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A review of various methods for the detection of foodborne pathogens and toxins

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Abstract
Pathogens and toxins in foods pose significant health risks and economic consequences worldwide. This review explores various techniques for detecting microbial pathogens and their toxins, emphasizing the importance of early screening in preventing foodborne illnesses. Conventional methods, relying on culturing microorganisms, are sensitive but time-consuming. Immunological-based methods, such as ELISA and lateral flow immunoassay, provide reliable results but can be affected by interfering molecules. Nucleic acid-based methods, including PCR and real-time PCR, offer rapid and specific detection of DNA or RNA sequences. Biosensor-based techniques, which make use of mass-based, optical, and electrochemical biosensors, offer quick and easy substitutes without requiring sample pre-enrichment. Nanomaterial-based biosensors show promise in enhancing stability and lowering detection limits. The review concludes, that the future developments will strengthen the importance of methods for the detection and monitoring of foodborne pathogens and toxins, with continuous research aimed at enhancing their precision and efficacy.

Keywords: Biosensor, detection, ELISA, foodborne pathogens, PCR, toxins

Introduction
Food safety is a major global concern. For centuries, food have been implicated as sources of foodborne diseases due to their susceptibility to contamination by pathogenic microorganisms and their toxins, which serve as the primary causative factors. Hence, food safety is intricately linked to both pathogenic microorganisms and microbial toxins [1]. Various microorganisms, such as bacteria, viruses, and fungi, are accountable for contaminating food and water. When ingested, these microorganisms can result in foodborne illnesses and are commonly referred to as foodborne pathogens [2]. Bacteria are the most common cause of foodborne illness. Microbial toxins are the substances produced by micro-organisms, such as bacteria and fungi, that are of high molecular weight and have antigenic properties. These toxins facilitate the onset of infection or disease through their ability to impair the immune system and the direct harm they cause to the host tissues [3]. Foodborne illnesses caused by bacterial toxins can arise from either consuming preexisting toxins in the food released by these bacteria or by consuming food containing an adequate concentration of pathogens that subsequently release their toxins within the digestive system of the consumer [4]. The toxin-producing organisms of major concern to food safety microbiologists include verotoxic *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, *Vibrio parahemolyticus*, *Streptococcus* spp., *Staphylococcus aureus*, *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter jejuni* and *Listeria monocytogenes*. At present, there is ongoing development of new approaches for detecting bacterial toxins, aiming to enhance their understanding and isolation. Aside from the health issues, the presence of pathogenic foodborne organisms and their toxins can cause significant economic losses in the food industry and the public health system [5]. Scientific advancements, contributing to a deeper understanding of the mechanisms underlying microbial toxins, have led to a more comprehensive categorization of these toxins. Presently, toxins are broadly classified into three major categories based on their specific modes of action [1]. Those are:

A. Toxins that exhibit their action by targeting the cell membrane through distinct means, such as the specific hydrolysis of phospholipids present in the membrane, attacking membrane proteins, or assembling multiple toxin molecules to create a hole or pore within the membrane.
B. Toxins which exert their influence on the cell surface by disrupting the cellular communication with the surrounding environment. They achieve this by binding to various signaling molecules such as kinases, G-proteins, cAMP, and cGMP.
C. Toxins that operate within the cell to impede the process of protein synthesis.

However, Bacterial toxins are typically classified into two main types: exotoxins (peptides and proteins) produced by both Gram-positive and Gram-negative bacteria, and endotoxins (lipopolysaccharides) produced specifically by Gram-negative bacteria. These toxins encompass a wide spectrum of molecular weights, ranging from under 1000 Da to over 100,000 Da, and demonstrate diverse physicochemical characteristics [6]. To proactively address the potential risks associated with bacterial toxins, precise and dependable detection methods are crucial across various domains, including clinical diagnostics, food and water monitoring, and biosecurity [3]. The detection of toxins is imperative to ensure robust quality control across diverse industries. The progress made in understanding the mechanisms of bacterial toxins has facilitated the development of improved techniques for their identification and detection. Consequently, the reliable, rapid, and cost-effective detection, identification, and quantification of pathogenic microorganisms and their toxins in food remain an ongoing challenge for ensuring food safety. Microbiologists and scientists consistently strive to optimize the existing methods while exploring novel approaches to meet this challenge [1]. The majority of foodborne pathogens of microbial origin can proliferate discreetly within food without causing noticeable changes in flavor, color, or texture. As a result, the identification, monitoring, and quantification of these microorganisms and their toxic substances are crucially important. Methods for pathogen detection in food must fulfill several essential criteria: (i) They should be rapid to accommodate perishable food items and the fast-paced nature of food production and distribution (ii) They must be selective to distinguish pathogens from the larger population of non-harmful microorganisms in food and (iii) They need to be sensitive as even a single pathogenic cell can constitute an infectious dose [5]. This review aims to provide an overview of the many methods used to identify foodborne pathogens and their toxins.

Overview of Techniques for the Detection of Foodborne Pathogens and Toxins

1. Conventional Detection Methods

Isolation and Identification of Causative Agent

This method utilizes specific media to count and isolate viable bacterial cells in food samples. This method is highly sensitive, cost-effective, and provide both qualitative and quantitative information about the micro-organisms present in the samples [7]. Isolation and identification of causative agent is time consuming as it takes several days to yield results as they rely on visible growth of bacterial colonies [8]. Moreover, the labour-intensive nature of culture medium preparation, plate inoculation, and colony counting contributes to the time-consuming aspect of these methods [7]. It generally considered as gold standard test for the detection and identification of bacteria.

Biological Assay

The initial approaches employed for bacterial toxin detection involved in-vivo experiments such as animal challenge tests or in-vitro methods utilizing tissue cultures. These traditional techniques are still in use despite being time-consuming and difficult, particularly for some bacterial toxins like botulinum neurotoxins (BoNT) [1]. Furthermore, these tests are the sole means of obtaining information regarding the biological activity of the toxins [4]. The mouse bioassay has been widely employed as a prominent method for toxin determination. It offers several advantages such as high sensitivity, the capability to detect various serotypes and subtypes, the ability to measure different aspects of active toxins, and responsiveness across diverse food matrices. However, the development of more affordable, quick, and sensitive alternatives to biological assays has been spurred by the latter's inconsistent results, high cost, time-consuming nature, ethical issues, and requirement for specialised animal facilities and trained personnel [1].

2. Rapid Detection Methods

In recent times, various rapid methods with improved sensitivity and specificity have been developed to overcome the limitations associated with conventional methods for detecting and identifying foodborne pathogens. Innovative molecular approaches for identifying pathogens are under development across several dimensions of detection including enhancing sensitivity, speed, selectivity, differentiation of viable cells, and suitability for on-site analysis [8, 9]. Rapid detection methods hold significance, especially in the food industry, as they can promptly identify the presence of pathogens in both raw and processed food [9]. In general, rapid detection methods are typically classified into three categories: nucleic acid-based methods, biosensor-based methods, and immunological-based methods [8, 9]. Every rapid method comes with its own set of advantages and limitations.

Nucleic Acid Based Methods

An encouraging advancement in microbial diagnosis was the incorporation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) probes. The challenges related to the specificity of antibodies can be addressed by employing RNA or DNA probes that directly pinpoint the nucleic acid of an organism. Nucleic acid-based methods function by detecting specific DNA or RNA sequences in the target pathogen. This is achieved through hybridization, where a synthetic oligonucleotide (probes or primers) that is complementary to the target sequence is used to bind with the target nucleic acid sequence [8]. Numerous bacterial pathogens, such as *C. botulinum* and *E. coli* O157, produce toxins that can lead to foodborne diseases. The genes responsible for production of toxins of such pathogens can be detected using nucleic acid-based methods [9]. A variety of nucleic acid-based assays, such as amplification, hybridization, microarrays, and biochips, have been developed as rapid methods for detecting foodborne pathogens.

Polymerase Chain Reaction (PCR) Assay

It allows the detection of even a single foodborne pathogen (bacteria, virus or fungi) present in the food by detecting a specific DNA sequence of the target pathogen. PCR operates

by amplifying a specific target DNA sequence in a cyclic three steps process [7]. The amplified target sequences are visualized on agarose gel as bands by staining with ethidium bromide dye [9]. Identification based on PCR amplification and sequencing of target genes is a reliable technique [8]. With the distinct advantages of rapidity, specificity, sensitivity, and less samples over culture-based methods, many PCR assays have been developed for the detection and validation of foodborne bacteria and viruses in food. PCR can be employed in the detection of bacterial toxins by amplifying specific genes responsible for their encoding. PCR techniques for identifying toxins have been created for various bacterial species, such as *Vibrio cholera*, *B. cereus*, *E. coli*, and *S. aureus* [8].

Multiplex PCR

By amplifying multiple gene targets at once, multiplex PCR offers a faster detection than conventional PCR. In multiplex PCR assays, multiple sets of specific primers are utilized, whereas only one set of specific primers is employed in conventional PCR assays [9]. Precision in primer design is crucial for crafting a successful multiplex PCR assay. Interaction among multiple primer sets may require adjusting concentrations to ensure consistent yields. Additionally, the primer sets should have similar annealing temperatures [8].

Real-time or Quantitative PCR (qPCR)

Real-time PCR, or quantitative PCR, differs from simple PCR in that it does not necessitate agarose gel electrophoresis for the detection of PCR products. In real-time PCR, the detection of PCR products occurs as they accumulate [8, 10]. The technique allows continuous monitoring of PCR product formation throughout the entire reaction by measuring the fluorescent signal produced by specific labelled probes or intercalating dyes. The fluorescence intensity correlates with the quantity of PCR amplicons. Numerous fluorescent systems have been developed for qPCR, the most often used ones are SYBR Green, TaqMan probes, and molecular beacons. Among the fluorescent systems for qPCR, SYBR Green is simpler and inexpensive compared to TaqMan probes or molecular beacons [9]. Real-time PCR offers a significant advantage in routine laboratories, as the PCR and detection are conducted in a single-tube system, eliminating the issue of carryover contamination [10].

Nucleic Acid Sequence Based Amplification (NASBA)

NASBA functions by amplifying nucleic acids under isothermal conditions. The typical reaction involves three enzymes: T7 RNA polymerase, RNase H, and avian myeloblastosis virus (AMV) reverse transcriptase (RT). These enzymes collectively work to amplify sequences from an initial single-stranded RNA template [8, 9]. The amplicons produced by NASBA can be detected through agarose gel electrophoresis. NASBA exhibits specificity for target RNA or DNA sequences, and its popularity has been growing due to its broad applicability for pathogen detection in clinical, environmental, and food samples. Real-time NASBA employs fluorescently labeled probes, specifically molecular beacons, to detect single-stranded RNA amplicons, resulting in a homogeneous NASBA assay. Real-time NASBA has been applied for the detection of diverse foodborne pathogens, including *Salmonella enterica*, *V. cholerae*, *S. aureus*, *C. jejuni*, and *C. coli* [8, 9]. Real-time NASBA can identify viable microorganisms in food samples through mRNA amplification. The detection of RNA targets indicates the presence of viable cells. NASBA provides high-throughput analysis and it has been brought into commercial use in the form of kits [9].

Loop-Mediated Isothermal Amplification (LAMP)

A novel nucleic acid amplification method, known as LAMP, has been shown to be a rapid, cost-effective, user-friendly, highly sensitive, and specific detection technique applied in various fields [8]. In LAMP, a set of four primers, consisting of two inner primers and two outer primers, is employed to pinpoint six specific regions of the target DNA. LAMP can generate a substantial quantity of amplicons within 60 minutes, typically at least 10^3-fold higher than simple PCR. The resulting LAMP amplicons can be identified through agarose gel electrophoresis or using SYBR Green I dye [8, 9]. Due to its speed and sensitivity, LAMP has been employed in detecting various foodborne pathogens. LAMP has demonstrated greater specificity and sensitivity compared to PCR assays for the identification of foodborne pathogens [11]. Moreover, various types of LAMP assays have been created for the identification of foodborne pathogens. Examples include multiplex LAMP, reverse-transcription LAMP, real-time LAMP, and in-situ LAMP. Real-time monitoring of LAMP amplification products through turbidity or fluorescence eliminates the requirement for agarose gel electrophoresis for visualization of products. Consequently, this enables high-throughput analysis while maintaining high sensitivity and specificity.

Oligonucleotide DNA Microarray

Recent advancements in multi-gene detection technology encompass the utilization of microarray technology. The DNA microarray emerges as a potential tool for identifying and characterizing either a single organism or multiple organisms, relying on the presence of one or more marker genes [9, 12]. By integrating with bioinformatics, DNA microarrays offer extensive possibilities for pinpointing the target gene or sequence and establishing a pathway for the analysis of foodborne microorganisms [13]. Microarrays consist of glass slides or chips coated with hundreds of specific oligonucleotide probes. These probes are chemically synthesized short sequences ranging from 25 to 80 base pairs. In this technique, nucleic acid fragments (DNA, cDNA, or RNA) are fluorescently labelled then denatured to form single-stranded fragments, and are hybridized to the array by binding with their respective oligonucleotide probes. The results are obtained by visualizing the fluorescence signal produced by the probe-sample complex [7, 9, 12]. The effective use of microarray techniques for detecting microbes in foods is constrained by various factors. These include challenges in isolating, concentrating, and purifying high-quality DNA from food sources, as well as the need for standardizing the optimal hybridization conditions due to diverse array probes required for detecting multiple pathogens. As of right now, the method works better as a research tool than as a workable diagnostic [12].

Immunological Based Methods

The widely adopted method for identifying foodborne pathogens relies on immunological detection through antigen-antibody bindings. These assays primarily depend on the specific interaction between an antibody and an antigen. Various types of antibodies have been utilized across different assay formats to detect foodborne pathogens and microbial toxins [8]. The sensitivity and specificity of immunological-

based methods are determined by the binding strength of a specific antibody to its antigen [9]. Antibodies specific to an antigen, whether it is the bacteria itself or a compound secreted by the bacteria, are generated by exposing animals to these antigens [14]. Immunological methods involve the application of polyclonal and monoclonal antibodies [8]. Enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay are among the immunological-based methods currently employed for detecting foodborne pathogens [9].

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA stands out as one of the most frequently employed immunological methods for identifying foodborne pathogens. Enzyme-linked immunosorbent assay (ELISA) is a highly accurate and sensitive immunological method widely utilized for the detection of foodborne pathogens, specifically for identifying antigens or haptens. Traditional ELISA typically involves chromogenic reporters and substrates that produce some kind of observable colour change to indicate the presence of antigen or analyte. Sandwich ELISA represents the most efficient form of ELISA, involving the use of two different types of antibodies [8, 9]. Various enzymes can be employed in ELISA such as horseradish peroxidase (HRP), alkaline phosphatase, and beta-galactosidase. Enzyme-linked immunosorbent assay (ELISA) has been applied for the detection of various BoNT serotypes in a broad spectrum of milk product matrices, such as whole milk and low-fat yogurt [15]. ELISA is also widely employed for detecting toxins found in foods, including *C. perfringens* α, β, and ε toxins; *Staphylococcal* enterotoxins A, B, C, and E; botulinum toxins and *E. coli* enterotoxins [9]. ELISA proves to be a cost-effective method that can substitute other techniques due to its high specificity, reproducibility, simplicity, and sensitivity [13].

Lateral Flow Immunoassay

While ELISA has found extensive use in numerous laboratories, its implementation demands various equipment and trained personnel. Hence, there is a need for rapid, cost-effective, yet reliable methods that can be executed and interpreted on-site. Lateral flow immunoassays (LFIA) fulfil these criteria and play a crucial role in diagnostic applications for food safety, facilitating the identification of contamination by specific pathogens and toxins, including biowarfare agents in food [16]. Lateral flow immunoassays, such as dipsticks and immunochromatographic strips, have been created for the rapid on-site detection of foodborne pathogens [9]. LFIA is an immunoassay variant in which the test sample moves, by capillary forces, along an analytical nitrocellulose membrane [16]. LFIA can be either qualitative with a specified cutoff level or quantitative when utilized with a photometric strip reader. LFIA come in two different fundamental formats: the competitive assay that are employed for testing analytes with a single epitope, and the sandwich assay which are used for testing analytes with multiple epitopes. The detection of foodborne pathogens using LFIA involves the use of labels such as monodisperse latex, colloidal gold, carbon, and fluorescent tags. While LFIA are simple and rapid, they are designed primarily for individual tests rather than high-throughput screening [9].

Biosensor Based Methods

The development of biosensors is among the rapidly expanding fields in the detection of foodborne pathogens [2]. The sensor measures physical and chemical quantities, transforming them into an electrical signal. Sensors function as transducers, converting one form of energy into another [8, 9]. Biosensors are the fusion of both transducers and biological elements. The bioreceptor, responsible for recognizing the target analyte, can be either biological material (enzymes, antibodies, nucleic acids, and cell receptors), biologically derived material (aptamers and recombinant antibodies), or biomimic (imprinted polymers and synthetic catalysts) [9, 13, 15]. The primary benefits of biosensors include rapid or real-time detection, portability, and the capability for multi-pathogen detection in both field and laboratory analyses. Fast or real-time detection allows for nearly immediate interactive information on food materials, enabling users to take corrective measures before consumption or the risk of further contamination [8]. Biosensors are user-friendly and do not necessitate sample pre-enrichment, unlike nucleic acid-based and immunological methods, which require pre-enrichment to concentrate pathogens before detection [9]. Owing to their remarkable specificity and sensitivity, biosensors have garnered interest in medicine, agriculture, food safety, and bioprocess industries [13].

Optical Biosensors

Optical biosensors are employed for the rapid detection of microbes and food contaminants [13]. Optical transducers operate by detecting changes in optical properties. Transducers such as optical fibres, Raman infrared spectroscopy, surface plasmon resonance (SPR), and others are employed in the development of optical biosensors. The SPR biosensor is the most commonly utilized optical biosensor for the detection of foodborne pathogens due to its high sensitivity. SPR measures alterations in the reflected angle of light resulting from the binding of cells to a specific receptor [7]. SPR has proven effective in detecting both bacteria and toxins. Most commercially available biosensors for the detection of foodborne pathogens are primarily optical-based. The commercialization of biosensors faces delays compared to other rapid methods due to factors like cost, quality assurance, stability issues, sensitivity issues, and instrumentation design.

Electrochemical Biosensors

Biosensors are characterized by the detection of altered potential and current when the sensing electrode interacts with the sample [14]. Based on how they operate, electrochemical biosensors come in several varieties, including potential (potentiometric), current (amperometric), conductance (conductometric), and impedance (impedimetric) [8, 9]. These biosensors are advantageous due to their low cost, small size, and resilience to liquid samples. The amperometric biosensor has been employed for the detection of *E. coli*, *Salmonella* and other pathogens [2].

Mass-based Biosensors

Mass-based or mass-sensitive biosensors function by detecting small changes in mass [14]. Biosensors like the quartz crystal microbalance (QCM) and surface acoustic wave (SAW) fall into this category [2]. Mass-based biosensors utilize piezoelectric crystals that vibrate at a specific frequency when stimulated by an electrical signal of a corresponding frequency [9].

QCM-based Biosensors

A biosensor based on QCM operates according to the

piezoelectric principle. An electric signal is employed between two gold plates on a quartz crystal to induce vibrations at a specific resonance frequency, which is subsequently measured. The biosensor based on QCM has been utilized for the detection of pathogens and endotoxins [2].

SAW-based Biosensors

The SAW based interdigital transducer generates acoustic waves on the surface through the piezoelectric substrate, and resulting changes are then detected. Biosensors based on SAW technology have been applied in diverse scenarios, including the detection of pathogens and endotoxins [2].

Nanomaterial-based Sensors

Recent years have witnessed significant progress in nanomaterial-based biosensors, wherein the sensing electrode is modified by nanomaterials to facilitate rapid electron transfer, triggered by various biomarkers. Leveraging this advantage, extensive research has linked nanomaterials with biomolecules for the creation of biosensors capable of detecting hazardous pathogens. Pathogens such as *Salmonella, E. coli,* and *L. monocytogenes* have been extensively studied for food detection methods. Additionally, respiratory syncytial virus and parasites like *Giardia* and *Cryptosporidium* pose threats to food contamination. Nanomaterial based sensors can avoid interference from other foodborne infections and shows remarkable stability with a low detection limit. Hence, nano-materials have been utilized to create a cost-effective biosensor for on-site measurements [2].

Conclusion

The early screening of food products is a crucial measure to prevent epidemics associated with food poisoning/foodborne pathogens. Traditional methods for detecting foodborne pathogens, which rely on culturing microorganisms, are selective but can be time consuming and labour intensive. Consequently, various rapid detection methods have been developed to overcome the limitations of conventional detection approaches. Traditional, immunological, and nucleic acid-based methods offer reliable results but are slow and demand specialized equipment and personnel. Biosensor based methods are user-friendly and do not necessitate trained personnel. Moreover, these techniques can be employed for the detection of foodborne pathogens without the need for sample pre-enrichment. Immunological methods, including ELISA and lateral flow immunoassay, are employed for detecting foodborne bacterial pathogens and their toxins. These methods perform optimally in the absence of interfering molecules in the samples, such as non-targeted cells, DNA, or proteins. Every developed method comes with its own set of advantages and disadvantages. In the future, these techniques will significantly underscore their importance in the detection and surveillance of foodborne pathogens. Additional research on the impact of various combinations of rapid methods for detecting foodborne pathogens is necessary to develop the most effective and accurate detection method.

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