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Current and Emerging Technologies for Rapid Detection of Pathogens

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Abstract

Foodborne diseases, caused by pathogenic bacteria, have become an important social issue in the field of food safety. It presents a widespread and growing threat to human health in both developed and developing countries. As such, techniques for the detection of foodborne pathogens and waterborne pathogens are urgently needed to prevent the occurrence of human foodborne infections. Although traditional culture-based bacterial isolation and identification are the “gold standard” methods with high preciseness, their drawbacks in time-consuming are inadequate for rapid detection of pathogen to reduce foodborne disease occurrence. Fortunately, with the development of biotechnologies and nanotechnologies, various kinds of new technologies for rapid detection of pathogens have been developed so far, such as nucleic acid-based methods, antibody-based methods, and aptamer-based assays. In this chapter, we summarized the principles and the application of some recent rapid detection technologies for pathogenic bacteria. Moreover, the advantages and disadvantages of the established and emerging rapid detection methods are addressed here.

Keywords: pathogen, rapid detection, nucleic acid, antibody, aptamer

1. Introduction

Foodborne pathogens, which are widely responsible for many foodborne diseases, constitute a serious threat to human health. In recent years, foodborne and waterborne pathogenic microorganisms have caused numerous epidemic diseases in the world [1]. *Salmonella*, *Shiga bacillus*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes* are the primary pathogens that are responsible for most foodborne disease [2–6]. Centers for Disease Control and Prevention (CDC) reported that approximately 73,000 cases of foodborne disease

occur annually. In 2013, a total of 19,056 are infected with foodborne pathogen, of which 4200 are hospitalizations, and 80 are deaths in the United States [7]. The foodborne diseases are even high prevalence in many developing countries. Worldwide, there are 600 million foodborne illnesses with 420,000 deaths in 2010, which is estimated by the World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group (FERG). A great proportion of these cases are due to the contamination of raw or undercooked foods and drinking water [4, 6, 8, 9]. Hence, it is urgent to detect foodborne pathogens in order to control foodborne pathogen spread and reduce foodborne disease occurrence.

Currently, culture-based bacterial isolation and identification are the “gold standard” methods for laboratory detection of foodborne pathogens [10]. However, they suffer from time consumption, which requires 2–3 days for initial culture and enrichment, and more than 1 week for confirming the target pathogenic bacteria [11, 12]. Moreover, it requires expensive instruments and professional technicians and remains problematic due to the lack of phenotypic characteristics

Method	Advantages	Disadvantages	Sensitivity	Ref.
Real-time PCR ^a	<ul style="list-style-type: none"> — Amplification can be monitored at real time — Confirmation of specific amplification by melting curve — Accurate quantification 	<ul style="list-style-type: none"> — Difficulty in multiplex assay — Need skilled person and support — False-positive results 	10 CFU/mL	[25]
Multiplex PCR ^a	<ul style="list-style-type: none"> — Highly efficient (detection of several pathogens at a time) — Systematic (suitable for detection of groups of pathogens) 	<ul style="list-style-type: none"> — Difficulty in distinguishing live and dead cells — Requires post-PCR processing of products (electrophoresis) — Need skilled person and support — Costs more than culture-based methods and ELISA 	1 CFU/mL	[26]
Antibody-based method (ELISA ^b and LFIA ^c)	<ul style="list-style-type: none"> — More rapid than culture-based methods (1–2 h vs. 5–7 d) — Can be automated to reduce assay time and manual labor input — Able to handle large numbers of samples — Convenient and suitable for the on-site testing 	<ul style="list-style-type: none"> — Difficulty to differentiate damaged or stressed cells — Need for pre-enrichment — High cross-reactivity with close antigens in bacteria 	60 CFU/mL	[35]
Aptamer-based method (optical and electrochemical methods)	<ul style="list-style-type: none"> — Inexpensive, stable, and can be chemically synthesized than antibody — Time-saving (2 h vs. 5–7 d of culture-based methods) — Automated to reduce manual labor input — High throughput — Multiplex assays 	<ul style="list-style-type: none"> — High false positive — Difficulty in detecting damaged or stressed cells — Need for pre-enrichment — Possibility of cross contamination 	1.5 CFU/mL	[78]

^a PCR, polymerase chain reaction; ^b ELISA, enzyme-linked immunosorbent assay; ^c LFIA, lateral flow immunoassays.

Table 1. Advantages and disadvantages of detection methods.

to distinguish between generic pathogens, which may largely restrict its application. It is evident that culture and colony-counting methods are inadequate for rapid detection of foodborne pathogens, especially for reduce foodborne disease occurrence. The frequent outbreak of foodborne diseases and the economic and social implications indicate that analytical methodologies that can rapidly detect and identify pathogens are urgently needed. As such, many researchers devote themselves to developing more advanced detection methods that can identify pathogens accurately and rapidly in a timely manner in the food industry [13–20].

In this chapter, we summarize the recent trends, developments, advantages, and disadvantages (listed in **Table 1**) about rapid detection of pathogens based on nucleic acid, antibodies, and aptamers and then give a perspective on the future directions of rapid analysis of pathogens.

2. Methodologies for pathogen detection

2.1. Nucleic acid-based assays

Culture- and colony-based methods are the standard methods for the detection of pathogens. They rely on the ability of microorganisms to multiply to visible colonies [21]. The major drawbacks of these microbiological methods are their labor intensiveness and time consumption as it usually takes 2–3 days for initial results and up to 7–10 days for confirmation. In comparison, nucleic acid-based assays can greatly shorten the testing time.

2.1.1. Real-time PCR

Real-time PCR technology is a reliable method in identification and quantitative detection of bacteria due to its accuracy, rapidity, specificity, and low detection limit. In addition, it is a promising alternative approach to estimating the number of bacteria [22, 23]. For example, Gyawali et al. [22] presented a specific and sensitive real-time PCR method to detect *Ancylostoma caninum* ova in wastewater matrices. This method exhibited high sensitivity with the ability to detect *Ancylostoma caninum* DNA up to dilution of 10^{-4} (equivalent to 500 fg) consistently. Moreover, the precise copy number of a specific nucleic acid sequence can be quantified with the real-time PCR technique based on a calibration curve created with known concentrations of DNA [24, 25]. Gokduman et al. [25] established a recombinant plasmid-based quantitative real-time PCR assay for *Salmonella enterica* serotypes with the detection limit of 10 CFU/mL. Obviously, the real-time quantitative PCR has already been a promising quantitative method for the quantitative detection of bacteria, due to its lower cost than that of culture-based method.

2.1.2. Multiplex PCR

Multiplex PCR, also known as multiple primer PCR, which is a PCR reaction system with two or more primers, can amplify a plurality of nucleic acid fragments in a system. Compared to other methods, multiplex PCR is very useful as it allows the simultaneous detection of several

pathogenic bacteria by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted [26]. Methods for multiplexing PCR have considerably improved over the last years, thereby decreasing genotyping costs and increasing throughput. Examples of multiplex PCR technique for the simultaneous detection pathogens include multiplex PCR assay for rapid and simultaneous detection of *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus* [27]; simultaneous detection of bacteria of the genus *Listeria*, *Listeria monocytogenes*, and major serotypes and epidemic clones of *Listeria monocytogenes* [28]; and simultaneous detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* [29]. Multiplex real-time PCR-based assay can rapidly detect 25 clinically important pathogens directly from whole blood in <6 h [30].

2.2. Antibody-based assays

Antibodies are a unique natural family of immune system-related glycoproteins known as immunoglobulins, produced by differentiated B cells in response to the attendant of an immunogen during an immune response. Because of the specific interactions and the extremely high equilibrium association constants ($10^{10}/\text{M}$ and greater) attainable between an antibody and its corresponding antigen, antibodies are employed as an excellent biorecognition element for the highly sensitive and selective immunoassays [31]. Their utilization in biosensors brings new tools for analysis in the biochemical, clinical, and environmental fields. Without exception, antibody-based assays such as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), and so on are very popular for the detection of pathogens.

2.2.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA-based approaches are the most prevalent antibody-based assay for pathogen detection [32]. Compared with the culture-based methods, this immunological approach has been used to detect pathogens in poultry production (poultry feed, feces, litter, carcass rinsing, and water samples) and has provided a better sensitivity and shorter time frame [33]. Recently, improvements by combination with other advanced nanomaterials such as novel enzyme-based signal probes have been made in the basic ELISA method for pathogen detection. For example, by using silica nanoparticles (NPs) carrying poly(acrylic acid) brushes as a “catalase (CAT) container” to increase enzyme loading, Chen et al. [34] presented an improved plasmonic ELISA (pELISA) method for detection of *Listeria monocytogenes* at ultralow concentrations with the sandwich format. The limit of detection (LOD) obtained by this method (80 CFU/mL) was two and five orders of magnitude lower than that of conventional CAT-based pELISA and horseradish peroxidase (HRP)-based conventional ELISA, respectively. To further simplify the preparation of enzyme-labeled antibody, Lin’s group innovatively prepared an all-in-one organic-inorganic nanoflower, which integrated biorecognition unit (concanavalin A or antibody), signal amplification unit (glucose oxidase or HRP), and carrier unit within a one-pot reaction. And then, it was used for a portable sensitive ELISA detection of *Escherichia coli* O157:H7. Under the optimal conditions, the detection sensitivity can reach as low as 10 CFU/mL for the case of concanavalin A-glucose oxidase [17] and 60 CFU/mL for the case of antibody-HRP [35].

2.2.2. Lateral flow immunoassay (LFIA)

LFIA-based methods are a form of immunoassay, which emerge for the first time at the end of the 1960s and consist of a chromatographic system and immunochemical reaction [36–38]. The principle of LFIA is based on antibody–antigen specific interaction. After the sample is applied to the sample pad, it migrates along the test strip *via* capillary action, and a signal response is obtained about 5–10 min later [39, 40]. Due to its simplicity, rapidity, low cost, portability, and facile interpretation without external reagent or external instrumentation, LFIA has held great potential for foodborne pathogen detection [15, 16, 41]. In addition, the LFIA can realize visual detection and quantitative detection by employing different labels, such as colloid gold, fluorescent materials, and magnetic beads [40, 42, 43]. Descriptions on some of the labels that are applied for pathogenic bacteria detection are presented in the following sections.

2.2.2.1. Colloid gold as label

Colloid gold is the most widely used label of LFIA due to its intense color and direct visualization [44], and it has been widely used for the detection of foodborne pathogens [45–48]. Jung et al. [45] used a colloid gold-based LFIA to detect *Escherichia coli* O157:H7 in enriched samples, and the LOD was 1.8×10^5 CFU/mL without enrichment and 1.8 CFU/mL after enrichment. Preechakasedkit et al. [49] also developed a colloid gold immunochromatographic strip for the detection of *Salmonella typhimurium* with a minimum detection limit of 1.14×10^5 CFU/mL. Park et al. [15] presented a detection method of *Escherichia coli* O157:H7 and *Salmonella typhimurium* with a pressed paper-based dipstick by employing colloid gold as labels. The detection limit of *Escherichia coli* O157:H7 was around 10^5 CFU/mL, while that of *Salmonella typhimurium* was around 10^6 CFU/mL. In the case of the work of Song et al. [41], *Shigella boydii*, and *Escherichia coli* O157:H7 can be detected simultaneously in bread, milk, and jelly samples using colloid gold-based LFIA, and the detection limit of 10^6 CFU/mL for both *Shigella boydii* and *Escherichia coli* O157:H7 was achieved.

2.2.2.2. Quantum dots as label

As the low sensitivity of colloid gold, fluorescent materials have gained more and more interest due to their higher sensitivity than colloid gold in the field of lateral flow assay [50]. Furthermore, the fluorescent materials enable lateral flow assay to detect the target quantitatively. Compared with colloid gold, which can only provide qualitative or semiquantitative results, quantitative detection can offer more information [42, 51, 52]. In particular, quantum dots show unique fluorescence properties, such as high and stable fluorescence signal [53–55]. During the last decade, quantum dot-based lateral flow assays have been applied to the detection of foodborne pathogen [56–58]. Bruno [56] utilized quantum dot-conjugated antibody as the signal reporter of the lateral flow assay to detect *Escherichia coli* O157:H7. With the assay, the detection limit of *Escherichia coli* O157:H7 is calculated to be 600 cells per test, while that of colloid gold-based lateral flow assay is 6000 cells per test, indicating the higher sensitivity of quantum dots than colloid gold as labels of lateral flow assay. Chen et al. [58] also developed a competitive format lateral flow assay with quantum dots for the detection of *Staphylococcus aureus* in food. The detection limit is 3 CFU/mL.

2.2.2.3. Magnetic beads as label

Magnetic beads are another type of label, which can realize quantitative detection of targets by measuring the magnetic signal [40, 57, 59]. Due to the fact that they are strongly colored and can enrich and separate targets from complex matrix, magnetic beads are new attractive materials to construct a lateral flow assay, which will probably replace traditional labels. Especially, magnetic beads can simultaneously provide visual signal and magnetic signal. Several researches have recently focused on the use of magnetic bead-based lateral flow assay to detect pathogenic bacteria [60–62]. Wang et al. [60] employed antibody-coated magnetic beads with the diameter of 300 nm as signal reporter of lateral flow assay for *Bacillus anthracis* spore detection. A detection limit of 6×10^4 spores/g of milk powder, 2×10^5 spores/g of starch, and 5×10^5 spores/g of baking soda was obtained, respectively. Suaifan et al. [63] described a magnetic bead-based lateral flow assay, which can specifically and simultaneously detect *Escherichia coli* O157:H7 proteases in complex food matrices. The limits of detection were 12 CFU/mL in broth and 30–300 CFU/mL in food matrices. Xia et al. [64] developed a gold magnetic bifunctional nanobead-based lateral flow assay for the detection of *Salmonella choleraesuis*. Results indicated that the assay was specific and rapid with the detection limit of 5×10^5 CFU/mL, which was much more sensitive than that of colloid gold-based LFIA (5×10^6 CFU/mL), suggesting that magnetic beads were indeed superior to colloid gold.

2.3. Aptamer-based assays

Besides antibodies, other biomolecules have been investigated to selectively capture and enrich pathogens from cultures, among which aptamer is the most prevalent one [65]. Aptamers, as short single-stranded nucleic acids (DNA or RNA), can bind with high affinity and specificity to a wide range of target molecules, such as ions, small organic molecules, and proteins [66–68]. The affinities of aptamers for their targets are comparable to, or even higher than most monoclonal antibodies. More importantly, compared with antibodies, they also exhibit a number of advantages. First of all, aptamers can be routinely produced by chemical synthesis, avoiding the use of animals required for antibody production. Furthermore, they are generally more chemically stable, and their binding properties are easier to manipulate. To this end, a number of aptasensors based on optics and electrochemistry have been recently reported for pathogenic microorganism typing and detection.

2.3.1. Optical strategies

Surface-enhanced Raman scattering (SERS) possesses several attractive properties, such as ultrahigh sensitivity, high speed, comparatively low cost, and multiplexing ability and portability [69–71], which enable SERS to be widely used for sensitive detection of chemical and biological agents [72, 73]. Since Holt and Cotton first reported the SERS spectrum of bacteria, the identification and detection of microorganism by SERS have attracted high interest recently due to the spectroscopic fingerprint and nondestructive data acquisition in aqueous environment [74]. To date, there have been many SERS biosensors developed, especially based on a “magnetic separation” approach, which focus on bacterial pathogen detection. Wang et al. [75] reported a magnetically assisted SERS biosensor for single-cell detection of *Staphylococcus aureus* on the basis of aptamer recognition. The biosensor consists of two basic elements

including a SERS substrate (Ag-coated magnetic nanoparticles) and a novel SERS tag (Au nanorod-5,5-dithiobis-(2-nitrobenzoic acid) (AuNR-DTNB)@Ag-DTNB core-shell plasmonic NPs or DTNB-labeled inside-and-outside plasmonic NPs (DioPNPs)). Based on these, the LOD of 10 cells/mL can be achieved for *Staphylococcus aureus* detection. Similarly, through combined gold NPs (GNPs) modified with Raman molecules and Fe₃O₄ magnetic GNPs immobilized with aptamer, Zhang et al. [76] successfully fabricated GNP-enhanced SERS aptasensor for the simultaneous detection of *Salmonella typhimurium* and *Staphylococcus aureus*. In comparison with these label-based SERS methods, label-free methods do not require a secondary label dye and can directly obtain the intrinsic fingerprint of bacteria, which relies on the mutual interaction of bacteria cell with the SERS substrate [77]. With this regard, a lot of label-free methods have been developed for the detection of pathogens. For example, Gao's group [78] successfully achieved intuitive label-free SERS detection of bacteria using aptamer-based in situ Ag NP synthesis. The biosensor as prepared can recognize bacteria quickly and directly by SERS with the formation of well-defined bacteria-aptamer@Ag NPs. The detection limit is down to 1.5 CFU/mL.

As another typical spectroscopic method, fluorescence resonance energy transfer (FRET, a homogeneous signal transduction technique), has been gradually employed for the determination of pathogenic bacteria. Yu et al. [79] presented a universal and facile one-step strategy for sensitive and selective detection of pathogenic bacteria using a dual-molecular affinity-based FRET platform based on the recognition of bacterial cell walls by antibiotic and aptamer molecules, respectively. Within 30 min, the FRET signal shows a linear variation with the concentration of *Staphylococcus aureus* in the range from 20 to 10⁸ CFU/mL with a detection limit of 10 CFU/mL. Moreover, Duan's group [80] further achieved simultaneous detection of *Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *Salmonella typhimurium* through using multicolor dyes as donors and carbon NPs as a sole acceptor in FRET.

2.3.2. Electrochemical strategies

Compared with optical-based biosensors, electrochemical methods, in general, show the potential for construction of fast, simple, low-cost, sensitive, and high-throughput biosensors that can be miniaturized [81–84]. To date, electrochemical aptasensors are widely used for identification and quantification of pathogens. For example, Labib et al. [85] developed an impedimetric sensor *via* assembling their selected highly specific DNA aptamers onto a gold NP-modified screen-printed carbon electrode for the highly sensitive detection of live *Salmonella typhimurium*. This aptasensor is very simple and highly selective. It can successfully detect *Salmonella typhimurium* down to 600 CFU/mL (equivalent to 18 live cells in 30 µL of assay volume). Moreover, to further improve the sensitivity, Abbaspour et al. [86] innovatively combined the magnetic beads' fast separation with the Ag NPs' signal amplification. They successfully fabricated an electrochemical dual-aptamer-based sandwich detection method for *Staphylococcus aureus*. The aptasensor as prepared shows an extended dynamic range from 10 to 1 × 10⁶ CFU/mL with a low detection limit of 1.0 CFU/mL (*S/N* = 3). Despite much progress has been made, these methods always require probe labeling and aptamer immobilization, which may affect the binding affinities between bacteria and their aptamers. With this respect, Ding's group [87] constructed a label-free potentiometric aptasensor for rapid, sensitive, and selective detection of *Listeria monocytogenes*. In this strategy, the target-binding event prevents

the aptamer from electrostatically interacting with protamine, which can be sensitively detected using a polycation-sensitive membrane electrode.

2.4. Conclusion

Culture-based foodborne pathogen detection methods, although sensitive enough, are often too time-consuming to reduce foodborne disease occurrence. Therefore, a large number of innovative methods have been developed to overcome this performance limitation. These rapid detection methods can be classified into nucleic acid-based methods, antibody-based methods, and aptamer-based methods. All these rapid methods for foodborne pathogen detection are superior to culture-based methods. However, some of them still require improvement in sensitivity, selectivity, simplicity, or accuracy to be of any practical use. Nucleic acid-based methods, as a replacement method for culture-based methods, have high sensitivity and require a shorter time than conventional culture-based techniques for foodborne pathogen detection. Most of them still require highly trained personnel and expensive instruments, which limit their use in a practical environment. The development of antibody-based methods helped improve the time required to yield results. The specific binding of antibody to its antigen results in its high specificity and sensitivity of antibody-based methods, and they work well in food matrices without being interfered by other DNAs, proteins, or nontarget cells. Aptamer-based methods are similar to antibody-based methods, which also exhibit high sensitivity and selectivity. However, they still need to be improved for food matrix detection. Increasing detection accuracy and decreasing detection time are the eternal themes in rapid detection. In the future, new nanomaterials and rational biosensing strategies would be developed to approach the goal.

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