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Critical Reviews in Biotechnology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibty20

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To cite this article: Surbhi Panwar, K. Siddaardha Duggirala, Pooja Yadav, Nabendu Debnath, Ashok Kumar Yadav & Ashwani Kumar (2022): Advanced diagnostic methods for identification of bacterial foodborne pathogens: contemporary and upcoming challenges, Critical Reviews in Biotechnology, DOI: 10.1080/07388551.2022.2095253

To link to this article: https://doi.org/10.1080/07388551.2022.2095253

Published online: 22 Aug 2022.

CRITICAL REVIEWS IN BIOTECHNOLOGY
https://doi.org/10.1080/07388551.2022.2095253

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REVIEW ARTICLE

Advanced diagnostic methods for identification of bacterial foodborne pathogens: contemporary and upcoming challenges

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ABSTRACT

It is a public health imperative to have safe food and water across the population. Foodborne infections are one of the primary causes of sickness and mortality in both developed and developing countries. An estimated 100 million foodborne diseases and 120 000 foodborne illness-related fatalities occur each year in India. Several factors affect foodborne illness, such as improper farming methods, poor sanitary and hygienic conditions at all levels of the food supply chain, the lack of preventative measures in the food processing industry, the misuse of food additives, as well as improper storage and handling. In addition, chemical and microbiological combinations also play a key role in disease development. But recent disease outbreaks indicated that microbial pathogens played a major role in the development of foodborne diseases. Therefore, prompt, rapid, and accurate detection of high-risk food pathogens is extremely vital to warrant the safety of the food items. Conventional approaches for identifying foodborne pathogens are labor-intensive and cumbersome. As a result, a range of technologies for the rapid detection of foodborne bacterial pathogens have been developed. Presently, many methods are available for the instantaneous detection, identification, and monitoring of foodborne pathogens, such as nucleic acid-based methods, biosensor-based methods, and immunological-based methods. The goal of this review is to provide a complete evaluation of several existing and emerging strategies for detecting food-borne pathogens. Furthermore, this review outlines innovative methodologies and their uses in food testing, along with their existing limits and future possibilities in the detection of live pathogens in food.

ARTICLE HISTORY
Received 14 January 2022
Revised 31 May 2022
Accepted 9 June 2022

KEYWORDS
Food safety; foodborne pathogens; immuno-based techniques; nucleic acid-based techniques; biosensor-based methods; food products

Introduction

The origination of new diseases and the reemergence of established infectious diseases are two of the most serious public health issues. Also, food-borne infections continue to be the leading cause of sickness and mortality, resulting in significant socio-economic losses throughout the world. Food safety is an issue of inordinate concern to everyone. Healthy food is a basic necessity that can be ensured by prioritizing food safety. However, disease surveillance systems, such as the Centers for Disease Control and Prevention (CDC) have reported the most concerning situation regarding food safety. Food-borne infections cause 54 million illnesses, 128 000 hospitalizations, and 3000 deaths in the United States each year [1]. In developing countries, such as India, between 2009 and 2018, there were 2688 food-borne outbreaks recorded [2]. Microorganisms that live in the environment cause illnesses in humans and easily contaminate consumable food items. In addition, most of the food varieties are exposed to microbial contamination or spoilage at any point during the: growing, harvesting, processing, storage, transportation, and preservation processes. Moreover, several environmental contexts, such as tropical, humid, and unhygienic conditions may enhance the risk of food contamination. Studies have found several bacterial species, such as *Escherichia coli, Salmonella sp., Listeria sp., Cyclospora sp., Clostridium sp., Campylobacter sp., Vibrio sp., Shigella sp., Pseudomonas sp.,* and *Acinetobacter sp.,* to contaminate food, making it unsafe to consume and causing food-borne disease [3,4]. Foodborne diseases are intermittent and are often ignored. A national-level study reported that the prevalence of food-borne diseases in Indian households was

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13.2% [5]. Therefore, it is critical to test food for the presence of foodborne pathogens to reduce the risk of foodborne illness.

Culturing microorganisms on agar plates followed by routine biochemical identifications are the traditional methods for identifying foodborne bacterial pathogens contained in food [4,6]. Traditional techniques are typically affordable and straightforward, but they can take a long time since they rely on the microorganisms' capacity to thrive in various culture media, such as pre-enrichment media, selective enrichment medium, and selective plating media. Traditional techniques are time-consuming since they need the preparation of culture medium, plate inoculation, and colony counting. Conventional procedures often take 2–3 days for preliminary identification and more than a week for pathogen species confirmation [7].

Furthermore, because of their low sensitivity, traditional methods may be limited. Viable but non-culturable (VBNC) bacteria can cause false-negative findings. Failure to detect foodborne pathogens would raise the danger of pathogen spread. In addition to the culture-based traditional techniques, culture-independent detection techniques, such as nucleic acid sequence-based detection techniques, which include different formats of polymerase chain reaction (PCR), biochemical detection techniques, immunological detection techniques, and various biosensor-based methods, such as electrical and optical biosensors, instrumental-based detection technologies, such as flow cytometry, gas chromatography with or without mass spectroscopy, have been frequently used for better and early results. However, PCR-based methods for the identification of foodborne pathogens involve post-PCR processing and have a greater risk of false-positive results [8]. One of the major techniques of PCR, such as Quantitative PCR (qPCR) and Real-Time PCR, was used efficiently to identify foodborne pathogens where the measurement and quantification of the amplified product are carried out by incorporating fluorescence molecules. Real-Time PCR reduces the chance of false-positives results, by incorporating intercalating fluorescence dyes during the quantification [9]. The recent developments can be effective; however, the ideal diagnostic method to detect food pathogens should be focusing on: higher sensitivity and specificity for particular pathogens, reduced assay time, and abundant types of test samples. On the contrary, the diversity of food creates various challenges in sampling, preparation, and analysis. Many inhibitors in food matrices demonstrate their ability to obstruct detection methods, such as DNA-based assays, PCR techniques, and antigen-antibody-specific assays (ELISA). Sample preparation is a difficult process that must be completed before the sample is exposed to detection. Furthermore, several techniques are utilized to identify pathogen DNA and toxin in food; however, many assays have failed to owe to a low recovery rate, which leads to decreased assay accuracy. This study focuses on detection technologies and procedures for microorganisms that cause food-borne diseases. Biochemical and biological detection methods, using various types of biosensors, nucleic acid sequence-based detection techniques, such as DNA microarray, polymerase chain reaction (PCR), and immunological methods are discussed in detail in this review paper. The limits and alternate detection approaches are also thoroughly addressed.

Scope of the review

Firstly, this review shed the light on the conventional detection methods and their limitations. Subsequently, it was focused on: the concept, advantages, disadvantages, applications, and comparative analysis of the recent and advanced detection techniques based on: immunological, nucleic acids, microarrays, biosensors, and aptamers, along with their several variants. Therefore, an attempt has been made in this review to put together collective and comprehensive information as well as a comparative analysis of all the detection methods in terms of concept, applications, knowledge gap along with merits and demerits, on one platform. This review would be certainly helpful for exploring the possibility of combinational chemistry for effective, accurate, and speedy detection of high-risk food pathogens.

Food-borne pathogen detection methodologies

The methodologies for the detection of pathogens are being developed through decades of investigation. In the 1970s the conventional method, i.e., selective media plating was developed to identify food pathogens. Later different research groups began to develop several rapid detection techniques. These rapid detection technologies are roughly classified into three types: immunological approaches, nucleic acid-based tests, and biosensors [10,11]. Moreover, nanoparticles in pathogen detection, multilocus sequence typing, repetitive extragenic palindromic PCR (REP-PCR), and other advanced techniques are also developed for rapid and accurate detection of foodborne pathogens [12]. Most of the above-mentioned techniques can assess a sample in a few minutes to a few hours, but they lack

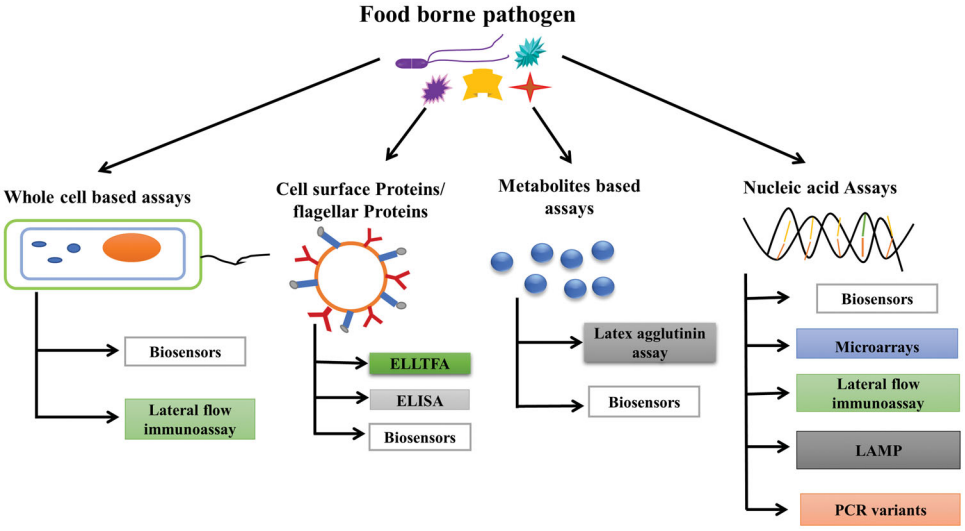

Figure 1. Schematic of techniques categorized according to the biological targets used for the detection of food-borne pathogens.

the sensitivity and specificity required for direct food testing, thus the sample must still be culture-enriched before analysis. Although the enrichment stage is frequently viewed as the major speed limitation; however, it may provide indirect advantages, such as dilution of inhibitors commonly found in food samples, the distinction of viable from non-viable cells, and revival of stressed or wounded cells in food samples.

Different conventional methods, such as cultured-based detection and the more recently developed rapid detection techniques like immunoassays, PCR technique, nucleic acid assays, and biosensors have been emphasized in the following sections, as well as in Figure 1. Additionally, an overview of recent advancements in rapid detection techniques is also presented.

Conventional methods

An abundant number of both selective and differential media plating techniques have been developed to assist in the recovery of pathogens from foodstuffs. A low number of pathogenic bacteria, often in the presence of competing microorganisms, has typically warranted prior enrichment procedures for accurate detection [13]. Isolated microorganisms are then confirmed by various biochemical tests which may take several days [14]. Another technique used to enhance the number of targeted pathogenic bacteria to a quantifiable amount is supplementing the food sample in both selective and nonselective broth cultures. This entirely depends on the food product being investigated. The enrichment generally requires 8–24 h before detection or estimation of a pathogen [15]. All these conventional methods have some drawbacks (Table 1) and, for these reasons, more advanced, accurate, and less time-consuming techniques are required.

Drawbacks of conventional methods

For many years the conventional methods have been in practice for the identification of pathogenic bacterial isolates from foods; however, they are: extremely labor-intensive, tedious, time-consuming, and complex. Furthermore, these methods are not able to differentiate between viable culturable cells and viable but non-culturable cells which may arise due to injury inflicted on the cells as a result of stressed conditions [16]. The number of enumerated bacteria may be lower than the actual number in foods because these cells are highly sensitized to the constituents and the environment they create in selective microbiological media and the failure of selective plating media to resuscitate damaged cells. Therefore, there is a chance of erroneous results being prepared when standard microbiological methods are used to detect them [16,17].

Rapid detection techniques for food-borne pathogens

Scientific advances in biotechnology have opened new vistas for the emergence of rapid diagnostic methods that include: simple miniaturized biochemical assays, physio-chemical tests, highly specific-DNA and immunological methods, and even fully automated diagnostic systems. The major benefit of rapid testing is that food processors can obtain results before the

Table 1. Outlook of merits and demerits of methods used to detect food pathogens.

Methods employed	Merits	Demerits
Conventional methods	• Specificity • Sensitivity • Valid results • Inexpensive	• Prior enrichment of sample • Extensive labor • Less efficient in selecting viable cells from non-viable cells
Immunoassays	• Specificity • Sensitivity • Valid results • Inexpensive	• Prior enrichment of sample • Cross-reactivity of anti-sera • Inadequate expression of antigens
Nucleic acid methods	• Sensitivity	• Prone to inhibitors, requires pure DNA
Simple PCR	• Specificity • Automated • Valid results	• Hard to discriminate between viable and non-viable cells
Multiplex PCR	• Sensitivity • Accuracy • Multiple pathogen identification • Labor savor	• Prone to inhibitors • Hard to discriminate between viable and non-viable cells • Designing of primers are necessary
Real-time PCR	• Sensitivity • Precision • Fast cycling • Replicable • Post-processing of amplified products is not required. • PCR products can be observed at any point of amplification procedure	• Expensive. • Difficult for multiplex real-time PCR assay • Pretentious to PCR inhibitors • Hard to distinguish viable and non-viable cells • Requires skilled workforce • Chances of cross-contamination
NASBA	• Sensitivity • Specificity • Less money is required • No requirement for thermal cycling system • Detection capability feasible microbes.	• Feasible microorganism required • Difficult to handle RNA
LAMP	• Sensitivity • Accuracy • Money saver • Trouble-free handling • Requirement of thermal cycling system is not necessary	• Difficult to design primer • Detection accuracy of unknown or un-sequenced targets is inadequate
Microarray	• Sensitivity • Specificity • High yield • Multiple pathogen detection • Serotype detection • Labor-saving	• Expensive • Hard to differentiate between viable and non-viable cells • Adequate skilled workforce is necessary • Requirement of labeled target genes
Biosensors	• Sensitivity • Precision • Multiple pathogen detection • Labor-saving • Speed detection • Low cost	• Nonspecific bindings • Stability of biological components, such as transducers

product leaves the manufacturing location; thereby, preventing the possible outbreak risks of food-borne illness.

Methods based on immunological reactions

The search for a rapid, accurate, economical, and easy performance led to the development of immunological assays. Therefore, before using PCR-based techniques, immune assays are preferred. In, this method, the selective interaction between antigen and antibody has been extensively utilized in the recognition process of bacterial strains. Distinct antibodies are used for the identification of antigens present on the surface of bacteria or flagella, thereby determining the specific bacterial strains. By utilizing this principle, numerous immune-assay-based methods have been developed by various scientific groups for the rapid discovery of pathogens in foods.

Enzyme-linked immunosorbent assay

One of the most widely used immunoassay techniques is the Enzyme-Linked Immunosorbent Assay (ELISA) [18], which typically consists of chromogenic reporters and substrates, and their interactions specify the presence of antigen or analyte based on the color change. Farber and Spiers in 1987 developed monoclonal antibodies (MAbS) specific for the identification of *Listeria monocytogenes* and later this method was applied against genus-specific antigens of *Listeria* [19,20]. These antigens, which have been identified as heat stable and

flagellar proteins, are employed in the commercially available Listeria-Tek ELISA kit developed by the Organan Teknika Corporation for the *Listeria* species detection. Sandwich ELISA is one of the efficient formats due to its sensitivity, where the antigen to be identified is sandwiched in between the detection antibody and the capture antibody. Sandwich ELISA techniques have been used to identify several pathogenic bacteria, such as *Vibrio parahaemolyticus* in seafood utilizing monoclonal antibodies against the TDH-related hemolysin (TRH). The detection limit of this technique is as low as 10^3 pathogenic *V. parahaemolyticus* cells in a sample [21]. For the identification of *Salmonella* in food items, commercial ELISA test kits, such as the BIOLINE *Salmonella* ELISA Test are developed and the detection limit was 1 CFU/25g of the sample [22]. Additionally, the ELISA technique can also be implemented for the detection of several toxins from food items [4].

Recently, high-throughput and automated ELISA systems have been developed for better and faster detection. For example, VITEK immunodiagnostic assay system (VIDAS, BioMerieux) is a system that automates the whole ELISA operation. It reports the results using an enzyme-linked fluorescent immunoassay (ELFA), which is comparable to ELISA but a more sensitive fluorescent immunoassay and can be performed in 45 min to 2 h [23]. The VIDAS system consists of a reagent strip as well as a plastic tube known as a solid phase receptacle (SPR) [24,25]. By applying the VIDAS detection system, some pathogenic microorganisms have been detected, such as *Salmonella, L. monocytogenes, E. coli* O157:H7, *Campylobacter* spp., staphylococcal enterotoxin from pork samples, fruits and vegetables, fish samples, Minas Frescal cheese, and raw milk cheese, respectively [26–29]. Furthermore, the Assurance Enzyme Immunoassay (EIA) kit has been created to enable automated and high-throughput testing of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *Campylobacter* from a variety of samples, including raw and cooked beef, alfalfa sprouts, and chicken meat [25,30]. Pang et al. [31] developed a new economical approach to ELISA assay that was paper-based with less required time for the detection of *E. coli* O157:H7 from Chinese cabbage. The technique was very accurate and sensitive with a limit of detection of 1×10^4 CFU/ml. Febo et al. [32] established an antibody-based method for the identification of *Salmonella enterica* subsp. *enterica*, where distinct monoclonal antibodies (1B6D9, 1B6C11, 1D12F11) were made to bind with *S. enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028 and another antibody (4E6F11) with *S. enterica* flagellin. Further, the more advanced characterization was done by other techniques, such as mass spectrometry and immunoblotting. Testing of food samples was done by *S. enterica* capture ELISA method and further checked by official ISO 6579:2002. The results obtained from the *Salmonella* capture ELISA technique are significant compared to the ISO 6579:2002 method in terms of sensitivity (100%), specificity (81%), and accuracy (90.5%). Thus it can be concluded that this immune assay is a possible substitute for the older methods employed for foodborne pathogen detection.

Enzyme-linked long tail fibers assay

This phage-based assay is similar to that of ELISA but varies by using phages Long Tail Fibers (LTFs) instead of traditional antibodies. LTFs determine the host range and, further, can be exploited to mediate adsorption of the virus particle to the potential pathogenic (bacteria) cells by binding to specific cell surface receptors. Their highly specific nature can be used to develop recombinant affinity molecules. Denyes et al. [33] developed this technique to detect *Salmonella* in liquid culture. The assay took as little as 2 h to detect 10^2 CFU/ml bacteria. Horseradish peroxidase mediated conversion of the chromogenic substrate also revealed that color intensity is largely proportional to a bacterial concentration between 10^2 and 10^7 CFU/ml. The methodology employed can be adapted and enhanced to detect other relevant bacterial pathogens.

Lateral flow immunoassay

Lateral Flow Immunoassay (LFI), also known as lateral flow immunochromatographic assays, has shown promising potential for instantaneous identification of foodborne pathogens [34,35]. LFI is simple, fast, accurate, and usually designed for individual diagnostics [36]. This device comprises four segments arranged in the form of four different parts, viz. sample pad, conjugate pad, nitrocellulose membrane, and an absorption pad. The LFI device also consists of a control line (for confirmation of whether the test is working properly) and a test or target line. The test sample will flow along with all the parts through capillary action and during the migration of the samples, it encounters a conjugate pad and later into the nitrocellulose membrane and then onto the absorbent pad. The conjugate pad contains conjugated labels and antibodies and if the sample has the target, it will bind to the conjugated antibodies and labels and carry on migrating toward the nitrocellulose membrane. Binding reagents present in the nitrocellulose membrane will subsequently bind

to the target and a colored line will form, which dictates the quantity of the target. Assays like dipstick and immunochromatographic strips are constructed using the principle of LFI for instantaneous on-site identification of foodborne harmful microorganisms, such as *Listeria* spp. and *Salmonella* [37]. Kim et al. [38] developed a strip sensor using the principle of the sandwich assay for *E. coli* O157:H7 detection. In 2019, Kim et al. [39] developed an ultra-fast assay for the detection of *Salmonella enteritidis* and *Salmonella typhimurium*, and *E. coli* O157:H7 from spiked milk. The reported LOD without pre-enrichment was 4.5×10^3 CFU, 4.5×10^4 CFU/ml, and 2.3×10^3 CFU/ml for *Salmonella sp., Salmonella enteritidis*, and *E. coli O157:H7*, respectively. The assay time of the procedure was 20 min.

Immuno magnetic separation

In this segregation process, immuno-magnetic particles/beads (capturing agents) are mixed with target cells and then separated by a suitable magnetic separator. The unbound cells are removed from the so-formed complex by washing it many times [40]. DeCory et al. [41] designed a procedure to detect *E. coli* O157:H7 in a rapid procedure from watery samples by using immuno-magnetic beads and immunoliposome (IMB/IL) fluorescence assay. Isothermal amplification is dependent on thermo helicase in combination with magnetic nanoparticles which are coated with silica (Si-MNPs) as a DNA separator for the faster and sensitized detection of *Cronobacter*. In 2014, Ma et al. [42] devised a procedure for the detection of three microbes: *Shigella, Salmonella,* and *S. aureus* by using coated magnetic beads with specified antibodies in 67 spiked pork samples. The LOD was 6.8, 2.0, and 9.6 CFU/g for *Shigella, Salmonella,* and *S. aureus*, respectively. The assay time was <8 h. Xu et al. [43] used the technique to decrease the assay time to lower than 3 h, with sensitivities of 10° and 10^1 CFU/ml of *Cronobacter* in pure culture and contaminated Powdered Infant Formula (PIF), respectively. In addition, Wang et al. [44] developed immunomagnetic separation with the enzyme-catalyzed reaction for the identification of *Salmonella*. This method employs Mn-mediated immuno-magnetic sensor which allows Mn(VII)/Mn(II) interconversion, which reduces the noise interfering with the detection signal, further amplifying the sensitivity of *Salmonella* and ractopamine. The LOD (limit of detection) for ractopamine and *Salmonella* are 8.1 pg/ml and 20 CFU/ml, respectively. This magnetic immuno-sensor maintains good stability and also greatly enhances the sensitivity of conventional paramagnetic ion-mediated magnetic sensors.

Latex agglutination assay

The first-ever developed assay was with an anti-Listeriolysin O (LLO) monoclonal antibody (HD5E12D7; IgG2b) covalently bound to polystyrene amidine modified latex is used in a slide agglutination assay. This assay can detect 0.1 ng/ml of listeriolysin in foods [45]. Later, Hajra et al. [46] invented a simplex latex agglutination method by using polyclonal antibodies with 98% specificity for the identification of Shiga toxin-producing *E. coli* (STEC). Listeriolysin O (LLO), a preformed hemolysin, serves as a suitable recipient for the identification of *L. monocytogenes* in foods.

Drawbacks of immuno assays

When compared to traditional plating methods, immunoassays have a substantially faster turnaround time, which means we might obtain a "not detected" result much sooner than the several days to a week required by cell culture. Despite their endurance, immunoassays can have a greater rate of false positives than classical plating (Table 1). In principle, antibodies should only respond to antigens found on the organism being tested. However, this is not always the case.

The sensitivity of ELISA is of the order of 10^3–10^6 cells for bacteria. Moreover, it requires prior enrichment to amplify the number of targeted bacteria above the threshold limit. With the requirement of such a high number of target cells against a large background of non-targeted cells in immunological-based assays, there is every possibility of cross-reactivities of the antisera [47,48]. Further, antibodies to cell surface antigens can allow the detection up to genus level only, making it necessary to perform confirmative biochemical tests on presumptive bacterial isolates. Pre-enrichment of samples is required for most of the techniques. Further, relatively limited availability of specific serological reagents and inadequate expression of antigens under certain conditions may also limit the application of immunological-based techniques.

Nucleic acid-based methods for foodborne pathogen detection

The advancement in the field of biotechnology has led to the development of many DNA/RNA-dependent rapid tests for sensitive, specific, accurate, and prompt detection of food-borne pathogens.

Gene probe assays

The DNA probe-based methods are more accurate and specific compared to other rapid methods as described above. These gene-probe assays can detect nucleic acid targets, such as genomic DNA, ribosomal RNA, and messenger RNA which are unique to the organism. These assays achieve specificity by hybridizing nucleic acid probes to unique sequence regions in the organism of interest. However, the inherently low number of cells and the high number of particles in the food matrix will push the current technology limit on sensitivity. Therefore, there is a need for at least 10^6 CFU of targeted organisms for giving a positive signal with a DNA probe [49]. These are some of the drawbacks that are limiting the usefulness of these remarkable DNA-based techniques from the truly rapid aspect.

Polymerase chain reaction

The discovery of PCR in 1985, by Kary Mullis, is one of the landmarks in the recombinant DNA technology field. PCR has many advantages which make it the most preferred technique over culture-based methods and immunoassays [50]. DNA segments are amplified by a very sensitive, specific, and rapid technique that has been extensively exploited for the identification of pathogens. It is an *in vitro* enzymatic amplification of a target sequence under controlled thermal conditions. The target region is amplified during a cyclic three-step process, i.e., denaturation, annealing, and extension. One of the most outstanding applications of this technique is in the area of diagnostics. It allows the analysis of food samples and clinical specimens within 3–4 h. One of the positive factors of PCR based technique is its applicability, directly to the food sample by extracting template DNA from the food sample without the need for isolating the target organism from the food, which can save a lot of time to make it a real rapid method for identification of foodborne pathogens.

Extensive work on PCR-dependent tests has been carried out for the detection of pathogens. One of the assays named multiplex polymerase chain reaction (mPCR) was implemented for the identification of *L. monocytogenes* in paneer samples (Spiked and non-spiked). In the spiked sample, the detection limit was observed to be 10^4 cells. It was optimized to detect 10 cells after 6 h enrichment [51]. The major application of mPCR is that in one reaction it can simultaneously amplify more target genes of distinct pathogens [52]. A foodborne pathogen panel consisting of 25 bacterial targets and 49 selected PCR primers which contain 12 oligonucleotide pairs was developed. This food pathogen panel in mPCR increases the: reliability, sensitivity, and specificity of pathogens in about two days from sample collection to the bioinformatics-based identification [53]. A duplex PCR-ELISA was developed which was specific when 25 nontarget bacteria strains were used as controls. The sensitivity was up to 1 CFU/ml, which was 1000 times greater than the normal duplex PCR method (10^3 CFU/ml) [54]. Other examples of PCR application in food-borne pathogens are tabulated in Table 2.

Polymerase chain reaction variants for foodborne pathogen detection

The various formats of PCR have been described for possible application in the detection of foods under different conditions. These variants differ in methodologies being used and thus have a separate application in detecting foodborne pathogens. These PCR formats are intended to provide additional information to substantiate PCR results.

Nested polymerase chain reaction

Inadequate quality and the copy number of the nucleic acid template lead to compromised PCR sensitivity. To solve this problem, the more advanced version of conventional PCR, i.e., nested PCR/nested RT-PCR can be used to refine the precision and reactivity of DNA/RNA amplification. The nested PCR/nested RT-PCR format utilizes two successive primers known as "outer primers" and "inner primers or nested primers" for the exact targets comprising two consecutive PCR reactions. During the first amplification process, outer primers get bind with upstream sequences from the inner primers or nested primers. In this first amplification process, a large portion of the targeted sequence gets elongated which later serves as a template and is amplified by inner primers or nested primers. The main objective of using nested PCR is to confirm the authenticity of the PCR products obtained after amplification of the targeted gene. This can be used as an alternative to southern hybridization and hence can save a lot of time for detection of the targeted pathogen in the foods. Kumar et al. [55,56] have validated the results of normal PCR to detect *L. monocytogenes* with that of nested PCR where internal primers were used for the *iap* and *hly* gene.

Reverse transcription polymerase chain reaction (RT-PCR)

Even being highly specific and sensitive, conventional PCR cannot discriminate between viable and nonviable

Table 2. Nucleic acid-based methods for the detection of foodborne pathogens.

Technique employed	Targeted pathogens	Targeted genes	Limit of detection	Assay time	References
Multiplex PCR	*L. monocytogenes*	16S rRNA and *hly*	10 ng pure DNA 1–10 cells (after enrichment)	4–6 h	[55]
Multiplex PCR	*L. monocytogenes*	*iap*, *hly* A, *hly* K9 and *prf*	2–20 CFU/ml	NA	[56]
Multiplex PCR	*B. cereus, L. monocytogenes,* and *S. aureus*	*groEL*, *iap* and *nuc*	10^3 CFU/ml	NA	[57]
Multiplex RT-PCR and Monoplex RT-PCR	*S. epidermidis*, *S. haemolyticus*, *S. aureus*, *S. viridans*, *E. coli* and *P. aeruginosa*	Bacterial 16S rRNA gene	Qualitative assay No limit mentioned	~3 h	[58]
SYBR-PCR and Multiplex RT-PCR	Shiga toxin-producing *E. coli* (STEC)	*stx*1 and *stx*2	1×10^2 CFU/ml	6–9 h	[59]
	E. coli O157:H7 and *L. monocytogenes*	*rfbE* and *hylA*	10^3–10^8 CFU/ml (modified tryptic soy broth) 10^3–10^4 CFU of *E. coli*/g (without enrichment) 1.4–2.2 CFU/g (without enrichment)	4 h	[60]
	E. coli O157:H7	*eaeA*	1.3×10^4 cells/g	NA	[61]
	S. typhi	Flagellin	$\geq 10^3$copies/ml	NA	[62]
	Salmonella species	*iagA*	2 ± 1 CFU (after enrichment)	16–18 h	[63]
	L. moncytogenes and *Salmonella spp.*	*hly*, *invA*	1 log CFU/ml (Pre-enrichment)	6 h	[64]
	E. coli O157:H7, S. aureus and *Salmonella*	*rfb*E, *nuc*, and *inv*A	10^3 CFU/mL	NA	[65]
DNA purification/Real Time PCR chip	*L. monocytogenes*	*hlyA*	10^4 and 10^7 cells/ml	45 min	[66]
Molecular beacon-based duplex RT PCR	*L. moncytogenes* and *E. coli* O157:H7	*RfbE*, *hlyA*	1 log CFU/ml 3 log CFU/ml (spiked sample)	6 h	[67]
Oligonucleotide microarray	*Salmonella* spp., *E. coli, B. cereus, Vibrio cholerae, S. dysenteriae, S. aureus, L. monocytogenes* and *E. faecalis*	16S rDNA and 23S rDNA	10^3 CFU/ml	2–4 h	[68]
LAMP	*Salmonella* spp., *S. aureus* and *E. coli* O157:H7	*invA*, *nuc*, and *eaeA*	3.0×10^1 CFU/sample of *Salmonella* spp. and *E. coli* O157:H7) and 3.0×10^2 CFU/sample of *S. aureus*)	1.15 h	[69]
	V. parahaemolyticus	*tlh*	5.3×10^2 CFU per ml/g	NA	[70]
	S. aureus	*orfX*	10 DNA copies and 10 CFU/reaction	~2 h	[71]
	E. faecalis	*mtlf*	3.2 CFU/250 ml after 10 h prior enrichment	~1.5 h	[72]

cells expected to be present in processed foods which can invariably give false results. In many cases, these false-positive results are overcome by the inclusion of the enrichment step, which further prolongs analysis time, and eliminates much of PCR's advantage. RT-PCR has one added advantage. It can discriminate viable cells from non-viable ones with extreme specificity and high sensitivity. RT-PCR is the molecular tool used for the detection of various bacterial species (pathogens and non-pathogens) which amplify the mRNA. Most bacterial mRNAs have a shorter life span of 0.5–2 min because they are rapidly degraded by endogenous RNases. Cellular mRNAs are also degraded in non-viable cells. Hence, any assay system which can detect mRNA will also be sensitive enough to discriminate between viable and non-viable cells. However, RT-PCR assay requires extensive post-amplification handling and, at the same time, they will not yield quantitative results. To find a solution to this problem, a group targeted the hemolysin gene (*hly*) and devised an assay called a fluorogenic 5′ nuclease assay to detect viable *L. monocytogenes* [73]. In this technique, an oligonucleotide probe is designed to bind with the target sequence. This probe is cleaved because of the endogenic 5′-3′ nuclease activity of *Thermus aquaticus* DNA polymerase and generates a quantifiable signal of product amplification.

Real-time polymerase chain reaction/quantitative polymerase chain reaction (qPCR)

Real-time PCR, a more advanced version of standard PCR cuts the probability of false positive by 99.9%. This dynamic range (over six log-linear) of quantifying DNA can help in detecting single target DNA. Real-time identification is done by involving six chemical techniques: SYBR Green, Molecular Beacons, TaqMan probes (Hydrolysis probes), FRET Probes, Scorpion Probes, and Ampliflour Probes. Among all these chemistries,

molecular beacons, the stem-loop structure nucleotides, are the most specific and sensitive, so they can easily discriminate target sequences that differ from one another by a single nucleotide substitution [74]. On the other hand, a quicker method had been added for the Shiga toxin-producing *E. coli* (STEC) and others which are comprised of the Real-Time fluorescences [75]. Molecular beacon probes were designed to be specific for *stx1* and *stx 2* genes responsible for toxin production. The sensitivity and specificity of the assay were determined to be 100 and 92%, respectively. Additional applications of Real-time PCR are given in Table 2.

Multiplex real-time polymerase chain reaction

Multiplex Real-Time polymerase chain reaction assay has long been used for simultaneous identification of too many disease-causing microorganisms in one go. Wang et al. [76] established an innovative method for concomitant identification of *L. monocytogenes* and *Salmonella spp*. in raw meat samples by multiplex Real-Time PCR, where they have targeted *hly* and *inv A* genes from *L. monocytogenes* and *Salmonella spp.*, respectively. Nurjayadi et al. [77] established a PCR technique for the identification of *Salmonella typhi* using the primers targeted against the *Salmonella typhi fimC* gene. They designed fimC-F (forward primer) and fimC-R (reverse primer). They reported an increase in amplification of fim-C to 56 ng with Cycle threshold (Ct) 14.783 and 14.923 at 4.528 pg/μL template concentration with Ct 23.9. The test is highly specific, as the primer can discriminate between target and non-target bacteria specifically *Shigella* and *E. coli* with Ct 27 949 and Ct 26 036, respectively. It can also detect 10^{-6} CFU/ml of *S. typhi* in eggs. Additional examples of the application of multiplex Real-Time PCR in various food matrices are listed in Table 2.

Microarrays

Microarray is as powerful as PCR as it can characterize gene expression and mutational analysis and, most importantly, have applications in diagnostics [78]. DNA microarray is also a powerful tool for the detection of food-borne pathogens. Microarrays are glass slides or chips that are covered with a high-density matrix of DNA fragments/oligonucleotides in a specific order [79]. The conventional microbial diagnostic microarray consists of three probes: Short, Long oligonucleotides, and PCR amplicons. The probe design plays a major role in the detection specificity of the targeted pathogen. Microarray was modified over the years, out of which a planar glass slide with various modifications on the surface was widely accepted by researchers. Each oligonucleotide probe can direct a discrete region of a gene sequence. This method of detection involves two steps: first, nucleic acid pieces are labeled with fluorescent dye followed by a denaturation step which gives single-stranded filaments. The generated single-stranded filaments are further hybridized to the array by binding to their matching oligonucleotide probes. The probe sample complex generates a fluorescence signal which is further analyzed to determine the concentration of labeled nucleic acid fragments. This concentration is directly dependent on fluorescence intensity [80]. The microarray in the detection of food pathogens is illustrated below in Figure 2.

Li et al. [81] have reported pathogenic *E. coli* serotypes and *Shigella* detection by oligonucleotide DNA microarray. Microarray assay which is developed by Wang et al. [82] could detect 22 foodborne pathogens. Some of the pathogens are *C. perfringens*, *S. aureus*, *L. monocytogenes*, *Vibrio cholerae*, *V. parahaemolyticus*, *C. jejuni*, *Salmonella spp*., *Shigella spp*., and *B. cereus*. Ranjbar et al. [83] developed a microarray assay system consisting of long oligonucleotide probes which are specific for 10 pathogenic bacteria. The unique regions are identified *in silico*. Staphy Chips is the first commercial microarray developed by Affymetrix and its collaborators can detect 15 *Staphylococcus* species, which includes methicillin-resistant *Staphylococcus aureus* (MRSA) [84,85]. Companies, namely: Affymetrix Inc., Agilent Technologies Inc., Sequenom Inc., Roche NimbleGen, and Illumnia Inc., have a huge share in the market of DNA microarray (CET, 2018).

Loop-mediated isothermal amplification (LAMP)

In 2000, Notomi come up with a technique called LAMP, which was a method of nucleic acid amplification and the enzyme used as Bst DNA polymerase. It maintained an isothermal condition range from 59 to 65 °C for the synthesis of DNA [86]. Unlike PCR, four primers are used for targeting 6 precise locations of desired DNA, two are internal primers and two are external primers for those locations. LAMP amplicons have multiple loops, cauliflower-like structures, and stem loops of various sizes. A large number of amplicons can be generated using LAMP within an hour which is 1000-fold higher than a simple PCR. This great yield of amplicons enhances detection limits which can be very low compared to PCR assays [87]. SYBR green I dye or agarose gel electrophoresis can be employed to

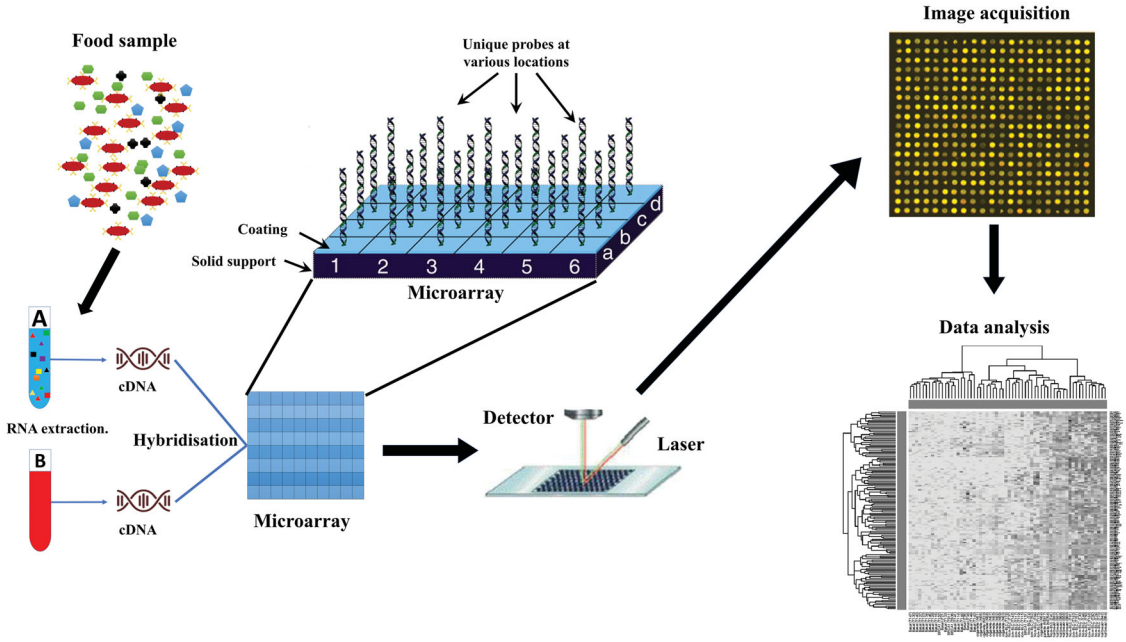

Figure 2. Microarray technique for foodborne. (a) Food matrix/sample. (b) RNA extraction and hybridization of control and sample. (c) Introduction sample on the microarray. (d) Scan and image acquisition of microarray. (e) Quantification of the signal. (f) Data analysis.

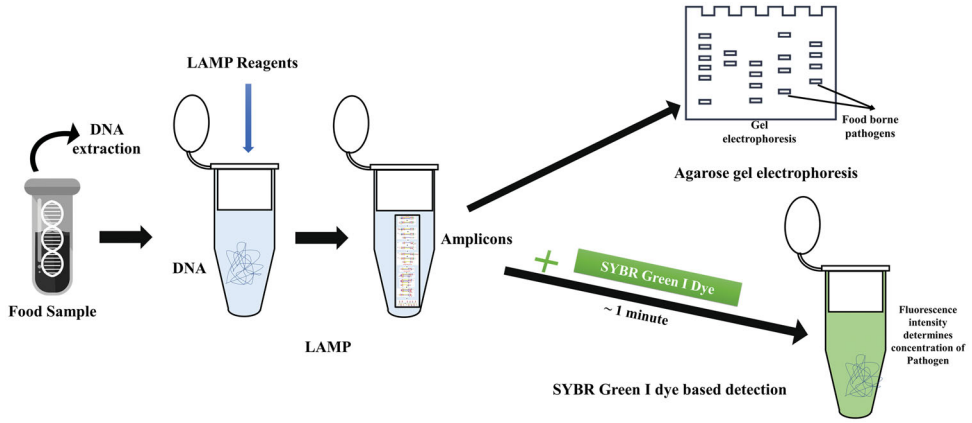

Figure 3. LAMP assay schematic for detection of foodborne pathogens. (a) Extraction of potential pathogen DNA from a food sample. (b) LAMP assay. (c) LAMP amplicons. (d) Agrose gel electrophoresis-based detection. (e) SYBR Green I-based detetction.

detect amplicons of LAMP [4]. Food pathogen detection using LAMP is illustrated in Figure 3.

Similar to PCR, various formats of the LAMP assay, such as Real-Time LAMP, Reverse transcriptase LAMP, and *in situ* LAMP have been developed [88–91]. The commercially available Loopamp detection kit by Eiken chemical can detect *S. enterica*, *Shigella*, entero-invasive *E. coli*, verotoxigenic *E. coli* (O157 & O26), and *Campylobacter* [92–95]. A molecular detection system (MDS) based on LAMP can detect *Salmonella* in egg products [96].

Other instances of LAMP in various food samples are tabulated below (Table 2).

Limitations of nucleic acid-based techniques

The capacity of nucleic acid-based detection techniques has several limitations (Table 1). PCR testing to detect dead cells is a typical source of worry. Though pathogenic cells are present in raw materials before processing, the inactivated cells of these original cells can nevertheless give a positive effect, even if there are no longer live cells capable of causing sickness. To address this issue, the sample is treated with DNA degrading enzymes, such as DNAse to successfully remove DNA contamination. Mostly, the PCR-based detection has been applied on pure cultures of pathogens isolated

from food samples, which requires an initial enrichment step to extract DNA. Notwithstanding the advantages of PCR, problems have been encountered when it is applied to naturally contaminated foods, resulting in a delay in widespread application. False-negative results can also arise due to the presence of magnesium cation chelators, nucleases that degrade nucleic acid targets or primers, or Taq polymerase inhibitors that further inhibit PCR. False-positive results may also arise because DNA/RNA amplification is highly sensitive and thus prone to cross-contamination. The most likely sources of contamination include other samples and carry-over of PCR products from previous amplifications.

Biosensor-based methods

Biosensors are devices that require biological input and a transducer that can convert that biological interaction into a quantifiable signal [4,97]. Biosensors provide quick and instantaneous identification of multiple disease-causing microorganisms. Biosensors have been optimized and developed for: *E. coli* O157:H7, *S. aureus*, *Salmonella*, and *L. monocytogenes*. Biosensor-based detection is illustrated in Figure 4.

Optics based biosensors

Surface plasmon resonance (SPR) is most popular and widely accepted by researchers. In SPR, bio-receptors are attached to very fine metal surfaces. In this system, the thin metal is exposed to electromagnetic radiation of a specific wavelength which produces a strong resonance. Pathogen binding to the metal surface of the sensor can alter the refractive index of metal, further modifying its wavelength for the electron resonance [98]. Masdor et al. [99] used subtractive immune assay (SIA) with Surface Plasmon Resonance (SPR), which showed excellent sensitivity along with high specificity for *C. jejuni* with a LOD of 131 ± 4 CFU/ml and a 95% confidence interval from 122 to 140 CFU/ml. The chances of cross-reactivity in the employed method to other foodborne pathogens include 7.8% in *S. typhimurium*, 3.88% in *L. monocytogenes*, and 1.56% in *E. coli*. *C. jejuni* in the real chicken sample at a concentration of <500 CFU/ml, was observed by combining culturing (plating) and the SIA-SPR method. Additional examples of optics-based biosensors are mentioned in Table 3.

Piezoelectric biosensors

Piezoelectric biosensing is based on a linear relationship between the frequency response of the deposited mass. Vaughan et al. [112] employed a Quartz crystal microbalance (QCM) sensor for detecting *L. monocytogenes* and the achieved LOD was 1×10^7 cells/ml. Si et al. [76] used a piezoelectric immunosensor for the detection of *Salmonella enteritidis* and LOD 1×10^5 cells/ml was achieved. Pohanka et al. [113] detected LOD of 10^6–10^9 CFU/ml in *E. coli*.

Electrochemical biosensors

The type of transducers used, classify electrochemical biosensors into four types. They are: potentiometric, amperometric, impedimetric, and conductometric which measure resultant signals obtained from antigen-bioreceptor interactions, such as voltage, current, impedance, and conductance, respectively [98]. Examples of the application of electrochemical biosensors in various food matrices are listed in Table 3.

Drawbacks of biosensors-based methods

Biosensors are sensitive and complex systems that need utmost care. Cross-reactivity and unwanted interactions can always cause errors (Table 1). Sample preparation is not always easy. There are challenges in the methods of generating low-priced dependable sensors and their reposition, stabilization, calibration, and integration.

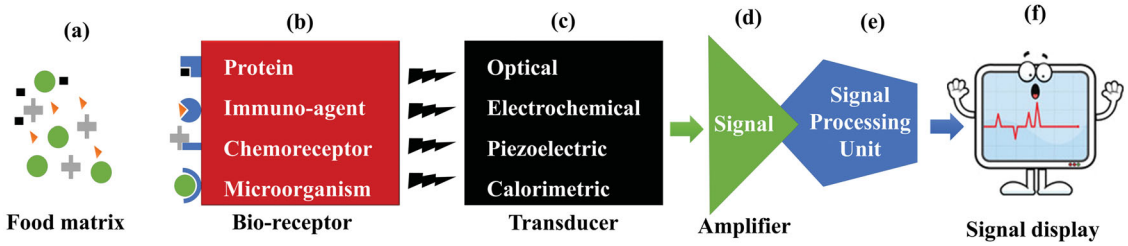

Figure 4. Schematic of bio-sensors for pathogen detection. (a) Food matrix. (b) Bioreceptor; the receptacles on the receptor recognize the target pathogen and signal to the transducer. (c) Transducer; transmits the signal received from the bio-receptor. (d) Amplifier; receives a signal from the transducer and amplifies the received signal. (e) Signal processing unit. (f) Signal display; displays signal in digital format to estimate levels.

Table 3. Biosensors-based detection methods of various causative agents for foodborne illness.

Technique employed	Targeted pathogens	Used active substrate	Detection limits	Duration of assay	References
Optical biosensor	*S. typhimurium*	Gold nanoparticles (AuNPs) with diameter of 20 nm	10^4 CFU/ml	30–35 min	[100]
	V. parahaemolyticus	A gold-coated polydimethylsiloxane (PDMS)	1.2×10^2–1.2×10^6 CFU/ml	NA	[101]
	L. monocytogenes	Anti-Fab antibody was immobilized on a CM5 sensor chip surface	1×10^5 cells/ml	<30 min	[102]
	E. coli O157:H7 H7	Lyophilized affnity-puried targeted against the O157 lipopolysaccharide	10^2–10^3 CFU/ml	30 min	[103]
	E. coli *S. enteritidis*	4-methylumbelliferyl- -d-glucuronide (MUG)	25 CFU/ml 23 CFU/ml	<1 h	[104]
Impedance-based biosensor	*Salmonella* serotypes B, D, and E	SU-8 (Microchem), Chromium (Cr) and gold (Au)	8 cells/ml	45 min	[105]
	Salmonella B and D	SU-8 2005 (Microchem), Chromium (Cr), and gold (Au)	300 cells/ml	1 h	[106]
	Vibrio cholerae O1	CeO_2 nanowires	1.0×10^2–1.0×10^4 CFU/ml	<1 h	[107]
Amperometric electrical biosensor	*E. coli* O157:H7	Cadmium sulfide quantum dots (CdS QDs)	3 CFU/ml	<3 h	[108]
	Vibrio cholerae O1	Screen-printed electrodes fabricated from Gwent carbon ink	10^5 cells/ml	55 min	[109]
Potentiometric electrical biosensor	*B. cereus*	Carbon paste electrode modified with spore-imprinted polymer (CPE/SIP)	10^2–10^5CFU/ml	NA	[110]
Luminescence electrical biosensor	*C. perfringens*	CHI 101 gold electrode with Hemin as a substrate	1.0×10^{-14} to 1.0×10^{-7} Mol/L (DNA)	~1 h	[111]

Aptamer-based detection technologies

Aptamers are small nucleotide sequences of 25–90 bases, very specific to their targets, and have great affinity comparable to antibodies. Aptamers are distinguished by the high affinity toward their target, which is measured in terms of a high dissociation constant ranging from 10^{-12} to 10^{-6} M [114]. These nucleotide sequences can fold to form stable two-dimensional or three-dimensional structures with a high surface area for target binding, which further adds to their affinity. Aptamers have several characteristic properties, such as chemical stability, specificity, ease to engineer according to target, high dissociation constant, and target variability from small ions to whole microorganisms, environment-friendly, etc. which make them a suitable method of pathogen detection. In 1990, two independent groups developed an *in vitro* selection method for aptamers called systematic evolution of ligands by exponential enrichment (SELEX) [115,116]. In the conventional SELEX method, three steps are followed: firstly synthesis of large *in-vitro* synthesized nucleic acid sequences library is allowed to bind with the target followed by aptamer-target complexes separation from others. Then this complex is used for amplification by PCR and selected for the next round. The SELX method was used to find aptamer specific for IVB pili encoded by a potential pathogen that causes typhoid in humans. The RNA-aptamer was found which not only specifically bound with type IVB pili but it also inhibited the entry of this strain in human cells [117]. The SELEX method was reportedly used for the aptamer selection specific to *V. parahemolyticus*. This was the whole-cell selection method where the selected aptamer showed high dissociation constant (K (d)) of 16.88 ± 1.92 nM and a great affinity (76%) toward this pathogen [118]. The same group reported the aptamers selected and characterized against *S. typhimurium* using the SELEX method [119]. A Schematic of the SELEX technique has been shown in Figure 5.

Based on the detection system employed, numerous aptamer-based biosensors have been developed, such as optical biosensors: fluorescence-based biosensors, surface-enhanced Raman scattering, colorimetric, electrochemical biosensors, lateral chromatography test strips detection.

Fluorescence biosensors

The basic principle behind fluorescence-based aptamer biosensors is that, once the target and the fluorescently tagged aptamers form a conjugate, it changes the intensity of fluorescence and is measured [120]. In 2016, a fluorescent-aptamer-based sandwich-type model was developed for the detection of *E. coli* O157, *S. entrica,* and Shiga-like toxin-1 in spinach, tomato, and beef. The detection limit of the said model was 100 CFU/ml for the *E. coli* O157 and *S. entrica* whereas 100 pg/ml for *S. enterica* and the reported assay time was 4 h for *E. coli* [121]. The same kind of model with slight modifications was applied by Wang et al. [122] for the detection of *S. aureus* and *S. typhimurium*, where

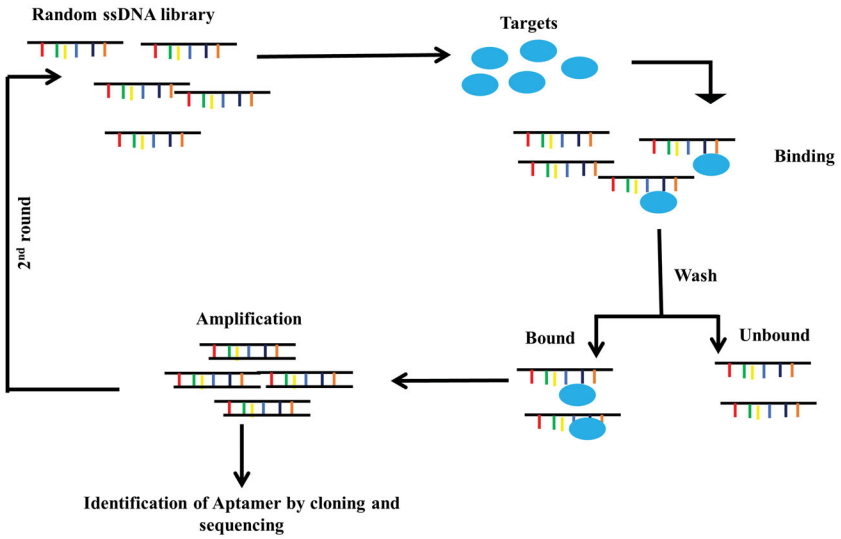

Figure 5. Schematic of SELEX technique for food-borne pathogen detection.

the magnetic nanoparticles conjugated with aptamer acted as a probe for capturing, and multi-color lanthanide-doped fluorescent nanoparticle aptamer conjugate acted as signal transducers. The reported detection limit was 15 CFU/ml.

In very recent times, an assay was generated for the detection of toxins found in foodstuff due to the biofilm formation using aptamer-graphene oxide conjugate with tagged fluorophores, i.e., Alexa Fluor 488 and Cy3. In the presence of the target, these fluorophores detached from the conjugate and hence can be quantified. It can detect up to a range of 1.797–1.484 ng/ml. This method has wider applications in detecting toxins present in the food [123].

Colorometric biosensors

Colorimetric-based optical biosensors generally exploit the ability to change the color of colloidal gold nanoparticles when they transit from one dispersion state to an aggregation state. Once the state changes, the size, and interparticle distance reduce which converts the nanoparticle from red to blue. For the calorimetric detection, there should substrate-specific substance that can oxidize the substrate to give visual color for detection. In a variety of applications of colorimetric biosensors, Horse Radish Peroxidase (HRP) has been used to produce visible sequences. Recently, Sun et al. [124] developed a platform for the detection of *V. parahaemolyticus*, in which both aptamer and its complementary sequence are made to bound with the magnetic beads. In the presence of a pathogen, G-quadruplex bounded with complementary sequence will dissociate from the bead and will further provide the concentration of *V. parahaemolyticus*. In this method, TMB and H_2O_2 catalyzed by G-quadruplex will give visible color. Wen-He Wu et al. [125] have developed a colorimetric method for the recognition of *E. coli* O157:H7 and *S. typhimurium* with the use of gold nanoparticles. The color change is monitored for the detection of the target bacterial strain with a low CFU count (10^5 CFU/ml) with less detection time of 20 min. Another research group used a gold nanoparticle-based two-stage aptasensing platform, to distinguish the live *C. jejuni* and *Campylobacter coli* in 50 chicken carcass samples only 30 min after pre-enrichment of the sample [126].

Electrochemical biosensor

Electrochemical biosensors are devices that measure the changes in the electrical properties of a platform which is developed to bind specifically with the target. This method has advantages over optical sensors, such as being cheap and easy to handle, and workable on turbid media; less amount of sample is required, though it is not as sensitive and specific as optical biosensors [127]. Suh and Jaykus [128] tried to detect a food-borne pathogen *L. monocytogenes* by using DNA aptamers ligated with biotin; whereas other groups developed a sensor by using two aptamers specific to the *S. aureus*. They conjugated aptamers with magnetic beads, and once the target binds with it, electrochemical change occurs which shows the presence of *S. aureus*.

In 2016, Sheikhzadeh et al. [129] reported a label-less electrochemical biosensor for the detection of *S. typhimurium* with an LOD of 3 CFU/ml. In that

biosensor, they conjugated poly- [pyrrole-co-3-carboxyl-pyrrole] polymer with the aptamer. When this conjugate and the target meet, the change in electrical properties is measured. Very recently, a highly sensitive and faster method was reported where recognition of *E. coli* O157:H7 was carried out by immobilizing aptamer and urease enzyme onto gold-coated nanoparticles. The electrons required for change in electrical properties are released from the hydrolysis of urea by the urease enzyme and this gold nanoparticle is there to measure the change in impedance. This change in impedance is directly proportional to the concentration of the pathogen. The detection limit and assay time observed were 101 CFU/ml and 3 h, respectively [130].

Surface plasmon resonance

In comparison to other optical biosensors, this biosensor has the advantage that it is a label-free detection method which makes the process easy and simple. This technique has been used for several years in different fields but recently it got researchers' attention to be used for single-cell and faster detection of a pathogen named *S. aureus* [131]. Yoo et al. [132] have devised a localized surface plasmon resonance (LSPR) biosensor with an embedded aptamer to it for the detection of bacterial species including *S. typhimurium*. The limit of detection of this method was noted as 30 CFU/ml. They reported the method to be a sensitive, accurate, and fast one for identifying several contagious diseases in a very simple way. A variable chip based on localized surface plasmon resonance with aptamers attached to gold nanoparticles immobilized on a substrate to detect *S. typhimurium* in pork meat (LOD 10^4 CFU/ml) was developed [100]. The time of detection assay is 30–35 min. In 2016, Vaisocherová-Lísalová and the group reported the implementation of an SPR biosensor for the fast and precise capturing of *E. coli* and *Salmonella sp*. from cucumber and hamburger extracts. The reported LODs for *E. coli* in cucumber were 57 CFU/ml and in hamburger 17 CFU/ml; whereas for *Salmonella sp*. it was 7.4×10^3 CFU/ml in cucumber and 11.7×10^3 CFU/ml in hamburger [133].

Conclusion

From the foregoing review, it can be concluded that advanced methods of detection are more: rapid, efficient, accurate, automatic, and less time-consuming, with the least or no false positives. In this review, various techniques developed during the period for foodborne pathogen detection are discussed with their advantages and limitations (Figure 6). Immunological-based methods have been widely utilized for the identification of disease-causing microorganisms and their secreted toxins found in the foodstuff. However, in immunological-based methods, the chances of cross-reactivity of antisera are very high. Biosensors-based methods also need more refinements for the recognition of on-site food pathogens from food matrixes. The PCR-based methods have always been widely utilized for the direct identification of disease-causing microorganisms found in the foods but the success of most of these techniques was achieved only when such foods

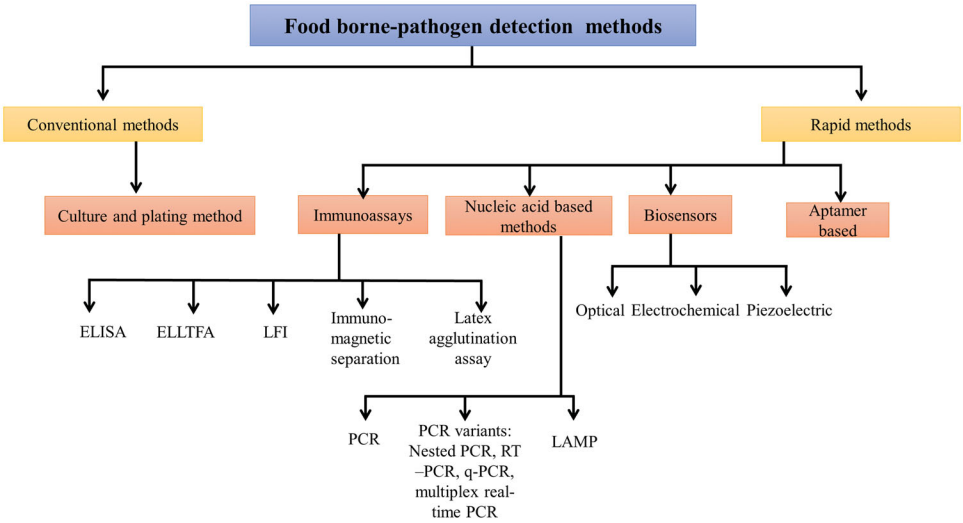

Figure 6. Schematic diagram depicting methods used for foodborne pathogen detection. ELISA: enzyme-linked immunosorbent assay; ELLTFA: enzyme-linked long tail fiber assay; LFI: lateral flow immunoassay; PCR: polymerase chain reaction; RT-PCR: reverse transcription-polymerase chain reaction; q-PCR: real-time polymerase chain reaction quantitative PCR; LAMP: loop-mediated isothermal amplification; biosensors are categorized according to the transducer being used.

were spiked with the targeted pathogens. When these techniques were applied to naturally contaminated foods, the results were not consistent. Real-time PCR techniques have found great acceptance in the detection and quantification of various high-risk pathogens in food samples. Real-time reduces the false positives and is more accurate than convention techniques. Nevertheless, the possibility of a combination of these rapid methods should be exploited as the combinatorial chemistry that can work synergistically for the most effective and accurate detection of these high-risk food pathogens.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

[1] Scharff RL, Besser J, Sharp DJ, et al. An economic evaluation of PulseNet: a network for foodborne disease surveillance. Am J Prev Med. 2016;50(5 Suppl 1):S66–S73.
[2] Bisht A, Kamble MP, Choudhary P, et al. A surveillance of food borne disease outbreaks in India: 2009–2018. Food Control. 2021;121:107630.
[3] Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States–major pathogens. Emerg Infect Dis. 2011;17(1):7–15.
[4] Zhao X, Lin C-W, Wang J, et al. Advances in rapid detection methods for foodborne pathogens. J Microbiol Biotechnol. 2014;24(3):297–312.
[5] Kohli C, Garg S. Food safety in India: an unfinished agenda. MAMC J Med Sci. 2015;1(3):131.
[6] Mandal P, Biswas A, K C, et al. Methods for rapid detection of foodborne pathogens: an overview. Am J Food Technol. 2011;6(2):87–102.
[7] Lee N, Kwon KY, Oh SK, et al. A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157:H7, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-Eat food. Foodborne Pathog Dis. 2014;11(7):574–580.
[8] Wolffs P, Norling B, Rådström P. Risk assessment of false-positive quantitative real-time PCR results in food, due to detection of DNA originating from dead cells. J Microbiol Methods. 2005;60(3):315–323.
[9] Stephenson FH. Real-time PCR. In: Calculations for molecular biology and biotechnology. UK: Elsevier; 2016. p. 215–320.
[10] Umesha S, Manukumar HM. Advanced molecular diagnostic techniques for detection of food-borne pathogens: current applications and future challenges. Crit Rev Food Sci Nutr. 2018;58(1):84–104.
[11] Mangal M, Bansal S, Sharma SK, et al. Molecular detection of foodborne pathogens: a rapid and accurate answer to food safety. Crit Rev Food Sci Nutr. 2016;56(9):1568–1584.
[12] Valderrama WB, Dudley EG, Doores S, et al. Commercially available rapid methods for detection of selected food-borne pathogens. Crit Rev Food Sci Nutr. 2016;56(9):1519–1531.
[13] Brackett RE, Beuchat LR. Methods and media for the isolation and cultivation of *Listeria monocytogenes* from various foods. Int J Food Microbiol. 1989;8(3):219–223.
[14] Adzitey F, Huda N, Ali GRR. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. 3 Biotech. 2013;3(2):97–107.
[15] Zhao T, Doyle MP. Evaluation of universal preenrichment broth for growth of heat-injured pathogens. J Food Prot. 2001;64(11):1751–1755.
[16] Busta FF. Practical implications of injured microorganisms in food. J Milk Food Technol. 1976;39(2):138–145.
[17] Hurst A. Bacterial injury: a review. Can J Microbiol. 1977;23(8):935–944.
[18] Walker JM. 1987. The enzyme linked immunosorbent assay (ELISA). In: Walker JM, Gaastra W, editors. BT – techniques in molecular biology. Vol. 2. Boston (MA): Springer US. p. 82–97.
[19] Farber JM, Speirs JI. Monoclonal antibodies directed against the flagellar antigens of *Listeria* species and their potential in EIA-Based methods. J Food Prot. 1987;50(6):479–484.
[20] Butman BT, Plank MC, Durham RJ, et al. Monoclonal antibodies which identify a genus-specific *Listeria* antigen. Appl Environ Microbiol. 1988;54(6):1564–1569.
[21] Kumar BK, Raghunath P, Devegowda D, et al. Development of monoclonal antibody based sandwich ELISA for the rapid detection of pathogenic *Vibrio parahaemolyticus* in seafood. Int J Food Microbiol. 2011;145(1):244–249.
[22] Bolton FJ, Fritz E, Poynton S, et al. Rapid enzyme-linked immunoassay for detection of *Salmonella* in food and feed products: performance testing program. J AOAC Int. 2000;83(2):299–304.
[23] Glynn B, Lahiff S, Wernecke M, et al. Current and emerging molecular diagnostic technologies applicable to bacterial food safety. Int J Dairy Tech. 2006;59(2):126–139.
[24] Law J, Mutalib A, Chan N-S, et al. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol. 2014;5:770.
[25] Fung DYC. Rapid methods and automation in microbiology. Compr Rev Food Sci Food Saf. 2002;1(1):3–22.
[26] Vieira-Pinto M, Oliveira M, Fernando B, et al. Rapid detection of *Salmonella* sp. in pork samples using fluorescent *in situ* hybridization: a comparison with VIDAS®-SLM system and ISO 6579 cultural method

ISO 6579. Arq Bras Med Vet Zootec. 2007;59(6): 1388–1393.
[27] Gomez-Govea M, Solis L, Heredia N, et al. Analysis of microbial contamination levels of fruits and vegetables at retail in Monterrey, Mexico. J Food Agric Environ. 2012;10:152–156.
[28] Carvalho RN, Oliveira A d, Mesquita A D, et al. PCR and ELISA (VIDAS ECO O157®) *Escherichia coli* O157:H7 identification in minas frescal cheese commercialized in Goiânia, GO. Braz J Microbiol. 2014;45: 7–10.
[29] Meyer C, Fredriksson-Ahomaa M, Sperner B, et al. Detection of *Listeria monocytogenes* in pork and beef using the VIDAS® LMO2 automated enzyme linked immunoassay method. Meat Sci. 2011;88(3):594–596.
[30] Taha EG, Mohamed A, Srivastava KK, et al. Rapid detection of *Salmonella* in chicken meat using immunomagnetic separation, CHROmagar, ELISA and real-time polymerase chain reaction (RT-PCR). Int J Poultry Sci. 2010;9(9):831–835.
[31] Pang B, Zhao C, Li L, et al. Development of a low-cost paper-based ELISA method for rapid *Escherichia coli* O157:H7 detection. Anal Biochem. 2018;542: 58–62.
[32] Di Febo T, Schirone M, Visciano P, et al. Development of a capture ELISA for rapid detection of *Salmonella enterica* in food samples. Food Anal Methods. 2019;12(2):322–330.
[33] Denyes JM, Dunne M, Steiner S, et al. Modified bacteriophage S16 long tail fiber proteins for rapid and specific immobilization and detection of *Salmonella* cells. Appl Environ Microbiol. 2017;83(12):e00277-17.
[34] Kim G, Lim J, Mo C. A review on lateral flow test strip for food safety. J Biosyst Eng. 2015;40(3): 277–283.
[35] Shan S, Lai W, Xiong Y, et al. Novel strategies to enhance lateral flow immunoassay sensitivity for detecting foodborne pathogens. J Agric Food Chem. 2015;63(3):745–753.
[36] De Boer SH, López MM. New Grower-Friendly methods for plant pathogen monitoring. Annu Rev Phytopathol. 2012;50:197–218.
[37] Shukla S, Leem H, Kim M. Development of a liposome-based immunochromatographic strip assay for the detection of *Salmonella*. Anal Bioanal Chem. 2011;401(8):2581–2590.
[38] Kim G, Moon J-H, Park SB, et al. Image analysis of a lateral flow strip sensor for the detection of *Escherichia coli* O157:H7. J Biosyst Eng. 2013;38(4): 335–340.
[39] Kim T-H, Hwang HJ, Kim JH. Ultra-Fast on-Site molecular detection of foodborne pathogens using a combination of convection polymerase chain reaction and nucleic acid lateral flow immunoassay. Foodborne Pathog Dis. 2019;16(2):144–151.
[40] Husakova M, Dziedzinska R, Slana I. Magnetic separation methods for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in various types of matrices: a review. Biomed Res Int. 2017;2017: 5869854.
[41] DeCory TR, Durst RA, Zimmerman SJ, et al. Development of an immunomagnetic Bead-Immunoliposome fluorescence assay for rapid detection of *Escherichia coli* O157:H7 in aqueous samples and comparison of the assay with a standard microbiological method. Appl Environ Microbiol. 2005; 71(4):1856–1864.
[42] Ma K, Deng Y, Bai Y, et al. Rapid and simultaneous detection of *Salmonella*, *Shigella*, and *Staphylococcus aureus* in fresh pork using a multiplex real-time PCR assay based on immunomagnetic separation. Food Control. 2014;42:87–93.
[43] Xu D, Ming X, Gan M, et al. Rapid detection of *Cronobacter* spp. in powdered infant formula by thermophilic helicase-dependent isothermal amplification combined with silica-coated magnetic particles separation. J Immunol Methods. 2018;462: 54–58.
[44] Wang Z, Xianyu Y, Zhang Z, et al. Background signal-free magnetic bioassay for food-borne pathogen and residue of veterinary drug via Mn(VII)/Mn(II) interconversion. ACS Sens. 2019;4(10):2771–2777.
[45] Beumer RR, te Giffel MC, de Boer E, et al. Growth of *Listeria monocytogenes* on sliced cooked meat products. Food Microbiol. 1996;13(4):333–340.
[46] Hajra TK, Bag PK, Das SC, et al. Development of a simple latex agglutination assay for detection of Shiga toxin-producing *Escherichia coli* (STEC) by using polyclonal antibody against STEC. Clin Vaccine Immunol. 2007;14(5):600–604.
[47] Ito H, Terai A, Kurazono H, et al. Cloning and nucleotide sequencing of vero toxin 2 variant genes from *Escherichia coli* O91 : H21 isolated from a patient with the hemolytic uremic syndrome. Microb Pathog. 1990;8(1):47–60.
[48] Willshaw GA, Smith HR, Scotland SM, et al. Heterogeneity of *Escherichia coli* phages encoding vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J Gen Microbiol. 1987;133(5): 1309–1317.
[49] Bej AK, Mahbubani MH. Applications of the polymerase chain reaction in environmental microbiology. PCR Methods Appl. 1992;1(3):151–159.
[50] Priyanka B, Patil RK, Dwarakanath S. A review on detection methods used for foodborne pathogens. Indian J Med Res. 2016;144(3):327–338.
[51] Kumar A, Grover S, Batish V. Monitoring paneer for *Listeria monocytogenes*-a high risk food pathogen by multiplex PCR. Afr J Biotech. 2012;11:9452–9456.
[52] Latha C, Anu CJ, Ajaykumar VJ, et al. Prevalence of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus*, and *Salmonella enterica typhimurium* in meat and meat products using multiplex polymerase chain reaction. Vet World. 2017;10(8): 927–931.
[53] Ferrario C, Lugli GA, Ossiprandi MC, et al. Next generation sequencing-based multigene panel for high throughput detection of food-borne pathogens. Int J Food Microbiol. 2017;256:20–29.
[54] Hu J, Huang R, Wang Y, et al. Development of duplex PCR-ELISA for simultaneous detection of *Salmonella* spp. and *Escherichia coli* O157: H7 in food. J Microbiol Methods. 2018;154:127–133.

[55] Kumar A, Grover S, Batish VK. A multiplex PCR assay based on 16S rRNA and hly for rapid detection of *L. monocytogenes* in milk. Food Meas. 2014;8(3):155–163.
[56] Kumar A, Grover S, Batish VK. Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. 3 Biotech. 2015;5(3):261–269.
[57] Wei S, Daliri EB-M, Chelliah R, et al. Development of a multiplex real-time PCR for simultaneous detection of *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus* in food samples. J Food Saf. 2019;39(1):e12558.
[58] Yang J, Qi X-M, Wu Y-G. The application analysis of multiplex real-time polymerase chain reaction assays for detection of pathogenic bacterium in peritoneal dialysis-associated peritonitis. Blood Purif. 2019;47(4):337–345.
[59] Brusa V, Galli L, Linares LH, et al. Development and validation of two SYBR green PCR assays and a multiplex real-time PCR for the detection of Shiga toxin-producing *Escherichia coli* in meat. J Microbiol Methods. 2015;119:10–17.
[60] Nguyen LT, Gillespie BE, Nam HM, et al. Detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in beef products by real-time polymerase chain reaction. Foodborne Pathog Dis. 2004;1(4):231–240.
[61] Fu Z, Rogelj S, Kieft TL. Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real-time PCR. Int J Food Microbiol. 2005;99(1):47–57.
[62] Massi N, Shirakawa M, Gotoh T, et al. Quantitative detection of *Salmonella enterica* serovar *typhi* from blood of suspected typhoid fever patients by real-time PCR. Int J Med Microbiol. 2005;295(2):117–120.
[63] Patel JR, Bhagwat AA, Sanglay GC, et al. Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. Food Microbiol. 2006;23(1):39–46.
[64] Singh J, Batish VK, Grover S. Simultaneous detection of *Listeria monocytogenes* and *Salmonella* spp. in dairy products using real time PCR-melt curve analysis. J Food Sci Technol. 2012;49(2):234–239.
[65] Wei C, Zhong J, Hu T, et al. Simultaneous detection of *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Salmonella* by multiplex PCR in milk. 3 Biotech. 2018;8(1):76.
[66] Cady NC, Stelick S, Kunnavakkam MV, et al. Real-time PCR detection of *Listeria monocytogenes* using an integrated microfluidics platform. Sensors Actuators B Chem. 2005;107(1):332–341.
[67] Singh J, Batish VK, Grover S. A molecular beacon-based duplex real-time polymerase chain reaction assay for simultaneous detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in milk and milk products. Foodborne Pathog Dis. 2009;6(10):1195–1201.
[68] Nasrabadi Z, Ranjbar R, Poorali F, et al. Detection of eight foodborne bacterial pathogens by oligonucleotide array hybridization. Electron Physician. 2017;9(5):4405–4411.
[69] Trinh KTL, Trinh TND, Lee NY. Fully integrated and slidable paper-embedded plastic microdevice for point-of-care testing of multiple foodborne pathogens. Biosens Bioelectron. 2019;135:120–128.
[70] Yamazaki W, Ishibashi M, Kawahara R, et al. Development of a loop-mediated isothermal amplification assay for sensitive and rapid detection of *Vibrio parahaemolyticus*. BMC Microbiol. 2008;8:163.
[71] Su J, Liu X, Cui H, et al. Rapid and simple detection of methicillin-resistance *Staphylococcus aureus* by orfX loop-mediated isothermal amplification assay. BMC Biotechnol. 2014;14:8.
[72] Xu X, Zhang S, Wu Q, et al. Development and application of a loop-mediated isothermal amplification (LAMP) method for rapid and sensitive detection of *Enterococcus faecalis* in drinking water. J Food Saf. 2014;34(2):103–110.
[73] Norton DM, Batt CA. Detection of viable *Listeria monocytogenes* with a 5′ nuclease PCR assay. Appl Environ Microbiol. 1999;65(5):2122–2127.
[74] Goel G, Kumar A, Puniya AK, et al. Molecular beacon: a multitask probe. J Appl Microbiol. 2005;99(3):435–442.
[75] Bélanger SD, Boissinot M, Ménard C, et al. Rapid detection of Shiga toxin-producing bacteria in feces by multiplex PCR with molecular beacons on the smart cycler. J Clin Microbiol. 2002;40(4):1436–1440.
[76] Wang X, Jothikumar N, Griffiths M. Enrichment and DNA extraction protocols for the simultaneous detection of *Salmonella* and *Listeria monocytogenes* in raw sausage meat with multiplex real-time PCR. J Food Prot. 2004;67(1):189–192.
[77] Nurjayadi M, Pertiwi YP, Islami N, et al. Detection of the *Salmonella typhi* bacteria in contaminated egg using real-time PCR to develop rapid detection of food poisoning bacteria. Biocatal Agric Biotechnol. 2019;20:101214.
[78] Kumar A, Goel G, Fehrenbach E, et al. Microarrays: the technology, analysis and application. Eng Life Sci. 2005;5(3):215–222.
[79] Umesha S, H MM. Advanced molecular diagnostic techniques for detection of food-borne pathogens; current applications and future challenges. Crit Rev Food Sci Nutr. 2016;58:84–104.
[80] Lauri A, Mariani PO. Potentials and limitations of molecular diagnostic methods in food safety. Genes Nutr. 2009;4(1):1–12.
[81] Li Y, Liu D, Cao B, et al. Development of a serotype-specific DNA microarray for identification of some *Shigella* and pathogenic *Escherichia coli* strains. J Clin Microbiol. 2006;44(12):4376–4383.
[82] Wang X-W, Zhang L, Jin L-Q, et al. Development and application of an oligonucleotide microarray for the detection of food-borne bacterial pathogens. Appl Microbiol Biotechnol. 2007;76(1):225–233.
[83] Ranjbar R, Behzadi P, Najafi A, et al. DNA microarray for rapid detection and identification of food and water borne bacteria: from dry to wet lab. Open Microbiol J. 2017;11:330–338.
[84] Mothershed EA, Whitney AM. Nucleic acid-based methods for the detection of bacterial pathogens:

present and future considerations for the clinical laboratory. Clin Chim Acta. 2006;363(1–2):206–220.
[85] Hamels S, Gala J-L, Dufour S, et al. Consensus PCR and microarray for diagnosis of the genus *Staphylococcus*, species, and methicillin resistance. Biotechniques. 2001;31(6):1364–1372.
[86] Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28(12):e63.
[87] Xu Z, Li L, Chu J, et al. Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. Food Res Int. 2012;47(2):166–173.
[88] Chen H, Zhang J, Sun D, et al. Development of reverse transcription loop-mediated isothermal amplification for rapid detection of H9 avian influenza virus. J Virol Methods. 2008;151(2):200–203.
[89] Han F, Ge B. Quantitative detection of *Vibrio vulnificus* in raw oysters by real-time loop-mediated isothermal amplification. Int J Food Microbiol. 2010;142(1–2):60–66.
[90] Shao Y, Zhu S, Jin C, et al. Development of multiplex loop-mediated isothermal amplification-RFLP (mLAMP-RFLP) to detect *Salmonella* spp. and *Shigella* spp. in milk. Int J Food Microbiol. 2011;148(2):75–79.
[91] Ye Y, Wang B, Huang F, et al. Application of *in situ* loop-mediated isothermal amplification method for detection of *Salmonella* in foods. Food Control. 2011;22(3–4):438–444.
[92] Hara-Kudo Y, Konishi N, Ohtsuka K, et al. Detection of verotoxigenic *Escherichia coli* O157 and O26 in food by plating methods and LAMP method: a collaborative study. Int J Food Microbiol. 2008;122(1–2):156–161.
[93] Ohtsuka K, Yanagawa K, Takatori K, et al. Detection of *Salmonella enterica* in naturally contaminated liquid eggs by loop-mediated isothermal amplification, and characterization of *Salmonella* isolates. Appl Environ Microbiol. 2005;71(11):6730–6735.
[94] Song T, Toma C, Nakasone N, et al. Sensitive and rapid detection of *shigella* and enteroinvasive *Escherichia coli* by a loop-mediated isothermal amplification method. FEMS Microbiol Lett. 2005;243(1):259–263.
[95] Yamazaki W, Taguchi M, Kawai T, et al. Comparison of loop-mediated isothermal amplification assay and conventional culture methods for detection of *Campylobacter jejuni* and *Campylobacter coli* in naturally contaminated chicken meat samples. Appl Environ Microbiol. 2009;75(6):1597–1603.
[96] Hu L, Ma LM, Zheng S, et al. Evaluation of 3M molecular detection system and ANSR pathogen detection system for rapid detection of *Salmonella* from egg products. Poult Sci. 2017;96(5):1410–1418.
[97] Velusamy V, Arshak K, Korostynska O, et al. An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol Adv. 2010;28(2):232–254.
[98] Zhang G. Foodborne pathogenic bacteria detection: an evaluation of current and developing methods. Meducator. 2013;1(24):28–30.
[99] Masdor NA, Altintas Z, Shukor MY, et al. Subtractive inhibition assay for the detection of *Campylobacter jejuni* in chicken samples using surface plasmon resonance. Sci Rep. 2019;9(1):13642.
[100] Oh SY, Heo NS, Shukla S, et al. Development of gold nanoparticle-aptamer-based LSPR sensing chips for the rapid detection of *Salmonella typhimurium* in pork meat. Sci Rep. 2017;7(1):10130.
[101] Wu S, Duan N, Shen M, et al. Surface-enhanced Raman spectroscopic single step detection of *Vibrio parahaemolyticus* using gold coated polydimethylsiloxane as the active substrate and aptamer modified gold nanoparticles. Mikrochim Acta. 2019;186(7):401.
[102] Leonard P, Hearty S, Quinn J, et al. A generic approach for the detection of whole *Listeria monocytogenes* cells in contaminated samples using surface plasmon resonance. Biosens Bioelectron. 2004;19(10):1331–1335.
[103] Demarco D, Lim D. Detection of *Escherichia coli* O157:H7 in 10- and 25-gram ground beef samples with an evanescent-wave biosensor with silica and polystyrene waveguides. J Food Prot. 2002;65(4):596–602.
[104] Hesari N, Alum A, Elzein M, et al. A biosensor platform for rapid detection of *E. coli* in drinking water. Enzyme Microb Technol. 2015;83:22–28.
[105] Liu J, Jasim I, Abdullah A, et al. An integrated impedance biosensor platform for detection of pathogens in poultry products. Sci Rep. 2018;8(1):16109.
[106] Liu J, Jasim I, Shen Z, et al. A microfluidic based biosensor for rapid detection of *Salmonella* in food products. PLOS One. 2019;14(5):e0216873.
[107] Tam PD, Thang CX. Label-free electrochemical immunosensor based on cerium oxide nanowires for *Vibrio cholerae* O1 detection. Mater Sci Eng C Mater Biol Appl. 2016;58:953–959.
[108] Zhong M, Yang L, Yang H, et al. An electrochemical immunobiosensor for ultrasensitive detection of *Escherichia coli* O157:H7 using CdS quantum dots-encapsulated metal-organic frameworks as signal-amplifying tags. Biosens Bioelectron. 2019;126:493–500.
[109] Rao VK, Sharma MK, Goel AK, et al. Amperometric immunosensor for the detection of *Vibrio cholerae* O1 using disposable screen-printed electrodes. Anal Sci. 2006;22(9):1207–1211.
[110] Ait Lahcen A, Arduini F, Lista F, et al. Label-free electrochemical sensor based on spore-imprinted polymer for *Bacillus cereus* spore detection. Sensors Actuators B Chem. 2018;276:114–120.
[111] Jiang D, Liu F, Liu C, et al. Induction of an electrochemiluminescence sensor for DNA detection of *Clostridium perfringens* based on rolling circle amplification. Anal Methods. 2014;6(5):1558–1562.
[112] Vaughan RD, O'Sullivan CK, Guilbault GG. Development of a quartz crystal microbalance (QCM) immunosensor for the detection of *Listeria monocytogenes*. Enzyme Microb Technol. 2001;29(10):635–638.
[113] Pohanka M, Skládal P, Pavliš O. Label-free piezoelectric immunosensor for rapid assay of *Escherichia coli*. J Immunoassay Immunochem. 2008;29(1):70–79.

[114] Hamula CLA, Guthrie JW, Zhang H, et al. Selection and analytical applications of aptamers. Trends Anal Chem. 2006;25(7):681–691.
[115] Ellington AD, Szostak JW. *In vitro* selection of RNA molecules that bind specific ligands. Nature. 1990; 346(6287):818–822.
[116] Tuerk C, MacDougal S, Gold L. RNA pseudoknots that inhibit human immunodeficiency virus type 1 reverse transcriptase. Proc Natl Acad Sci USA. 1992;89(15): 6988–6992.
[117] Pan Q, Zhang X-L, Wu H-Y, et al. Aptamers that preferentially bind type IVB pili and inhibit human monocytic-cell invasion by *Salmonella enterica* serovar *typhi*. Antimicrob Agents Chemother. 2005;49(10): 4052–4060.
[118] Duan N, Wu S, Chen X, et al. Selection and identification of a DNA aptamer targeted to *Vibrio parahemolyticus*. J Agric Food Chem. 2012;60(16):4034–4038.
[119] Duan N, Wu S, Chen X, et al. Selection and characterization of aptamers against *Salmonella typhimurium* using whole-bacterium systemic evolution of ligands by exponential enrichment (SELEX). J Agric Food Chem. 2013;61(13):3229–3234.
[120] Wang YX, Ye ZZ, Si CY, et al. Application of aptamer based biosensors for detection of pathogenic microorganisms. Chinese J Anal Chem. 2012;40(4):634–642.
[121] Bruno J. Fluorescent DNA aptamer-magnetic bead sandwich assays and portable fluorometer for sensitive and rapid foodborne pathogen detection and epidemiology. J Infect Dis Epidemiol. 2016;2(1):1–6.
[122] Wang X, Huang Y, Wu S, et al. Simultaneous detection of *Staphylococcus aureus* and *Salmonella typhimurium* using multicolor time-resolved fluorescence nanoparticles as labels. Int J Food Microbiol. 2016; 237:172–179.
[123] Wang Q, Yang Q, Wu W. Graphene-Based steganographic aptasensor for information computing and monitoring toxins of biofilm in food. Front Microbiol. 2019;10:3139.
[124] Sun Y, Duan N, Ma P, et al. Colorimetric aptasensor based on truncated aptamer and trivalent DNAzyme for *Vibrio parahemolyticus* determination. J Agric Food Chem. 2019;67(8):2313–2320.
[125] Wu W, Li M, Wang Y, et al. Aptasensors for rapid detection of *Escherichia coli* O157:H7 and *Salmonella typhimurium*. Nanoscale Res Lett. 2012;7(1):658.
[126] Kim Y-J, Kim H-S, Chon J-W, et al. New colorimetric aptasensor for rapid on-site detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass samples. Anal Chim Acta. 2018;1029: 78–85.
[127] Muniandy S, Dinshaw IJ, Teh SJ, et al. Graphene-based label-free electrochemical aptasensor for rapid and sensitive detection of foodborne pathogen. Anal Bioanal Chem. 2017;409(29):6893–6905.
[128] Suh SH, Jaykus L-A. Nucleic acid aptamers for capture and detection of *Listeria* spp. J Biotechnol. 2013; 167(4):454–461.
[129] Sheikhzadeh E, Chamsaz M, Turner APF, et al. Label-free impedimetric biosensor for *Salmonella typhimurium* detection based on poly [pyrrole-co-3-carboxylpyrrole] copolymer supported aptamer. Biosens Bioelectron. 2016;80:194–200.
[130] Xu M, Wang R, Li Y. Electrochemical biosensors for rapid detection of *Escherichia coli* O157:H7. Talanta. 2017;162:511–522.
[131] Chang Y-C, Yang C-Y, Sun R-L, et al. Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. Sci Rep. 2013;3:1863.
[132] Yoo S, Kim D-K, Lee SY. Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species. Talanta. 2015;132:112–117.
[133] Vaisocherová-Lísalová H, Visova I, Ermini ML, et al. Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples. Biosens Bioelectron. 2016;80:84–90.