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Advanced Detection Techniques of Foodborne Pathogens

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Food safety safeguards consumer health by preventing foodborne diseases. Several key factors such as microbial contamination, chemical and nutritional alterations, biodiversity, water activity, climate variations, and environmental hygiene can influence the safety of food [1]. Among the various factors, foodborne pathogens are the main contributors to reducing the acceptability of food for consumption, often causing foodborne illnesses. These diseases, caused by pathogenic organisms, pose significant health risks on a global scale [2].

Bacteria, viruses, fungi, yeast, and parasites are the major pathogens responsible for foodborne illnesses. They contaminate food items such as fresh produce, raw fish, meat, poultry, eggs, and dairy at different stages, including cultivation, harvesting, processing, storage, transportation, and preservation. Once ingested, these pathogens enter the body through the gastrointestinal tract and trigger various foodborne diseases [3]. Escherichia coli, Salmonella spp., Clostridium spp., Bacillus spp., Vibrio spp., Shigella spp., Pseudomonas spp., Listeria spp., Cyclospora spp., Campylobacter spp., Staphylococcus spp., Klebsiella spp., and Acinetobacter spp. are major bacterial species that cause foodborne illnesses in humans through their pathogenic effects. Among these, *Escherichia coli* is a significant pathogen linked to diseases such as thrombotic thrombocytopenic purpura (TTP), hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Common sources of *E. coli* include raw or undercooked meat, unpasteurized milk, fresh produce, and vegetables. Notably, the strain *E. coli* O157:H7 produces Shiga toxin, which is responsible for severe human infections[4]

Detecting foodborne pathogens is essential for maintaining food safety within the food industry. Research plays a vital role in identifying and controlling these pathogens before they cause major outbreaks. Pathogen detection is also a key requirement for regulatory compliance in food production and processing. Over time, several methods have been developed to detect foodborne pathogens, and these techniques are classified into different categories based on their main strengths and limitations[5]. While traditional methods offer good selectivity and sensitivity, they are time-consuming and require intensive labor. To overcome these limitations, several advanced approaches have been developed for detecting and identifying foodborne pathogens. These include biosensor-based techniques, nucleic acid sequence-based methods such as DNA microarray and DNA hybridization, spectroscopic and instrument-based methods, aptamer-based techniques, loop-mediated isothermal amplification (LAMP), and metagenomic assays.

Advanced Methods

Hybridization-based method

The hybridization-based method is a sophisticated molecular technique that detects specific pathogen genes by utilizing complementary DNA and RNA sequences. In this approach, synthetic complementary DNA fragments either single- or double-stranded known as probes, are tagged with fluorescent dyes and used to bind to the nucleic acids of targeted pathogens.

It is a fast, stable, and highly sensitive fluorescence-based technique that detects pathogens by signaling their presence during amplification [6]. Hybridization can be applied in various assays, including fluorometric, colorimetric [7], electrochemical and chemiluminescent methods[8]. In this method, a cascade reaction generates two hairpins that form long-nicked DNA double helices with the probes. The modified hairpin probes produce signals upon binding with the hybridized product, indicating the presence of pathogens. Listeria monocytogenes in ready-to-eat foods can be detected using two probes—MNP and 250-probe—through a magnetic DNA-based hybridization method, achieving a detection limit of 50 CFU/mL within 2 hours. E. coli O157:H7 can be detected by hybridizing the target single-stranded DNA with an aptamer and probes, achieving a detection limit of 8.35×10^2 CFU/mL in milk [9].

Array-Based Method

Array techniques detect pathogens by analyzing DNA sequences, RNA transcripts, and proteins through in situ or ex situ synthesis of biomolecules on a solid substrate. This method identifies interactions between the target and probe, enabling spatial screening in a microarray format . Array technology offers advantages such as speed, sensitivity, high accuracy, and the ability to process many samples simultaneously [10]. DNA-based arrays (microarrays) and other array-based approaches are widely used for detecting pathogens in food samples.

DNA Microarray: DNA microarray is a widely used technique for detecting and quantifying pathogens by assessing gene expression levels. This advanced technology involves immobilizing nucleic acids such as oligonucleotides, genomic DNA, or cDNA on solid surfaces like nylon membranes, glass slides, or silicon chips, which are then exposed to complementary nucleic acid probes [11].

Alternative Array-Based Detection: Carbohydrate-based arrays are effective tools for detecting pathogens through carbohydrate–protein interactions. For instance, mannose-coated microarrays can identify E. coli and Salmonella spp., highlighting allelic variations among pathogens . Lectin-based microarrays detect pathogens by exploiting the interaction between glycans and bacterial lipopolysaccharides. These lectin arrays allow rapid identification and differentiation of various bacteria based on glycosylation patterns. Gram-negative bacteria, including Campylobacter jejuni, Escherichia coli, Lactobacillus spp., and Pseudomonas spp., can be identified using lectin-based microarrays [12].

Spectroscopy Technique

Spectroscopy is an analytical method that examines the interaction between electromagnetic radiation and matter to perform qualitative and quantitative analysis . It is commonly employed for the rapid detection of pathogens in food. Techniques such as Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and hyperspectral imaging have been used to identify microbial contamination [13]. While this method is highly sensitive to molecular surfaces, it has drawbacks, including being time-consuming and susceptible to interference from fluorescence.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a method that utilizes the infrared (IR) spectrum, specifically in the mid-infrared range (400–4000 cm^{-1}). It provides a rapid and fairly accurate biochemical fingerprinting approach for detecting foodborne pathogens, often combined with various statistical analysis techniques. The FTIR spectroscopy technique works by detecting the vibrations of molecules excited by an infrared light beam, with the resulting absorbance spectrum corresponding to specific biochemical or chemical compounds. It can assess biochemical changes in food and reveal the presence of microbial metabolites. This method is effective for rapid bacterial strain typing , evaluating meat quality, and monitoring microbiological spoilage in seafood. FTIR has been used to detect *Bacillus cereus*, *Bacillus cytotoxicus*, *Bacillus thuringiensis*, *Bacillus mycoides*, and *Bacillus weihenstephanensis* [14].

Raman Spectroscopy: Raman spectroscopy is a spectroscopic technique that analyzes vibrational, rotational, and low-frequency modes within a system. It relies on the principle

that when laser light ranging from visible to near-infrared or near-ultraviolet is elastically scattered by molecules, a small portion of the light undergoes energy transfer between the incident photons and the molecules [15].

Hyperspectral Imaging Technique (HSI): Hyperspectral imaging (HSI) is an emerging technology used for real-time monitoring of food quality. It captures the spectrum of each pixel in an image to identify specific analytes. By detecting microbial contamination or food spoilage during processing, HSI enables rapid and precise assessment of food quality [16].

Biosensor

A biosensor is an analytical tool designed to detect specific analytes (target pathogens) using biological recognition elements that generate a measurable signal, which is then captured, amplified, processed, and analyzed by a physical transducer. Biosensor-based detection is simple, cost-effective, fast, and highly selective for identifying foodborne pathogens. Unlike conventional methods—which are slower, labor-intensive, less specific, and unable to detect VBNC (viable but nonculturable) organisms—biosensors offer a more efficient and reliable approach for pathogen detection in food [17].

Electrochemical Biosensor: An electrochemical biosensor converts chemical information into an analytical signal. In this system, bioreceptors are immobilized on the electrode surface to recognize the target analyte. When the analyte binds to the receptors, it alters the electrical properties, generating a signal through chemical reactions such as oxidation and reduction. This technique enables both qualitative and quantitative analysis of the target. Key advantages include simplicity, quick response, high sensitivity, and enhanced stability [18].

Optical Biosensor: An optical biosensor detects pathogens by measuring optical signals such as changes in light phase, frequency, or amplitude through interactions between an analyte and a bioreceptor . The bioreceptor binds to the analyte, and the transducer converts this molecular interaction into a measurable optical signal. Detection relies on different optical phenomena, including absorption, reflection, refraction, infrared response, polarization, dispersion, chemiluminescence, fluorescence, and phosphorescence. The main components of an optical biosensor include a light source, a transmission medium, a biofunctional surface containing the bioreceptor, and an optical detection system [19].

Advanced Biosensors for Detecting Foodborne Pathogens

The biosensors discussed above enable rapid and specific detection of foodborne pathogens in food samples. However, they face certain challenges, including high cost, limited stability, reproducibility issues, and complex instrumentation. Recent progress in nanomaterials and interdisciplinary multifunctional approaches has led to the development of advanced biosensors for pathogen detection in food [20]. These include nanomaterial-based, microfluidics-based, aptamer-based, portable device-integrated, and smartphone-based biosensors, which offer fast, sensitive, and user-friendly on-site detection.

Nanomaterial-based biosensor: Nanomaterial-based biosensors are innovative devices that utilize unique electrical, optical, mechanical, and thermal properties of nanomaterials to detect foodborne pathogens. These biosensors enable rapid, portable, highly sensitive, and on-site detection of target microorganisms [194]. The types of nanomaterials employed include: (1) metallic nanomaterials such as gold (AuNPs), silver (AgNPs), platinum (PtNPs), and palladium (PdNPs); (2) metal oxide nanomaterials like cerium dioxide (CeO_2) and copper oxide (CuO); (3) magnetic nanomaterials (MNPs) including NiO, Co_3O_4, and Fe_2O_3; (4) carbon nanomaterials such as carbon nanotubes (CNTs) and graphene; (5) polymer-based nanomaterials including dendrimers, conducting polymers, and molecularly imprinted polymers; (6) quantum dots (QDs); (7) upconverting nanomaterials (UCNPs); (8) transition metal dichalcogenides (TMDs); and (9) other carbon-based nanostructures [21].

Conclusions

This review highlights both conventional and advanced techniques for detecting foodborne pathogens. Timely detection is critical to maintaining food safety and preventing foodborne illnesses. Conventional approaches are limited to laboratory settings and face drawbacks such as being time-intensive, resource-demanding, prone to contamination, and requiring skilled

personnel. In contrast, advanced methods offer benefits like speed, simplicity, cost-effectiveness, sensitivity, and rapid data analysis. Nonetheless, both approaches carry their own merits and limitations. Hence, the chosen method must ensure accuracy, reliability, cost-efficiency, selectivity for specific pathogens, and consistency in results.

Despite progress, challenges remain in scaling these techniques for industrial use, particularly in achieving rapid and dependable sample preparation and developing intelligent detection platforms. Moreover, designing new detection devices must account for the type of food and its nutritional composition (proteins, fats, fibers, and carbohydrates). Thus, tailored sample preparation strategies and analytical tools are necessary for pathogen identification in diverse food products. Future investigations should focus on overcoming these analytical gaps to create more comprehensive detection systems. Furthermore, attributes such as precision, accuracy, validation, sustainability, affordability, and commercial applicability should guide the development of next-generation pathogen detection methods.

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