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Review article

RAPID METHODS FOR DETECTION OF FOODBORNE PATHOGENS AND THEIR TOXINS - A REVIEW OF CURRENT TRENDS AND FUTURE PERSPECTIVES

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ABSTRACT:

Background: Food safety is of utmost importance to sustaining life and promoting good health and is a main problem in any country, regardless of economic and social development. Unsafe food containing harmful pathogens and their toxins cause hundreds of diseases, particularly affecting infants, young children, the elderly and sick people. Therefore, the rapid detection of foodborne pathogens is of great significance for public health. The aim of this review was to provide comprehensive information regarding methods for the detection of foodborne pathogens as well as genus *Fusarium* and their toxins, from fundamental to state-of-the-art, presenting their advantages and limitations in order to update current knowledge.

Review Results: The traditional culture-based methods for the detection of pathogens in food are usually laborious, limited by complex sample preparation procedures, time-consuming, slow to provide results, and require well-trained professional staff. However, compared to these methods, the new ones - immunological methods, nucleic acid-based assays and biosensor-based methods are rapid, accurate, highly sensitive and specific, and easy to use. Precisely providing accurate real-time results would help limit foodborne disease outbreaks, ensure compliance with legislation setting maximum levels of pathogens in certain food categories, detect and improve the import of food contaminated with dangerous levels of pathogenic microorganisms, and ensure public health safety.

Conclusion: The development of rapid and automated methods that detect real-time, small numbers of viable microbial cells within a given volume of food is vital in the prevention, transmission and treatment of foodborne diseases. Such methods would also offer a great commercial advantage in the food industry and related fields.

Keywords: foodborne pathogens, Genus *Fusarium*, bacterial toxins, mycotoxins, rapid methods,

BACKGROUND

Food safety is of utmost importance to sustaining life and promoting good health and is a main problem in any country, regardless of economic and social development. Foodborne diseases have become a major public health concern worldwide due to the significantly increased incidence of cases over the last 20 years. A wide variety of microorganisms can cause foodborne illness - bacteria, viruses, fungi and parasites. Pathogenic bacteria and viruses, for example, Norovirus, Hepatitis E virus, Salmonella species, Campylobacter species, Vibrio species, Listeria monocytogenes, Staphylococcus aureus and pathogenic Escherichia coli, are responsible for the highest number of foodborne illness outbreaks worldwide [1]. A number of microorganisms are also able to produce toxins causing foodborne diseases. These are mainly *S. aureus, Vibrio cholerae, Clostridium* species, *Bacillus cereus*, some of the *E. coli* species, and genus *Fusarium* [2]. Some fungal species produce toxic metabolites (mycotoxins) that commonly contaminate staple foods and feed and are an unavoidable problem due to their presence in globally consumed cereals such as rice, maize and wheat [3]. Additionally, these compounds have a huge financial impact, causing money losses to producers, processors, and also food and feed consumers [4]. Mycotoxins are thermally stable and demonstrate high levels of bioaccumulation [5]. Due to their severe effects observed in humans and animals - cytotoxicity, carcinogenicity, mutagenicity, neurotoxicity, hepatotoxicity, immunosuppressive and estrogenic effects, mycotoxins have been more precisely observed in recent decades. Since mycotoxin toxicity in food products can occur at very low concentrations, appropriate detection of their presence requires sensitive and reliable methods for their early detection [6]. Unsafe food containing harmful pathogens and their toxins cause hundreds of diseases, particularly affecting infants, young children, the elderly and sick people. Therefore, the rapid detection of foodborne pathogens is of great significance for public health.

The aim of this review was to provide comprehensive information regarding methods for the detection of

foodborne pathogens as well as genus *Fusarium* and their toxins (including mycotoxins), from fundamental to state-of-the-art, presenting their advantages and limitations in order to update current knowledge.

REVIEW RESULTS

It is essential to analyze food for the presence of pathogens and their toxins to ensure a safe and reliable food supply and to minimize the occurrence of foodborne pathogen diseases among consumers.

1.1. Culture-based methods

Culture-based methods still represent the first choice for many food-testing laboratories. The traditional culture-based methods for the detection of pathogens in food are usually laborious, limited by complex sample preparation procedures, time-consuming and slow to provide results, and require well-trained professional staff. They are based on culturing the microorganisms on agar plates, followed by standard biochemical identifications and serological confirmation of the results [7], which usually require 2 to 3 days for preliminary identification and more than a week for confirmation of the species of the pathogens [8]. Furthermore, false negative results may occur due to viable but uncultivable pathogens, which means these methods may be limited by their low sensitivity [9]. Cultural approaches are available in both qualitative and quantitative formats [10]. Qualitative methods are used when only the presence or absence of a pathogen in a food sample must be determined. Presumptive colonies are grown on selective media from a known amount of food. Pure cultures are raised, and the pathogen is identified using various biochemical or serological tests [11]. The quantitative methods are based on serial dilution procedures and are used to count the microorganisms present in the food sample by culture method. Although these methods are reasonably affordable, sensitive, and still considered gold standards, their main disadvantage is their long analysis time (7 and 10 days) and labor-intensive nature [12].

Compared to traditional culture-based methods, the new ones - immunological methods, nucleic acid-based assays and biosensor-based methods are rapid, accurate, highly sensitive, specific, and easy to use. Regarding mycotoxins, including those produced by the genus *Fusarium*, rapid mycotoxin analysis shares many common advantages, including speed, low cost, simplicity, and ease of use [13]. The portability and possibility of multitoxins detection are also key aspects of the rapid methods. Mobility is also important given the growing demand for on-site testing, which can be carried out on-site in the food production process [14]. Results are obtained relatively quickly, as samples do not need to be sent to and analyzed in laboratories, preventing the food production process from slowing. The detection of multiple toxins eliminates the need to perform multiple tests for a single toxin per batch of sample [15]. The main limitations of these methods are matrix interference, antibody cross-reactivity and the need for matrix validation [16].

1.2. Immunological methods

The detection of foodborne pathogens by immunological assays is widely used. These methods are based on antibody-antigen interactions, where a specific antibody will bind to its specific antigen. The binding strength of a particular antibody to its antigen determines the sensitivity and specificity of immunological methods. A variety of antibodies has been employed in different assay types for the detection of foodborne pathogens and microbial toxins [8]. Because monoclonal antibodies provide an unlimited supply of a single antibody, they are often more useful than polyclonal ones in specific detection of a molecule. The detection of microbial contamination by immunological methods using monoclonal antibodies is specific, sensitive, reproducible and reliable, and numerous commercial immunological assays exist to detect a wide variety of microbes and their products [17]. Immunoassays are available in various configurations, but Enzyme-linked immunosorbent assay (ELISA) and Lateral flow immunoassay (LFIA) are among the immunological methods recently most often used for the detection of foodborne pathogens.

ELISA is very accurate and sensitive and one of the most widely used and rapid immunological assay for detecting foodborne pathogens [18]. Most commercially available immunological kits use sandwich assays that are very sensitive and robust and involve two antibodies, which bind to the tested toxin. ELISA is also the most commonly used immunological method for foodborne toxins detection - staphylococcal enterotoxins, botulinum toxins, *E. coli* enterotoxins [2, 8]. Recently, high-throughput and automated systems have been available for the detection of foodborne pathogens, which are more sensitive, can complete an assay for up to 2 hours, and the result will is automatically analyzed [19]. ELISA is also widely used in the detection of mycotoxins in various types of food, such as wheat, corn, soybeans, spearmint [14]. Regarding the detection of mycotoxins produced by the genus *Fusarium*, the method has been shown to be a rapid and simple technique for screening and analysis of mycotoxins on-site [20]. ELISA is simple in design, allows simultaneous testing of multiple samples, and its detection is precise [21]. It is a high-throughput assay with small sample volume requirements and fewer clean-up procedures compared to chromatographic methods [16]. However, this technique has some drawbacks – cross-reactivity with related mycotoxins, matrix interference problems, which may lead to underestimation or overestimation of mycotoxins concentrations in tested specimens, and possible false-positive/negative results [16].

Due to the disadvantages of the enzyme-linked immunosorbent assay, such as enrichment requiring at least 16-24 hours and the requirement of special equipment and trained staff, rapid, cheap, and still reliable methods that can be conducted and interpreted at the site of the contamination are needed [8]. More and more on-site immunological techniques based on lateral flow immunoassays, such as dipstick, immunochromatography,

and immunofiltration, are gaining attention in the area of pathogen and disease detection in the food industry and medicine [22]. Lateral flow strip assay has also developed as the widely and commercially consumed immunoassays for fast analysis of mycotoxins, including those produced by the genus *Fusarium* (in rice, maize, cereals) [16]. LFIAs are very fast assays and suitable for large-scale on-site screening. Their advantages include simple step procedures, manageable setup, rapidness of analysis, high sensitivity, low cost and less interference, and a fast detection of mycotoxins for mass screening. Nevertheless, they are designed for individual tests rather than high-throughput screening [23].

1.3. Nucleic-acid based methods

These methods rely on the detection of certain gene sequences in the target pathogen's genotype in order to detect specific genes characteristic of a certain genus, species, or even strain of the microbe and to detect toxins and toxin-related genes as well. One of the most important advantages of nucleic-acid-based food pathogen detection assays is the high level of specificity. There are many other types of DNA-based assays, but probes and nucleic acid amplification techniques are the most common and have been commercially developed for the rapid detection of foodborne pathogens. also

Probe-based tests are easy to administer. They are often used in the food industry. Nucleic acid samples are immobilized to inorganic substrates in these experiments so that they can be easily manipulated without being damaged or lost [11]. The DNA probes involve using a labeled, known DNA probe to hybridize the DNA sequence of an unknown microbial pathogen.

The polymerase chain reaction (PCR) method is currently widely used. It is an *in vitro* enzymatic multiplication (amplification) of a selected section of a DNA sequence, restricted by known short, specially synthesized oligonucleotides (primers), complementary to the sections limiting the amplified sequence. DNA synthesis in each new cycle of the PCR reaction starts precisely from the primers. The method provides various advantages over culture and other traditional procedures, including sensitivity, specificity, accuracy, rapidity, and the ability to detect small amounts of target nucleic acid in a sample [11]. PCR is available in various formats to detect foodborne pathogens, [24]. Over the last two decades, many different advances in the original PCR protocol have been described, and many PCR assays for the detection and validation of foodborne bacteria and viruses in food have been developed and applied in food samples [25]. PCR reactions could be affected by the presence of inhibitory compounds in food and selective microbiological media [7]. A problem with routine PCR use in food testing laboratories is the complicated procedures and the very clean environment needed to perform the tests [7]. In addition, PCR can not distinguish between live and dead cells, therefore providing more false negative results [26]. Certain PCR methods described are Ligase chain reaction (LCR) PCR, Nucleic acid sequence-based amplification (NASBA), Strand displacement amplification, Nested polymerase chain reaction, Real-time PCR, Multiplex PCR, Low-stringency single-specific-primer PCR, Restriction fragment length polymorphism, Amplified fragment length polymorphism (AFLP), Random amplified polymorphic DNA technique, Loop-mediated isothermal amplification (LAMP), Repetitive extragenic palindromic PCR, and DNase treated DNA (DTD) PCR.

LCR is a new technology that uses DNA amplification to detect the nucleic acid sequence of bacteria. The assay is commonly used for the specific recognition of *L. monocytogenes* but has one drawback in terms of food pathogen detection: it can detect DNA from dead species [27]. NASBA is an isothermal amplification reaction (which eliminates the need for expensive thermal cyclers) for the detection of RNA or DNA and were originally developed to identify viruses, but have also been used in food testing to detect bacterial pathogens in various foods [28]. The innovative nested PCR is a more sensitive and specific method than traditional PCR because it reduces non-specific binding in the products caused by the amplification of unexpected primer binding sites. It is used to detect a variety of foodborne pathogens, including *Vibrio parahaemolyticus, E. coli O157:H7, Salmonella, Listeria monocytogenes*, and *Staphylococcus aureus*, as well as to identify *Fusarium culmorum* contamination in cereal samples [29]. Real-time PCR is a technique for the amplification and quantification of a specific DNA molecule in real-time. It has the ability to detect as well as quantify - an ideal number of copies or a relative amount [11]. The real-time monitoring of the process means no need for post-amplification treatment of the samples, such as gel electrophoresis, which reduce the time of analysis [8]. Nowadays, this method is increasingly applied in food microbiology, and a number of commercial kits based on real-time PCR technology for the identification and characterization of foodborne diseases are available [30]. The multiplex PCR method gives more information because many genes are targeted simultaneously. The other advantages of this assay are that multiple sets of primers are included in one reaction tube, allowing more than one target sequence to be amplified in one reaction system; fewer reagents and enzyme are used; sample preparation and results are performed in a short time, as pathogens are assessed individually. The only disadvantage is that amplified fragments of the same length cannot be distinguished, and a smaller amount of amplified product may not appear on an agarose gel. However, this drawback can be avoided by developing longer and higher melting temperature primers than those used in conventional PCR [27, 28]. LAMP for detecting target genes in food samples is a rapid, easily operating, accurate and cost-effective method with high sensitivity and exceeding specificity [31]. Under isothermal conditions, LAMP is a one-step reaction that amplifies a target DNA sequence. It uses a strand-displacement DNA polymerase, and during the reaction, six different sites in the target DNA are recognized [4]. LAMP products can be visually counted rather than by electrophoresis, which simplifies detection. The

method is used to detect a variety of pathogens that cause foodborne illness - *Legionella, Salmonella, Campylobacter, Listeria, S. aureus,* and verotoxin-producing *E. coli* [32]. DTD PCR avoids the problems of the rapid detection of foodborne pathogens the PCR-based techniques, and the method itself has been shown to be sufficiently specific. To escape the problem of false positive results obtained by amplifying DNA from dead cells, DNase I enzyme treatment followed by PCR is applied. The technique can be used for the identification of viable cells of pathogens *Listeria monocytogenes, E. coli O157:H7, Salmonella enterica* and *Vibrio parahaemolyticus* [33].

1.4. Biosensors

Biosensors are the most promising new tool to overcome the slow (1-2 days) determination of pathogenic microbes by standard procedures in foods such as milk, cheese, meat (pork, chicken), raw vegetables and fruits [4]. Biosensors are analytical devices that consist of a bioreceptor responsible for recognizing the target analyte and a transducer that converts the biological interactions into a measurable electrical signal [8]. They are easy to operate and require no pre-enrichment of the sample, in contrast to methods based on nucleic acids and immunological methods [34]. One of the greatest advantages of biosensors is that very rapid or real-time detection, portability, and detection of multiple pathogens are possible for both field and laboratory analyses, which would provide almost immediate interactive information about food materials to consumers, and they could take corrective measures before consumption or further contamination [35]. Biosensors have been developed and applied for microbial analysis of foodborne pathogens, including *Escherichia coli O157:H7, S. aureus, Salmonella* species and *L. monocytogenes*, as well as various microbial toxins such as staphylococcal enterotoxins and mycotoxins produced by genus *Fusarium* in peanuts, corn, wheat [36].

Various types of biosensors exist – Immunosensors, Enzyme-based biosensors, Electrochemical biosensors, Amperometric biosensors, Potentiometric biosensors, Magnetoelastic biosensors. Immunosensors are based on specific antibody-antigen interactions. They have been developed to detect *Salmonella* species in eggs, chicken meat and milk and *S. aureus* enterotoxin. The results of the immunosensors are read *via* digital signals and, therefore, do not depend much on personal factors such as bias, fatigue, level of training or visual impairment [8]. The enzyme-based immunoassay reagents are non-hazardous, stable and sensitive [11]. Storage stability, sensitivity, high selectivity, short reaction time, and high reproducibility are all advantages of enzyme immobilization. By labeling an antibody with enzymes, pathogenic bacteria such as *L. monocytogenes, E. coli,* and *Campylobacter jejuni* can be detected [11]. Electrochemical biosensors measure electrochemical responses and provides label-free, online, high-throughput bacterial detection devices [37]. Experiments with electrochemical biosensors are extremely fast and can sometimes be completed in just over an hour [38]. Such a biosensor exists for the detection of foodborne pathogens such as *S. aureus, B. cereus, E. coli*.

1.5. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS allows highly sensitive and rapid analysis of femtomole or even atomole levels of organic and/or macromolecular biomolecules without their destruction [5]. It offers the possibility of rapid and sensitive detection of bacteria, viruses and even mycotoxins [39]. The method is independent of the culture conditions, culture formulations, cultivation time, and quantity of inoculum required for identification [40]. Other advantages over conventional techniques are its ability to analyze samples within minutes by using only a small sample, accuracy, less expensive cost than molecular and immunological-based detection methods, and the fact that trained laboratory staff is not required [41]. MALDI-TOF MS produces singly charged ions; thus, interpretation of data is easy and prior separation by chromatography is not required [40]. Consequently, the high throughput and speed associated with complete automation has made the method an obvious choice for proteomics work on large-scale [40]. MALDI-TOF MS has been successfully applied in food microbiology for various purposes: the identification of bacteria isolated from dairy cows' milk, the identification of pathogenic bacteria contaminating powdered infant formula food, the characterization of biogenic amine-producing bacteria responsible for food poisoning, and the identification of causative agents of seafood-borne bacterial gastroenteritis [42]. Various researchers have reported that MALDI-TOF MS was a reliable and time-saving approach for the identification of various human fungal pathogens and for rapid and sensitive detection of numerous mycotoxins [39].

1.6. Ultrafast liquid chromatography connected with tandem mass spectrometry (UFLC-MS/MS) for rapid detection of mycotoxins, including produced by the genus *Fusarium*

UFLC is a process of separating analyte components in a liquid mixture at ultra-high pressure. The liquid sample is injected into the solvent stream and flows through a column containing greatly reduced particle size. A process of differential migration separates sample components from each other as they pass through the column. UFLC has achieved significant improvements in column technology, dramatically increasing speed, resolution, and separation performance independent of pressure, as in liquid chromatography, thus significantly reducing the analysis time [43]. The method provides good sensitivity and multiple opportunities for mycotoxins detection, including a wide range of toxins produced by the genus *Fusarium*, simultaneous analysis of mycotoxins [16]. The very expensive cost and the need for specialists' expertise can be mentioned as disadvantages of UFLC [16].

CONCLUSION

Traditional foodborne pathogen detection methods that rely on culture methods are effective and the gold standard but less sensitive and specific, time-consuming and labor-intensive. Therefore, to overcome the limitations, new methods are required. The development of rapid and automated methods that can detect real-time, small numbers of viable microbial cells within a given volume of food is vital in the prevention, transmission and treatment of foodborne diseases. Such methods would also offer a great commercial advantage in the food industry and related fields. Precisely providing accurate real-time results would help not only limit foodborne disease outbreaks but also ensure compliance with legislation setting maximum levels of pathogens in certain food categories, detect and improve the import of food contaminated with dangerous levels of pathogenic microorganisms and their toxins, and ensure public health safety.

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