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Review

Accelerated Detection of Foodborne Pathogens and Toxins: Techniques and Applications – A Review

Rozalina Yordanova[1], Daniela Stoeva[2], Deyana Gencheva[3], Albena Andonova[4], Georgi Beev[2*]

[1]*Medical College, Trakia University, Stara Zagora, Bulgaria*
[2]*Department of Biological Sciences, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria*
[3]*Department Fundamental Sciences in Animal Husbandry, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria*
[4]*Department of Health Care, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

Abstract

Pathogens associated with food and their corresponding toxins, including mycotoxins, pose a significant threat to public health, leading to various health issues worldwide, particularly affecting infants, young children, and the elderly. The rise in foodborne illnesses highlights the crucial importance of food safety in ensuring a reliable food supply and promoting health. Timely detection of foodborne pathogens, toxins, and mycotoxins is essential for reducing the incidence of foodborne diseases. This review aims to provide comprehensive insights into the methodology for rapid detection, detailing the principles, applications, advantages, and limitations to enhance current knowledge. Conventional culture-based methods for foodborne pathogen identification are selective and are hindered by significant time demands, labor intensity, challenges from complex sample preparation, delayed results, and the need for specialized personnel. In contrast, the new emerging detection techniques such as immunological, nucleic acid-based, and biosensor-based methods offer time efficiency, reduced labor, improved accuracy, sensitivity, specificity, and reliability, and are user-friendly. Recently, numerous novel techniques have been developed for the rapid identification of foodborne pathogens, toxins, and mycotoxins, transforming food safety practices. Providing rapid and accurate results is crucial for controlling and managing foodborne disease outbreaks while ensuring food safety. As the global food supply chain becomes more complex, advancing rapid and automated detection methods remains essential for safeguarding food safety and quality, as well as enhancing public health.

Keywords: foodborne pathogens, rapid detection methods, bacterial toxins, mycotoxins

Резюме

Бактериалните патогени и техните токсини в храните представляват значителен риск за общественото здраве, тъй като причиняват широк спектър от заболявания в световен мащаб, които заягат предимно бебета, малки деца и възрастни хора. Честотата на заболяванията с хранителен произход се увеличава през годините, поради което безопасността на храните е от съществено значение за осигуряване снабдяването с безопасна храна и насърчаване на оптимално човешко здраве. Бързият анализ на храните за наличие на пренасяни с храна патогени/токсини е от съществено значение за минимизиране появата на заболявания. Целта на този обзор е да предостави подробна информация за методите за бързо откриване на пренасяни с храни патогени и техните токсини, акцентирайки на принципите и приложението им и на ползите и недостатъците им. Конвенционалните методи, използвани за откриване на патогени в храните са селективни, но също така време отнемащи, трудоемки, ограничени са от сложни техники за подготовка на пробите и изискват добре обучен персонал. В сравнение с тези методи, новите методи – имунологични, базирани на нуклеинови киселини и базирани на биосензори са бързи, точни, спестяващи труд, по-чувствителни и специфични,

*Corresponding author: georgi.beev@trakia-uni.bg
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надеждни и лесни за използване. През последните години са разработени няколко иновативни техники за бързо откриване на пренасяни в храната патогени/токсини, които революционизират областта на безопасността на храните. Бързото и точно предоставяне на резултати е от решаваща роля за ограничаване възникването на огнища от заболявания, пренасяни с храните и за осигуряване безопасността на храните. Тъй като глобалната верига за снабдяване с храни става все по-сложна, разработването на бързи и автоматизирани методи за откриване на хранителни патогении/токсини, ще продължи да бъде приоритет за гарантиране безопасността и качеството на хранителните продукти и подобряване на общественото здраве.

Introduction

The assurance of food safety is essential for the preservation of life and the promotion of optimal public health outcomes. The prevalence of foodborne illnesses has risen significantly over the last two decades, positioning food safety as a critical public health concern across nations, irrespective of their economic and social progress. A substantial proportion of global outbreaks of foodborne illnesses can be attributed to a diverse array of pathogenic microorganisms, including various species of *Salmonella* species, *Campylobacter* species, *Vibrio* species, *Listeria monocytogenes, Staphylococcus aureus,* pathogenic *Escherichia coli, Norovirus,* and *Hepatitis E virus* (https://www.who.int/news-room/fact-sheets/detail/food-safety). Additionally, certain microorganisms, for example, *S. aureus, Vibrio cholerae, Clostridium* species, *Bacillus cereus*, some species of *E. coli*, and the genus *Fusarium*, can synthesize toxins that contribute to foodborne diseases (Fusco *et al.,* 2011). Some fungal species are capable of producing toxic heat-stable metabolites that can accumulate to significant levels (Hleba *et al.,* 2017; Gencheva and Beev, 2021). These compounds, referred to as mycotoxins, frequently contaminate staple foods and animal feed, presenting a formidable challenge due to their prevalence in widely consumed grains such as rice, maize, and wheat (Marroquín-Cardona *et al.,* 2014; Stoeva *et al.,* 2023a). also have significant The economic implications of mycotoxins are substantial, leading to financial losses for producers, processors, and consumers of food and feed products (de Oliveira *et al.,* 2014). In recent decades, mycotoxins have garnered increased attention due to their harmful effects on both humans and animals, which encompass cancer-causing potential, genetic alterations, toxic effects on the nervous system, hepatotoxicity, immunosuppressive properties, and estrogenic effects. Given the potential toxicity of mycotoxins even at minimal concentrations in food products, it is imperative to implement effective and trustworthy techniques for their prompt detection (Kolesarova *et al.,* 2012).

A variety of diseases that particularly affect vulnerable populations such as infants, young children, the elderly, and individuals with compromised immune systems, can arise from consumption of food contaminated with harmful pathogens and their toxins resulting in numerous health complications (Stoeva *et al.,* 2023b). Consequently, initiatives aimed at safeguarding food safety through the prompt identification of foodborne pathogens and toxins are critical for the protection of public health.

The objective of this review is to provide comprehensive information regarding the methodologies employed for the rapid detection of foodborne pathogens and their toxins (including mycotoxins), elucidating their underlying principles, applications, benefits, and drawbacks to enhance the current state of knowledge.

Methods for detection of foodborne pathogens

The incidence of foodborne pathogen infections among consumers can be mitigated, and a secure and reliable food supply can be ensured through the regular examination of food for the presence of pathogens and their associated toxins.

Culture-based methods

These methods are traditional but remain prevalent techniques for the detection and identification of foodborne microorganisms. They rely on the cultivation of microorganisms in selective and differential culture media under controlled environmental conditions to promote their growth and subsequent identification (biochemical and serological). Typically, these methods are characterized by being time-consuming, labor-intensive, limited by complicated sample preparation protocols, and slow to generate results (requiring 2 to 3 days for initial identification and over a week for the pathogens species to be confirmed) (Blodgett, 2010; Mandal *et al.,* 2011; Zhao *et al.,* 2014). Moreover, the occurrence of false negative results attributable to viable but uncultivable pathogens suggests that the limited sensitivity of these methods may present a significant constraint limitation (Li *et al.,*

2014). Cultural approaches can be categorized into qualitative and quantitative (Jasson *et al.,* 2010). Qualitative methods are utilized to determine the presence of a pathogen in a food sample, whereas to enumerate microorganisms in the food sample and are based on serial dilution techniques.

Immunological methods

Immunological assays are widely used in the detection of foodborne pathogens. They rely on interactions between antibodies and antigens. The affinity of this binding significantly affects the sensitivity and specificity of the immunological methods employed. Different types of antibodies are utilized in various assays to identify foodborne pathogens and microbial toxins (Zhao *et al.,* 2014). Monoclonal antibodies, which provide a steady supply of a single type of antibody, are frequently more advantageous compared to polyclonal antibodies for the precise detection of specific molecules. The incorporation of monoclonal antibodies in immunological techniques for microbial contamination detection ensures specificity, sensitivity, reproducibility, and reliability, with numerous commercial assays available for the detection of a broad spectrum of microorganisms and their products (Leonard *et al.,* 2003). Immunoassays are available in multiple forms, with Enzyme-linked immunosorbent assay (ELISA) and Lateral flow immunoassay (LFIA) being the most commonly employed recently for the detection of foodborne pathogens.

ELISA represents one of the most extensively utilized and rapid immunological techniques for detecting and quantifying specific proteins, including both antigens and antibodies, within a sample matrix. Its high specificity, sensitivity, and adaptability render it particularly effective in identifying foodborne pathogens (Vernozy-Rozand *et al.,* 2004). There are different types of ELISA, including direct, indirect, sandwich, and competitive. The predominant immunological tests available commercially employ the highly specific and sensitive sandwich assay format, which effectively encapsulates the antigen between two distinct layers of antibodies (capture and detection antibodies). Unique antibodies are strategically employed to identify specific antigens associated with various foodborne pathogens such as *Salmonella, Listeria, E. coli*, and *Campylobacter*. Furthermore, ELISA is the most frequently employed immunological approach for detecting foodborne toxins, including staphylococcal enterotoxins, botulinum toxins, and *E. coli* enterotoxins (Fusco *et al.,* 2011; Zhao *et al.,* 2014). Another prevalent application of ELISA involves the detection of mycotoxins across different food matrices, including wheat, maize, soybeans, and spearmint (Xu *et al.,* 2010; Janik *et al.,* 2021). Concerning the detection of mycotoxins produced by the genus *Fusarium*, the method has been proven to be a rapid and simple technique for their on-site screening and evaluation (Al-Jaal *et al.,* 2019; Liang *et al.,* 2018). ELISA kits are commercially available and relatively user-friendly, facilitating routine testing. The method can detect low concentrations of pathogens due to the strong binding affinity between antibodies and antigens and provides both quantitative (pathogens concentration) and qualitative (presence/absence) outcomes. It is suitable for screening multiple samples simultaneously, making it efficient for extensive testing (Muldoon *et al.,* 2007). In comparison to chromatographic approaches, ELISA necessitates a small sample volume and fewer purification procedures (Oplatowska-Stachowiak *et al.,* 2018; Singh and Mehta, 2020). Nevertheless, the method exhibits certain limitations, particularly regarding mycotoxin detection - potential for cross-reactivity with non-target antigens, leading to false positive results, challenges related to matrix interference that may result in overestimation or underestimation of mycotoxin levels in analyzed specimens, and additionally, the process may be time-consuming and costly compared to some rapid testing alternatives (Thway and Salimi-Moosavi, 2014; Singh and Mehta, 2020).

Given the limitations associated with the enzyme-linked immunosorbent assay, which necessitates an enrichment period of 16-24 hours and requires specialized equipment alongside trained personnel, there exists a demand for rapid, cost-effective, and reliable methods that can be conducted and interpreted directly at contamination sites (Zhao *et al.,* 2014).

On-site immunological methods employing lateral flow immunoassays (LFIA), including dipstick, immunochromatography, and immunofiltration, are gaining prominence in pathogen and disease detection in the food industry and medicine (Muldoon *et al.,* 2007). The lateral flow strip assay is characterized by its rapidity, high sensitivity, and suitability for large-scale on-site screening, having emerged as a widely utilized and commercially popular immunoassay for rapid analysis of mycotoxins, particularly those generated by the *Fusarium* genus in rice, maize, and cereals (Kolosova *et al.,* 2008; Singh and Mehta, 2020). Nevertheless, it is primarily designed for singular tests rather than high-throughput screening (De Boer and López,

2012; Liu *et al.,* 2020).

Nucleic-acid based methods

Nucleic-acid-based methods serve to identify specific gene sequences within a pathogen's genotype to detect genes unique to a particular genus, species, or strain, as well as genes associated with toxin production. A significant benefit of these diagnostic assays is their remarkable specificity. A variety of DNA-based assays are available, with nucleic acid probes and amplification techniques being the most prevalent. These methodologies have undergone commercial development to enable the expedited identification of foodborne pathogens. Probe-based assays are easy to utilize and frequently employed in the food industry. In these tests, nucleic acid samples are fixed on inorganic substrates, permitting manipulation without degradation or loss (Chowdary Akkina *et al.,* 2023). The application of DNA probes entails the use of a labeled, known DNA probe that hybridizes with the DNA sequence corresponding to an unidentified microbial pathogen.

Numerous enhancements to the original polymerase chain reaction (PCR) technique have been documented, resulting in the development and application of various PCR tests on food samples to identify and confirm foodborne bacteria and viruses (Alvarenga *et al.,* 2012; Priyanka *et al.,* 2016). Among these PCR methods are Real-time PCR, Multiplex PCR, Nested PCR, Ligase chain reaction (LCR) PCR, Loop-mediated isothermal amplification (LAMP), Strand displacement amplification (SDA), Nucleic acid sequence-based amplification (NASBA), Amplified fragment length polymorphism (AFLP), Random amplified polymorphic DNA technique (RAPD), Repetitive extragenic palindromic PCR (REP-PCR), and DNase treated DNA (DTD) PCR.

The polymerase chain reaction and its derivative Real-Time PCR (qPCR) are extensively utilized techniques for the detection of foodborne pathogens and come in various configurations (Cook, 2003). PCR facilitates the in vitro amplification of distinct DNA sequences, enabling the detection of pathogens even in minimal concentrations. qPCR, an advanced version of the conventional PCR, allows real-time quantification of DNA, yielding expeditious results. The methods provide numerous advantages over the traditional approaches, including high sensitivity and specificity, rapidity, quantitative outcomes (in qPCR), and capacity to concurrently detect multiple pathogens (multiplex PCR) (Chowdary Akkina *et al.,* 2023). Furthermore, multiplex PCR assay utilize fewer reagents and enzymes. However, the implementation of routine PCR in food testing laboratories is hindered by complex protocols, the presence of inhibitory substances in food matrices and selective culture media that could compromise the assay, the risk of contamination leading to false positive results, and the necessity for sophisticated equipment and trained personnel (Mandal *et al.,* 2011). Moreover, due to PCR's inability to differentiate between viable and non-viable cells, false-negative results may also occur (Biswas *et al.,* 2008). An additional limitation of multiplex PCR is its inability to distinguish between amplified fragments of equivalent length, as well as the potential inability to visualize small quantities of amplified product on an agarose gel. Nevertheless, this disadvantage can be eliminated by designing primers with longer sequences and elevated melting temperatures compared to those used in traditional PCR (Min and Baeumner, 2002; Kawasaki *et al.,* 2005).

The novel nested PCR represents an advancement of the conventional PCR technique enhancing both sensitivity and specificity (Yan and Surmeier, 1997). It involves two sequential sets of PCR reactions. The initial set is responsible for amplifying the target DNA, while the subsequent set employs a distinct pair of primers to further amplify a subset of the primary product. It has proven effective in detecting various foodborne pathogens, including *E. coli O157:H7, Salmonella, Vibrio parahaemolyticus, L. monocytogenes*, and *S. aureus*, as well as in identifying *Fusarium culmorum* contamination in cereal samples (Abravaya *et al.,* 2003). The higher sensitivity of the method is attributed to its ability to amplify low quantities of DNA, making it possible to detect low-abundance targets. The increased specificity results from minimized non-specific amplification, as the second set of primers is designed to target a specific subset of the initial PCR product.

LCR represents an innovative DNA amplification technique similar to PCR. However, it employs a DNA ligase enzyme to connect contiguous nucleotides on a DNA strand instead of relying on polymerase-mediated synthesis for amplification (Fakruddin *et al.,* 2013). LCR is especially useful for the detection of specific DNA sequences, single nucleotide polymorphisms (SNPs), or mutations with high specificity and sensitivity. The amount of ligated product is directly proportional to the concentration of target DNA, facilitating quantitative analysis. Furthermore, multiple target sequences can be amplified and detected simultaneously in

a single reaction (Gerry *et al.,* 1999). The method is commonly utilized to specifically identify *L. monocytogenes*, but it has a limitation regarding the identification of foodborne pathogens, as it can also detect DNA from non-viable organisms (Min and Baeumner, 2002).

LAMP constitutes a novel amplification technique that operates at a constant temperature, eliminating the need for thermal cycling as required in PCR (Notomi *et al.,* 2000). The visual assessment of LAMP products simplifies detection processes, eliminating the need for electrophoresis. LAMP is a rapid and efficient method, with results available within an hour (Fakruddin *et al.,* 2013). It is characterized by high sensitivity and exceeding specificity alongside minimal equipment requirements (Notomi *et al.,* 2000). The technique is employed for the identification of several microorganisms responsible for foodborne illnesses, including *Salmonella, Campylobacter, Listeria, Legionella, S. aureus,* and verotoxin-producing *E. coli* (Mori and Notomi, 2009; Yang *et al.,* 2011). It is important to note that the method has certain limitations since optimizing for different pathogens can be challenging and because the interpretation of the results without adequate training can be problematic.

SDA is an isothermal DNA amplification technique that facilitates the rapid amplification of nucleic acids without the need for thermal cycling, as used in traditional PCR (Walker *et al.,* 1992). The method relies on the unique property of specific DNA polymerases to displace downstream DNA strands during synthesis (Walker *et al.,* 1992). The isothermal nature of the method eliminates the requirement for a thermal cycling apparatus, simplifying the procedure and reducing associated costs. Additionally, it offers high sensitivity due to the ability to detect low-abundance targets, making it suitable for diagnostic applications. SDA is employed for the detection of specific foodborne pathogens, such as *Salmonella, E. coli, L. monocytogenes*, and *Campylobacter* directly from food samples, for monitoring pathogens presence in food processing environments, and for quality control to ensure the absence of harmful microorganisms in food products before distribution. In contrast to LAMP, SDA uses nickase enzymes for strand displacement, whereas LAMP relies on loop primers and a DNA polymerase with strand-displacing activity.

NASBA represents a transcription-based amplification technique that operates under isothermal conditions, specifically developed to amplify RNA sequences (Fakruddin *et al.,* 2013). Unlike PCR, which requires thermal cycling, NASBA operates at a constant temperature, making it suitable for amplifying RNA targets without reliance on cycling apparatus (de Boer and Beumer, 1999; Kawasaki *et al.,* 2005). This characteristic simplifies the procedure and diminishes the associated. Nucleic Acid Sequence-Based Amplification serves as an effective for the rapid and sensitive detection of RNA sequences, offering notable advantages in diagnostic contexts, gene expression studies, and other fields requiring RNA examination. The technique is capable of producing substantial amounts of RNA in a short period and is capable of identifying low-abundance RNA targets. Originally conceived for viral identification, it has been extended for the detection of bacterial pathogens in diverse food products (Kawasaki *et al.,* 2005). NASBA proves to be a valuable method for the detection of RNA viruses, such as *Norovirus* and *Hepatitis A virus* in food samples, as well as monitoring of RNA-based pathogens in food processing environments thereby ensuring the absence of harmful RNA-based microorganisms in food products before distribution.

AFLP is a robust molecular marker technique employed across various applications, including DNA fingerprinting, genetic mapping, assessing genetic diversity, and phylogenetic analysis (Vos *et al.,* 1995). The method combines the principles of restriction enzyme cleavage and selective amplification of resultant restriction fragments, generating a unique set of DNA fragments that facilitate differentiation among diverse genotypes. The method is characterized by several advantages – it offers high resolution (generates numerous polymorphic markers, providing high-resolution genetic fingerprints) and applies to any organism due to the lack of prior DNA sequence data required (Paun and Schönswetter, 2012). AFLP can be utilized in the detection and characterization of foodborne microorganisms, enabling the identification of specific DNA markers unique to particular strains or species of microorganisms. Such markers can be used to identify pathogens present in food samples, even at low concentrations or when coexist with other microorganisms. AFLP can also distinguish strains within a species based on their genetic variability, which is crucial in epidemiological studies to trace the origin of foodborne outbreaks and to understand the genetic relatedness among isolates from varied sources or geographic locations. In research contexts, AFLP is utilized to examine the genetic diversity and evolutionary patterns of foodborne

microorganisms. Furthermore, it supports surveillance initiatives by public health organizations to monitor the prevalence and distribution of foodborne pathogens across different food sources and environments, holding relevance in both research and practical settings related to food safety and public health.

RAPD is a PCR-based technique employed for genetic fingerprinting profiling and identifying genetic polymorphisms without prior genomic information (Franklin *et al.,* 1999; Comeau *et al.,* 2004). The method utilizes short, random primers to amplify anonymous segments of genomic DNA, resulting in unique distinctive patterns of DNA fragments that facilitate the differentiation of individuals, strains, or species (Franklin *et al.,* 1999; Comeau *et al.,* 2004). A notable limitation of RAPD is its sensitivity to variations in experimental parameters, including PCR reaction conditions and primer selection. These factors can significantly influence the reproducibility and dependability of outcomes. Therefore, despite the method's simplicity, careful optimization and validation of protocols are essential for achieving consistent and reliable results. RAPD can be used in epidemiological studies to trace the origin of foodborne disease outbreaks (Gurakan *et al.,* 2008). By comparing the RAPD patterns of isolates from food samples and infected individuals, it is possible to determine whether the same microorganism strain was responsible for the illness. Analogous to AFLP, RAPD can be employed in quality control processes in food production establishments. It helps monitor the presence of specific pathogens or contaminants in food products, ensuring adherence to safety standards before consumer distribution.

REP-PCR is a molecular typing technique employed to differentiate bacterial strains based on the presence of repetitive extragenic palindromic sequences in their genomes (Versalovic *et al.,* 1994; Olive and Bean, 1999). These sequences are short, highly conserved, and dispersed throughout bacterial genomes, providing unique fingerprint patterns when amplified by PCR. The approach exhibits considerable discriminatory power (generates unique fingerprint patterns capable of distinguishing closely related bacterial strains) (Spigaglia and Mastrantonio, 2003), simplicity (an uncomplicated and rapid protocol that does not necessitate advanced equipment), and reproducibility (provides consistent results under optimized conditions). However, like most other PCR-based techniques, it requires careful optimization of experimental conditions and primer design to ensure results reliability. REP-PCR demonstrates greater reproducibility and specificity compared to RAPD due to the use of specific primers. It is capable of identifying particular specific strains or species of pathogens (De Brulin, 1992; Alves *et al.,* 2002) in complex food matrices, even at low concentrations. The method can be employed in quality control processes in food production facilities to monitor the presence of specific pathogens or to verify that food products are free from contamination by harmful microorganisms.

DTD PCR is a technique designed to ensure the elimination of contaminating DNA, particularly in RNA samples intended for reverse transcription PCR (RT-PCR) or other applications where DNA contamination may compromise the results (Nadugala and Rakshit, 2007). DNase treatment degrades any contaminating DNA, preserving only the RNA. This is particularly important in applications such as quantitative RT-PCR (qRT-PCR) to ensure accurate measurement of RNA levels. The technique can be applied to diverse sample types and downstream applications, avoids challenges associated with the prompt detection of foodborne pathogens, and the method has demonstrated sufficient specificity (Mukhopadhyay and Mukhopadhyay, 2002). Using DNase I enzyme treatment and PCR, false positive results obtained by amplifying DNA from dead cells are avoided. DTD PCR can be employed for the identification of viable cells of *L. monocytogenes, E. coli O157:H7, Salmonella Enterica,* and *V. parahaemolyticus* (Nadugala and Rakshit, 2007).

Biosensors-based methods

Biosensors are analytical devices that integrate a biological recognition element with a physicochemical transducer to detect the existence of specific analytes. In contrast to techniques based on nucleic acids and immunological approaches, biosensors are simple to use and do not require pre-enrichment of the sample (Singh *et al.,* 2013). Typically, a biosensor consists of three main elements: a biological recognition component which could be enzymes, antibodies, nucleic acids, or microorganisms that specifically interact with the target pathogen; a transducer (optical, electrochemical, piezoelectric or thermal) that converts the biological interaction into a measurable signal; and a signal processor that amplifies and interprets the signal to produce a comprehensible output, which indicates the presence or concentration of the pathogen. Biosensors represent a highly promising novel instru-

ment to overcome the prolonged standard procedures for detection of pathogenic microorganisms in foods products such as milk, cheese, meat (pork, chicken), raw vegetables and fruits (de Oliveira *et al.,* 2014). They have been developed and implemented to assess foodborne pathogens, including *E. coli O157:H7, S. aureus, Salmonella* species and *L. monocytogenes*, as well as various microbial toxins like staphylococcal enterotoxins and mycotoxins produced by genus *Fusarium* in peanuts, corn, wheat (Asiello and Baeumner, 2011).

The advantages of biosensors are high sensitivity and specificity (they can detect low concentrations of pathogens encompass high sensitivity and specificity due to the selectivity of the biological recognition component); rapidity (they can provide rapid results within minutes to hours, in contrast to traditional methods that may extend over several days, which is vital for timely intervention and prevention of foodborne illness outbreaks); portability (numerous biosensors are compact and portable, enabling on-site testing in food production facilities reducing the need for laboratory-based testing and enabling immediate corrective measures prior to consumption or further contamination) (Rasooly and Herold, 2006); continuous monitoring (they can be integrated into food production processes for continuous surveillance, ensuring real-time detection of contamination); multiplex detection (some of biosensors are capable of detecting multiple pathogens simultaneously, improving efficiency and comprehensive safety evaluations); and ease of operation (they are designed for user-friendly operation, often requiring minimal technical training). Notable challenges and considerations pertinent to biosensors include the stability of the biological recognition element, which can affect the shelf life and reproducibility of the sensors; the potentially high costs associated with the development and production of biosensors, although advancements in technology are reducing these expenses; and the possibility of complex food matrices interfering with sensor efficacy, necessitating careful sample preparation and calibration. Examples of biosensors in practical applications representing a significant advancement in detection technology include immuno sensors and enzyme-based biosensors, electrochemical biosensors, potentiometric biosensors, etc. Immunosensors exhibit minimal dependence on personal factors such as bias, fatigue, level of training, or visual impairment and have been developed to detect *Salmonella* species in various food products including eggs, chicken meat, and milk, as well as *S. aureus* enterotoxin (Zhao et al., 2014).

Concerning enzyme-based biosensors, the immobilization of enzymes offers several benefits such as elevated selectivity, rapid reaction time, high reproducibility, and enhanced storage stability. Moreover, the reagents employed in enzyme-based immunoassay are non-hazardous, stable, and sensitive (Chowdary Akkina *et al.,* 2023). This category of biosensors is used for the identification of foodborne pathogens including *L. monocytogenes, E. coli,* and *Campylobacter jejuni* (Chuang *et al.,* 2012; Chowdary Akkina *et al.,* 2023). Electrochemical biosensors are effectively employed for *Listeria* species, *S. aureus, B. cereus, E. coli* detection. They provide label-free, real-time, and rapid bacterial detection, often completed within a time frame exceeding just one hour (Pedrero *et al.,* 2009; Yue *et al.,* 2016). Additionally, the piezoelectric biosensors are used for the detection of *E. coli O157* in aqueous and food matrices. As technology advances, biosensors are expected to assume an increasingly critical role in food safety surveillance and quality assurance protocols.

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

MALDI-TOF MS represents a highly sensitive and rapid analytical technique employed for the assessment of biomolecules, particularly proteins, peptides, nucleic acids, and other large biomolecules while preserving their integrity (Hleba *et al.,* 2017). In the field of Microbiology, the method is used for the identification and classification of microorganisms (bacteria, viruses, fungi) based on their protein or peptide profiles, as well as the broad spectrum of mycotoxins (Elosta *et al.,* 2007). The applications in food microbiology for MALDI-TOF MS encompass the identification of bacteria isolated from dairy cow's milk, the detection of pathogenic bacteria in 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 (Fernández-No *et al.,* 2010; Böhme *et al.,* 2011). The advantages of MALDI-TOF MS compared to traditional culture methods include its unaffectedness by the culture conditions, formulations, required cultivation duration, and the inoculum amount needed for identification (Singhal *et al.,* 2015). Furthermore, the technique offers an easy results interpretation, and cost-effectiveness, and does not necessitate highly skilled laboratory personnel, in contrast to molecular and immunological detection methods (Everley

et al., 2010; Cherkaoui *et al.,* 2010).

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

UFLC-MS/MS serves as a sophisticated analytical methodology for the prompt identification of mycotoxins, including those produced by the *Fusarium* genus (Xing *et al.,* 2016; Singh and Mehta, 2020).

Ultrafast liquid chromatography refers to high-performance liquid chromatography (HPLC) systems that have been optimized for high-speed separations with enhanced resolution and sensitivity compared to traditional HPLC systems. UFLC systems typically use shorter columns filled with smaller particles (sub-2 μm), which facilitate expedited separations while preserving peak resolution (Gangadasu *et al.,* 2015). In the context of mycotoxin assessment, tandem mass spectrometry involves two sequential stages of mass spectrometry to enhance both sensitivity and specificity: ionization of analytes (mycotoxins) and selection of specific precursor ions by the fragmentation of these selected precursor ions, and detection of resultant fragment ions to produce a mass spectrum unique to the mycotoxin. The advantages of UFLC-MS/MS include rapid separation and analysis due to the ultrafast chromatographic system; enhanced sensitivity and selectivity afforded by tandem mass spectrometry; accurate identification and quantification of mycotoxins, including those produced by *Fusarium* spp., based on their unique fragmentation patterns; and its appropriateness for a wide range of mycotoxins and sample matrices encountered in food safety and agricultural assessments (Xing *et al.,* 2016; Singh and Mehta, 2020). Nevertheless, the exorbitant costs and the requirement for specialized expertise may be considered as limitations of this approach (Singh and Mehta, 2020).

Conclusion

The rapid identification of foodborne pathogens and their associated toxins is paramount for the assurance of food safety, the prevention of outbreaks, and the protection of public health. Innovations in molecular, immunological, and biosensor-oriented methodologies present distinct advantages over traditional foodborne pathogen detection strategies and have substantially enhanced the speed, sensitivity, and specificity of pathogen identification. Emerging technologies such as microfluidics, clustered regularly interspaced short palindromic repeats-based assays, and nanotechnology exhibit considerable potential for further enhancing detection capabilities.

Ongoing research and development in this field will facilitate the establishment of more efficient and sensitive detection methods, improving monitoring and control of foodborne diseases, ultimately reducing their societal impact.

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