (Figure 6).[53]

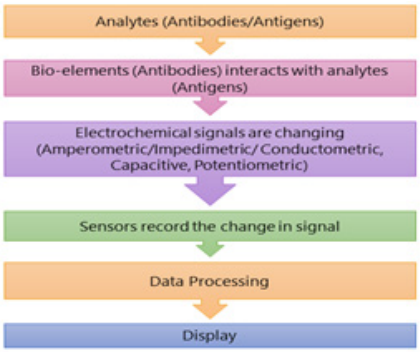

Figure 6 Schematic diagram of immunosensor.

Foodborne pathogenic bacteria

A pathogen can be defined as an agent that causes diseases in humans and along with animals and plants. Foodborne pathogens cause intestinal disorders in humans causing a huge economic burden followed by serious health problems.[54] Diseases caused because of food-borne pathogens have serious outcomes on food safety and quality assurance to public health issues. A recent report shows more than 250 diseases that are already known, are caused due to various food-borne pathogenic micro-organisms e.g. pathogenic bacteria, viruses, parasites, fungi, etc. Among all of these food-borne pathogenic bacteria causes 91% of serious illness in the USA.[54,55] In India, states like West Bengal (31.22%), Gujrat (22.67%), and Karnataka (29.11%), are reported to have severe food-borne illnesses which leads to 31.5% serious illnesses, and 8.7% within the 2008-2018 period.[56]

There are so many traditional approaches for detection of the food-borne pathogenic bacteria but these methods are expensive and take several days to give the result both qualitatively and quantitatively.[52] In the food industry, it is a must to detect this pathogen in food very rapidly within a short period, so here comes immuno-sensing technology as an alternative to the conventional detection process. By using this sensor technology, we can get accurate results within a very limited period.[40]

Some of the common foodborne illness-causing bacteria that are detected through impedimetric immunosensors are *E. coli, Salmonella, Listeria monocytogens, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus pyogenes*.[7] The routes of infection, viability, modes of transmission, incubation period, and symptoms of these mentioned pathogens are discussed thoroughly in the following section:

Escherichia coli

Humans and warm-blooded animals both have the bacteria *E. coli* in their guts. E. coli belongs to different categories according to their epidemiology, serotype, and virulence mechanisms. According to WHO, most strains of *E. coli* do not cause severe diseases but a strain producing the Shiga toxin of E. coli (STEC) may cause severe foodborne illness.[57] It is transmitted in the human body by contaminated food consumption like raw milk, undercooked meats, and raw, unwashed vegetables. E. coli can be classified into six different sub-categories.[58]

These are:

a) coli with enterotoxin (ETEC)

b) coli Enteroinvasive (EIEC) and enteropathogenic (EPEC)

c) coli that is enteroaggregative

d) E. coli that is widely adherent (DACE)

e) E. coli with hemorrhagic symptoms (EHEC) EC is known as Enteric E. coli which is classified because of virulence properties and serological characteristics.[57]

Viability

i. **Growing temperature:** 7°C-50°C.

ii. **Optimum temperature:** 37°C.

iii. **Growing nature:** in acidic foods.

iv. **PH:** 4.4 o Water activity [minimum] (aW): 0.95

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Mode of transmission

1) The serotype *E. coli* has been recognized as the route of various sporadic cases of foodborne illness in the human body and it was recognized as a pathogen in 1982.
2) Sometimes the complication of illness leads to death.
3) Cattle are a natural reservoir of Enterohemorrhagic *Escherichia coli* (EHEC), 75% of EHEC are linked to consuming tainted goods generated from or connected to cattle.[58]
4) *coli* or (O157:H7) which is a serotype that produces Shiga toxin may cause human sickness through the consumption of contaminated milk(raw) derived from products like yogurt, unpasteurized fresh-pressed apple cider, and undercooked ground meat products.[59]
5) The largest outburst of *E. coli 0157:H7* was reported in Sakai, Japan in 1996 due to the excessive consumption of radish sprouts which are washed by contaminated water. Approx, 3 children and 6000 people died because of this outbreak of infection.
6) *coli 0157:H7* is often found in the feces of healthy cattle and it is transmitted by the water, mostly by food and direct contamination with infected people.
7) The infectious dose is very little, less than 100 cells. So, by these people transmission occurs sometimes[60]

Incubation period

i. The incubation period of *E. coli 0157:H7* is about 1 week or less for adults but it may be longer for children.
ii. In most cases, the disease is self-limiting in 5-10 days.

Signs and symptoms

a) Benign symptoms are fever, abdominal cramping, and diarrhea.
b) In severe cases, bloody diarrhea may be observed.
c) Hemolytic uremia syndrome (HUS), and kidney failure may also occur over a long time of infectious period.
d) Fever is absent or low grade.

Detection through impedimetric immunosensor

It is a gram-negative bacterium. Detection through impedimetric immunosensor of E. coli is most popular nowadays because it is simple to manipulate in the lab. For the detection, impedimetric immunosensors are employed with gold interdigitated array microelectrodes (IDAMs), and are inserted in a microfluidic device.[28,61,62] This system can detect low concentrations of [1.2×10^3 CFU/mL] from the beef sample (ground). And detect [1.6×10^2 CFU/mL] from the culture (pure) within 35 minutes only. E. coli was conjugated with specific antibodies to magnetic nanoparticles by linking with biotin-streptavidin. As a result, magnetic nanoparticles and antibody conjugates are formed which arrest bacteria and concentrate them. A microfluidic device was then used to place the sample due to impedimetric immune-sensing.[28] Gold is the most useful material for integrated array microelectrodes. Dimensions that may be used for IDAMs are 0.1 to 0.2 μm, high for the finger of each electrode and a length of 1-20 mm with inner space of electrode 1-20 μm is used.[28,62] In river water, the gold electrodes are covered with mercaptoacetic acid, or (MACA) SAM, to detect the presence of E. coli. The formation of peptide bonds with antibodies catalyzed by the compounds N-hydroxy succinimide (NHS) and N-ethyl-N-dimethylaminopropyl-carbodiimide (EDC) which are treated with the sample construction.[63] The addition of NHS and EDC leads to a carboxylic group (terminal) of MACA replacement by the esterification of NHS. That may cause a nucleophilic attack through an amine group. The limit of detection of [1×10^3 CFU/mL] was attained by 20 μL sample volume within 1 hour. Self-assembled monolayer (SAM) forms a barrier between the analyte solution and electrode and serves as a dielectric obstacle to the investigation of the transfer of electrons.[61] The incorporation of AuNPS in immunosensor construction creates an ab microenvironment for immobilization and stabilization of biomolecules (i.e., Antibodies) and arouses electron transfer among electrode and sample material.[28]

Salmonella

It is a facultative, rod-shaped, gram-negative bacteria, family Enterobacteriaceae, as *E. coli*, also known as "enteric" bacteria. Salmonella produces two different illnesses in people. These are acute gastroenteritis and salmonellosis (enteric fever typhoid).[64] Salmonellosis is caused by the invasion of bacteria into the bloodstream and acute gastroenteritis caused due to foodborne intoxication. When a human or other animal becomes clinically diseased or continues to harbor the infection, they may expel salmonella. Salmonella can be divided into two categories[65]:

I. Non-typhoidal *Salmonella* i.e., *Salmonella javiana*, Salmonella enteritidis, Salmonella typhimurium
II. Typhoidal *Salmonella* i.e., Salmonella typhi, *Salmonella schottmulleri*, *Salmonella paratyphi*-A .

Viability

a) Disseminated in the natural environment as soil, water, or plant foods through animal or human excretion.
b) Multiply significantly in a natural environment.
c) They can survive in water for several weeks as well as the year in the soil if temperature, pH, and humidity are in favor.

Mode of transmission

1) It can spread the fecal-oral route.
2) Transmitted by water and food, direct contact with an infected animal.
3) Most of the transmission happens by food. For example, consuming poultry, milk, eggs, etc.
4) Animals' digestive tracts are home to most salmonella species and thus by eating tainted food of animal origin can be passed to humans.[66]
5) Consumption of uncooked or superficially cooked seafood also caused salmonellosis.
6) Vegetables and fruits (unwashed) cause salmonella because they may be contaminated with fertilizers of fecal origin.[65,67]

Incubation period

I. Disease symptoms may occur within 12-72 hours after consuming foods containing enough *Salmonella*.
II. Symptoms may last for 2-5 days.[65,66]

Signs and symptoms

i. Diarrhea (in severe cases sometimes blood-tinged may be observed).

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ii. Abdominal cramps, fever, nausea, vomiting.

iii. If the infection spreads to the bloodstream and other distant organs, the duration of illness is increased from 2-5 days, and inflammation, focal infection, and septicemia may occur.[67]

iv. Arthritis may occur in long-term infection (although it is a sporadic case)

v. The *Salmonella* serotype is associated with three types of human illness including enterocolitis, typhoid fever, and bacteremia.[65-67]

Detection through impedimetric immunosensor

The most common types of salmonella that infect humans are predominantly *Salmonella typhi* and *Salmonella typhimurium*. Symptoms of S. typhimurium appear within 12-72 hours after ingestion.[68] This serotype of Salmonella is mainly found in milk. It can be detected by an immunosensor using a working electrode (gold), covered with SAM (thiol-based). When the detection processing occurs, antibodies opposed to salmonella are indulgent with the cross-linkage of glutaraldehyde in SAM. LOD of 10^2 CFU/mL in a 2-hour (2mL) milk sample is detected through an impedimetric immunosensor.[69,70] Gold IDAMs are used to construct an immunosensor that can find certain strains of salmonella with the help of novel magnetic silica nanotubes (MSNTs), due to the capacity to capture bacteria using electrostatic interaction.[71,72] MSNTs are used due to their multifunctional structure and for that, a gold IDAM can detect a LOD of [10^2 CFU/mL] in a [50 µL] volume of sample within 1 hour only.[69,70] For the detection of S. typhi, AuNPs were used covered with antibodies and can detect LOD i.e., the detection limit is [10^2 CFU/mL] in [10 µL] of sample within 1 hour.[28,73,74]

Listeria monocytogenes

It is a rod-shaped, facultative, intracellular, gram-positive bacterium. The reason behind the listeriosis infection is the consumption of contaminated food. Listeria monocytogenes is common among pregnant women, neonates, and elderly people also.[75] Listeriosis is manifested by perinatal infection, systemic infection, and febrile gastroenteritis, and affects the central nervous system also. Invasive listeriosis is very severe and causes meningoencephalitis, abortion, and sepsis. The Listeria family comprises 10 different species including Listeria monocytogenes. Listeria monocytogenes has 13 different types of serotypes because of the flagellar varieties and surface antigens. In humans only three types of serotypes of *Listeria monocytogenes* cause the disease, these are 1/2a, 1/2b, 4a.[76]

Viability

1) Eating contaminated food containing a higher number of *Listeria monocytogenes* is the main cause of infection.

2) L. monocytogenes may survive in low temperatures, found in refrigerators (if sufficient time is available).

3) Also found in soil, water, and animal digestive tract.[77]

Mode of transmission

a) Infection can be transmitted from human to human, and from pregnant women to fetal.

b) Ready-to-eat food may be contaminated during the processing step then the bacteria may multiply to dangerous levels when it undergoes the distribution and storage step.

c) Mainly 2 types of listeriosis are observed: invasive and non-invasive listeriosis. Invasive listeriosis is more severe than non-invasive listeriosis.[75,77]

Incubation period

a) The incubation period is usually 1-2 weeks, and can be varied between 2-3 days and up to 90 days.

b) Non-invasive listeriosis incubation is much shorter than invasive.[77]

Signs and symptoms

i. Non-invasive listeriosis can also be called febrile listerial gastroenteritis, symptoms include headache, fever, diarrhea, and muscular pain.[75]

ii. Invasive listeriosis shows severe symptoms like myalgia, septicemia, fever, and meningitis.

iii. Chills, fatigue, nausea, and vomiting were also observed.[75,77]

Detection through impedimetric immunosensor

The cheaper and faster process of detecting *Listeria monocytogenes* by an impedimetric immunosensor is frequently popular and widely used these days. TiO2 as a nanowire bundle of the micro-electrode is employed to immunosensor because of the detection of that bacteria. LOD of [4.7×10^2 CFU/mL] in [15 µL] of the sample can be detected within 50 min, by using dot-blot assay LODs of [2.2×10^4 CFU/mL] and [2.2×10^5 CFU/mL] can be detected accordingly.[78] Magnetic nanoparticles (30nm) are used to coat the antibodies in opposition to specific pathogenic bacteria like *L. monocytogenes* by biotin-streptavidin coupling. The system can detect LOD of [10^4 CFU / mL] from the food samples milk, lettuce, or ground beef within 3 hours of immunoreaction. Because of the favorable biocompatibility, photochemical stability, and good chemical stability TiO2 nanowires in immunosensors are readily popular.[79]

Staphylococcus aureus

It is a gram-positive bacterium, belonging to the group of Micrococcaaceae family. A severe form of food poisoning caused by Staphylococcal is globally widespread. It occurs because of the ingestion of improperly stored, cooked, or uncooked food having many *S. aureus*.[80] It contains different protein toxins and thus is responsible for infections and S. aureus may secrete two types of toxins, having superantigen activity.

I. One is enterotoxins (six types of antigenic types that is SE-A, D, B, C., G, E)

II. Another type is toxin-causing toxic shock syndrome (TSST-1). TSST-1 is a superantigen that may cause toxic shock symptoms during infection.

Viability

i. It is resistant to heat, drying, and radiation.

ii. Food can be heated if the bacteria have begun to produce the toxin; however, this only kills the bacteria and not the toxin. These types of organisms can grow in 10% salt and the colonies are mainly Gold or yellowish (aureus: gold/yellow) in colour.

iii. These organisms can develop both aerobically and anaerobically, and in facultative conditions also and the temperatures range between 18° C to 40°C.[80]

Mode of transmission

i. Commonly found in processed meats, chicken, salad, pastries, ice cream, ham, etc.

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ii. It is also found in the nasal passages (skin of humans); through these sources, it can easily enter food.

iii. Illness can be passed through a person.

Incubation period

i. Symptoms usually develop within 30 min-8 hours of consuming an item containing staph toxin.

ii. Severe illness is rare.

Signs and symptoms:

i. Sudden start of nausea, vomiting

ii. Stomach cramps

iii. Diarrhea

iv. Skin and soft tissue infection

v. Osteomyelitis and septic arthritis

vi. Pulmonary infections, gastroenteritis and meningitis also.

vii. Toxic-shock syndrome (TSS),

viii. Urinary tract infections.[80]

Detection through impedimetric immunosensor

To detect *S. aureus*, an impedimetric immunosensor was created by attaching a gold electrode to it and using the 6-mercapto hexadecenoic acid, also known as SAM. This is very specific to antibodies against *S. aureus*, which are subsequently connected for additional immune response.[28,81]

Streptococcus pyogenes and Pseudomonas aeruginosa

Gram-positive *Streptococcus pyogenes* bacteria cause infections and is an oxidase-negative, catalase-negative, and facultative anaerobe—also, a β-hemolytic streptococci. Based on post-infectious sequelae of *S. pyogenes* i.e., divided into 2 categories: Class I, Class II[82,83]

a) Class I strains are responsible for rheumatic fever.

b) Class II strains responsible for acute glomerulonephritis.

c) Pseudomonas aeruginosa is a rod-shaped, mono-flagellated, gram-negative bacterium[84] with two antigens.

d) O-Ag: This common polysaccharide-based antigen, also known as the A-band, is made of the homopolymer d-rhamnose.

e) O-specific antigen, or B-band, which is made up of a hetero-polymer with repeat units that range from three to five different sugars.

Viability

a) Streptococcus pyogenes grows best in the presence of 5-10% carbon dioxide.

b) Streptococcus pyogenes form pinpoint colonies on the blood agar plates.[83]

c) Pseudomonas aeruginosa grows best at 25°C-37°C, ability to grow: 42°C.

d) Pseudomonas aeruginosa's show the ability to survive under various extreme conditions of environments.[84]

Mode of transmission:

a) Streptococcus pyogenes colonizes in the anus, pharynx, and genital mucosa.

b) Infections caused due to *S. pyogenes* are very contagious and harmful.

c) Transmission may occur by the droplets of airborne, hand contact, and nasal discharge.[83]

d) Pseudomonas aeruginosa colonizes the surfaces of packaged food, water taps, and medical devices in the form of biofilm.[84]

Incubation period: *Streptococcus pyogenes* and *Pseudomonas aeruginosa*

A LOD of 10^2 cells in a 10 µL volume sample was detected within 30 min only by this process.

Pseudomonas aeruginosa can be detected by an impedimetric immunosensor modified with immobilizing polyclonal antibodies concerning *Pseudomonas aeruginosa* on the screen-printed carbon-electrode also known as SPCE.[28,85]

With the evolution of the impedimetric immunosensor, the identification or detection of foodborne pathogenic bacteria uses different kinds of parameters for the detection process.[86] Both types of bacterial incubation periods in the human body varied according to their dose or amount of ingestion.[87]

Symptoms may usually appear within 1-2 days after ingestion through food or water.

Signs and symptoms

In the case of *Streptococcus pyogenes* symptoms are fever, pharyngeal exudate, malaise, enlarged tonsils, and tender cervical lymphadenopathy.[86] In children most common symptom is non-bullous impetigo (skin infection). It is an itchy reddish-colored rash naturally grown around the surface area of the nose and mouth.[83] Patient with scarlet fever also develops strawberry-like tongue and sore throat, pharyngitis.[87]

In the event of *Pseudomonas aeruginosa* symptoms are ulcerative keratitis of the eye, folliculitis (it is a type of skin or soft tissue infection), pneumonia, nosocomial infections, urinary tract infections and severe in those who are suffering from cancer and cystic fibrosis.[88]

Detection through impedimetric immunosensor

Streptococcus pyogenes can be detected through an impedimetric immunosensor using Dropsens gold SPEs modified with the polytyramine (Ptyr) deposition layer. Biotin-targeted antibodies were attached with biotin-neutraAvidin.

Immunosensing technology has opened a new field in modern research as well as food processing industries, as an alternative to the conventional method of detecting the pathogen in a limited time. So, the popularity and usage of Immunosensing technology are increasing day by day.[89] The different impedimetric immunosensors used to detect the mentioned pathogens found in various literature are summarized below Table 1.[90]

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Table 1 A summary of the different types of electrodes and analytics used in impedimetric immunosensors

Immunosensor electrodes	Analyte	Volume of sample	LOD	Time duration for the detection process	Reference
Au	*E. coli* 0157:H7	20 µL	1×10^3 CFU/mL	1 hour	29,89
Au	*E. coli* 0157:H7	-	2 CFU/mL	45-50 min	29
Au IDAM	*E. coli* 0157:H7	100 µL	-1.6×10^3 CFU/mL (Pure culture)	30-35 min	90
Au microelectrode	*E. coli* 0157:H7	-	102 CFU/mL	-	91
AuNPs	*E. coli* 0157:H7	-	10^2 CFU/mL	2 hours	53
Au	*S.Typhimurium*	2mL	-10^2 CFU/mL (in milk) and -10 CFU/mL (in pure culture)	3-10 hours	69
Au IDAM	*S.Typhimurium*	50 µL	10^2 CFU/mL	1 hour	28,72
Au microelectrode	*Listeria monocytogenes*	15 µL	4.7×10^2 CFU/mL	50-55 min	28,79
Au SPE	*S. pyogenes*	10 µL	10^2 CFU/mL	30 min	85
Au	*S. aureus*	5 mL	10 CFU/mL	-	81
SPCE	*P. aeruginosa*	-	10 CFU/mL	-	28,92
Pt interdigitated microelectrode	*S.Typhi*	10 µL	10^2 CFU/mL	1 hour	73
IDAM	*Listeria monocytogenes*	20 µL	10^4 CFU/mL (lettuce, milk)	3 hours	93
Ag/AgCl	*E. coli* 0157:H7	-	83.7 CFU/mL (in milk)	-	28,94
Au SPIM	*E. coli* 0157:H7	25 µL	1.4×10^3 CFU/mL	-	28,95
Pt wire	*E. coli* 0157:H7 and *S. aureus*	-	10^2 CFU/mL	2 hours	96
Ti-Au IDAM	*S.Typhimurium*	-	10^3 CFU/mL	30-35 min	71
Au (Au-MBA-Ab) [MBA=4-mercaptobenzoic acid)	*E. coli* 0157:H7	1 mL	3 CFU/mL	90 min	28,96

Publication of reviews on impedimetric immunosensors

The number of impedimetric immunosensor reviews published every year and associated matters of interest published every month as well as the year about impedimetric immunosensor are shown in the following figures. These statistical representations highlight the importance of immunosensors in the field of research in this decade (Figure 7) (Figure 8).[96]

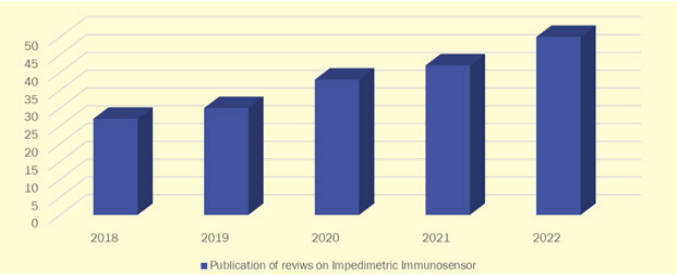

Figure 7 Publication of review on impedimetric immunosensors.

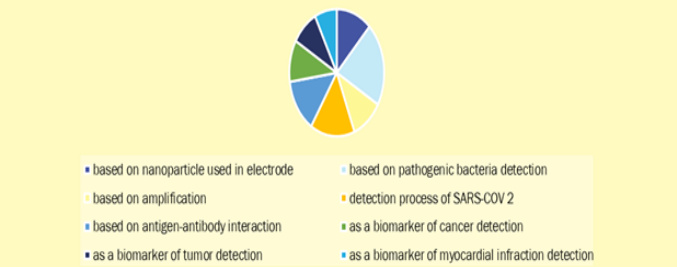

Figure 8 Areas of impedimetric immunosensors related reviews published within last years [2018-2022].

Conclusion and future perspectives

Impedimetric immunosensor is developed to detect bacterial cells by employing immobilizing antibodies that are specific to target bacterial cells over the surface of an electrode. This sensor detects pathogenic bacteria by using electrochemical impedance spectroscopy (EIS). The impedance of this type of sensor is influenced by the type of electrode, analyte, antigen-antibody interaction, LOD, capacitance, and resistance of electron transfer. The impedimetric immunosensor mechanism or working procedure is very similar to a well-known rapid detection method (ELISA) Enzyme-Linked Immunosorbent Assay. Impedimetric Immunosensing gives a label-free method of rapid and non-destructive detection, that may determine the desirable molecule in a very low concentration and gives an accurate automated result within 1-24 hours, using much less time than any other conventional methods. Some limitations may arise because of the expensive cost of electrodes and antibodies. The prospects of Immunosensing technology are immense and different types of nanomaterials AuNPs or MWCNTs may be used on the electrode. Affimers, are non-antibody proteins for detection of the pathogenic bacteria within the food may be developed in the future. So, electrochemical methods especially, impedimetric spectroscopy-based ones provide the best choice for food processing industries.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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