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Advances and challenges in molecular detection of foodborne pathogens

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ABSTRACT

Foodborne pathogens remain a major global public health pandemic, contributing majorly to morbidity, mortality, and economic burden across the food supply chain. Contamination occurs at multiple stages from production to consumption, from microorganisms including bacteria, viruses, fungi, and parasites. Rapid and accurate detection of these pathogens is therefore critical to ensure food safety and prevent outbreaks. Traditional detection methods such as culture-based, biochemical, and immunological assays, although dependable, are often time-consuming, labor-intensive, and limited in sensitivity and specificity. These limitations have led to the development of advanced molecular detection techniques.

This review aims to evaluate current molecular approaches for the detection of foodborne pathogens, with a focus on nucleic acid-based methods, hybridization techniques, biosensors, and emerging technologies. Techniques such as polymerase chain reaction (PCR) and its variants, loop-mediated isothermal amplification (LAMP), DNA microarrays, and spectroscopy-based methods have enhanced detection speed, accuracy, and multiplexing capability. Also, biosensor-based platforms and nanotechnology technologies offer promising solutions for rapid, on-site detection. But several challenges including the presence of inhibitory substances, distinguishing viable from non-viable cells, high operational costs, and lack of standardization techniques are present.

Also, issues related to reproducibility, sensitivity under field conditions, and regulatory validation continue to restrict widespread implementation. Molecular detection technology represents transformative methods in food safety diagnostics. Future integration with emerging tools such as CRISPR-based systems, artificial intelligence, and multi-omics possess major potential for improving detection accuracy.

KEY WORDS

Foodborne pathogens; Molecular detection; DNA microarrays; Polymerase Chain Reaction; CRISPR-based systems

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Introduction

Food safety is a critical aspect of public health, ensuring food consumption does not have risks to human health. However, foodborne diseases continue as global burden due to contamination by pathogenic microorganisms. These include bacteria, viruses, fungi, and parasites that enters the food chain at various stages [1]. The consumption of contaminated food can lead to diseases and other severe systemic conditions. Microbial contamination is controlled by factors such as environmental hygiene, water activity, climate conditions, and human interventions. Pathogens like Escherichia coli, Salmonella spp., Listeria monocytogenes, and Campylobacter spp. are associated with foodborne diseases, majorly in animal-derived products and fresh produce [2]. Also, viruses such as norovirus and hepatitis A virus, along with fungi and parasites, contribute to food spoilage and disease transmission. These microorganisms often exhibit diverse survival strategies, including resistance to environmental stress and preservation techniques, making their detection increasingly complex [3].

Modern food systems involve complex distribution networks, which increase the chances of cross-contamination. Furthermore, the emergence of antibiotic-resistant strains and viable but non-culturable (VBNC) microorganisms poses additional challenges for detection and control. Traditional methods as culture-based methods allow for the detection of viable organisms through selective enrichment and differential plating. Similarly, biochemical tests and immunoassays such as enzyme-linked immunosorbent assay (ELISA) provide valuable information on metabolic and antigenic characteristics of pathogens. But these are time-consuming and may lack the sensitivity required to detect low levels of pathogens [4].

Molecular biotechnology has improved the detection methods through rapid, sensitive, and specific identification at the genetic level. These techniques are primarily based on the analysis of nucleic acids, allowing for the detection of pathogen-specific genes, virulence factors, and genetic markers. These methods offer advantages, including reduced detection time, high throughput capability, and the ability to detect non-culturable organisms [5]. Polymerase chain reaction (PCR) and its variants, such as real-time PCR and multiplex PCR, have become fundamental tools in molecular diagnostics due to their ability to amplify target DNA sequences with high specificity. Isothermal amplification techniques, including loop-mediated isothermal amplification (LAMP), further improves detection efficiency by eliminating the need for thermal cycling. Also, DNA microarrays, biosensors, and spectroscopic methods have improved the pathogen detection by simultaneous analysis of multiple targets and real-time monitoring. The addition of nanotechnology and biosensing platforms has also improved detection sensitivity and portability, helping on-site analysis in food processing [6].

Previous studies have proved the effectiveness of PCR-based methods in detecting specific pathogens such as E. coli, Salmonella, and Listeria through amplification of genetic

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regions such as 16S rRNA and virulence-associated genes. Real-time PCR has improved quantification accuracy by correlating fluorescence signals with DNA amplification, enabling rapid and precise pathogen identification. Similarly, isothermal amplification methods and DNA microarray technology has helped high-throughput detection of multiple pathogens, providing data into gene expression and pathogen diversity [7]. Spectroscopic techniques, including Fourier transform infrared (FTIR) and Raman spectroscopy, have been used for microbial fingerprinting and rapid identification of contaminants in food samples. Biosensor-based detection systems have also emerged as a promising alternative, combining biological elements with physicochemical transducers to produce measurable signals. These systems offer advantages such as high sensitivity, portability, and real-time analysis [8].

Although molecular detection techniques provide major improvements several limitations remain. One major limitation is the presence of inhibitory substances in complex food samples, which can interfere with nucleic acid amplification and reduce detection sensitivity. Also, many molecular methods are unable to distinguish between viable and non-viable cells, leading to potential false-positive results. High operational costs and the requirement for specialized equipment and trained individuals also restrict the widespread use of advanced detection technologies [9].

The objective of this review is to provide a comprehensive analysis of molecular detection methods for foodborne pathogens, highlighting recent advances, methodological strengths, and existing challenges. The review aims to compare conventional and modern detection techniques, with a particular focus on nucleic acid-based approaches, biosensor technologies, and emerging molecular tools [10].

Conventional Detection Methods

Culture-based methods

Culture-based methods are the most traditional approach for the detection of foodborne pathogens. These methods rely on the growth of microorganisms on selective and differential media under controlled environmental conditions. The detection process typically involves sequential steps, including pre-enrichment to revive stressed or injured cells, selective enrichment to increase the concentration of target pathogens, and subsequent plating on selective agar for isolation and identification. The growth characteristics, colony morphology, and biochemical behaviour of microorganisms are used to confirm pathogen identity. These are highly reliable and enable detection and characterization such as antimicrobial susceptibility testing and strain typing. However, these methods are time-consuming, often requiring 18-48 hours or longer depending on the organism. These are also labour-intensive and require skilled individuals and controlled laboratory conditions. The inability to detect viable but non-culturable (VBNC) organisms also limit their usage [11].

Biochemical and immunological methods

Biochemical and immunological methods act as alternative for pathogen detection by targeting metabolic activities and antigen–antibody interactions, respectively. Biochemical assays involve the identification of microorganisms based on enzymatic reactions and metabolic characteristics, such as carbohydrate fermentation, enzyme production, and substrate utilization. These are used with culture-based techniques to confirm microbial identity [12]. Immunological methods, including enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays, are based on the specific interaction between antigens and antibodies. ELISA helps the detection of pathogen-specific proteins or toxins through enzyme-mediated colorimetric reactions, allowing both qualitative and quantitative analysis. Lateral flow assays offer rapid, portable, and user-friendly detection, making them suitable for on-site testing. With these advantages, biochemical and immunological methods have limitations. Cross-reactivity between antibodies and non-target antigens can lead to false-positive results, while low antigen concentrations may reduce sensitivity. Also, these methods require pre-enrichment steps to achieve detectable levels of pathogens. Immunoassays also fail to detect damaged or stressed cells, and variability in antigen expression can affect accuracy [13].

Molecular Detection Techniques

Nucleic acid-based methods

Polymerase chain reaction (PCR) and variants

Polymerase chain reaction (PCR) is widely used molecular techniques for the detection of foodborne pathogens due to their high sensitivity, specificity, and rapid amplification capability. It is based on the amplification of target DNA sequences through cyclic processes of denaturation, annealing, and extension, helping the detection of micro quantities of pathogen-specific genetic material. Traditional PCR targets conserved regions such as the 16S rRNA gene for bacterial identification, as well as virulence-associated genes including stx, invA, hlyA, and uidA, which provide species-level specificity [14].

Multiplex PCR represents development of traditional PCR, allowing amplification of multiple target genes within a single reaction. This approach increases diagnostic efficiency with the detection of multiple pathogens or virulence markers concurrently, thus reducing time and reagent consumption. However, multiplex PCR requires careful optimization of primer design and reaction conditions to prevent preferential amplification and non-specific interactions [15].

Real-time PCR (qPCR) further improves detection by using fluorescent dyes or probes such as SYBR Green and TaqMan, allowing real-time monitoring and quantification of amplified DNA. The fluorescence intensity correlates with the amount of target DNA, helping both qualitative and quantitative analysis. It is useful for detecting RNA viruses, where RNA is first converted into complementary DNA (cDNA) before amplification (Table 1).

Table 1. Nucleic acid-based detection methods.

Method	Pathogen	Target Gene	Product Size
PCR	*E. coli*	stx gene	300 bp
PCR	*Salmonella*	MDH gene	261 bp
Real-time PCR	*E. coli*	stx2	115 bp
qPCR	*Listeria*	hlyA	2000 bp
RT-PCR	*Hepatitis A virus*	VP1	192 bp
Multiplex PCR	*Clostridium*	Multiple genes	Variable

Isothermal amplification techniques

Isothermal amplification techniques have emerged as alternatives to PCR, eliminating the need for thermal cycling. Loop-mediated isothermal amplification (LAMP) is one of the most widely practiced methods for detecting foodborne

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pathogens. LAMP uses four to six primers targeting distinct regions of DNA, along with a strand-displacing DNA polymerase, to obtain high amplification efficiency within a short time. The method generates a large amount of DNA product, often visible through turbidity or colorimetric changes [16].

Recombinase polymerase amplification (RPA) is another isothermal technique that operates at low temperatures (37-42 °C) and provides rapid amplification within minutes. RPA uses recombinase enzymes for primer binding to target DNA, followed by strand displacement and amplification. Both LAMP and RPA are highly sensitive and suitable for point-of-care and field applications due to their minimal equipment requirements [17].

Hybridization and microarray-based methods

DNA hybridization techniques

DNA hybridization techniques rely on the specific binding of complementary nucleic acid sequences for the detection of foodborne pathogens. In these methods, labeled single-stranded DNA or RNA probes are designed to hybridize with target sequences present in the pathogen genome. The high specificity of probe-target interaction helps accurate identification of microorganisms [18].

Fluorescent labeling is commonly used in hybridization assays, where probes are tagged with fluorophores that emit signals upon successful binding to the target nucleic acid. Detection is achieved through fluorescence-based systems, allowing rapid and sensitive identification of pathogens. Various detection formats, including colorimetric, electrochemical, and chemiluminescent assays, have also been developed to improve versatility and sensitivity [19].

DNA microarray and high-throughput detection

DNA microarray technology provides a high-throughput extension of hybridization-based detection, ensuring simultaneous analysis of multiple pathogens and genetic markers. In this method, thousands of immobilized probes are arranged on solid substrates such as glass slides or silicon chips, allowing parallel hybridization with labeled nucleic acid targets. This facilitates the detection of diverse microbial species, virulent genes, and antibiotic resistance determinants within a single assay. Microarrays are useful for gene expression profiling and comparative genomic analysis, providing insights into pathogen behavior, diversity, and adaptation in food environments. The ability to detect multiple targets simultaneously makes it highly suitable for large-scale food safety monitoring (Table 2).

Table 2. Array-Based Detection Methods.

Pathogen	Target	Platform
E. coli	ssrA gene	Microfluidic device
Salmonella	Sdf gene	DVD chip
Campylobacter	hipO gene	DVD chip
Listeria	iap gene	Strip plate

Spectroscopy-based molecular detection

FTIR and Raman spectroscopy

Spectroscopy-based techniques have developed as powerful tools for the rapid and non-destructive detection of foodborne pathogens through molecular-level analysis. Fourier Transform Infrared (FTIR) spectroscopy and Raman spectroscopy are widely used due to their ability to generate unique biochemical fingerprints of microbial cells. FTIR operates in the mid-infrared region and measures the vibrational transitions of molecular bonds, producing specific absorption spectra corresponding to cellular components such as lipids, proteins, nucleic acids, and carbohydrates. This ensures identification and differentiation of microbial species based on their biochemical composition [20].

Raman spectroscopy depends on inelastic scattering of monochromatic light, providing detailed information about molecular structure and composition. Advanced variants such as Surface-Enhanced Raman Scattering (SERS) improves signal sensitivity for detection of low concentrations of pathogens [21].

Hyperspectral imaging

Hyperspectral imaging (HSI) is an advanced analytical technique that integrates imaging and spectroscopy to provide both spatial and spectral information for each pixel in a sample. This ensures real-time monitoring of food quality and microbial contamination during processing and storage. HSI operates across multiple spectral regions, including near infrared and mid-infrared wavelengths, allowing detection of biochemical and structural changes in food products. The use of hyperspectral imaging with artificial intelligence and machine learning algorithms has improved its capability for rapid and automated pathogen detection. These can analyze large datasets to identify contamination patterns with high accuracy [22].

Biosensor-based detection

Biosensors are analytical devices designed for the rapid and specific detection of foodborne pathogens. The core components of a biosensor include bioreceptors and transducers. Bioreceptors, such as antibodies, nucleic acids, enzymes, aptamers, or bacteriophages, bind to target analytes, including microbial cells, toxins, or genetic material. This interaction generates a biochemical signal, which is then converted by the transducer into a measurable output, such as electrical, optical, or mechanical signals. The efficiency of a biosensor depends on the nature of the bioreceptor, surface immobilization techniques, and signal amplification mechanisms, ensuring highly sensitive detection even at low pathogen concentrations [23].

Biosensors used in food pathogen detection are classified into electrochemical, optical, and piezoelectric types based on the nature of the signal transduction. Electrochemical biosensors are among the most widely used due to their high sensitivity, rapid response, and ease of miniaturization. These sensors detect changes in current voltage, or impedance resulting from biochemical interactions between the target pathogen and the bioreceptor. Variants such as amperometric, potentiometric, and impedimetric biosensors are commonly employed for detecting pathogens like Escherichia coli and Salmonella spp [24].

Optical biosensors operate by detecting changes in light properties, including absorption, fluorescence, and refractive index, upon analyte binding. Techniques such as surface plasmon resonance (SPR), fluorescence-based detection, and

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colorimetric assays enable real-time and label-free detectionof pathogens with high specificity. Piezoelectric biosensors, including quartz crystal microbalance (QCM) systems, detect mass changes on sensor surfaces by measuring variations in frequency, allowing sensitive detection of microbial binding events (Table 3).

Table 3. Biosensors for Pathogen Detection.

Biosensor Type	Pathogen	Detection Limit	Time
Amperometric	*E. coli*	10 cells/mL	—
Voltammetric	*Listeria*	9 CFU/mL	1 min
Potentiometric	*Salmonella*	5 cells/mL	1 h
Impedimetric	*E. coli*	20 CFU/mL	30 min
Optical (SPR)	Campylobacter	~130 CFU/mL	—
SERS	*Salmonella*	4 CFU/mL	40 min
Piezoelectric	*Campylobacter*	20–30 CFU/mL	30 min

Comparative Analysis

Performance comparison

The performance of detection methods for foodborne pathogens is generally evaluated on sensitivity, specificity, and detection speed. Traditional culture-based methods are highly specific and dependable for confirming viable microorganisms but has low sensitivity when pathogen concentrations are minimal and require extended incubation periods. Biochemical and immunological methods improve detection speed and allow identification of pathogen-specific metabolic or antigenic markers but, their sensitivity may be limited by low analyte concentrations and potential cross-reactivity [25].

Nucleic acid-based methods such as PCR and its variants demonstrate superior sensitivity and specificity by targeting conserved genetic sequences and virulence-associated genes. These detect even low levels of pathogens within hours, significantly reducing turnaround time. Isothermal amplification and biosensor-based detection further enhances speed and allow near real-time analysis. However, variations in assay performance may arise due to matrix interference, primer design, and platform limitations [26].

Industrial comparison

Detection methods in industrial food safety systems depends on factors such as cost, scalability, ease of use, and suitability for field deployment. Culture-based methods remain widely used in regulatory frameworks due to their low cost and established standardization. Biochemical and immunological assays offer moderate cost and improved output, making them suitable for daily screening in food processing environments [27].

Molecular methods often require expensive instrumentation, skilled individuals, and controlled laboratory conditions, which may limit their widespread usage in controlled environment. But advancements in portable PCR systems and isothermal amplification techniques have improved their usage for field applications. Biosensor-based platforms and microfluidic devices provide promising solutions for real-time, on-site detection, offering scalability and automation. Table 4 provides comparative analysis of detection methods.

Table 4. Comparison between Pathogens Detection Methods.

Pathogen	Source	Conventional Methods	Advanced Methods
E. coli	Milk, meat, fruits, vegetables	ELISA, lateral flow, PCR	Biosensors, LAMP, aptamers
Salmonella spp.	Meat, poultry, dairy	Immunomagnetic assay, PCR	Biosensors, LAMP
Clostridium spp.	Milk, canned foods	Culture, ELISA, PCR	Biosensors, LAMP
Vibrio spp.	Seafood, dairy	qPCR, ELISA	LAMP, DNA hybridization
Shigella spp.	Milk, seafood	ELISA, PCR	Aptamers, nanobiosensors
Bacillus spp.	Meat, dairy	PCR, immunoassay	Silicon biosensor
Campylobacter spp.	Meat, poultry	PCR, immunoassay	Nanoplasmonic sensor
Adenovirus	Fruits, vegetables	RT-PCR	LAMP, biosensor
Norovirus	Meat, dairy	qPCR, RT-PCR	Aptamer-based detection
Hepatovirus	Meat products	RT-PCR, ELISA	Biosensor, LAMP

Challenges And Limitations

Technical challenges

One of the primary issues is the complexity of food matrices, which contain diverse components such as fats, proteins, polysaccharides, and polyphenols that can interfere with nucleic acid extraction and amplification. These substances act as inhibitors in PCR-based assays, reducing amplification efficiency and potentially leading to false-negative results. Another major challenge is the false-positive and false-negative results. False positives may arise due to contamination, non-specific amplification, or cross-reactivity with non-target organisms. False negatives can occur due to low pathogen load, inefficient DNA extraction, or the presence of inhibitory compounds. Many molecular methods also fail to differentiate between viable and non-viable cells, which may result in inaccurate assessment of contamination risks [28].

Economic and infrastructure barriers

The widespread implementation of advanced molecular detection techniques is often limited by economic and infrastructural complexities. High costs associated with specialized equipment, reagents, and maintenance reduce accessibility, particularly in developing regions and small-scale food industries. Molecular platforms such as real-time PCR, sequencing technologies, and advanced biosensors require well-equipped laboratories and trained individuals. Also, the need for continuous calibration, quality control, and technical

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expertise restricts the scalability of these methods for daily use [29].

Standardization and regulatory issues

A major barrier in the adoption of molecular detection methods is the lack of standardized protocols and regulatory frameworks. Variability in sample preparation, primer design, amplification conditions, and detection platforms can lead to inconsistent results across laboratories. Many molecular methods, despite demonstrating high sensitivity and specificity in research, require extensive validation before being accepted for daily use in food safety monitoring. The absence of universally accepted guidelines for validation and quality assurance delays their implementation [30].

Future Perspectives

Integration with AI and big data

The integration of artificial intelligence (AI) and big data analytics is expected to transform foodborne pathogen detection by using smart, data-driven diagnostics. Advanced computational algorithms can analyse complex datasets generated from molecular techniques such as PCR, sequencing, and spectroscopy, improving detection accuracy and predictive capability. Machine learning models can identify contamination patterns, predict outbreak risks, and enhance decision-making in food safety management systems. Furthermore, AI-assisted interpretation of spectral and genomic data helps rapid identification of pathogens with minimal human intervention [31].

Portable and point-of-care systems

The development of portable and point-of-care diagnostic systems represents a major advancement toward rapid and on-site detection of foodborne pathogens. Technologies such as lab-on-a-chip platforms and microfluidic devices integrate sample preparation, amplification, and detection into compact systems, enabling efficient analysis outside traditional laboratory settings. These systems reduce detection time, minimize reagent consumption, and require minimal technical expertise. Along with isothermal amplification techniques and biosensor-based detection, portable devices provide results in real-time [32].

Multi-omics and precision food safety

The application of multi-omics methods, including genomics, transcriptomics, proteomics, and metabolomics, provide detailed insights into pathogen diversity, virulence mechanisms, and interactions with food matrices and environmental factors. Whole-genome sequencing and metagenomics support the identification of emerging pathogens and antimicrobial resistance genes, while proteomic and metabolomic analyses reveal functional and metabolic states of microorganisms. Integration of multi-omics data allows for precise characterization of contamination sources and monitoring of microbial communities [33].

Conclusion

The detection of foodborne pathogens had significant transformation with molecular biotechnology. Traditional methods, including culture-based, biochemical, and immunological approaches, have acted as foundational tools for pathogen identification, but their limitations in application, sensitivity, and labour intensity restrict their effectiveness in modern food safety systems. In response, molecular detection techniques such as PCR and its variants, isothermal amplification, hybridization-based methods, and biosensor technologies have demonstrated superior performance in terms of rapidity, specificity, and sensitivity. Despite these developments, several challenges remain, including interference from complex food structures, inability to differentiate between viable from non-viable cells, high operational costs, and lack of standardized protocols. Addressing these limitations is essential for the widespread implementation of molecular diagnostics in routine food safety monitoring. Future research should focus on using emerging technologies such as artificial intelligence, portable diagnostic platforms, and multi-omics approaches to develop cost-effective, robust, and field-deployable systems.

Disclosure Statement

No potential conflict of interest was reported by the author.

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